Peripheral blood mucosal-associated invariant T (MAIT) cells in tuberculosis patients and healthy *Mycobacterium tuberculosis*-exposed controls

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Abstract (200 words)

Background: In human blood, mucosal-associated invariant T (MAIT) cells are abundant T cells, which recognize antigens presented on ~~the~~ non-polymorphic major histocompatibility complex-related 1 (MR1) ~~molecule~~molecules. MAIT cells are activated by mycobacteria, and prior human studies indicate that blood frequencies of MAIT cells, defined by cell surface markers, decline during TB disease, consistent with redistribution to the lungs.

Methods: We tested whether frequencies of blood MAIT cells were altered in patients with TB disease relative to healthy *Mycobacterium tuberculosis* (*Mtb*)-exposed controls from Peru and South Africa. We quantified their frequencies using MR1 tetramers loaded with 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU).

Results: Unlike findings from prior studies, frequencies of blood MAIT cells were similar among TB-disease patients, latent and uninfected controls. In both cohorts, frequencies of MAIT cells defined by MR1-tetramer staining and co-expression of CD161 and the T cell receptor alpha variable gene TRAV1-2 were strongly correlated. Disease severity captured by body mass index or TB disease transcriptional signatures ~~of TB disease~~ did not correlate with MAIT cell frequencies in TB patients.

Discussion: ~~Our data indicate that blood frequencies of~~ MR1-restrictied MAIT cells~~, unlike~~ are detected at similar levels with tetramers or surface markers. Unlike MHC-restricted T cells, blood frequencies of MAIT cells are poor correlates of TB disease~~. The findings do not preclude~~, but may play roles ~~of MAIT cells~~ in ~~TB~~ pathophysiology.

Key Words: MR1, tetramer, MAIT, tuberculosis, household contacts

Introduction

According to the World Health Organization, *Mycobacterium tuberculosis* (*Mtb*) is the leading cause of death from infectious disease globally[1], with one quarter of the world's population estimated to be infected with *Mtb*[2]. The commonly used interferon-γ release assay (IGRA), measures MHC-restricted αβ T cell responses to *Mtb* antigens as a reliable diagnostic test for infection[3]. Thus, it is broadly accepted that expansion of antigen-specific, MHC-restricted T cell populations in blood is the usual human response to *Mtb* infection.

Recent studies showed that non-MHC encoded, antigen presenting molecules can present mycobacterial antigens to activate αβ T cell responses in experimental *Mtb* infections[4, 5, 6]. Prominent among these T cell types are mucosal-associated invariant T (MAIT) cells, which recognize MR1 and are particularly abundant, comprising ~0.1 to 10% of circulating T cells in healthy individuals[7]. MAIT cell antigens include riboflavin derivatives and other metabolites[8, 9, 10]. Unlike highly polymorphic MHC genes, MR1 is nearly monomorphic in humans[6]. Thus, MAIT cells can recognize antigens presented by antigen presenting cells from any human, and hence are known as donor-unrestricted T cells (DURTs)[6].

The abundance of MAIT cells and reactivity towards mycobacterial antigens[11] raise the possibility that MAIT cells could play roles in controlling natural *Mtb* infection. Unlike conventional T cells, MAIT cell frequencies were reported to decline in the peripheral blood of TB patients, relative to *Mtb*-unexposed controls[12, 13, 14, 15, 16], or following *Mtb* infection of mice[15] and non-human primates[17]. This outcome is consistent with their suspected relocation to the lungs or other sites of infection *in vivo*. ~~Consistently~~Consistent with this prediction, a recent study reported that MAIT cell frequencies were enriched in the bronchoalveolar lavage of TB patients[18]. However, these studies are relatively small and rely on assays that detect activation by MR1[16] and expression of the TRAV1-2 variable region of the T cell receptor alpha (TCRα),

which is frequently rearranged with the TCRα joining region TRAJ33 in MAIT cell[19, 20]. However, there are examples of TRAV1-2$^-$ T cells that recognize MR1[21, 22, 23]. Conversely, TRAV1-2$^+$ TCRs can also recognize antigens presented by MHC or CD1b proteins[4]. Thus, TCR sequence-independent methods to unequivocally identify MR1-binding T cells are important. MR1 tetramers loaded with the vitamin B-like metabolite 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU) allow direct identification of MAIT cells based on binding specificity to MR1 and the antigenic ligand[7, 8, 20, 23].

A third definition of MAIT cells relies on expression of cell surface markers rather than activation by MR1 or TRAV1-2 expression. MAIT cells are predominantly CD8$^+$ or CD4$^-$CD8$^-$ T cells, with a small CD4$^+$ fraction[7, 24], and co-express the C-type lectin CD161[25] and CD26 ectopeptidase[7, 26, 27]. Thus, clinical studies have tracked cells co-expressing CD3 and CD161, [13,12] or CD26[14], usually in combination with TRAV1-2[7]. These cell surface marker-defined MAIT cells, sometimes called 'phenotypic' MAIT cells, emerged mainly from human studies, where functional responses to MR1-ligand complex were not feasible, or before MR1-tetramer development.

The emergence of parallel TCR-, tetramer- and phenotype-based criteria raises basic questions about the best MAIT cell definition and the concordance of these measurements in humans. MR1-tetramers are now well validated to identify and characterize sub-populations of MAIT cells[7], or in mechanistic studies to identify MAIT cell functions *in vitro*[21]. However, MR1 tetramers have not been applied to large cohorts of TB patients. Since prior studies of MAIT cells in TB patients and controls relied on expression of TRAV1-2 and CD161[12, 13, 14], we undertook a study of patients with TB disease and healthy *Mtb*-exposed participants from two geographically distinct populations to measure peripheral blood MAIT cells using either TRAV1-2 and CD161 co-expression or 5-OP-RU-loaded MR1 tetramers to test whether blood MAIT cell frequencies were altered in TB disease.

Materials and Methods

Participants were enrolled in two cross-sectional studies in Peru and South Africa. Adults and parents or legal guardians of minors provided informed consent, while minors provided assent.

Peruvian Household Contacts Cohort

Bacillus Calmette-Guérin (BCG)-vaccinated HIV-uninfected participants were recruited through Socios En Salud (SES), from settlements around Lima, Peru~~,~~[28]. Participants included adults with recently diagnosed sputum culture-positive, drug-sensitive pulmonary TB disease (TB disease, n=50) and asymptomatic household contacts assessed within two-weeks of diagnosing the index case (n=100). Contacts were evaluated for TB disease symptoms at enrolment and excluded if clinical symptoms of TB disease were present. Healthy household contacts were assessed for *Mtb* infection using the QuantiFERON TB-Gold In-Tube assay (Qiagen) and considered latently *Mtb*-infected if their IFNγ levels were ≥0.35 international units (IU)/mL (n=50) and uninfected if <0.35 IU/mL (n=50). Peripheral blood mononuclear cells (PBMC) were isolated from 50 mL of venous blood using ficoll, then cryopreserved and shipped to the Brigham and Women's Hospital for storage and analysis by flow cytometry. The Institutional Review Board of the Harvard Faculty of Medicine and Partners Healthcare (protocol number IRB16-1173), and the Institutional Committee of Ethics in Research of the Peruvian Institutes of Health approved this study protocol.

South African Cohort

We recruited HIV-negative adults who received BCG vaccination at birth from communities in the town of Worcester ~~town~~ near Cape Town, South Africa into the previously described Cross-sectional TB Cohort (CTBC)[28,29]. Individuals with newly diagnosed sputum Xpert MTB/RIF-positive TB disease (TB disease, n=19) and asymptomatic, QuantiFERON TB-Gold In-Tube-

positive latently *Mtb*-infected (latent, n=19) adults were enrolled. We did not recruit any uninfected participants in this study arm~~.~~ because high TB prevalence rates limit recruitment of reliably uninfected subjects. PBMC samples were processed from blood collected in Vacutainer CPT mononuclear cell separation tubes (BD) and cryopreserved for flow cytometry analysis in Cape Town. The CTBC study protocol was approved by the University of Cape Town Human Research Ethics Committee (HREC 761/2015).

Flow cytometry analysis

For Peruvian samples, MR1 monomers loaded with 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU), or 6-formylpterin (6-FP) as a negative control, were produced at The University of Melbourne, Australia as described[8, 20]. To generate MR1 tetramers, 1μg of MR1 protein was tetramerized using 6 aliquots of 1μL Biolegend Streptavidin-PerCP-Cy5.5 (Biolegend) diluted 1:4 in phosphate buffered saline (PBS). Cryopreserved samples from Peru were thawed at 37°C, and approximately $3X10^6$ cells were stained with a fixable blue viability cell stain (ThermoFisher Scientific) according to manufacturer's instructions, followed by MR1 tetramers in staining media (5% bovine serum albumin and 0.01% sodium azide in PBS) for 10 minutes at room temperature in the dark, followed by cell surface antibodies **(Supplementary Table 1A)** for 5 minutes. Subsequently, cells were treated with unconjugated OKT-3 antibody, and incubated for 5 minutes at room temperature followed by 10 minutes at 4°C. Cells were fixed in 2% paraformaldehyde (Electron Microscopy Sciences) in PBS for 20 minutes. For South African samples, MR1 tetramers were obtained from the National Institutes of Health (NIH) Tetramer Core Facility, and used to stain peripheral blood mononuclear ~~cells~~cell (PBMC) samples at a 1:200 dilution in 50μL at room temperature for 45 minutes, followed by antibodies at 4°C for 30 minutes **(Supplementary Table 1B)**.

RNA processing and TB Risk score analysis

For the Peruvian cohort, RNA samples were extracted from 10^6 PBMCs using the RNeasy kit (Qiagen), and frozen aliquots were shipped to the University of Cape Town. For the South African cohort, 2.5mL of venous blood was drawn directly into PAXgene blood RNA tubes and frozen. RNA was extracted using PreAnalytiX PAXgene Blood RNA extraction kit (Qiagen). All RNA samples were reverse transcribed to cDNA using EpiScript RNase H-Reverse Transcriptase (Lucigen), pre-amplified with a master mix of Taqman primer probes **(Supplementary Table 2)** in 2X PCR master mix (ThermoFisher), and analyzed by microfluidic real-time PCR using the Biomark 192.24 gene expression integrated fluidic circuit (IFC) system (Fluidigm) for multiplex analysis of 24 assays and 196 samples as previously described[29,30].

Data analysis

Flow cytometry data were analyzed in Flowjo version 10.4.2. Computation of transcriptomic signature scores from qRT-PCR cycle threshold values and generalized linear regression models were performed in R versions 3.5.1-3.6 for Mac. Other statistical analyses were performed in GraphPad Prism versions 7-8.

Results

From 145 enrollees, 135 PBMC samples passed quality control for yield, viability and sterility: 48 patients with TB disease, 48 asymptomatic uninfected and 49 latently *Mtb*-infected (latent) adults **(Table 1A and Supplementary Table 3A)**. Participants with latent TB were older than either uninfected participants (*Mann-Whitney p=0.022*), or patients with TB disease (*Mann-*

Whitney p=0.028). As expected from higher TB disease prevalence in adult males[1], the proportion of males in TB disease patients was higher than in other groups **(Table 1A)**.

Tetramer-based MAIT cell detection requires a multi-step process whereby 5-OP-RU-loaded MR1 monomers are assembled with labeled streptavidin for staining and flow cytometry. To our knowledge, large TB disease-focused human studies with MR1-5-OP-RU tetramers had not been carried out previously, so we designed quality controls to assess tetramer-staining reproducibility. After pre-gating to exclude dead lymphocytes and doublets **(Figure 1A)**, $CD3^+$ $tetramer^+$ cells were clearly distinguishable from $CD3^+$ $tetramer^-$ cells **(Figure 1A)**. Tetramer-based MAIT cell frequencies were defined as the proportion of $CD3^+$ $tetramer^+$ cells among all $CD3^+$ lymphocytes. From 135 analyzable Peruvian samples, we obtained a median MAIT cell frequency of 0.43% (interquartile range: 0.19% - 0.9%) **(Supplementary Table 3A)**. To assess tetramer staining reproducibility, we repeated analyses of the same PBMC samples in 10 participants using different tetramer batches assembled on different days measured an average of 117 days apart **(Supplementary Table 3A)**. Absolute frequencies of MAIT cells were highly reproducible **(**spearman rho = 0.96, **Figure 1B and 1C)**. As a negative control, we stained the samples with MR1 tetramers loaded with the inhibitory MR1 ligand 6-formylpterin (6-FP)[3031], which showed very low false positive staining **(Figure 1D)**.

Prior to MR1-5-OP-RU tetramer-based studies, MAIT cells were defined by antibodies specifically recognizing their TRAV1-2^+ TCRs, cell surface CD161 and CD26 expression, or combinations of these criteria[7, 24, 26, 27]. Since several key prior reports used CD161 and TRAV1-2 co-expression instead of MR1 tetramers to define MAIT cells in the blood of TB patients[12, 13, 14], we first sought to determine whether those two measurements were concordant ~~in our study.~~. All samples were concurrently stained with anti-TRAV1-2 and CD161 antibodies, and 5-OP-RU-loaded MR1 tetramers to define MAIT cells **(Figure 1E)**. Frequencies of MAIT cells defined as

TRAV1-2^{+}CD161^{+} or MR1-tetramer^{+} were highly correlated, and individuals with marked deviations in these two measures were not observed **(Figure 1F)**.

In order to test the association between MAIT cell frequencies and TB disease status in another population, we analyzed MAIT cell frequencies in independently recruited South African participants with recently diagnosed TB disease (n=19) or latent infection (n=19) **(Table 1A and Figure 2A)**. TB disease patients were younger and more likely to be male than latent counterparts **(Table 1A and Supplementary Table 3B)**. Frequencies of blood MAIT cells defined by MR1 tetramer binding or TRAV1-2/CD161 co-expression were also significantly correlated **(Figure 2B and 2C)**.

Next, we asked whether blood MAIT cell frequencies were different in TB patients compared to healthy controls. Unlike prior studies[12, 13, 14], we did not detect significant differences between frequencies of blood MR1-tetramer^{+} MAIT cells in Peruvian TB patients versus either uninfected or latent contacts **(**Kruskal-Wallis *p=0.14*, **Figure 3A)**. Similarly, the frequencies of blood MAIT cells in PBMC samples from South African participants with TB disease or latent *Mtb*-infection were nearly identical **(Figure 3A)**. We also could not detect an association between MAIT cell frequencies and TB disease status after adjustment for age and gender **(Supplementary Tables 4A-4C)**. Further, despite geographic and genetic differences between the two populations, which were analyzed in two different laboratories with MR1 tetramers from two different sources, median frequencies among participants from Peru and South Africa were highly similar (Mann-Whitney: *p=0.63*).

As predicted by the correlation between MAIT cell frequencies defined as CD3^{+}tetramer^{+} or CD3^{+}CD161^{+}TRAV1-2^{+} cells **(Figure 1F and 2C)**, frequencies of CD3^{+}CD161^{+}TRAV1-2^{+} MAIT cells were also not significantly different between TB patients and healthy participants from Peru (Kruskal-Wallis *p=0.11*) or South Africa (Mann-Whitney: *p=0.86*) **(Figure 3B)**.

Additionally, we included anti-CD26 surface staining to more accurately capture MAIT cells stained by MR1 tetramers in South African samples, as previously reported[7, 26, 27] **(Supplementary Figure 1A)**. Similarly, peripheral blood MAIT cell frequencies defined as $CD3^+CD26^+$MR1-tetramer$^+$ cells from latent participants and TB disease patients were not significantly different **(Supplementary Figure 1B)**. Thus, our inability to detect associations between tetramer-defined MAIT cell frequencies and TB status did not change after defining MAIT cells by widely used surrogate surface markers.

Since our results differed from previous studies, which reported lower frequencies of blood MAIT cells in TB patients compared to controls[12, 13, 14], we asked whether demographic factors confounded the association between MAIT cells and TB disease status. In previous studies, healthy control samples were randomly selected from community blood bank donors, and hence were not necessarily exposed to *Mtb*[12, 13]. Thus, we hypothesized that MAIT cell frequencies in healthy controls might depend on the extent of *Mtb* infection[3]. We used *Mtb*-specific IFNγ release as a surrogate for the extent of *Mtb* infection[3], but observed no correlation between MAIT cell frequencies and magnitude of *Mtb*-specific IFNγ release **(Supplementary Figure 2A and Supplementary Table 4A)**. Age was weakly positively correlated with *Mtb*-specific IFNγ release **(Supplementary Figure 2B)**, and negatively associated with MAIT cell frequencies in healthy Peruvian participants **(Supplementary Figure 2C)**. Frequencies of MAIT cells were lower in male TB patients than female counterparts **(*p=0.0074*, Figure 3C)**. Overall, both age and gender confounded the association between MAIT cell frequencies and TB status **(Supplementary Tables 4A-4C)**.

We considered that pooling patients of ranging degrees of TB disease severity could have weakened any association between MAIT cell frequencies and TB status. We hypothesized that stratification by disease severity might reveal an association, as reported in

Korean TB patients[13]. ~~Weight loss is a clinical indicator of TB disease severity[31]~~Weight loss is a clinical indicator of TB disease severity[32], but frequencies of MR1-tetramer$^+$ MAIT cells did not correlate with body mass index in TB patients in both populations **(Figure 4A and Supplementary Table 3)**. Blood transcriptional profiling of TB patients and controls had previously identified transcriptional signatures of TB diagnosis and disease severity[32,33], presence of progressive incipient TB disease in healthy *Mtb*-exposed individuals[29,30] and treatment outcome[33,34]. Hence, we hypothesized that patients with high scores for host transcriptional TB signatures[34, 35, 36, 37] **(Supplementary Tables 2 and 5)** as surrogates for disease severity[32,33], would show reduced frequencies of MAIT cells in the blood. DIAG3, a PCR-adapted TB disease signature, known to detect subclinical and TB disease, and correlates with TB disease severity [34, 35, 37,36, 38], did not correlate with MAIT cell frequencies in patients with TB disease **(Figure 4B)**. Similarly, RISK6, a 6-transcript signature that can detect incipient disease in *Mtb*-infected individuals as well as TB disease **(**under review [38,39]**)**, did not correlate with blood MAIT cell frequencies in TB patients from either cohort **(Figure 4C)**. Additional parsimonious signatures of incipient or subclinical TB (RISK4)[34,35], or TB disease (DIAG4)[35, 36, 37] also did not correlate with MAIT cell frequencies **(Supplementary Figure 3 and Supplementary Table 5)**. Collectively, our data did not support that variable disease severities among TB patients explains the poor association between MAIT cell frequencies in blood and TB status.

Although most MAIT cells are CD4$^-$, CD161$^+$ and CD45RO$^+$[7], recent studies have highlighted subpopulations of MAIT cells that lack some of these features and suggested that these “atypical” MAIT cells might have distinct functions[7, 21]. Therefore, we also tested whether frequencies of CD4$^+$ and CD161$^-$ MR1-tetramer$^+$ T cells were altered in TB patients **(Figure 5A)**. Frequencies of CD4$^+$ MR1-tetramer$^+$ MAIT cells in the blood were not different in TB patients and controls in either group **(Figure 5B)**. We obtained similar results when MAIT cells

were defined as $CD26^{+}MR1\text{-}tetramer^{+}$ MAIT cells in South African samples **(Supplementary Figure 4)**. Proportions of $CD161^{-}$ $MR1\text{-}tetramer^{+}$ T cells were significantly lower in the blood of Peruvian uninfected individuals compared to either latent (p= 0.014) or TB disease patient (p= 0.0014) counterparts **(Figure 5B)**.

MAIT cells have been reported to virtually universally express the memory marker CD45RO in the blood[39,40], which is thought to be part of their pre-programmed nature as innate T cells with effector functions[40,41], rather than infection-driven conversion to memory cells observed in classical MHC-restricted T cells. Consistent with this hypothesis, CD45RO expression on MAIT cells among Peruvian blood samples was uniformly high and similar among TB patients, latent and uninfected controls **(Figure 5C)**. $MR1\text{-}tetramer^{-}$ T cells showed a bimodal distribution of CD45RO that marks memory and non-memory T cells in MHC-restricted T cells. In contrast, MAIT cells showed a unimodal distribution and intermediate CD45RO expression levels compared to $CD45RO^{+}$ $MR1\text{-}tetramer^{-}$ T cells **(Figure 5C)**. Collectively, the data suggests that frequencies of blood MAIT cells with atypical cell surface markers were not associated with TB status.

Discussion

Here we report a large study profiling MR1-tetramer binding MAIT cells in TB disease in two genetically and geographically distinct populations. Using samples from 183 donors, we did not detect differences between MR1-tetramer$^+$ MAIT cell frequencies in the blood of TB patients and healthy *Mtb*-exposed controls. Measuring MAIT cell frequencies in a large-scale clinical study with tetramers, which identify all MR1-5-OP-RU-reactive cells[7, 8, 20, 42], was feasible and highly reproducible. Importantly, frequencies of MAIT cells defined as MR1 tetramer$^+$ or CD161$^+$TRAV1-2$^+$ T cells, as in prior studies[12], showed virtually identical outcomes. Therefore, the discordance between our finding and previous clinical studies reporting a decline in MAIT cell frequencies in the blood of TB patients relative to healthy controls was unlikely due to the method of MAIT cell detection.

Several factors may underlie the difference between our study and previous studies of TB disease and MAIT cells[12, 13, 14]. Firstly, prior studies recruited blood bank donors in non-endemic areas: France, Korea and China[12, 13, 14], and thus *Mtb* exposure was likely lower than controls in our study. In our study, Peruvian controls were recently exposed to *Mtb* in the household[41,43], and South African controls were IGRA-positive, and from a high *Mtb* transmission area[42,44,28,29]. We did not recruit IGRA-negative South African participants, due to the extremely high prevalence of latency in communities from which participants were recruited, which both limits available subjects and the reliability with which they can be assigned as truly uninfected[45]. *Mtb*-infected household contacts of TB patients were reported to have inflammatory profiles that more closely resemble ones observed in active TB patients than latently *Mtb*-infected individuals[31, 43, 32, 46]. Therefore, the high *Mtb* exposure rates in our control groups[44,47], might explain the lack of difference in MAIT cell frequencies between TB patients and healthy controls in this study, which we also observed in CD1b-reactive T cells[28]. Consistently, we detected lower median frequencies of blood MAIT cells in Peruvians (0.43%)

and South Africans (0.47%), compared to healthy Australians (2.6%)[7], which may have resulted from the infection history of participants in our study. ~~We, and others[45]~~We, and others[48], hypothesized that blood MAIT cell frequencies would decrease with increasing exposure to *Mtb*, or clinical severity of disease due to MAIT cell recruitment to the infection site[18]. Our data did not detect associations between MAIT cell frequencies and *Mtb* infection, estimated by IFNγ release[46,49]~~, or disease severity.~~, or disease severity, inferred through transcriptional risk scores, originally defined in whole blood RNA samples[30, 35]. Although risk scores in Peruvians were derived from PBMC samples, they accurately predicted TB disease[39, 50], yet still did not influence MAIT cell frequencies. Consistent with this finding, MAIT cell frequencies were reported to decline in blood samples from patients with non-tuberculous mycobacterial lung disease[13] or bacterial pneumonia[12]. This suggests that inflammation caused by infection with any lung pathogen may drive recruitment of MAIT cells to the lung from the blood, regardless of clinical progression to TB disease. However, TB status could still induce nuanced changes in MAIT cell functions, such as higher TCR-mediated responses in infected individuals in Haiti than uninfected counterparts[51] and changes in TCR clonotypes[52, 53]. Hence, systematic evaluation of MAIT cell functions using unbiased profiling approaches may yield insight.

Age and gender had detectable effects on MAIT cell frequencies in our study. Similar to cohorts from Australia[7], Czech Republic[47,54] and China[48,55], blood MAIT cell frequencies in Peruvians peaked in the third decade of life, then declined steadily. Interestingly, blood MAIT cell frequencies were reported to be lower in males than females only during reproductive years[47,54]. Since we detected lower MAIT cell frequencies in Peruvian male TB patients only, the combined age and gender effect might have been missed in the study of Korean TB patients, because participants were mostly elderly[13]. The higher TB disease severity[1] and lower MAIT cell frequencies in males than females[47,54] may suggest a role for MAIT cells in controlling *Mtb* infection. However, larger prospective studies would evaluate these interactions more reliably.

Several lines of evidence support the hypothesis that MAIT cells relocate from the blood to the lungs in individuals infected with *Mtb*. Oligoclonal expansion of lung-resident MAIT cells detected in bronchoalveolar lavage samples of TB patients was recently reported[18], consistent with a potential role for MAIT cells in controlling early *Mtb* infection. However, frequencies of MR1-tetramer^{+} MAIT cells in both peripheral blood and bronchoalveolar lavage samples were lower in children with TB disease compared to latent counterparts[49,56], arguing against MAIT cell migration to the alveolar space to explain their reported decline in the blood of TB patients. Additionally, two recent reports in non-human primates reported that MAIT cell accumulation in the lung following *Mtb* infection was transient[50,57], and did not correlate with disease outcome[17]. MR1-deficient *Mtb*-infected mice showed higher lung bacterial burdens than MR1-sufficient counterparts, suggesting a host protective role. Therefore, the role of MAIT cells in the lung following infection warrants additional investigation.

Our study of MR1-tetramer-detected MAIT cells, analyzed separately in South American and African cohorts, alludes to generalizable MAIT cell features. We show that unlike MHC-restricted T cells in IFNγ-release assays, peripheral MAIT cell frequencies are not reliable correlates of *Mtb*-infection, TB disease or severity in high *Mtb* transmission settings. ~~However, considering their diverse roles in microbial infections[45],~~However, considering their diverse roles in microbial infections[48] and their known response to mycobacterial antigens, it is likely that MAIT cells play roles in TB pathophysiology, particularly in the lung, the major disease site in pulmonary TB. Future studies should focus on their functional profiles in TB, and tissue-specific roles, in high and low endemic settings.

Table 1: Summary of demographic characteristics of participants in the (A) Peruvian cohort of index TB cases and household contacts and (B) South African CTBC cohort

(A)

Variable	**Uninfected (n=48)**	**Latent (n=49)**	**TB disease (n=48)**	**P-value**
Median age, years (Interquartile range)	28.5 (21.5-41)	38 (25-52)	28.5 (19-40.8)	Kruskal-Wallis 0.035
Gender, n (%) Male	23 (47.9)	19 (38.8)	31 (64.6)	χ^2 0.036

(B)

Variable	**Latent (n=19)**	**TB disease (n=19)**	**P-value**
Median age, years (Interquartile range)	41 (37-46)	32 (27-38)	Mann-Whitney 0.025
Gender, n (%) Male	6 (31.6)	13 (68.4)	χ^2 0.023

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Declaration: LKN, SBE, AJC, JMcC, and JR are named co-inventors on patents describing MR1 tetramers. For experiments using the NIH-supplied MR1 tetramer, the MR1 tetramer technology was developed jointly by Dr. James McCluskey, Dr. Jamie Rossjohn, and Dr. David Fairlie, and the material was produced by the NIH Tetramer Core Facility as permitted to be distributed by the University of Melbourne. SS and TJS are named co-inventors on a filed provisional patent application for biosignature for prediction of progression to tuberculosis.

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Figure Legends:

Figure 1: Frequencies of MAIT cells defined by MR1-tetramers are reproducible and correlate with CD161 and TRAV1-2 co-expression in Peruvian samples.

(A) A gating strategy defines 5-OP-RU loaded MR1-tetramer-binding MAIT cells in peripheral blood mononuclear cells (PMBC) from Peruvian samples.

(B) Two examples of flow cytometry plots of 5-OP-RU-loaded MR1 tetramer staining in T cells in the same PBMC samples from two Peruvian participants were acquired on the indicated dates.

(C) The frequencies of 5-OP-RU-loaded MR1 tetramer$^+$ cells among all CD3$^+$ lymphocytes from 10 PBMC samples were measured twice by flow cytometry on different dates. The correlation coefficient rho (ρ) and p-value are calculated using a two-tailed Spearman correlation test.

(D) Flow cytometry plot of 6-FP-loaded MR1 tetramer staining in T cells is shown from the same sample indicated in (A).

(E) A flow cytometry gating strategy defines CD3$^+$TRAV1-2$^+$CD161$^+$ phenotypically defined MAIT cells from on the sample depicted in (A).

(F) Spearman correlation is shown between MR1-tetramer$^+$ and phenotypically defined TRAV1-2$^+$CD161$^+$ MAIT cells as a proportion of total CD3$^+$ T cells in Peruvian PBMC samples analyzed.

Figure 2: Frequencies of MAIT cells defined by MR1-tetramers and CD161 and TRAV1-2 co-expression are highly correlated in South African samples.

(A) Gating strategy defines MAIT cells in South African PBMC samples.

(B) Flow cytometry plots show MR1-tetramer-binding MAIT cells and $CD3^+TRAV1\text{-}2^+CD161^+$ cells (phenotypic MAIT cells) among all $CD3^+$ lymphocytes.

(C) Spearman correlation between frequencies of MR1-tetramer$^+$ and phenotypic MAIT cells ($TRAV1\text{-}2^+CD161^+$) as a proportion of total $CD3^+$ T cells in 39 South African PBMC samples.

Figure 3: MAIT cell frequencies do not distinguish TB disease state among Peruvian or South African participants.

(A) Proportions of MR1-tetramer-binding MAIT cells among $CD3^+$ T cells in PBMC samples are shown with error bars denoting medians and interquartile ranges. P-values are calculated using Mann-Whitney *U* test.

(B) Plots shown as in (A) give proportions of $CD3^+TRAV1\text{-}2^+CD161^+$ cells out of all T cells.

(C) Comparison of frequencies of MR1-tetramer-binding cells are shown by gender in the two populations. Unadjusted p-values correspond to Mann-Whitney *U* test. Error bars denote medians and interquartile ranges.

Figure 4: Association between disease severity and MAIT cell frequencies.

Correlation between frequencies of MAIT cells and body mass index (A) or transcriptional signatures of TB including DIAG3 scores (B) and RISK6 (C) in Peruvian (left) or South African (right) patients with TB disease. Correlation coefficient rho (ρ) and p-values are calculated using Spearman non-parametric test.

Figure 5: MAIT cells with atypical phenotypes are not associated with TB status

(A) Flow cytometric gating strategy for MAIT cells with atypical phenotypes shows T cells positively staining with the 5-OP-RU-loaded MR1 tetramer, then gated to identify proportions of TRAV1-2^{-}, CD4^{+} and CD161^{-} MAIT cells.

(B) Proportions of MAIT cells showing a CD4^{+} or CD161^{-} phenotype are shown with error bars to denote medians and interquartile ranges. P-values are calculated using the Kruskal-Wallis test among the three Peruvian groups, or a Mann-Whitney *U* test between South African latent and TB disease samples.

(C) Top: Flow cytometry plot depicting expression of the CD45RO memory marker in MR1 tetramer^{+} T cells in a Peruvian sample compared to tetramer^{-} T cells. Bottom: Mean fluorescence index of PerCPCy5.5-conjugated anti-CD45RO antibody staining in MR1 tetramer^{+} T cells in the three Peruvian groups. Error bars denote median and interquartile range. The p-value is calculated using the Kruskal-Wallis test.

Peripheral blood mucosal-associated invariant T (MAIT) cells in tuberculosis patients and healthy *Mycobacterium tuberculosis*-exposed controls

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Abstract (200 words)

Background: In human blood, mucosal-associated invariant T (MAIT) cells are abundant T cells, which recognize antigens presented on non-polymorphic major histocompatibility complex-related 1 (MR1) molecules. MAIT cells are activated by mycobacteria, and prior human studies indicate that blood frequencies of MAIT cells, defined by cell surface markers, decline during TB disease, consistent with redistribution to the lungs.

Methods: We tested whether frequencies of blood MAIT cells were altered in patients with TB disease relative to healthy *Mycobacterium tuberculosis* (*Mtb*)-exposed controls from Peru and South Africa. We quantified their frequencies using MR1 tetramers loaded with 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU).

Results: Unlike findings from prior studies, frequencies of blood MAIT cells were similar among TB-disease patients, latent and uninfected controls. In both cohorts, frequencies of MAIT cells defined by MR1-tetramer staining and co-expression of CD161 and the T cell receptor alpha variable gene TRAV1-2 were strongly correlated. Disease severity captured by body mass index or TB disease transcriptional signatures did not correlate with MAIT cell frequencies in TB patients.

Discussion: MR1-restrictied MAIT cells are detected at similar levels with tetramers or surface markers. Unlike MHC-restricted T cells, blood frequencies of MAIT cells are poor correlates of TB disease, but may play roles in pathophysiology.

Key Words: MR1, tetramer, MAIT, tuberculosis, household contacts

Introduction

According to the World Health Organization, *Mycobacterium tuberculosis* (*Mtb*) is the leading cause of death from infectious disease globally[1], with one quarter of the world's population estimated to be infected with *Mtb*[2]. The commonly used interferon-γ release assay (IGRA), measures MHC-restricted αβ T cell responses to *Mtb* antigens as a reliable diagnostic test for infection[3]. Thus, it is broadly accepted that expansion of antigen-specific, MHC-restricted T cell populations in blood is the usual human response to *Mtb* infection.

Recent studies showed that non-MHC encoded, antigen presenting molecules can present mycobacterial antigens to activate αβ T cell responses in experimental *Mtb* infections[4, 5, 6]. Prominent among these T cell types are mucosal-associated invariant T (MAIT) cells, which recognize MR1 and are particularly abundant, comprising ~0.1 to 10% of circulating T cells in healthy individuals[7]. MAIT cell antigens include riboflavin derivatives and other metabolites[8, 9, 10]. Unlike highly polymorphic MHC genes, MR1 is nearly monomorphic in humans[6]. Thus, MAIT cells can recognize antigens presented by antigen presenting cells from any human, and hence are known as donor-unrestricted T cells (DURTs)[6].

The abundance of MAIT cells and reactivity towards mycobacterial antigens[11] raise the possibility that MAIT cells could play roles in controlling natural *Mtb* infection. Unlike conventional T cells, MAIT cell frequencies were reported to decline in the peripheral blood of TB patients, relative to *Mtb*-unexposed controls[12, 13, 14, 15, 16], or following *Mtb* infection of mice[15] and non-human primates[17]. This outcome is consistent with their suspected relocation to the lungs or other sites of infection *in vivo*. Consistent with this prediction, a recent study reported that MAIT cell frequencies were enriched in the bronchoalveolar lavage of TB patients[18]. However, these studies are relatively small and rely on assays that detect activation by MR1[16] and expression of the TRAV1-2 variable region of the T cell receptor alpha (TCRα), which is

frequently rearranged with the TCRα joining region TRAJ33 in MAIT cell[19, 20]. However, there are examples of TRAV1-2$^-$ T cells that recognize MR1[21, 22, 23]. Conversely, TRAV1-2$^+$ TCRs can also recognize antigens presented by MHC or CD1b proteins[4]. Thus, TCR sequence-independent methods to unequivocally identify MR1-binding T cells are important. MR1 tetramers loaded with the vitamin B-like metabolite 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU) allow direct identification of MAIT cells based on binding specificity to MR1 and the antigenic ligand[7, 8, 20, 23].

A third definition of MAIT cells relies on expression of cell surface markers rather than activation by MR1 or TRAV1-2 expression. MAIT cells are predominantly CD8$^+$ or CD4$^-$CD8$^-$ T cells, with a small CD4$^+$ fraction[7, 24], and co-express the C-type lectin CD161[25] and CD26 ectopeptidase[7, 26, 27]. Thus, clinical studies have tracked cells co-expressing CD3 and CD161, [13,12] or CD26[14], usually in combination with TRAV1-2[7]. These cell surface marker-defined MAIT cells, sometimes called 'phenotypic' MAIT cells, emerged mainly from human studies, where functional responses to MR1-ligand complex were not feasible, or before MR1-tetramer development.

The emergence of parallel TCR-, tetramer- and phenotype-based criteria raises basic questions about the best MAIT cell definition and the concordance of these measurements in humans. MR1-tetramers are now well validated to identify and characterize sub-populations of MAIT cells[7], or in mechanistic studies to identify MAIT cell functions *in vitro*[21]. However, MR1 tetramers have not been applied to large cohorts of TB patients. Since prior studies of MAIT cells in TB patients and controls relied on expression of TRAV1-2 and CD161[12, 13, 14], we undertook a study of patients with TB disease and healthy *Mtb*-exposed participants from two geographically distinct populations to measure peripheral blood MAIT cells using either TRAV1-2 and CD161 co-expression or 5-OP-RU-loaded MR1 tetramers to test whether blood MAIT cell frequencies were altered in TB disease.

Materials and Methods

Participants were enrolled in two cross-sectional studies in Peru and South Africa. Adults and parents or legal guardians of minors provided informed consent, while minors provided assent.

Peruvian Household Contacts Cohort

Bacillus Calmette-Guérin (BCG)-vaccinated HIV-uninfected participants were recruited through Socios En Salud (SES), from settlements around Lima, Peru[28]. Participants included adults with recently diagnosed sputum culture-positive, drug-sensitive pulmonary TB disease (TB disease, n=50) and asymptomatic household contacts assessed within two-weeks of diagnosing the index case (n=100). Contacts were evaluated for TB disease symptoms at enrolment and excluded if clinical symptoms of TB disease were present. Healthy household contacts were assessed for *Mtb* infection using the QuantiFERON TB-Gold In-Tube assay (Qiagen) and considered latently *Mtb*-infected if their IFNγ levels were ≥0.35 international units (IU)/mL (n=50) and uninfected if <0.35 IU/mL (n=50). Peripheral blood mononuclear cells (PBMC) were isolated from 50 mL of venous blood using ficoll, then cryopreserved and shipped to the Brigham and Women's Hospital for storage and analysis by flow cytometry. The Institutional Review Board of the Harvard Faculty of Medicine and Partners Healthcare (protocol number IRB16-1173), and the Institutional Committee of Ethics in Research of the Peruvian Institutes of Health approved this study protocol.

South African Cohort

We recruited HIV-negative adults who received BCG vaccination at birth from communities in the town of Worcester near Cape Town, South Africa into the previously described Cross-sectional TB Cohort (CTBC)[29]. Individuals with newly diagnosed sputum Xpert MTB/RIF-positive TB disease (TB disease, n=19) and asymptomatic, QuantiFERON TB-Gold In-Tube-positive

latently *Mtb*-infected (latent, n=19) adults were enrolled. We did not recruit any uninfected participants in this study arm because high TB prevalence rates limit recruitment of reliably uninfected subjects. PBMC samples were processed from blood collected in Vacutainer CPT mononuclear cell separation tubes (BD) and cryopreserved for flow cytometry analysis in Cape Town. The CTBC study protocol was approved by the University of Cape Town Human Research Ethics Committee (HREC 761/2015).

Flow cytometry analysis

For Peruvian samples, MR1 monomers loaded with 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU), or 6-formylpterin (6-FP) as a negative control, were produced at The University of Melbourne, Australia as described[8, 20]. To generate MR1 tetramers, 1μg of MR1 protein was tetramerized using 6 aliquots of 1μL Biolegend Streptavidin-PerCP-Cy5.5 (Biolegend) diluted 1:4 in phosphate buffered saline (PBS). Cryopreserved samples from Peru were thawed at 37°C, and approximately $3X10^6$ cells were stained with a fixable blue viability cell stain (ThermoFisher Scientific) according to manufacturer's instructions, followed by MR1 tetramers in staining media (5% bovine serum albumin and 0.01% sodium azide in PBS) for 10 minutes at room temperature in the dark, followed by cell surface antibodies **(Supplementary Table 1A)** for 5 minutes. Subsequently, cells were treated with unconjugated OKT-3 antibody, and incubated for 5 minutes at room temperature followed by 10 minutes at 4°C. Cells were fixed in 2% paraformaldehyde (Electron Microscopy Sciences) in PBS for 20 minutes. For South African samples, MR1 tetramers were obtained from the National Institutes of Health (NIH) Tetramer Core Facility, and used to stain peripheral blood mononuclear cell (PBMC) samples at a 1:200 dilution in 50μL at room temperature for 45 minutes, followed by antibodies at 4°C for 30 minutes **(Supplementary Table 1B)**.

RNA processing and TB Risk score analysis

For the Peruvian cohort, RNA samples were extracted from 10^6 PBMCs using the RNeasy kit (Qiagen), and frozen aliquots were shipped to the University of Cape Town. For the South African cohort, 2.5mL of venous blood was drawn directly into PAXgene blood RNA tubes and frozen. RNA was extracted using PreAnalytiX PAXgene Blood RNA extraction kit (Qiagen). All RNA samples were reverse transcribed to cDNA using EpiScript RNase H-Reverse Transcriptase (Lucigen), pre-amplified with a master mix of Taqman primer probes **(Supplementary Table 2)** in 2X PCR master mix (ThermoFisher), and analyzed by microfluidic real-time PCR using the Biomark 192.24 gene expression integrated fluidic circuit (IFC) system (Fluidigm) for multiplex analysis of 24 assays and 196 samples as previously described[30].

Data analysis

Flow cytometry data were analyzed in Flowjo version 10.4.2. Computation of transcriptomic signature scores from qRT-PCR cycle threshold values and generalized linear regression models were performed in R versions 3.5.1-3.6 for Mac. Other statistical analyses were performed in GraphPad Prism versions 7-8.

Results

From 145 enrollees, 135 PBMC samples passed quality control for yield, viability and sterility: 48 patients with TB disease, 48 asymptomatic uninfected and 49 latently *Mtb*-infected (latent) adults **(Table 1A and Supplementary Table 3A)**. Participants with latent TB were older than either uninfected participants (*Mann-Whitney p=0.022*), or patients with TB disease (*Mann-*

Whitney p=0.028). As expected from higher TB disease prevalence in adult males[1], the proportion of males in TB disease patients was higher than in other groups **(Table 1A)**.

Tetramer-based MAIT cell detection requires a multi-step process whereby 5-OP-RU-loaded MR1 monomers are assembled with labeled streptavidin for staining and flow cytometry. To our knowledge, large TB disease-focused human studies with MR1-5-OP-RU tetramers had not been carried out previously, so we designed quality controls to assess tetramer-staining reproducibility. After pre-gating to exclude dead lymphocytes and doublets **(Figure 1A)**, $CD3^+$ $tetramer^+$ cells were clearly distinguishable from $CD3^+$ $tetramer^-$ cells **(Figure 1A)**. Tetramer-based MAIT cell frequencies were defined as the proportion of $CD3^+$ $tetramer^+$ cells among all $CD3^+$ lymphocytes. From 135 analyzable Peruvian samples, we obtained a median MAIT cell frequency of 0.43% (interquartile range: 0.19% - 0.9%) **(Supplementary Table 3A)**. To assess tetramer staining reproducibility, we repeated analyses of the same PBMC samples in 10 participants using different tetramer batches assembled on different days measured an average of 117 days apart **(Supplementary Table 3A)**. Absolute frequencies of MAIT cells were highly reproducible **(**spearman rho = 0.96, **Figure 1B and 1C)**. As a negative control, we stained the samples with MR1 tetramers loaded with the inhibitory MR1 ligand 6-formylpterin (6-FP)[31], which showed very low false positive staining **(Figure 1D)**.

Prior to MR1-5-OP-RU tetramer-based studies, MAIT cells were defined by antibodies specifically recognizing their TRAV1-2^+ TCRs, cell surface CD161 and CD26 expression, or combinations of these criteria[7, 24, 26, 27]. Since several key prior reports used CD161 and TRAV1-2 co-expression instead of MR1 tetramers to define MAIT cells in the blood of TB patients[12, 13, 14], we first sought to determine whether those two measurements were concordant. All samples were concurrently stained with anti-TRAV1-2 and CD161 antibodies, and 5-OP-RU-loaded MR1 tetramers to define MAIT cells **(Figure 1E)**. Frequencies of MAIT cells defined as TRAV1-

2^+CD161^+ or MR1-tetramer$^+$ were highly correlated, and individuals with marked deviations in these two measures were not observed **(Figure 1F)**.

In order to test the association between MAIT cell frequencies and TB disease status in another population, we analyzed MAIT cell frequencies in independently recruited South African participants with recently diagnosed TB disease (n=19) or latent infection (n=19) **(Table 1A and Figure 2A)**. TB disease patients were younger and more likely to be male than latent counterparts **(Table 1A and Supplementary Table 3B)**. Frequencies of blood MAIT cells defined by MR1 tetramer binding or TRAV1-2/CD161 co-expression were also significantly correlated **(Figure 2B and 2C)**.

Next, we asked whether blood MAIT cell frequencies were different in TB patients compared to healthy controls. Unlike prior studies[12, 13, 14], we did not detect significant differences between frequencies of blood MR1-tetramer$^+$ MAIT cells in Peruvian TB patients versus either uninfected or latent contacts **(**Kruskal-Wallis *p=0.14*, **Figure 3A)**. Similarly, the frequencies of blood MAIT cells in PBMC samples from South African participants with TB disease or latent *Mtb*-infection were nearly identical **(Figure 3A)**. We also could not detect an association between MAIT cell frequencies and TB disease status after adjustment for age and gender **(Supplementary Tables 4A-4C)**. Further, despite geographic and genetic differences between the two populations, which were analyzed in two different laboratories with MR1 tetramers from two different sources, median frequencies among participants from Peru and South Africa were highly similar (Mann-Whitney: *p=0.63*).

As predicted by the correlation between MAIT cell frequencies defined as $CD3^+$tetramer$^+$ or $CD3^+CD161^+TRAV1\text{-}2^+$ cells **(Figure 1F and 2C)**, frequencies of $CD3^+CD161^+TRAV1\text{-}2^+$ MAIT cells were also not significantly different between TB patients and healthy participants from Peru (Kruskal-Wallis *p=0.11*) or South Africa (Mann-Whitney: *p=0.86*) **(Figure 3B)**.

Additionally, we included anti-CD26 surface staining to more accurately capture MAIT cells stained by MR1 tetramers in South African samples, as previously reported[7, 26, 27] **(Supplementary Figure 1A)**. Similarly, peripheral blood MAIT cell frequencies defined as $CD3^+CD26^+$MR1-tetramer$^+$ cells from latent participants and TB disease patients were not significantly different **(Supplementary Figure 1B)**. Thus, our inability to detect associations between tetramer-defined MAIT cell frequencies and TB status did not change after defining MAIT cells by widely used surrogate surface markers.

Since our results differed from previous studies, which reported lower frequencies of blood MAIT cells in TB patients compared to controls[12, 13, 14], we asked whether demographic factors confounded the association between MAIT cells and TB disease status. In previous studies, healthy control samples were randomly selected from community blood bank donors, and hence were not necessarily exposed to *Mtb*[12, 13]. Thus, we hypothesized that MAIT cell frequencies in healthy controls might depend on the extent of *Mtb* infection[3]. We used *Mtb*-specific IFNγ release as a surrogate for the extent of *Mtb* infection[3], but observed no correlation between MAIT cell frequencies and magnitude of *Mtb*-specific IFNγ release **(Supplementary Figure 2A and Supplementary Table 4A)**. Age was weakly positively correlated with *Mtb*-specific IFNγ release **(Supplementary Figure 2B)**, and negatively associated with MAIT cell frequencies in healthy Peruvian participants **(Supplementary Figure 2C)**. Frequencies of MAIT cells were lower in male TB patients than female counterparts **(***p=0.0074*, **Figure 3C)**. Overall, both age and gender confounded the association between MAIT cell frequencies and TB status **(Supplementary Tables 4A-4C)**.

We considered that pooling patients of ranging degrees of TB disease severity could have weakened any association between MAIT cell frequencies and TB status. We hypothesized that stratification by disease severity might reveal an association, as reported in

213 Korean TB patients[13]. Weight loss is a clinical indicator of TB disease severity[32], but frequencies
214 of MR1-tetramer$^+$ MAIT cells did not correlate with body mass index in TB patients in both
215 populations **(Figure 4A and Supplementary Table 3)**. Blood transcriptional profiling of TB
216 patients and controls had previously identified transcriptional signatures of TB diagnosis and
217 disease severity[33], presence of progressive incipient TB disease in healthy *Mtb*-exposed
218 individuals[30] and treatment outcome[34]. Hence, we hypothesized that patients with high scores
219 for host transcriptional TB signatures[35, 36, 37] **(Supplementary Tables 2 and 5)** as surrogates for
220 disease severity[33], would show reduced frequencies of MAIT cells in the blood. DIAG3, a PCR-
221 adapted TB disease signature, known to detect subclinical and TB disease, and correlates with
222 TB disease severity [35, 36, 38], did not correlate with MAIT cell frequencies in patients with TB
223 disease **(Figure 4B)**. Similarly, RISK6, a 6-transcript signature that can detect incipient disease
224 in *Mtb*-infected individuals as well as TB disease **(**under review [39]**)**, did not correlate with blood
225 MAIT cell frequencies in TB patients from either cohort **(Figure 4C)**. Additional parsimonious
226 signatures of incipient or subclinical TB (RISK4)[35], or TB disease (DIAG4)[36, 37] also did not
227 correlate with MAIT cell frequencies **(Supplementary Figure 3 and Supplementary Table 5)**.
228 Collectively, our data did not support that variable disease severities among TB patients
229 explains the poor association between MAIT cell frequencies in blood and TB status.

230 Although most MAIT cells are CD4$^-$, CD161$^+$ and CD45RO$^+$[7], recent studies have
231 highlighted subpopulations of MAIT cells that lack some of these features and suggested that
232 these “atypical” MAIT cells might have distinct functions[7, 21]. Therefore, we also tested whether
233 frequencies of CD4$^+$ and CD161$^-$ MR1-tetramer$^+$ T cells were altered in TB patients **(Figure**
234 **5A)**. Frequencies of CD4$^+$ MR1-tetramer$^+$ MAIT cells in the blood were not different in TB
235 patients and controls in either group **(Figure 5B)**. We obtained similar results when MAIT cells
236 were defined as CD26$^+$MR1-tetramer$^+$ MAIT cells in South African samples **(Supplementary**
237 **Figure 4)**. Proportions of CD161$^-$ MR1-tetramer$^+$ T cells were significantly lower in the blood of

Peruvian uninfected individuals compared to either latent (p= 0.014) or TB disease patient (p= 0.0014) counterparts **(Figure 5B)**.

MAIT cells have been reported to virtually universally express the memory marker CD45RO in the blood[40], which is thought to be part of their pre-programmed nature as innate T cells with effector functions[41], rather than infection-driven conversion to memory cells observed in classical MHC-restricted T cells. Consistent with this hypothesis, CD45RO expression on MAIT cells among Peruvian blood samples was uniformly high and similar among TB patients, latent and uninfected controls **(Figure 5C)**. MR1-tetramer$^-$ T cells showed a bimodal distribution of CD45RO that marks memory and non-memory T cells in MHC-restricted T cells. In contrast, MAIT cells showed a unimodal distribution and intermediate CD45RO expression levels compared to CD45RO$^+$ MR1-tetramer$^-$ T cells **(Figure 5C)**. Collectively, the data suggests that frequencies of blood MAIT cells with atypical cell surface markers were not associated with TB status.

Discussion

Here we report a large study profiling MR1-tetramer binding MAIT cells in TB disease in two genetically and geographically distinct populations. Using samples from 183 donors, we did not detect differences between MR1-tetramer$^+$ MAIT cell frequencies in the blood of TB patients and healthy *Mtb*-exposed controls. Measuring MAIT cell frequencies in a large-scale clinical study with tetramers, which identify all MR1-5-OP-RU-reactive cells[7, 8, 20, 42], was feasible and highly reproducible. Importantly, frequencies of MAIT cells defined as MR1 tetramer$^+$ or CD161$^+$TRAV1-2$^+$ T cells, as in prior studies[12], showed virtually identical outcomes. Therefore, the discordance between our finding and previous clinical studies reporting a decline in MAIT cell frequencies in the blood of TB patients relative to healthy controls was unlikely due to the method of MAIT cell detection.

Several factors may underlie the difference between our study and previous studies of TB disease and MAIT cells[12, 13, 14]. Firstly, prior studies recruited blood bank donors in non-endemic areas: France, Korea and China[12, 13, 14], and thus *Mtb* exposure was likely lower than controls in our study. In our study, Peruvian controls were recently exposed to *Mtb* in the household[43], and South African controls were IGRA-positive, and from a high *Mtb* transmission area[44,29]. We did not recruit IGRA-negative South African participants, due to the extremely high prevalence of latency in communities from which participants were recruited, which both limits available subjects and the reliability with which they can be assigned as truly uninfected[45]. *Mtb*-infected household contacts of TB patients were reported to have inflammatory profiles that more closely resemble ones observed in active TB patients than latently *Mtb*-infected individuals[32, 46]. Therefore, the high *Mtb* exposure rates in our control groups[47], might explain the lack of difference in MAIT cell frequencies between TB patients and healthy controls in this study, which we also observed in CD1b-reactive T cells[28]. Consistently, we detected lower median frequencies of blood MAIT cells in Peruvians (0.43%) and South Africans (0.47%),

compared to healthy Australians (2.6%)[7], which may have resulted from the infection history of participants in our study. We, and others[48], hypothesized that blood MAIT cell frequencies would decrease with increasing exposure to *Mtb*, or clinical severity of disease due to MAIT cell recruitment to the infection site[18]. Our data did not detect associations between MAIT cell frequencies and *Mtb* infection, estimated by IFNγ release[49], or disease severity, inferred through transcriptional risk scores, originally defined in whole blood RNA samples[30, 35]. Although risk scores in Peruvians were derived from PBMC samples, they accurately predicted TB disease[39, 50], yet still did not influence MAIT cell frequencies. Consistent with this finding, MAIT cell frequencies were reported to decline in blood samples from patients with non-tuberculous mycobacterial lung disease[13] or bacterial pneumonia[12]. This suggests that inflammation caused by infection with any lung pathogen may drive recruitment of MAIT cells to the lung from the blood, regardless of clinical progression to TB disease. However, TB status could still induce nuanced changes in MAIT cell functions, such as higher TCR-mediated responses in infected individuals in Haiti than uninfected counterparts[51] and changes in TCR clonotypes[52, 53]. Hence, systematic evaluation of MAIT cell functions using unbiased profiling approaches may yield insight.

Age and gender had detectable effects on MAIT cell frequencies in our study. Similar to cohorts from Australia[7], Czech Republic[54] and China[55], blood MAIT cell frequencies in Peruvians peaked in the third decade of life, then declined steadily. Interestingly, blood MAIT cell frequencies were reported to be lower in males than females only during reproductive years[54]. Since we detected lower MAIT cell frequencies in Peruvian male TB patients only, the combined age and gender effect might have been missed in the study of Korean TB patients, because participants were mostly elderly[13]. The higher TB disease severity[1] and lower MAIT cell frequencies in males than females[54] may suggest a role for MAIT cells in controlling *Mtb* infection. However, larger prospective studies would evaluate these interactions more reliably.

Several lines of evidence support the hypothesis that MAIT cells relocate from the blood to the lungs in individuals infected with *Mtb*. Oligoclonal expansion of lung-resident MAIT cells detected in bronchoalveolar lavage samples of TB patients was recently reported[18], consistent with a potential role for MAIT cells in controlling early *Mtb* infection. However, frequencies of MR1-tetramer^{+} MAIT cells in both peripheral blood and bronchoalveolar lavage samples were lower in children with TB disease compared to latent counterparts[56], arguing against MAIT cell migration to the alveolar space to explain their reported decline in the blood of TB patients. Additionally, two recent reports in non-human primates reported that MAIT cell accumulation in the lung following *Mtb* infection was transient[57], and did not correlate with disease outcome[17]. MR1-deficient *Mtb*-infected mice showed higher lung bacterial burdens than MR1-sufficient counterparts, suggesting a host protective role. Therefore, the role of MAIT cells in the lung following infection warrants additional investigation.

Our study of MR1-tetramer-detected MAIT cells, analyzed separately in South American and African cohorts, alludes to generalizable MAIT cell features. We show that unlike MHC-restricted T cells in IFNγ-release assays, peripheral MAIT cell frequencies are not reliable correlates of *Mtb*-infection, TB disease or severity in high *Mtb* transmission settings. However, considering their diverse roles in microbial infections[48] and their known response to mycobacterial antigens, it is likely that MAIT cells play roles in TB pathophysiology, particularly in the lung, the major disease site in pulmonary TB. Future studies should focus on their functional profiles in TB, and tissue-specific roles, in high and low endemic settings.

Table 1: Summary of demographic characteristics of participants in the (A) Peruvian cohort of index TB cases and household contacts and (B) South African CTBC cohort

(A)

Variable	**Uninfected (n=48)**	**Latent (n=49)**	**TB disease (n=48)**	**P-value**
Median age, years (Interquartile range)	28.5 (21.5-41)	38 (25-52)	28.5 (19-40.8)	Kruskal-Wallis 0.035
Gender, n (%) Male	23 (47.9)	19 (38.8)	31 (64.6)	χ^2 0.036

(B)

Variable	**Latent (n=19)**	**TB disease (n=19)**	**P-value**
Median age, years (Interquartile range)	41 (37-46)	32 (27-38)	Mann-Whitney 0.025
Gender, n (%) Male	6 (31.6)	13 (68.4)	χ^2 0.023

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Declaration: LKN, SBE, AJC, JMcC, and JR are named co-inventors on patents describing MR1 tetramers. For experiments using the NIH-supplied MR1 tetramer, the MR1 tetramer technology was developed jointly by Dr. James McCluskey, Dr. Jamie Rossjohn, and Dr. David Fairlie, and the material was produced by the NIH Tetramer Core Facility as permitted to be distributed by the University of Melbourne. SS and TJS are named co-inventors on a filed provisional patent application for biosignature for prediction of progression to tuberculosis.

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Figure Legends:

Figure 1: Frequencies of MAIT cells defined by MR1-tetramers are reproducible and correlate with CD161 and TRAV1-2 co-expression in Peruvian samples.

(A) A gating strategy defines 5-OP-RU loaded MR1-tetramer-binding MAIT cells in peripheral blood mononuclear cells (PMBC) from Peruvian samples.

(B) Two examples of flow cytometry plots of 5-OP-RU-loaded MR1 tetramer staining in T cells in the same PBMC samples from two Peruvian participants were acquired on the indicated dates.

(C) The frequencies of 5-OP-RU-loaded MR1 tetramer$^+$ cells among all CD3$^+$ lymphocytes from 10 PBMC samples were measured twice by flow cytometry on different dates. The correlation coefficient rho (ρ) and p-value are calculated using a two-tailed Spearman correlation test.

(D) Flow cytometry plot of 6-FP-loaded MR1 tetramer staining in T cells is shown from the same sample indicated in (A).

(E) A flow cytometry gating strategy defines CD3$^+$TRAV1-2$^+$CD161$^+$ phenotypically defined MAIT cells from on the sample depicted in (A).

(F) Spearman correlation is shown between MR1-tetramer$^+$ and phenotypically defined TRAV1-2$^+$CD161$^+$ MAIT cells as a proportion of total CD3$^+$ T cells in Peruvian PBMC samples analyzed.

Figure 2: Frequencies of MAIT cells defined by MR1-tetramers and CD161 and TRAV1-2 co-expression are highly correlated in South African samples.

(A) Gating strategy defines MAIT cells in South African PBMC samples.

559 (B) Flow cytometry plots show MR1-tetramer-binding MAIT cells and $CD3^+TRAV1\text{-}2^+CD161^+$
560 cells (phenotypic MAIT cells) among all $CD3^+$ lymphocytes.

561 (C) Spearman correlation between frequencies of MR1-tetramer$^+$ and phenotypic MAIT cells
562 ($TRAV1\text{-}2^+CD161^+$) as a proportion of total $CD3^+$ T cells in 39 South African PBMC samples.

563

564 **Figure 3: MAIT cell frequencies do not distinguish TB disease state among Peruvian or**
565 **South African participants.**

566 (A) Proportions of MR1-tetramer-binding MAIT cells among $CD3^+$ T cells in PBMC samples are
567 shown with error bars denoting medians and interquartile ranges. P-values are calculated using
568 Mann-Whitney *U* test.

569 (B) Plots shown as in (A) give proportions of $CD3^+TRAV1\text{-}2^+CD161^+$ cells out of all T cells.

570 (C) Comparison of frequencies of MR1-tetramer-binding cells are shown by gender in the two
571 populations. Unadjusted p-values correspond to Mann-Whitney *U* test. Error bars denote
572 medians and interquartile ranges.

573

574 **Figure 4: Association between disease severity and MAIT cell frequencies.**

575 Correlation between frequencies of MAIT cells and body mass index (A) or transcriptional
576 signatures of TB including DIAG3 scores (B) and RISK6 (C) in Peruvian (left) or South African
577 (right) patients with TB disease. Correlation coefficient rho (ρ) and p-values are calculated using
578 Spearman non-parametric test.

579

Figure 5: MAIT cells with atypical phenotypes are not associated with TB status

(A) Flow cytometric gating strategy for MAIT cells with atypical phenotypes shows T cells positively staining with the 5-OP-RU-loaded MR1 tetramer, then gated to identify proportions of TRAV1-2^{-}, CD4^{+} and CD161^{-} MAIT cells.

(B) Proportions of MAIT cells showing a CD4^{+} or CD161^{-} phenotype are shown with error bars to denote medians and interquartile ranges. P-values are calculated using the Kruskal-Wallis test among the three Peruvian groups, or a Mann-Whitney *U* test between South African latent and TB disease samples.

(C) Top: Flow cytometry plot depicting expression of the CD45RO memory marker in MR1 tetramer^{+} T cells in a Peruvian sample compared to tetramer^{-} T cells. Bottom: Mean fluorescence index of PerCPCy5.5-conjugated anti-CD45RO antibody staining in MR1 tetramer^{+} T cells in the three Peruvian groups. Error bars denote median and interquartile range. The p-value is calculated using the Kruskal-Wallis test.

Figure 1

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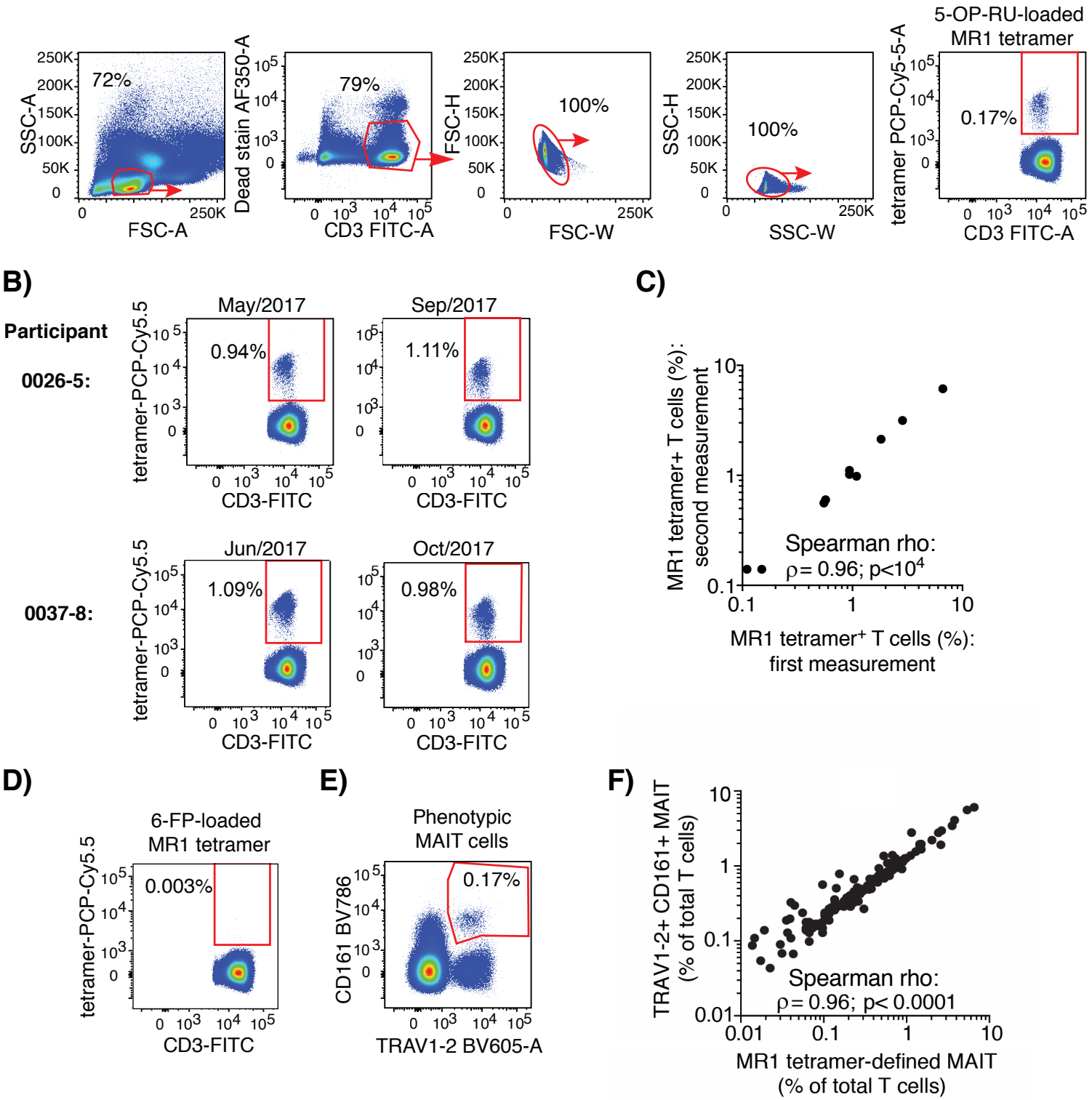
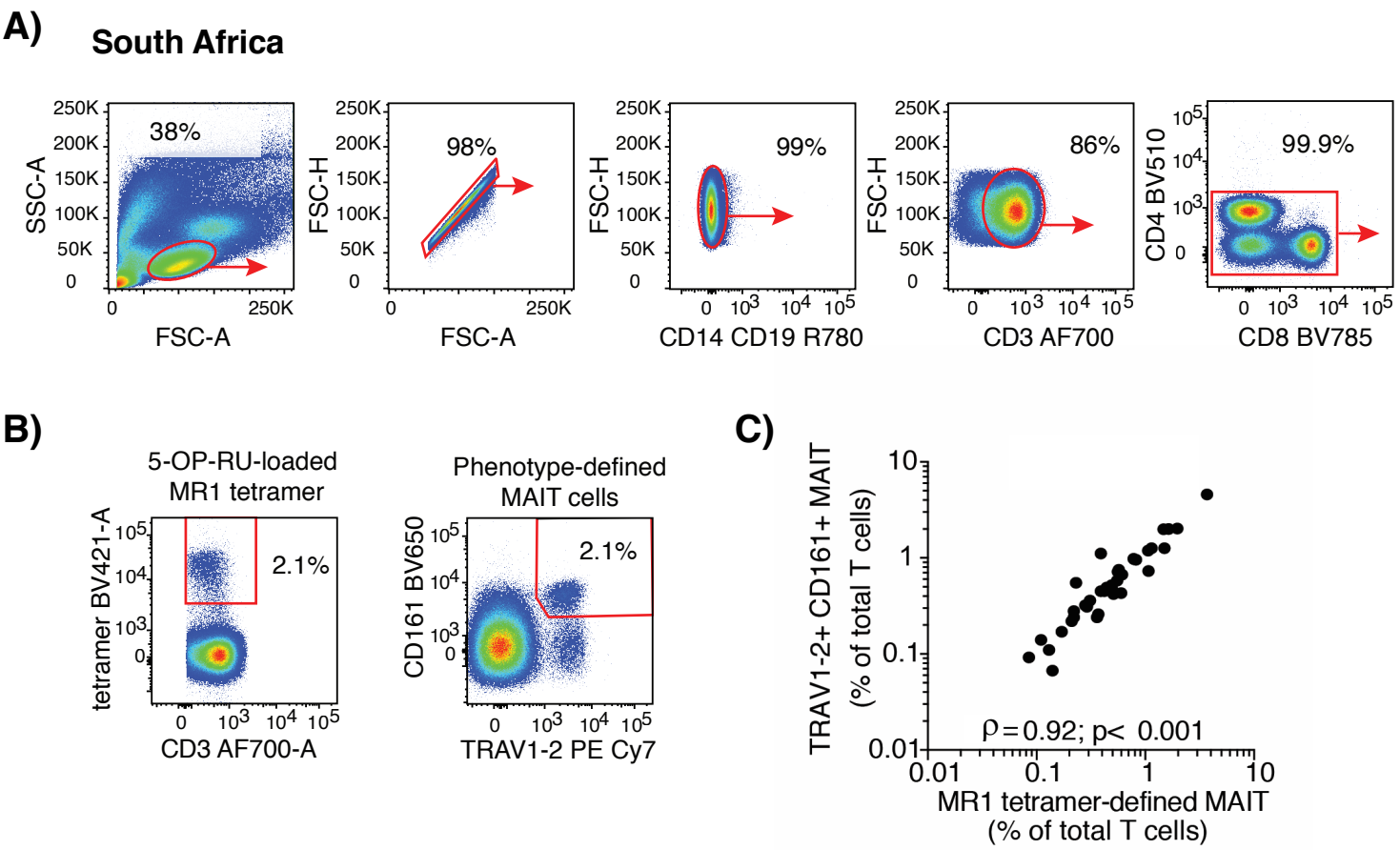
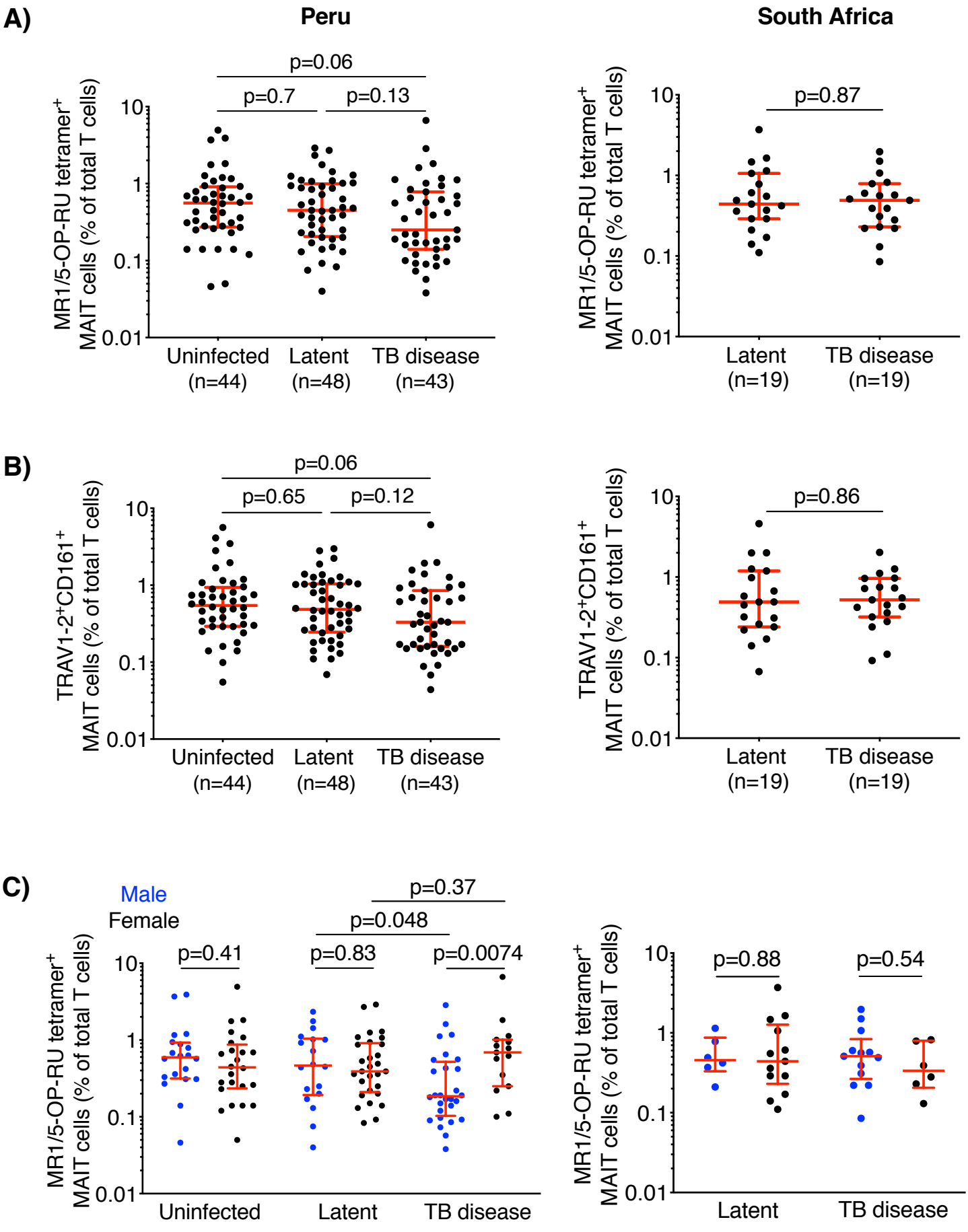
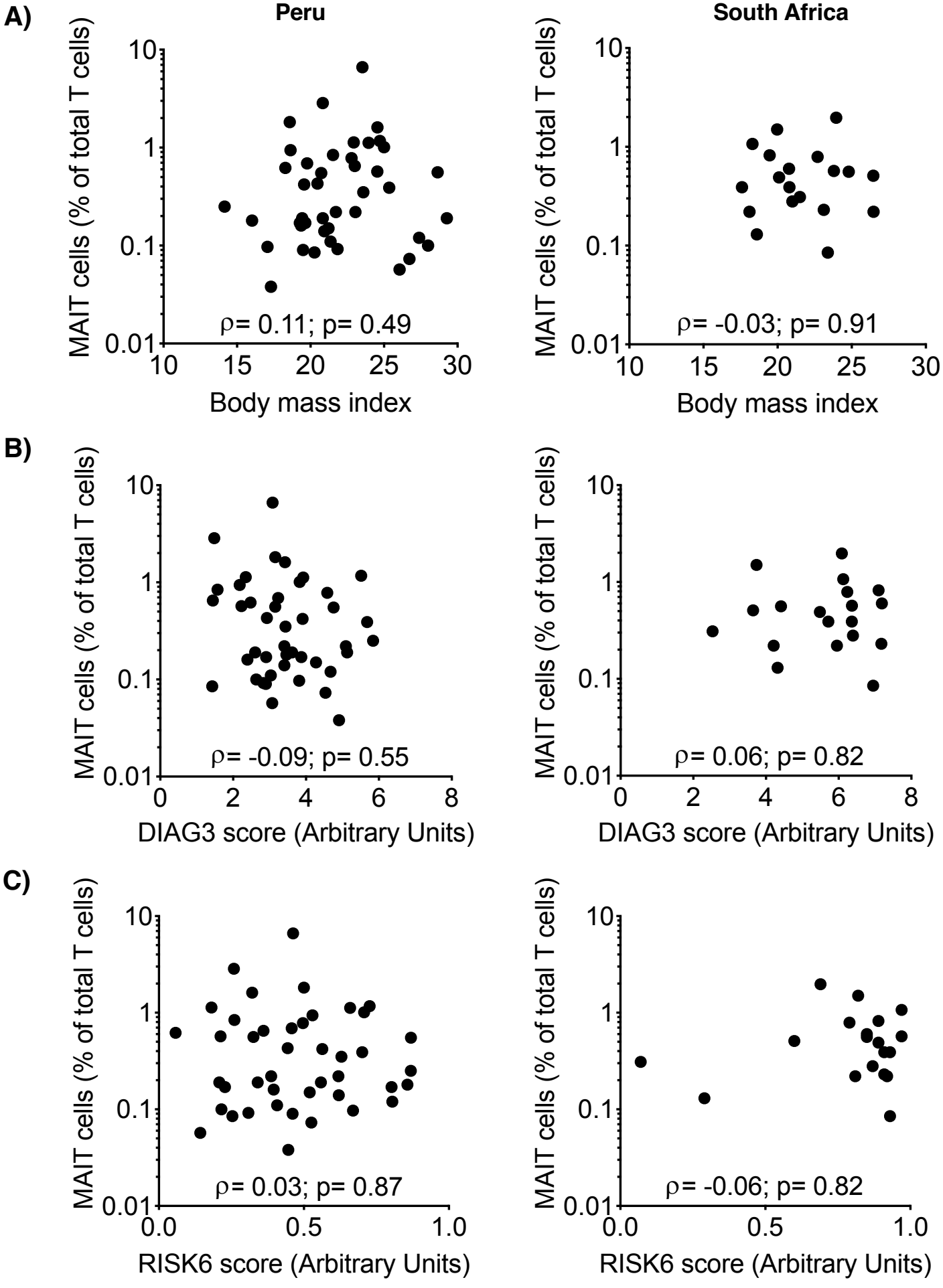
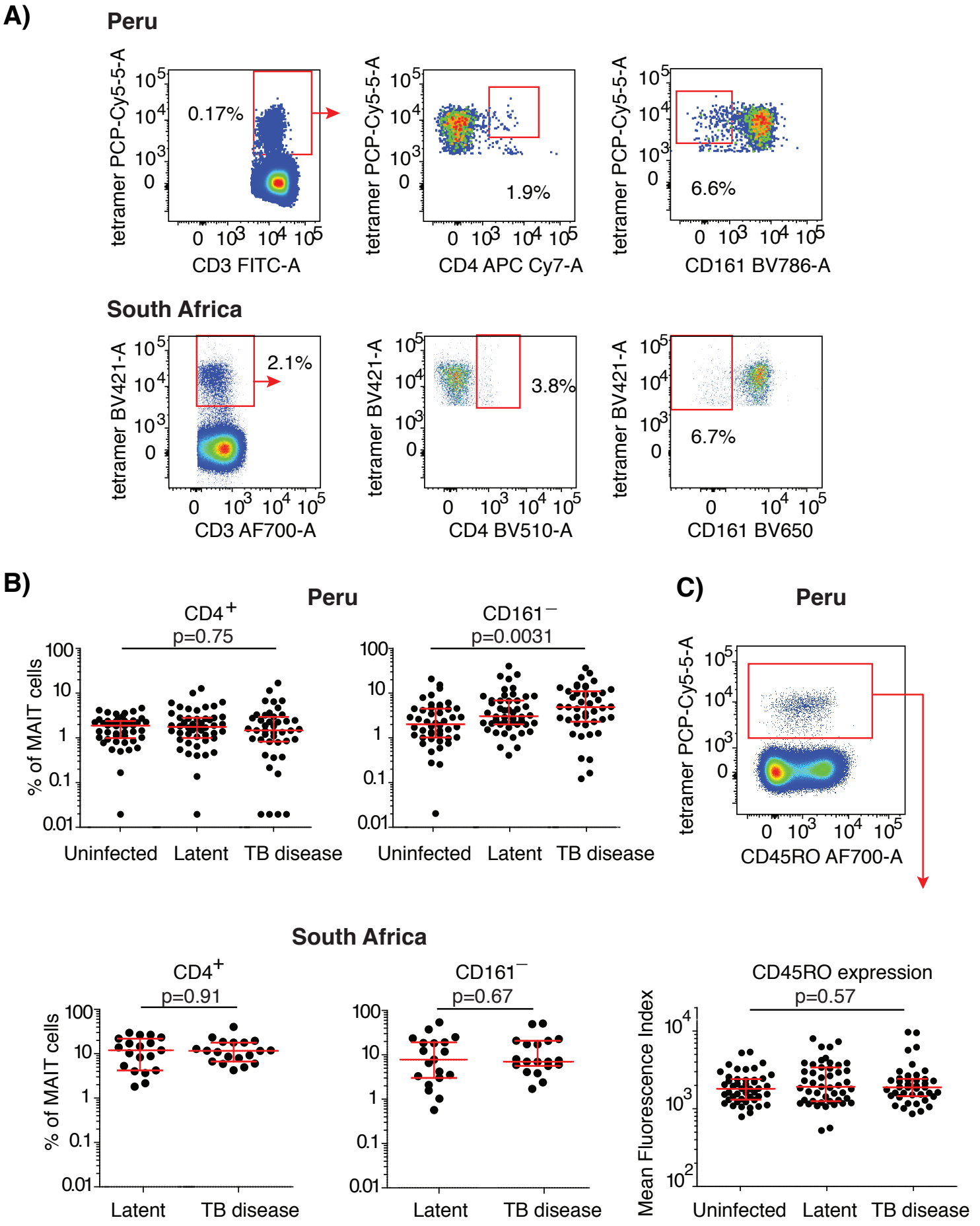
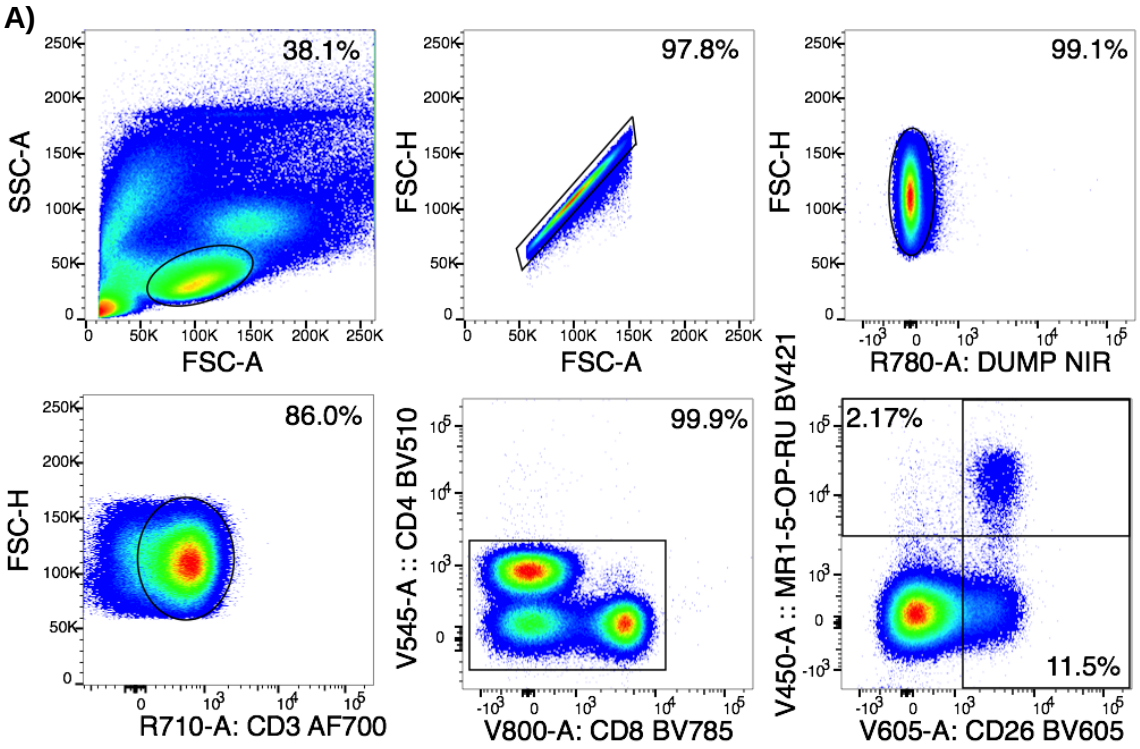


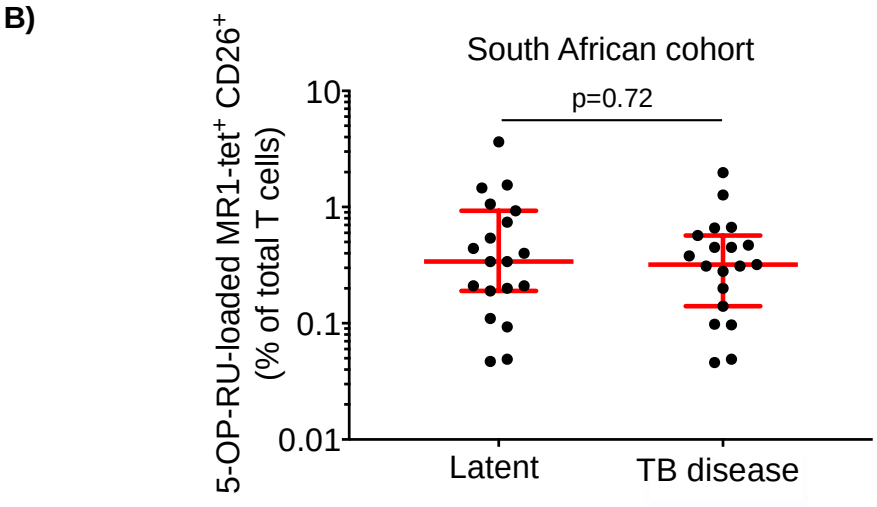

A)
South Africa
38%
98%
99%
86%
99.9%
SSC-A
FSC-H
FSC-A
CD14 CD19 R780
CD3 AF700
CD4 BV510
CD8 BV785
B)
5-OP-RU-loaded MR1 tetramer
2.1%
tetramer BV421-A
CD3 AF700-A
Phenotype-defined MAIT cells
2.1%
CD161 BV650
TRAV1-2 PE Cy7
C)
TRAV1-2+ CD161+ MAIT (% of total T cells)
ρ = 0.92; p< 0.001
MR1 tetramer-defined MAIT (% of total T cells)


A)
Peru
South Africa
p=0.06
p=0.7
p=0.13
p=0.87
MR1/5-OP-RU tetramer+ MAIT cells (% of total T cells)
10
1
0.1
0.01
Uninfected (n=44)
Latent (n=48)
TB disease (n=43)
Latent (n=19)
TB disease (n=19)
B)
p=0.06
p=0.65
p=0.12
p=0.86
TRAV1-2+CD161+ MAIT cells (% of total T cells)
C)
Male
Female
p=0.37
p=0.048
p=0.41
p=0.83
p=0.0074
p=0.88
p=0.54


A)
Peru
South Africa
MAIT cells (% of total T cells)
Body mass index
ρ= 0.11; p= 0.49
ρ= -0.03; p= 0.91
B)
DIAG3 score (Arbitrary Units)
ρ= -0.09; p= 0.55
ρ= 0.06; p= 0.82
C)
RISK6 score (Arbitrary Units)
ρ= 0.03; p= 0.87
ρ= -0.06; p= 0.82


A)
Peru
tetramer PCP-Cy5-5-A
0.17%
CD3 FITC-A
1.9%
CD4 APC Cy7-A
6.6%
CD161 BV786-A
South Africa
tetramer BV421-A
2.1%
CD3 AF700-A
3.8%
CD4 BV510-A
6.7%
CD161 BV650
B)
Peru
CD4+
p=0.75
CD161−
p=0.0031
% of MAIT cells
Uninfected
Latent
TB disease
C)
Peru
tetramer PCP-Cy5-5-A
CD45RO AF700-A
South Africa
CD4+
p=0.91
CD161−
p=0.67
CD45RO expression
p=0.57
Mean Fluorescence Index
Latent
TB disease
Uninfected

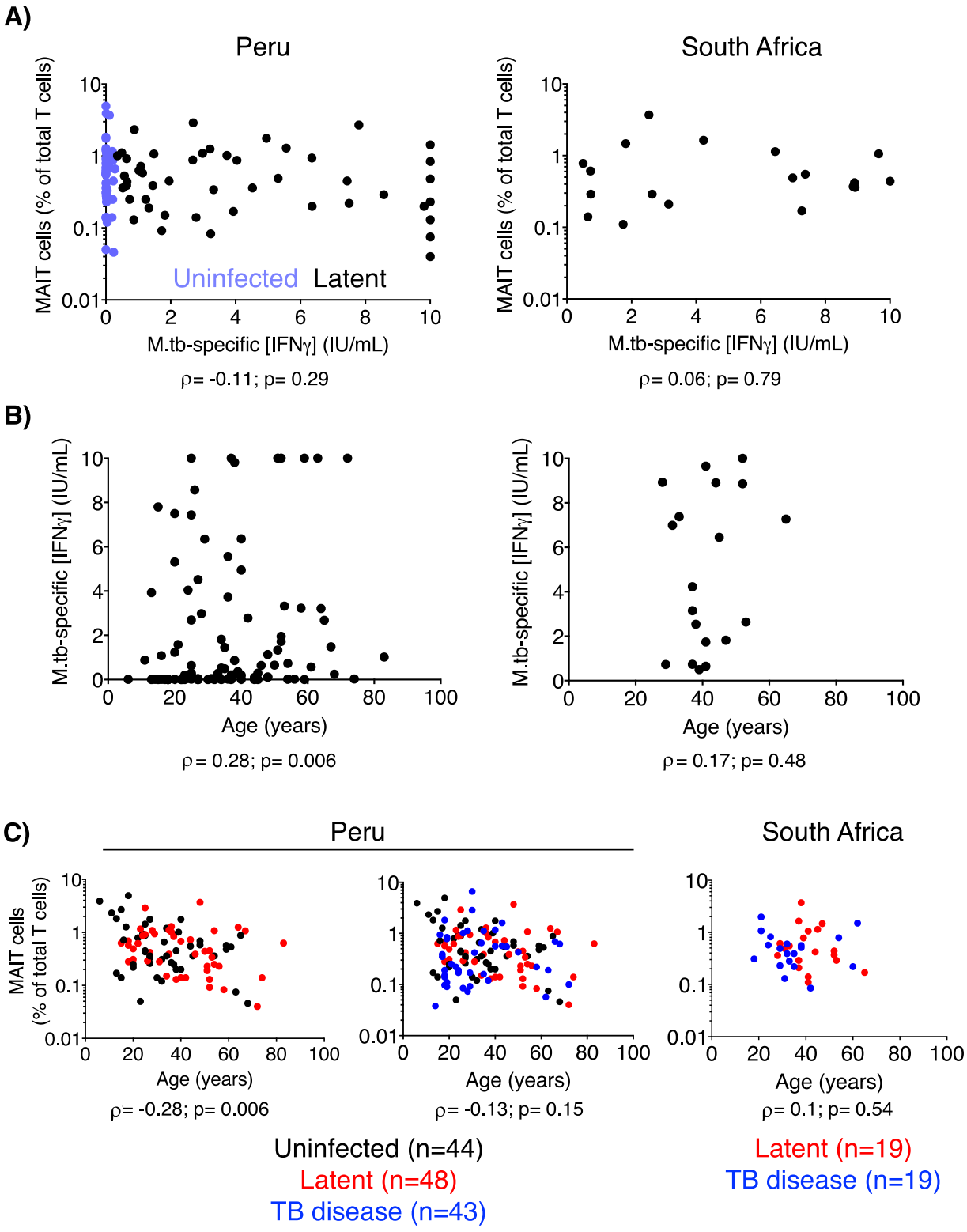




Supplementary Figure 1:

(A) Gating strategy for CD26$^+$ MR1-tetramer$^+$ double positive cells, as a stringent definition of MAIT cells.

(B) Frequencies of MR1-tetramer$^+$ MAIT cells that co-express CD26 out of all CD3$^+$ T cells in South African PBMC samples from participants with latent *Mtb* infection or active TB disease. Error bars denote medians and interquartile ranges.

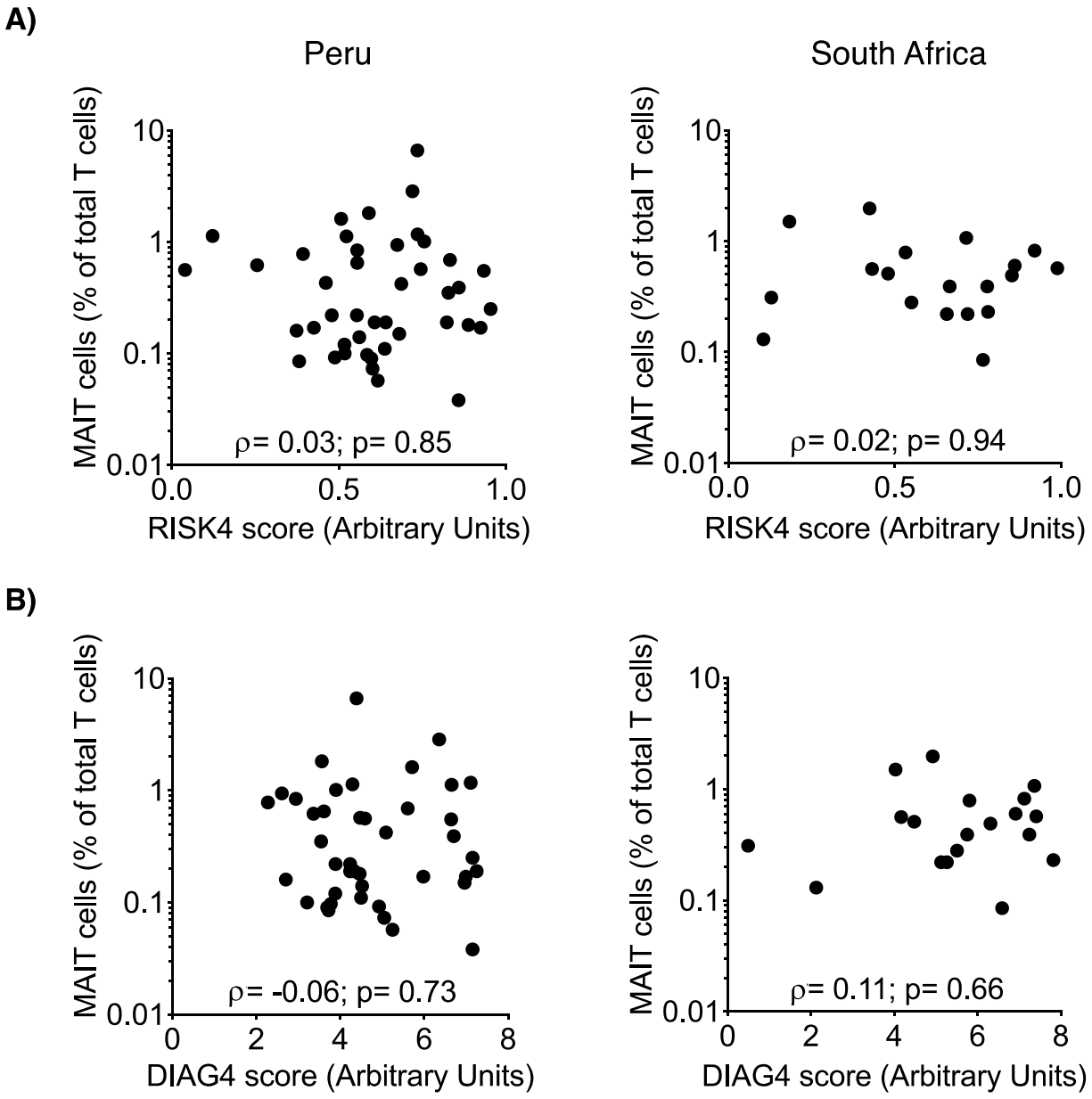

A)
Peru
South Africa
MAIT cells (% of total T cells)
M.tb-specific [IFNγ] (IU/mL)
Uninfected
Latent
ρ= -0.11; p= 0.29
ρ= 0.06; p= 0.79
B)
M.tb-specific [IFNγ] (IU/mL)
Age (years)
ρ= 0.28; p= 0.006
ρ= 0.17; p= 0.48
C)
Peru
South Africa
MAIT cells (% of total T cells)
Age (years)
ρ= -0.28; p= 0.006
ρ= -0.13; p= 0.15
ρ= 0.1; p= 0.54
Uninfected (n=44)
Latent (n=48)
TB disease (n=43)
Latent (n=19)
TB disease (n=19)

Supplementary Figure 2: MAIT cell frequencies decline with age in healthy individuals

(A) Correlation between background-subtracted concentrations of interferon gamma released in response to stimulation with *Mtb* antigens in the QuantiFERON TB-Gold assay and frequencies of MR1-tetramer^{+} cells in healthy Peruvian (left) and South African (right) participants. Correlation coefficient and p-values are calculated using Spearman non-parametric test.

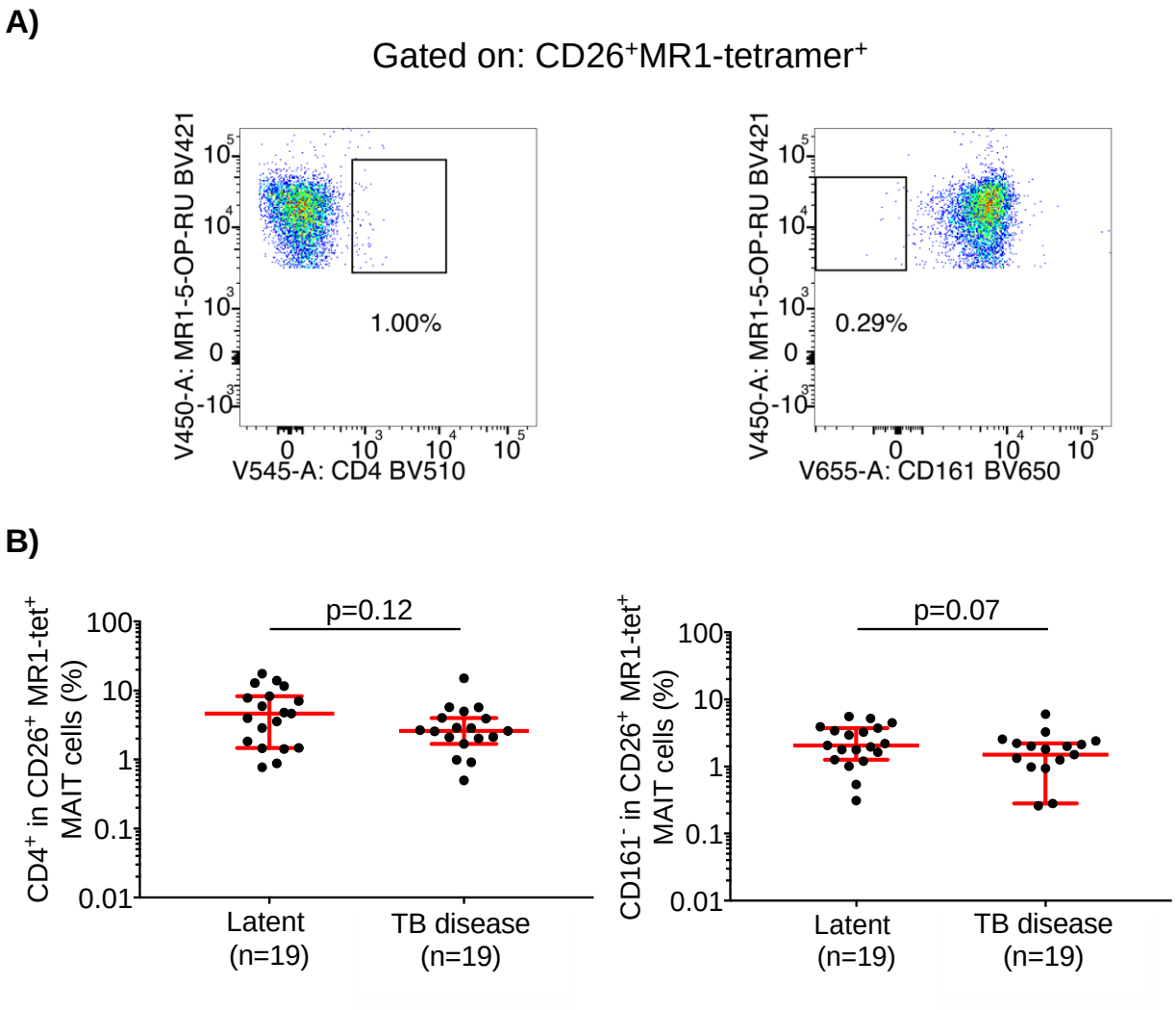
(B) Association between age and concentrations of *Mtb*-specific interferon gamma release measured by the QuantiFERON in-tube assay in healthy Peruvian household contacts of TB patients (left) or South African latently *Mtb*-infected individuals (right). Spearmen correlation coefficient and p-values are shown.

(C) Correlation between age and frequencies of 5-OP-RU loaded MR1-tetramer-binding MAIT cells in Peruvian participants when active TB patients are excluded (left), or included (middle), or in both latent and active TB South African participants (right). Correlation coefficient and p-values are calculated using Spearman non-parametric test.



Supplementary Figure 3: Transcriptional biomarkers of TB disease do not predict peripheral MAIT cell frequencies

Spearman correlation between transcriptional biomarkers of TB disease: RISK4 (top) and DIAG4 (bottom), with MAIT cell frequencies in Peruvian (left) or South African (right) TB patients. Spearmen correlation coefficients and unadjusted p-values are depicted for each association.



Supplementary Figure 4: Frequencies of atypical MAIT cells among CD26$^+$MR1-tetramer$^+$ defined MAIT cells in South African samples

(A) Gating strategy for CD4$^+$ (left) or CD161$^-$ (right) cells among CD26$^+$ MR1-tetramer$^+$ cells in South African PBMC samples.

(B) Frequencies of CD4$^+$ (left) or CD161$^-$ (right) among MR1-tetramer$^+$ MAIT cells that co-express CD26 in South African PBMC samples from participants with latent *Mtb* infection or active TB disease. Error bars denote medians and interquartile ranges. P-values correspond to Mann-Whitney *U* test results.

Supplementary Table 1: List of flow cytometry antibodies for Peruvian (A) or South African (B) samples

A)

Fluorochrome	Antigen	Clone	Catalogue no.	Supplier
AlexaFluor350	Viability		L34962	ThermoFisher
PerCP-Cy5.5	MR1-tetramer	-	-	in house*
FITC	CD3	SK7	340542	BD
Brilliant Violet 605	TRAV1-2	3C10	351720	Biolegend
APC-Cy7	CD4	RPA-T4	557871	BD
Brilliant Violet 786	CD161	DX12	744096	BD
AlexaFluor700	CD45RO	UCHL1	561136	BD

B)

Fluorochrome	Antigen	Clone	Catalogue no.	Supplier
APC-H7	Viability	-	L10119	ThermoFisher
APC-H7	CD14	MAB	563184	BD
APC-H7	CD19	SJ25C1	641395	BD
AlexaFluor700	CD3	UCHT1	300424	Biolegend
Brilliant Violet 510	CD4	RPA-T4	300546	Biolegend
Brilliant Violet 785	CD8	SK1	344740	Biolegend
Brilliant Violet 605	CD26	M-A261	344740	Biolegend
Brilliant Violet 650	CD161	DX12	563864	BD
PE-Cy7	TRAV1-2	3C10	351712	Biolegend
Brilliant Violet 421	5-OP-RU loaded MR1 tetramer	-	-	NIH tetramer core
AlexaFluor488	6-FP loaded MR1 tetramer	-	-	NIH tetramer core

5-OP-RU: 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil
6-FP: 6-formylpterin
* MR1-monomers loaded with either 5-OP-RU or 6-formylpterin were generated in the McCluskey lab

Supplementary Table 2: Taqman primer probes used for analysis of TB risk scores

Gene	Taqman probe ID	Model	Reference
GBP2	GBP2_Hs00894846_g1	RISK6	Penn-Nicholson (Manuscript under review). Preprint in *MedRxiv* manuscript ID: 19006197
FCGR1B	FCGR1B_Hs02341825_m1	RISK6	
SERPING1	SERPING1_Hs00934329_m1	RISK6	
TUBGCP6	TUBGCP6_Hs00363509_g1	RISK6	
TRMT2A	TRMT2A_Hs01000041_g1	RISK6	
SDR39U1	SDR39U1_Hs01016970_g1	RISK6	
GAS6	GAS6_Hs01090305_m1	RISK4	Suliman and Thompson, et al. *AJRCCM,* 2018
SEPT4	SEPT4_Hs00910208_g1	RISK4	
CD1C	CD1C_Hs00957534_g1	RISK4	
BLK	BLK_Hs01017452_m1	RISK4	
GBP5	GBP5_Hs00369472_m1	DIAG3	Suliman and Thompson, et al. *AJRCCM,* 2018- Signature adapted from Sweeney, et al. *Lancet Respiratory Medicine*, 2016
DUSP3	DUSP3_Hs01115776_m1	DIAG3	
KLF2	KLF2_Hs00360439_g1	DIAG3	
GBP1	GBP1_Hs00977005_m1	DIAG4	Suliman and Thompson, et al. *AJRCCM,* 2018- Signature adapted from Maertzdorf, et al. *EMBO Molecular Medicine* 2016
IFITM3	IFITM3_Hs03057129_s1	DIAG4	
P2RY14	P2RY14_Hs01848195_s1	DIAG4	
ID3	ID3_Hs00954037_g1	DIAG4	

Supplementary Table3A

Supplementary Table 3: Cohort demographics and transcriptional scores for Peruvian (A) or South African (B) participants. Highlighted samples in (A) have technical duplicates.

A) Peru

Participant ID	Group	Sex	Age (years)	Race (self-reported)	Frequency of 5-OP-RU-tet+ T cells	*M.tb*-specific interferon gamma release (IU/mL)*	Body mass index	Frequency of 5-OP-RU-tet+ T cells (repeat)	Time between two measurements (days)
LA2P-0004-3	TB disease	Female	19	American Indian	0.84	Not measured	21.53		
LA2P-0016-5	TB disease	Female	17	American Indian	0.35	Not measured	23.59		
LA2P-0022-2	TB disease	Female	66	American Indian	0.69	Not measured	19.77		
LA2P-0025-4	TB disease	Female	30	American Indian + white	6.63	Not measured	23.53	6.12	92
LA2P-0028-0	TB disease	Female	19	American Indian	0.11	Not measured	21.36	0.14	100
LA2P-0034-7	TB disease	Female	68	American Indian	0.62	Not measured	18.29		
LA2P-0045-6	TB disease	Female	72	American Indian + White	0.1	Not measured	28		
LA2P-0057-1	TB disease	Female	26	American Indian	1.01	Not measured	25		
LA2P-0062-1	TB disease	Female	52	American Indian	-	Not measured	-		
LA2P-0069-9	TB disease	Female	16	American Indian	0.94	Not measured	18.64		
LA2P-0074-6	TB disease	Female	43	American Indian + White	0.57	Not measured	24.54		
LA2P-0081-1	TB disease	Female	18	American Indian + White	1.82	Not measured	18.59		
LA2P-0105-9	TB disease	Female	35	American Indian	-	Not measured	-		
LA2P-0104-1	TB disease	Female	21	American Indian	0.25	Not measured	14.15		
LA2P-0128-2	TB disease	Female	23	American Indian + White	0.22	Not measured	21.72		
LA2P-0137-8	TB disease	Female	28	American Indian + White	1.13	Not measured	22.94		
LA2P-0157-5	TB disease	Female	19	American Indian + White	0.78	Not measured	22.79		
LA2P-0003-2	TB disease	Male	34	American Indian	0.65	Not measured	23.01		
LA2P-0010-5	TB disease	Male	14	American Indian + White	0.038	Not measured	17.31		
LA2P-0015-3	TB disease	Male	19	American Indian	0.09	Not measured	19.49		
LA2P-0019-7	TB disease	Male	23	Not reported	-	Not measured	-		
LA2P-0031-8	TB disease	Male	35	American Indian	1.12	Not measured	23.95		
LA2P-0037-8	TB disease	Male	18	American Indian + White	0.14	Not measured	20.94		
LA2P-0041-7	TB disease	Male	63	American Indian	0.19	Not measured	20.83		
LA2P-0042-3	TB disease	Male	29	American Indian	0.092	Not measured	21.84		
LA2P-0048-5	TB disease	Male	24	American Indian	0.17	Not measured	19.64		
LA2P-0053-0	TB disease	Male	55	American Indian	-	Not measured	-		
LA2P-0054-1	TB disease	Male	23	American Indian	-	Not measured	-		
LA2P-0063-1	TB disease	Male	37	White	0.12	Not measured	27.39		
LA2P-0066-1	TB disease	Male	24	American Indian	0.18	Not measured	16.02		
LA2P-0076-4	TB disease	Male	30	American Indian + white	2.85	Not measured	20.83	3.15	110
LA2P-0078-5	TB disease	Male	29	American Indian + White	0.17	Not measured	19.27		
LA2P-0087-4	TB disease	Male	40	American Indian + White	0.56	Not measured	28.65		
LA2P-0090-9	TB disease	Male	26	American Indian + White	0.085	Not measured	20.27		
LA2P-0095-4	TB disease	Male	58	American Indian + White	0.22	Not measured	23.05		
LA2P-0096-5	TB disease	Male	18	American Indian + White	0.097	Not measured	17.07		
LA2P-0099-2	TB disease	Male	62	American Indian + White	0.057	Not measured	26.06		
LA2P-0109-8	TB disease	Male	18	White	0.15	Not measured	21.22		
LA2P-0111-2	TB disease	Male	18	American Indian	0.19	Not measured	29.27		
LA2P-0115-7	TB disease	Male	50	American Indian + White	0.43	Not measured	20.47		
LA2P-0120-2	TB disease	Male	17	American Indian + White	0.39	Not measured	25.35		
LA2P-0131-1	TB disease	Male	24	American Indian + White	0.19	Not measured	19.43		
LA2P-0148-2	TB disease	Male	45	American Indian + White	0.55	Not measured	20.73		
LA2P-0200-7	TB disease	Male	31	American Indian + White	0.42	Not measured	19.56		
LA2P-0156-7	TB disease	Male	35	American Indian + White	0.16	Not measured	19.36		
LA2P-0141-5	TB disease	Male	28	American Indian + White	0.073	Not measured	26.73		
LA2P-0192-0	TB disease	Male	43	American Indian + White	1.61	Not measured	24.55		
LA2P-0189-7	TB disease	Male	40	American Indian + White	1.17	Not measured	24.72		
LA3P-0018-8	Uninfected	Female	33	American Indian + White	0.66	0.28	Not available		
LA3P-0041-7	Uninfected	Female	18	American Indian	4.94	0	Not available		
LA3P-0043-8	Uninfected	Female	44	American Indian	0.55	0	Not available	0.56	116
LA3P-0045-6	Uninfected	Female	13	American Indian	1.82	0	Not available	2.13	111
LA3P-0056-4	Uninfected	Female	25	American Indian + White	0.43	0.02	Not available		
LA3P-0061-2	Uninfected	Female	37	American Indian	0.92	0	Not available		
LA3P-0065-5	Uninfected	Female	27	American Indian	0.26	0.02	Not available		
LA3P-0075-5	Uninfected	Female	37	American Indian	0.25	0.21	Not available		
LA3P-0077-0	Uninfected	Female	15	American Indian + White	0.14	0	Not available		
LA3P-0079-1	Uninfected	Female	32	American Indian + White	0.12	0.04	Not available		
LA3P-0089-9	Uninfected	Female	27	American Indian + White	1.76	0	Not available		
LA3P-0091-5	Uninfected	Female	54	American Indian	0.36	0.02	Not available		
LA3P-0093-7	Uninfected	Female	38	American Indian	0.45	0.23	Not available		
LA3P-0115-7	Uninfected	Female	23	American Indian	0.05	0	Not available		
LA3P-0099-2	Uninfected	Female	48	American Indian	0.69	0.11	Not available		
LA3P-0135-8	Uninfected	Female	44	American Indian + White	0.73	0.1	Not available		
LA3P-0149-4	Uninfected	Female	54	American Indian + White	1.08	0.02	Not available		
LA3P-0151-9	Uninfected	Female	18	American Indian + White	0.69	0	Not available		
LA3P-0153-0	Uninfected	Female	31	American Indian	0.28	0.04	Not available		
LA3P-0161-3	Uninfected	Female	74	American Indian	0.14	0.03	Not available		
LA3P-0155-9	Uninfected	Female	25	American Indian	-	0.29	Not available		
LA3P-0175-1	Uninfected	Female	56	American Indian + White	0.23	0.02	Not available		
LA3P-0193-1	Uninfected	Female	17	American Indian + White	1.27	0	Not available		
LA3P-0195-4	Uninfected	Female	40	American Indian + White	0.14	0.19	Not available		
LA3P-0228-1	Uninfected	Female	18	American Indian + White	0.28	0.01	Not available		
LA3P-0004-3	Uninfected	Male	30	American Indian + White	0.14	0	Not available		
LA3P-0012-0	Uninfected	Male	16	American Indian	-	0.01	Not available		
LA3P-0015-3	Uninfected	Male	36	American Indian + White	0.27	0.02	Not available		
LA3P-0024-6	Uninfected	Male	59	American Indian + White	0.57	0	Not available		
LA3P-0060-2	Uninfected	Male	32	American Indian	0.31	0	Not available		
LA3P-0039-9	Uninfected	Male	6	American Indian + White	3.9	0.01	Not available		
LA3P-0050-5	Uninfected	Male	27	Black	0.33	0.04	Not available		
LA3P-0067-4	Uninfected	Male	24	American Indian	0.94	0.06	Not available	1.02	112
LA3P-0069-9	Uninfected	Male	22	American Indian	0.79	0	Not available		
LA3P-0113-0	Uninfected	Male	25	American Indian	0.88	0.22	Not available		
LA3P-0107-5	Uninfected	Male	68	American Indian	0.046	0.24	Not available		
LA3P-0101-2	Uninfected	Male	45	American Indian + White	-	0.29	Not available		
LA3P-0127-8	Uninfected	Male	34	American Indian	1.2	0	Not available		
LA3P-0121-8	Uninfected	Male	45	American Indian + White	0.36	0	Not available		
LA3P-0144-6	Uninfected	Male	15	American Indian + White	0.63	0.01	Not available		
LA3P-0131-1	Uninfected	Male	27	American Indian + White	0.42	0	Not available		
LA3P-0133-6	Uninfected	Male	14	American Indian + White	-	0.01	Not available		
LA3P-0138-4	Uninfected	Male	20	American Indian	0.31	0.01	Not available		
LA3P-0140-6	Uninfected	Male	18	American Indian + white	0.57	0	Not available	0.6	118
LA3P-0145-5	Uninfected	Male	23	American Indian	1.16	0.2	Not available		
LA3P-0197-0	Uninfected	Male	48	American Indian	3.69	0.11	Not available		
LA3P-0204-9	Uninfected	Male	40	American Indian + White	0.68	0.01	Not available		
LA3P-0230-1	Uninfected	Male	22	American Indian + White	0.62	0	Not available		
LA3P-0001-2	Latent *M.tb*-infected	Female	37	American Indian + White	0.84	> 10	Not available		
LA3P-0009-9	Latent *M.tb*-infected	Female	35	American Indian	0.39	1.45	Not available		
LA3P-0006-8	Latent *M.tb*-infected	Female	36	American Indian + White	1.29	5.56	Not available		
LA3P-0020-0	Latent *M.tb*-infected	Female	53	American Indian + White	0.34	3.32	Not available		
LA3P-0022-2	Latent *M.tb*-infected	Female	83	American Indian + White	0.63	1.02	Not available		
LA3P-0026-5	Latent *M.tb*-infected	Female	29	American Indian	0.94	6.35	Not available	1.11	120
LA3P-0033-8	Latent *M.tb*-infected	Female	51	American Indian + White	0.19	1.33	Not available		
LA3P-0035-9	Latent *M.tb*-infected	Female	34	American Indian	0.15	1.82	Not available	0.14	154
LA3P-0037-8	Latent *M.tb*-infected	Female	28	American Indian + White	1.09	2.98	Not available	0.98	139
LA3P-0029-5	Latent *M.tb*-infected	Female	26	American Indian + White	0.29	8.57	Not available		
LA3P-0048-5	Latent *M.tb*-infected	Female	54	American Indian + White	0.25	0.73	Not available		
LA3P-0063-1	Latent *M.tb*-infected	Female	58	American Indian	0.083	3.23	Not available		
LA3P-0073-6	Latent *M.tb*-infected	Female	52	American Indian + White	0.092	1.72	Not available		
LA3P-0084-1	Latent *M.tb*-infected	Female	67	American Indian + White	1.07	1.48	Not available		
LA3P-0082-5	Latent *M.tb*-infected	Female	38	American Indian + White	0.13	0.86	Not available		
LA3P-0087-4	Latent *M.tb*-infected	Female	20	American Indian + White	0.49	5.31	Not available		
LA3P-0095-4	Latent *M.tb*-infected	Female	52	American Indian + White	0.45	1.95	Not available		
LA3P-0109-8	Latent *M.tb*-infected	Female	24	American Indian	0.87	4.04	Not available		
LA3P-0111-2	Latent *M.tb*-infected	Female	64	Not reported	1.25	3.21	Not available		
LA3P-0106-1	Latent *M.tb*-infected	Female	46	American Indian	0.39	0.64	Not available		
LA3P-0117-6	Latent *M.tb*-infected	Female	42	American Indian	0.14	2.78	Not available		
LA3P-0120-2	Latent *M.tb*-infected	Female	25	American Indian	2.9	2.69	Not available		
LA3P-0129-5	Latent *M.tb*-infected	Female	20	American Indian	0.22	7.5	Not available		
LA3P-0141-5	Latent *M.tb*-infected	Female	48	American Indian + White	0.58	1.13	Not available		
LA3P-0173-3	Latent *M.tb*-infected	Female	27	American Indian + White	0.36	4.52	Not available		
LA3P-0190-1	Latent *M.tb*-infected	Female	15	American Indian + White	2.7	7.8	Not available		
LA3P-0179-8	Latent *M.tb*-infected	Female	61	American Indian + White	0.53	0.57	Not available		
LA3P-0199-5	Latent *M.tb*-infected	Female	34	American Indian + White	0.36	0.52	Not available		
LA3P-0185-0	Latent *M.tb*-infected	Female	38	American Indian + White	0.2	9.81	Not available		
LA3P-0206-0	Latent *M.tb*-infected	Female	65	American Indian + White	0.88	2.68	Not available		
LA3P-0007-3	Latent *M.tb*-infected	Male	50	American Indian + White	0.44	0.65	Not available		
LA3P-0013-3	Latent *M.tb*-infected	Male	35	American Indian	1.11	0.49	Not available		
LA3P-0051-5	Latent *M.tb*-infected	Male	21	American Indian + White	-	1.58	Not available		
LA3P-0058-1	Latent *M.tb*-infected	Male	51	American Indian	0.23	> 10	Not available		
LA3P-0071-1	Latent *M.tb*-infected	Male	72	American Indian	0.04	> 10	Not available		
LA3P-0086-8	Latent *M.tb*-infected	Male	52	American Indian + White	0.13	> 10	Not available		
LA3P-0097-5	Latent *M.tb*-infected	Male	25	American Indian	0.92	0.64	Not available		
LA3P-0103-8	Latent *M.tb*-infected	Male	36	American Indian + White	1.02	3.73	Not available		
LA3P-0125-3	Latent *M.tb*-infected	Male	40	American Indian	0.2	6.36	Not available		
LA3P-0123-9	Latent *M.tb*-infected	Male	25	American Indian	0.45	7.44	Not available		
LA3P-0147-4	Latent *M.tb*-infected	Male	11	American Indian	2.33	0.88	Not available		
LA3P-0164-3	Latent *M.tb*-infected	Male	13	American Indian + White	0.17	3.93	Not available		
LA3P-0166-3	Latent *M.tb*-infected	Male	39	American Indian	1.01	0.36	Not available		
LA3P-0168-8	Latent *M.tb*-infected	Male	20	American Indian	0.25	1.23	Not available		
LA3P-0188-6	Latent *M.tb*-infected	Male	40	American Indian + White	1.76	4.95	Not available		
LA3P-0177-4	Latent *M.tb*-infected	Male	63	American Indian + White	0.075	> 10	Not available		
LA3P-0181-0	Latent *M.tb*-infected	Male	16	American Indian + White	0.72	1.08	Not available		
LA3P-0183-1	Latent *M.tb*-infected	Male	59	American Indian + White	0.48	> 10	Not available		
LA3P-0202-0	Latent *M.tb*-infected	Male	25	American Indian + White	1.43	>10	Not available		

Supplementary Table3B

B) South Africa

Participant ID	Cohort	Sex	Age	Race (self-reported)	Frequency of 5-OP-RU-tet+ T cells	*M.tb*-specific interferon gamma release	Body mass index	CD161+TRAV1-2+CD3+ (%)
TB 15-01-0005 SHIP	TB disease	Female	29	Coloured	0.23	Not measured	26.46	0.098
TB 15-01-0007 SHIP	TB disease	Female	25	Black	0.82	Not measured	23.39	0.097
TB 15-01-0010 SHIP	TB disease	Female	54	Coloured	0.79	Not measured	23.95	0.66
TB 15-01-0012 SHIP	TB disease	Female	35	Black	0.39	Not measured	18.1	0.57
TB 15-01-0014 SHIP	TB disease	Female	33	Black	0.28	Not measured	20.77	0.67
TB 15-01-0016 SHIP	TB disease	Female	31	Coloured	0.13	Not measured	26.47	0.45
TB 15-01-0003 SHIP	TB disease	Male	60	Coloured	0.22	Not measured	18.6	0.28
TB 15-01-0008 SHIP	TB disease	Male	21	Black	1.07	Not measured	21.5	0.45
TB 15-01-0011 SHIP	TB disease	Male	38	Coloured	0.56	Not measured	18.98	0.14
TB 15-01-0013 SHIP	TB disease	Male	24	Black	0.57	Not measured	20.1	1.27
TB 15-01-0015 SHIP	TB disease	Male	62	Coloured	1.5	Not measured	17.6	0.049
TB 15-01-0019 SHIP	TB disease	Male	38	Coloured	0.51	Not measured	23.12	0.38
TB 15-01-0020 SHIP	TB disease	Male	42	Coloured	0.085	Not measured	19.46	0.046
TB 15-01-0021 SHIP	TB disease	Male	18	Coloured	0.31	Not measured	18.3	0.31
TB 15-01-0022 SHIP	TB disease	Male	21	Coloured	1.97	Not measured	22.7	1.98
TB 15-01-0025 SHIP	TB disease	Male	35	Black	0.22	Not measured	20.8	0.2
TB 15-01-0026 SHIP	TB disease	Male	29	Black	0.49	Not measured	23.8	0.31
TB 15-01-0027 SHIP	TB disease	Male	32	Black	0.6	Not measured	21	0.47
TB 15-01-0029 SHIP	TB disease	Male	32	Black	0.39	Not measured	19.97	0.32
LTBI 15-01-0001-SHIP	Latent *M.tb*-infected	Female	33	Coloured	0.55	7.38	33.3	0.44
LTBI 15-01-0002-SHIP	Latent *M.tb*-infected	Female	38	Coloured	3.7	2.54	26.2	3.64
LTBI 15-01-0003-SHIP	Latent *M.tb*-infected	Female	28	Coloured	0.36	8.92	23.6	0.19
LTBI 15-01-0005-SHIP	Latent *M.tb*-infected	Female	41	Coloured	0.14	0.65	31.6	0.2
LTBI 15-01-0006-SHIP	Latent *M.tb*-infected	Female	37	Coloured	1.64	4.23	40.3	0.047
LTBI 15-01-0007-SHIP	Latent *M.tb*-infected	Female	47	Coloured	1.47	1.82	34.5	1.55
LTBI 15-01-0009-SHIP	Latent *M.tb*-infected	Female	52	Coloured	0.44	11.5	26.9	1.46
LTBI 15-01-0015-SHIP	Latent *M.tb*-infected	Female	41	Coloured	1.06	9.65	33.3	0.34
LTBI 15-01-0018-SHIP	Latent *M.tb*-infected	Female	37	Caucasian	0.29	0.74	19.3	0.11
LTBI 15-01-0019-SHIP	Latent *M.tb*-infected	Female	41	Black	0.11	1.74	32.9	0.93
LTBI 15-01-0021-SHIP	Latent *M.tb*-infected	Female	65	Coloured	0.17	7.27	35.9	0.21
LTBI 15-01-0025-SHIP	Latent *M.tb*-infected	Female	29	Coloured	0.61	0.73	44	0.049
LTBI 15-01-0026-SHIP	Latent *M.tb*-infected	Female	53	Coloured	0.29	2.64	28.7	0.093
LTBI 15-01-0004-SHIP	Latent *M.tb*-infected	Male	52	Coloured	0.37	8.86	23.7	0.74
LTBI 15-01-0014-SHIP	Latent *M.tb*-infected	Male	37	Coloured	0.21	3.15	32.1	1.06
LTBI 15-01-0022-SHIP	Latent *M.tb*-infected	Male	39	Black	0.78	0.5	29.1	0.34
LTBI 15-01-0023-SHIP	Latent *M.tb*-infected	Male	45	Coloured	1.14	6.45	46.2	0.54
LTBI 15-01-0024-SHIP	Latent *M.tb*-infected	Male	44	Coloured	0.42	8.9	22.2	0.21
LTBI 15-01-0031-SHIP	Latent *M.tb*-infected	Male	31	Coloured	0.49	6.99	22.4	0.4

**Mtb*-specific interferon gamma release (IU/mL) is calculated as: *Mtb* antigen-stimulated IGRA - IGRA from unstimulated blood (Nil). Values below 0 are converted to 0, and values above 10 are outside the standard curve and recorded as >10

Supplementary Table4

Supplementary Table 4: Association between MAIT cell frequencies and TB status with and without adjustment for demographic co-variates using a generalized linear regression model

A) *Mtb* infection (healthy controls only):
Multivariate linear regression model: MAIT cell frequencies ~ IGRA + Age + Gender

Site	Variable	Regression coefficient		Significance
		Estimate	Standard Error	p-value
Peru only	IGRA (univariate)**	-0.02	0.03	0.449
	IGRA (multivariate)	-0.01	0.03	0.684
	Age	-0.01	0.01	0.007
	Gender (Male)	0.03	0.18	0.856
Both sites*	IGRA (univariate)	-0.02	0.02	0.371
	IGRA (multivariate)	-0.01	0.02	0.683
	Age	-0.01	0.01	0.006
	Gender (Male)	-0.02	0.16	0.909

B) TB disease status (all participants):
Multivariate linear regression model: MAIT cell frequencies ~ TB status + Age + Gender

Site	Variable	Regression coefficient		Significance
		Estimate	Standard Error	p-value
Peru only	TB disease (univariate)	-0.09	0.17	0.591
	TB disease (multivariate)	-0.10	0.17	0.555
	Age	-0.01	0.00	0.020
	Gender (Male)	-0.16	0.16	0.315
Both sites*	TB disease (univariate)	-0.11	0.14	0.413
	TB disease (multivariate)	-0.13	0.14	0.381
	Age	-0.01	0.00	0.015
	Gender (Male)	-0.14	0.14	0.323

C) Interaction model for TB disease status using log-transformed frequencies of MAIT cells:
Multivariate linear regression model: Log(MAIT cell frequencies) ~ TB status + Age + Gender + TB statusXGender

Site	Variable	Regression coefficient		Significance
		Estimate	Standard Error	p-value
Peru only	TB disease (univariate)	-0.15	0.08	0.068
	TB disease (multivariate)	0.02	0.11	0.835
	Age	-0.01	0.00	0.017
	Gender (male)	0.02	0.08	0.842
	TB status X Gender (interaction: male)	-0.26	0.14	0.073
	Gender (Female)	-0.02	0.09	0.806
	TB status X Gender (interaction: female)	0.42	0.17	0.013
Both sites*	TB disease (univariate)	-0.12	0.07	0.086
	TB disease (multivariate)	0.09	0.13	0.471
	Age	-0.01	0.00	0.021
	Gender (male)	0.02	0.09	0.806
	TB status X Gender (interaction: male)	-0.42	0.17	0.013
	Gender (Female)	-0.02	0.08	0.842
	TB status X Gender (interaction: female)	0.26	0.14	0.073

*Analysis includes both Peruvian and South African samples combined. Sample size does not permit a reliable independent analysis of South African samples.
**Linear regression result reported without adjustment (univariate) and after adjustment for age and gender as co-variates (multivariate)
***Cases are compared to all controls: Peruvian controls include both infected and uninfected household contacts

Supplementary Table 5: Transcriptomic TB signature scores in patients with active TB in Peruvian and South African cohorts

Participant ID	Cohort	Sample type	RISK6 score	RISK4 score	DIAG3 score	DIAG4 score
LA2P-0004-3	Peru	PBMC	0.26	0.56	1.57	2.95
LA2P-0016-5	Peru	PBMC	0.63	0.83	3.44	3.55
LA2P-0022-2	Peru	PBMC	0.46	0.83	3.24	5.61
LA2P-0025-4	Peru	PBMC	0.46	0.74	3.08	4.39
LA2P-0028-0	Peru	PBMC	0.41	0.64	3.04	4.50
LA2P-0034-7	Peru	PBMC	0.06	0.26	2.48	3.37
LA2P-0045-6	Peru	PBMC	0.22	0.52	2.64	3.21
LA2P-0057-1	Peru	PBMC	0.71	0.76	3.83	3.90
LA2P-0062-1	Peru	PBMC	0.16	0.48	2.22	4.44
LA2P-0069-9	Peru	PBMC	0.53	0.68	2.18	2.61
LA2P-0074-6	Peru	PBMC	0.21	0.75	2.23	4.48
LA2P-0081-1	Peru	PBMC	0.50	0.59	3.16	3.56
LA2P-0105-9	Peru	PBMC	0.81	0.83	4.71	5.79
LA2P-0104-1	Peru	PBMC	0.87	0.95	5.84	7.15
LA2P-0128-2	Peru	PBMC	0.62	0.55	5.09	4.24
LA2P-0137-8	Peru	PBMC	0.18	0.12	2.35	4.29
LA2P-0157-5	Peru	PBMC	0.50	0.39	4.58	2.28
LA2P-0003-2	Peru	PBMC	0.36	0.56	1.44	3.61
LA2P-0010-5	Peru	PBMC	0.45	0.86	4.91	7.15
LA2P-0015-3	Peru	PBMC	0.46	0.60	2.90	3.69
LA2P-0019-7	Peru	PBMC	0.53	0.79	3.31	5.21
LA2P-0031-8	Peru	PBMC	0.66	0.52	3.92	6.65
LA2P-0037-8	Peru	PBMC	0.62	0.56	3.41	4.52
LA2P-0041-7	Peru	PBMC	0.34	0.64	2.60	4.33
LA2P-0042-3	Peru	PBMC	0.31	0.49	2.83	4.92
LA2P-0048-5	Peru	PBMC	0.23	0.43	2.91	5.98
LA2P-0053-0	Peru	PBMC	0.47	0.88	3.13	4.88
LA2P-0054-1	Peru	PBMC	0.43	0.90	3.63	NA
LA2P-0063-1	Peru	PBMC	0.80	0.52	4.67	3.88
LA2P-0066-1	Peru	PBMC	0.86	0.89	3.46	4.46
LA2P-0076-4	Peru	PBMC	0.26	0.72	1.48	6.36
LA2P-0078-5	Peru	PBMC	0.80	0.92	3.87	6.99
LA2P-0087-4	Peru	PBMC	0.33	0.04	3.15	4.59
LA2P-0090-9	Peru	PBMC	0.25	0.38	1.42	3.72
LA2P-0095-4	Peru	PBMC	0.39	0.48	3.41	3.89
LA2P-0096-5	Peru	PBMC	0.67	0.58	3.81	3.78
LA2P-0099-2	Peru	PBMC	0.14	0.62	3.07	5.25
LA2P-0109-8	Peru	PBMC	0.52	0.68	4.27	6.96
LA2P-0111-2	Peru	PBMC	0.56	0.61	3.62	4.24
LA2P-0115-7	Peru	PBMC	0.44	0.46	2.93	NA
LA2P-0120-2	Peru	PBMC	0.70	0.86	5.68	6.71
LA2P-0131-1	Peru	PBMC	0.21	0.82	5.13	7.25
LA2P-0148-2	Peru	PBMC	0.87	0.93	4.76	6.64
LA2P-0200-7	Peru	PBMC	0.56	0.69	3.91	5.09
LA2P-0156-7	Peru	PBMC	0.40	0.37	2.39	2.71
LA2P-0141-5	Peru	PBMC	0.53	0.60	4.53	5.05
LA2P-0192-0	Peru	PBMC	0.32	0.51	3.42	5.71
LA2P-0189-7	Peru	PBMC	0.73	0.74	5.51	7.11
TB 15-01-0005 SHIP	South Africa	whole blood	0.91	0.78	7.18	7.82
TB 15-01-0007 SHIP	South Africa	whole blood	0.89	0.92	7.10	7.12
TB 15-01-0010 SHIP	South Africa	whole blood	0.79	0.53	6.24	5.81
TB 15-01-0012 SHIP	South Africa	whole blood	0.91	0.67	6.36	5.75
TB 15-01-0014 SHIP	South Africa	whole blood	0.87	0.55	6.39	5.51
TB 15-01-0016 SHIP	South Africa	whole blood	0.29	0.10	4.32	2.13
TB 15-01-0003 SHIP	South Africa	whole blood	0.81	0.72	4.21	5.13
TB 15-01-0008 SHIP	South Africa	whole blood	0.97	0.72	6.13	7.36
TB 15-01-0011 SHIP	South Africa	whole blood	0.85	0.43	4.41	4.17
TB 15-01-0013 SHIP	South Africa	whole blood	0.97	0.99	6.36	7.41
TB 15-01-0015 SHIP	South Africa	whole blood	0.82	0.18	3.74	4.03
TB 15-01-0019 SHIP	South Africa	whole blood	0.60	0.48	3.65	4.48
TB 15-01-0020 SHIP	South Africa	whole blood	0.93	0.77	6.95	6.59
TB 15-01-0021 SHIP	South Africa	whole blood	0.07	0.13	2.53	0.49
TB 15-01-0022 SHIP	South Africa	whole blood	0.69	0.42	6.09	4.92
TB 15-01-0025 SHIP	South Africa	whole blood	0.92	0.66	5.95	5.27
TB 15-01-0026 SHIP	South Africa	whole blood	0.89	0.85	5.48	6.31
TB 15-01-0027 SHIP	South Africa	whole blood	0.85	0.86	7.19	6.91
TB 15-01-0029 SHIP	South Africa	whole blood	0.93	0.78	5.72	7.25

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