

1 **DNA methylation patterns associated with prior tuberculosis infection in people with** 2 **HIV: a pilot cross-sectional study**

3 Joseph Baruch Baluku^{a,b}, Sharon Namiro^{c,a}, Daphine Kigongo^b, Brenda Namanda^a,
4 Hakiimu Kawalya^{d,n}, Irene Najjingo^a, Waiswa Geoffrey^e, Nixon Niyonzima^e, Naghib Bogere^e,
5 Edwin Nuwagira^f, Joshua Rhein^g, Nick Jones^g, Christian Kraef^h, Megan Shaughnessyⁱ, Arohi
6 Chauhan^j, Immaculate Nankya^k, Sayoki Mfinanga^l, Stanton Gerson^m, Bruce Kirenga^{a,c}.

7 ^aMakerere University Lung Institute, Kampala, Uganda

8 ^bDivision of pulmonology, Kiruddu National Referral Hospital, Kampala, Uganda

9 ^cDepartment of internal Medicine, Makerere University College of Health Sciences, Kampala,
10 Uganda

11 ^dThe African Center of Excellence in Bioinformatics and Data Intensive
12 Sciences, Kampala, Uganda

13 ^eUganda Cancer Institute, Kampala, Uganda

14 ^fDepartment of Internal Medicine, Mbarara University of Science and Technology

15 ^gDepartment of Medicine, University of Minnesota, Minnesota, USA

16 ^hDepartment of Infectious Diseases, Rigshospitalet, University of Copenhagen, Denmark

17 ⁱDivision of Infectious Disease, Department of Medicine, Hennepin Healthcare, Minnesota,
18 USA

19 ^jSouth Asian Institute of Health Promotion, Odisha, India

20 ^kJoint Clinical Research Center, Kampala, Uganda

21 ^lNational Institute for Medical Research, Muhimbili Center, Tanzania

22 ^mSchool of Medicine, Case Western Reserve University, USA

23 ⁿDepartment of Immunology and Molecular Biology, School of Biomedical Sciences, College
24 of Health Sciences, Makerere University, Kampala P.O. Box 7072, Uganda

25

26 ***Corresponding author**

27 PO Box 26343 Kampala, Uganda

28 bbjoe18@gmail.com

29 +256784088494

30

31 ABSTRACT

32 **Background:** Mechanisms by which prior tuberculosis (TB) increases long-term risk for
33 cancer, cardiovascular, and neurological disorders remain unclear, particularly in people with
34 HIV (PWH). This study investigated DNA methylation (DNAm) patterns and associated
35 pathways in PWH with and without prior TB infection.

36 **Methods:** DNAm was analyzed in blood samples from 30 PWH (10 with prior latent TB
37 infection [LTBI], 10 with previous successfully treated active TB, and 10 with no TB) using
38 the Illumina MethylationEPIC BeadChip covering over 850,000 CpG sites. Functional
39 enrichment analyses for Gene Ontology, KEGG pathways, and gene set enrichment analysis
40 were performed. Statistical significance was set at a false discovery rate of <0.05.

41 **Results:** A total of 25,084 differentially methylated CpGs (dmCpGs) were identified in the
42 prior active TB vs. no TB comparison, corresponding to 8 differentially methylated regions
43 (DMRs) in KCNC4-DT, GRAMD1C, ZNF44, FIGN, KCNN3, and PLA2G1B genes. In the
44 LTBI vs. no TB comparison, 7,682 dmCpGs were observed, corresponding to 18 DMRs in
45 SPATC1L, ZFP57, KCNN3, LRSAM1, PLEKHG5, MCF2L, BRSK2, SH3GL2, AP001468.58
46 and STK32C genes.

47 In both prior active TB vs. no TB and LTBI vs. no TB comparisons, DNAm changes were
48 enriched in pathways related to neurogenesis, neuron differentiation, glutamatergic synapse,
49 and neuroactive ligand-receptor interactions. The LTBI comparison showed additional
50 enrichment in pathways related to synaptic membrane and serotonergic synapse.

51 Cardiovascular pathways were specific to prior active TB, with significant enrichment in
52 vascular smooth muscle contraction, arrhythmogenic right ventricular cardiomyopathy,
53 hypertrophic cardiomyopathy, and dilated cardiomyopathy pathways.

54 Both TB groups showed enrichment in gene sets associated with lung, colorectal, gastric,
55 and breast cancers. The prior active TB group demonstrated additional enrichment for
56 prostate cancer and proteoglycans in cancer, while the LTBI group had additional
57 enrichment for endometrial, esophageal, liver cancers, and Ewing's sarcoma.

58 **Conclusion:** Prior TB infection in PWH is associated with DNAm changes in pathways
59 related to neural function, cardiovascular health, and cancer risk suggesting epigenetic
60 mechanisms for TB-related long-term complications.

61

62 **Key words:** DNA methylation, TB, HIV, cancer, cardiovascular disease, dementia

63

64 INTRODUCTION

65 Despite the improvement in diagnostic modalities and discovery of effective
66 chemotherapeutic agents, tuberculosis (TB) is the leading cause of death from a single
67 infectious agent, contributing 1.25 million deaths in 2023 [1]. Although the global tuberculosis
68 treatment success for drug sensitive TB is high, long-term complications of tuberculosis
69 contribute to significant disability and mortality even after TB treatment success [2,3]. After
70 completion of TB treatment, previously treated patients are 3 times more likely to die than
71 individuals without TB, and this is independent of age, sex, country income and type of TB
72 [4] or drug resistance profile [5]. For this reason, there are growing calls for programmatic
73 follow up of TB patients after TB cure or treatment completion [6,7]. While these calls draw
74 attention to the several post TB pulmonary complications resulting from structural changes in
75 the lung (such as chronic obstructive airway disease, bronchiectasis, and fibrosis) [7],
76 cardiovascular disease (CVD) and cancer account for almost 40% of deaths after TB
77 treatment completion, while respiratory causes contribute only 14% [4]. Evidence from
78 cohort studies shows that pulmonary TB (PTB) increases the risk for ischemic stroke [8],
79 acute coronary syndrome [9], myocardial infarction [10], and chronic kidney disease [11].
80 Additionally, PTB has been reported to increase the incidence of neurologic complications
81 even in the absence of central nervous system tuberculosis. Dementia [12], parkinsonism
82 [13] and depression [14] are reported in population-wide cohort studies. Finally, TB is
83 associated with an increased risk for cancer at ten sites, including head and neck cancer,
84 hepatobiliary cancer, Hodgkin's lymphoma, lung cancer, gastrointestinal cancer, non-
85 Hodgkin's lymphoma, pancreatic cancer, leukemia, kidney and bladder cancer, and ovarian
86 cancer [15]. Latent TB infection (LTBI) has been associated with similar risks for cancer,
87 CVD and mental health problems [16–18].
88 The mechanisms underlying the long-term complications of TB are not well elucidated.
89 Chronic inflammation and traditional CVD risk factors such as diabetes mellitus, smoking,
90 and age have been implicated in increasing CVD risk [19,20]. Active TB and LTBI could

contribute to cancer development by inducing chronic inflammation, genomic instability, and modulation of host cell signaling pathways [21]. Further, DNA methylation is thought to occur in genes and pathways involved in immune-regulation even after TB treatment. For example, this has been observed in genes involved in cytokine production (Interleukin (IL)-6), toll-like receptor signaling (TLR2), and other immune-related pathways (PI3K-AKT, MAPK, mTOR) [22]. However, these mechanisms have been demonstrated in predominantly HIV negative cohorts despite evidence that people with HIV (PWH) already experience ongoing accelerated aging driven by persistent inflammation, immune senescence, mitochondrial dysfunction, epigenetic alterations, and long term toxicities attributed to anti-retroviral therapy (ART) [23].

Our study aim was to determine whether prior TB infection is associated with DNA methylation (DNAm) patterns and pathways that could confer future risk for long term complications among PWH. By elucidating these mechanisms, the findings could inform targeted interventions to mitigate long-term complications, offering new avenues for improving the care of individuals with TB-HIV coinfection.

METHODS

Study design and population

In this cross-sectional study, 30 adult PWH on ART were randomly selected from three HIV clinics in Uganda. Detailed study methods are described elsewhere [24]. Participants were stratified into three groups: 10 with prior LTBI, 10 with previously treated active TB, and 10 with no history of TB infection. Previous treatment for active TB (diagnosed either by sputum GeneXpert, microscopy or urine lipoarabinomannan) was ascertained from the HIV care records and the unit TB register at the respective clinics. LTBI was defined as a positive Quantiferon (QFT)-plus assay, according to manufacturer's instructions, in an individual without previous treatment for active TB [25]. PWH with LTBI had all completed a course of TB preventive therapy according to national guidelines prior to enrollment [26]. Prior TB was

defined as either LTBI or previous treated active TB. PWH with no TB had never been treated for active TB and had a negative QFT-plus assay.

Sample collection and processing

Whole blood samples (5 ml) were collected from each participant using EDTA vacutainers. Genomic DNA was extracted from the blood using the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. DNA quantity was measured using Qubit 4 fluorometer (ThermoFisher Scientific, USA), following the manufacturer's instructions. For the methylation assay, 500 ng of DNA was used. DNA methylation (DNAm) levels were assayed using the Illumina Infinium MethylationEPIC v2.0 BeadChip array and the iScan system (Illumina, Inc., San Diego, CA, USA), in accordance with the manufacturer's instructions, generating IDAT files for downstream bioinformatics analysis to assess methylation levels.

Data processing and analysis

The raw data files obtained from the Illumina MethylationEPIC BeadChip were preprocessed and analyzed using the minfi package in the R statistical software environment. The minfi package is a comprehensive toolkit specifically designed for the analysis of Illumina Infinium methylation arrays [27]. The preprocessing steps involved an initial quality assessment of the raw data using various metrics, including visualization of beta value distributions and evaluation of control probes to ensure optimal bisulfite conversion efficiency. Subsequently, background correction and quantile normalization were performed using the preprocessQuantile function to minimize technical variation and ensure consistent methylation value distributions across samples. To ensure data quality, probes with a detection p-value greater than 0.01 were removed, and probes located on sex chromosomes (X and Y) were excluded to avoid confounding due to sex differences. Additionally, probes containing single nucleotide polymorphisms (SNPs) were removed using the dropLociWithSnps function to prevent methylation differences caused by genetic variation.

Blood cell composition was estimated using the estimateCellCounts2 function, referencing the FlowSorted.CordBloodCombined.450k dataset and utilizing the IDOLOptimizedCpGsCordBlood marker set. The cell types assessed included CD4+ T cells, CD8+ T cells, B cells, monocytes, natural killer (NK) cells, granulocytes, and nucleated red blood cells. The consistent proportion of blood cell types across samples, as detailed in **Supplementary Table 1**, indicated the absence of biological bias in the experimental setup. This consistency is important because the selected Horvath's methylation clock, used for age estimation, incorporates various cell types, including blood cells.

Methylation beta values were also used to estimate epigenetic age for each individual using Horvath, a first-generation DNAm epigenetic clock [28]. Age acceleration represented the extent to which the biological age of an individual exceeds their chronological age at the time of measurement. Age estimates were determined using dnaMethyAge package version 0.2.0 and the measure for age acceleration was defined as residuals yielded from regressing epigenetic age on chronological age from which root mean squared deviation (RMSD) and mean absolute deviation (MAD) were computable.

Identification of differentially methylated CpGs and regions

Differentially methylated CpGs (dmCpGs) and regions (DMRs) were identified using the dmpFinder function in minfi, which applies a linear model to compare methylation levels while adjusting for age and sex. This function performed pairwise comparisons between the three groups (LTBI, active TB, and no TB) to identify CpG sites that exhibit significant differences in methylation levels. Significant regions were defined by β -value differences >0.2 and permutation testing ($B=0$). The dmpFinder function utilizes statistical tests to assess the significance of methylation differences, adjusting for multiple comparisons to control the false discovery rate (FDR). An FDR <0.05 was considered statistically significant. The Comparisons included: 1. Previous Active TB vs. no TB; 2. Prior LTBI TB vs. no TB; 3. Prior TB infection (both LTBI and previous active TB) vs. no TB; and 4. Previous active TB vs. LTBI.

Pathway Analysis

Pathway and gene ontology (GO) enrichment analyses were conducted using the missMethyl package to identify biological pathways associated with significant DMRs. These included GO enrichment analysis [29] using the gometh function and pathway analysis through the gsmeth function, leveraging gene sets from the Kyoto Encyclopedia of Genes and Genomes (KEGG) [30], Molecular Signatures Database (MSigDB) [31] and PANTHER version 16 database [32]. All statistical analyses were carried out using R version 4.3.1.

RESULTS

Characteristics of study participants of PWH with and without prior TB

Of 30 participants, 17 (57%) were female and the median (interquartile range, IQR) age was 46.5 (40.0 – 50.0) years. All participants were on ART and had achieved viral load suppression (viral load <1,000 copies/ml). **Table 1** shows characteristics of the participants.

Chronological and epigenetic age of i PWH with and without prior TB

The median chronological and epigenetic ages for the whole study population were 46.5 and 51.5 years, respectively. When stratified by group, the median chronological and epigenetic ages were as follows: previous active TB (47.5 vs. 53.2 years), LTBI (42.0 vs. 47.4 years), and no TB groups (45.5 vs. 49.6 years). The comparison of epigenetic and chronological age between prior TB infection (both LTBI and active) and those without TB is shown in **figure 1**. The results demonstrate an overall trend toward accelerated aging among PWH that was more pronounced in people with prior TB infection (both LTBI and active TB).

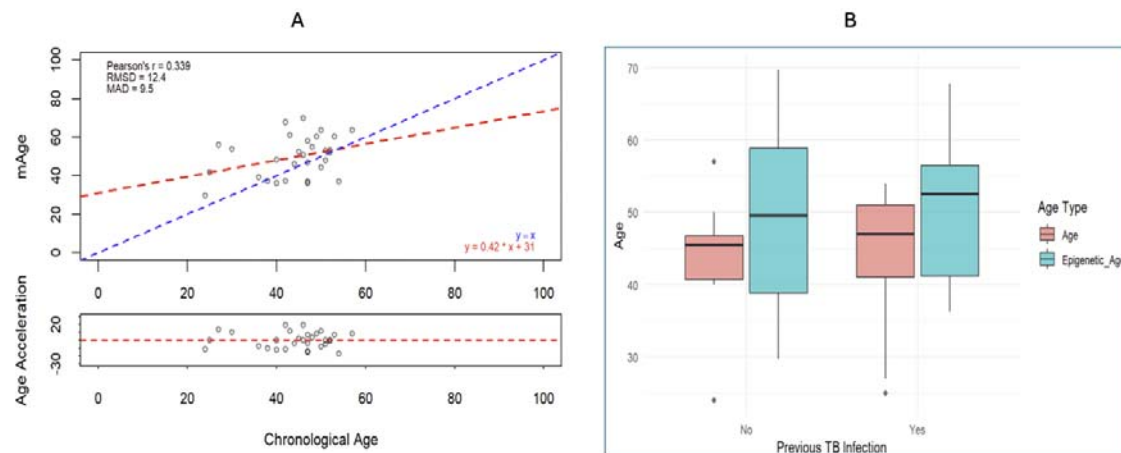


Figure 1: Epigenetic Age (DNAm) Compared to Chronological Age in Individuals with and without Prior Tuberculosis Infection. (A) Scatter plot of DNAm age (y-axis) versus chronological age (x-axis) estimated using Horvath's 2018 methylation clock. The blue dashed line represents perfect agreement between DNAm age and chronological age, while the red dashed line represents the regression line derived from the actual data. Most samples are above the blue line, indicating an overestimation of DNAm age. The bottom panel shows age acceleration values (DNAm age minus chronological age residuals), with positive values indicating accelerated aging (DNAm age > chronological age) and negative values indicating decelerated aging (DNAm age < chronological age). The results demonstrate a slight overall trend toward accelerated aging. (B) Box plot comparing DNAm age (Epigenetic_Age) and chronological age (Age) between individuals with previous TB infection and those without TB infection. DNAm age is higher than chronological age in both groups, and individuals with a history of TB show slightly higher DNAm age compared to the group without prior TB. This suggests potential epigenetic age acceleration associated with prior TB infection.

DNA methylation patterns in PWH with and without prior TB

A total of 25,084 differentially methylated CpGs (dmCpGs) were identified in the comparison between prior active TB vs. no TB groups (**Table 2 and Figure 2: A1&A2**). These dmCpGs corresponded to 8 differentially methylated regions (DMRs) associated with the following genes: KCNC4-DT (hypomethylation), FIGN (hypomethylation), MS4A4A (hypomethylation), GRAMD1C (hypermethylation), ZNF44 (hypermethylation), KCNN3 (hypermethylation), and PLA2G1B (hypermethylation).

In the comparison between LTBI vs. no TB, 7,682 dmCpGs were observed (**Table 2 and Figure 2: B1&B2**). These were associated with 18 DMRs, including genes such as ZFP57 (hypomethylation), PLEKHG5 (hypomethylation), STK32C (hypomethylation), MCF2L (hypomethylation), KCNN3 (hypermethylation), LRSAM1 (hypermethylation), AP001468.58

217 (hypermethylation), CTA-280A3.2 or EPIC1-LOC124905150 (hypermethylation), SH3GL2
218 (hypermethylation) as well as SPATC1L and BRSK2 gene regions with both hypo and
219 hypermethylated probes.

220 Overall, 38,558 dmCpGs were identified when comparing individuals with any history of TB

221 infection (both active and latent TB) to the control group (**Table 2 and Figure 2: C1&2**).

222 These dmCpGs corresponded to 8 DMRs associated with genes such as KCNC4-DT

223 (hypomethylation), PLEKHG5 (hypomethylation), SLC16A9 (hypomethylation), BRSK2
 224 (hypomethylation), MCF2L (hypomethylation) and KCNN3 (hypermethylation).

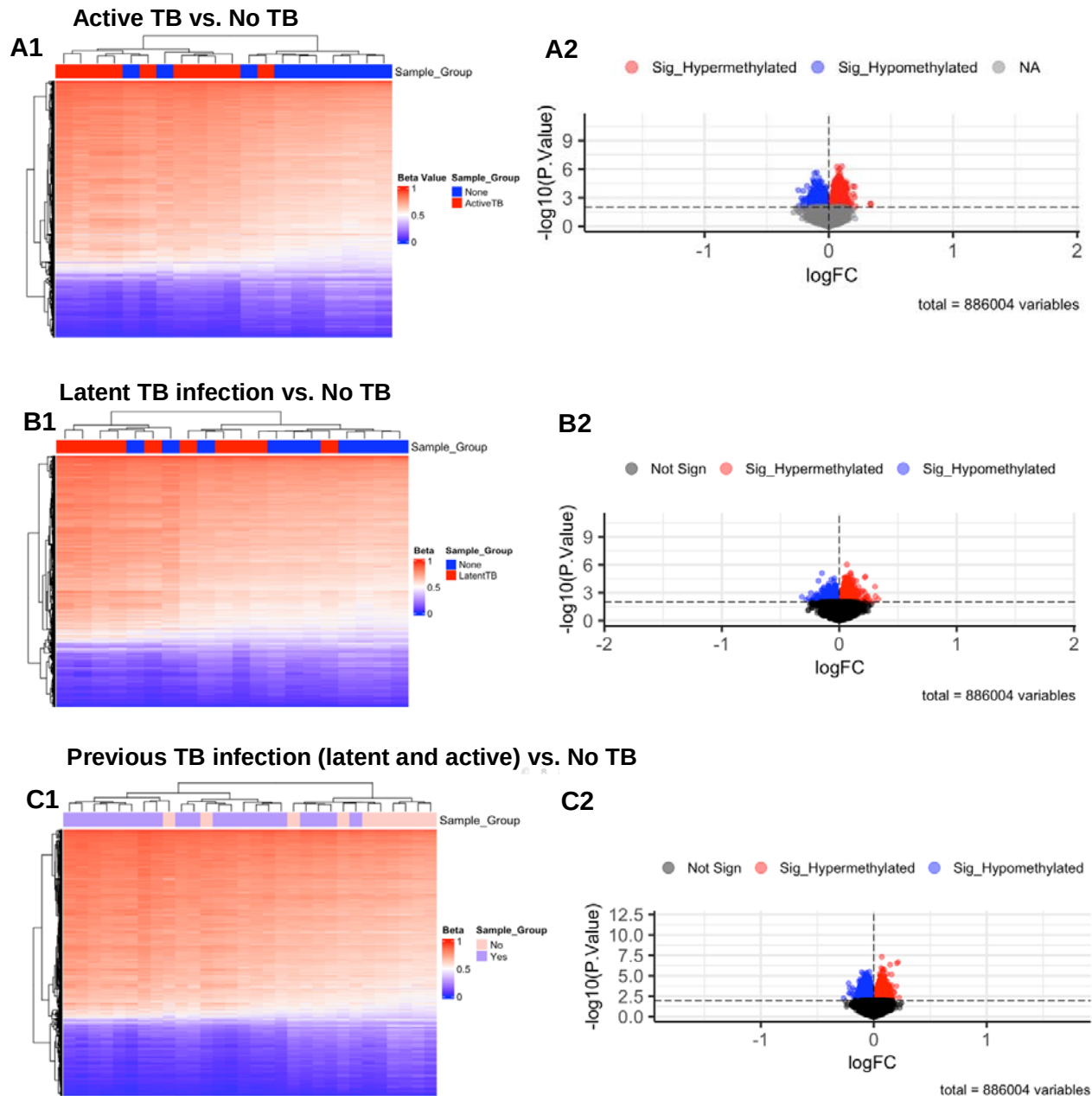


Figure 2: Heatmaps A1, B1, and C1 derived from beta values of the differentially methylated CpG sites identified in pairwise comparison across different groups with stringency criteria of adjusted $P < 0.01$ and mean methylation difference > 0.2 . Dendrogram shows separation based on groups. Enhanced Volcano plots A2, B2 and C2 of the differentially methylated CpG-sites separating different groups. The x-axis is the log fold change and the y-axis is the negative log10 of p-value with the cut off p-value of 0.05 shown with dash-dotted horizontal line. Blue CpGs indicate hypomethylated while red CpGs are hypermethylated.

225

226 **Enriched pathways associated with DNAm patterns in PWH with and without prior TB**

227 In both prior active TB vs. no TB and prior LTBI vs. no TB comparisons, DNAm changes
228 were enriched in pathways related to neurogenesis and neuron differentiation. Comparing
229 groups exposed to prior TB (both active and LTBI) to controls revealed enrichment in
230 pathways related to arrhythmogenic right ventricular cardiomyopathy, proteoglycans in
231 cancer, neuroactive ligand-receptor interactions, axonal guidance, glutamatergic synapses,
232 lung cancer, and breast cancer (**Figure 3A**).

233 LTBI showed additional enrichment in pathways related to glutamatergic and serotonergic
234 synapses, neuroactive ligand-receptor interactions (**Figure 3B**). Cardiovascular pathways
235 were specific to prior active TB, with enrichment in vascular smooth muscle contraction,
236 arrhythmogenic right ventricular cardiomyopathy, hypertrophic cardiomyopathy, and dilated
237 cardiomyopathy pathways (**Figure 3C**).

238 Both pairwise comparisons (prior active TB vs. no TB and prior LTBI vs. no TB comparisons)
239 showed enrichment in gene sets associated with lung, colorectal, gastric, and breast
240 cancers. The prior active TB group demonstrated additional enrichment for prostate cancer
241 and proteoglycans in cancer, while the LTBI group had additional enrichment for
242 endometrial, esophageal, liver cancers, and Ewing's sarcoma.

243 Analysis using the PANTHER database consistently showed enrichment in the Wnt signaling
244 pathway, with 34 associated genes in the active TV vs no TB comparison and 17 genes in
245 the latent TB vs. no TB comparison. Pathways involved in inflammation mediated by
246 chemokine and cytokine signaling, integrin signaling, and angiogenesis were also enriched
247 (**Table 3**).

248

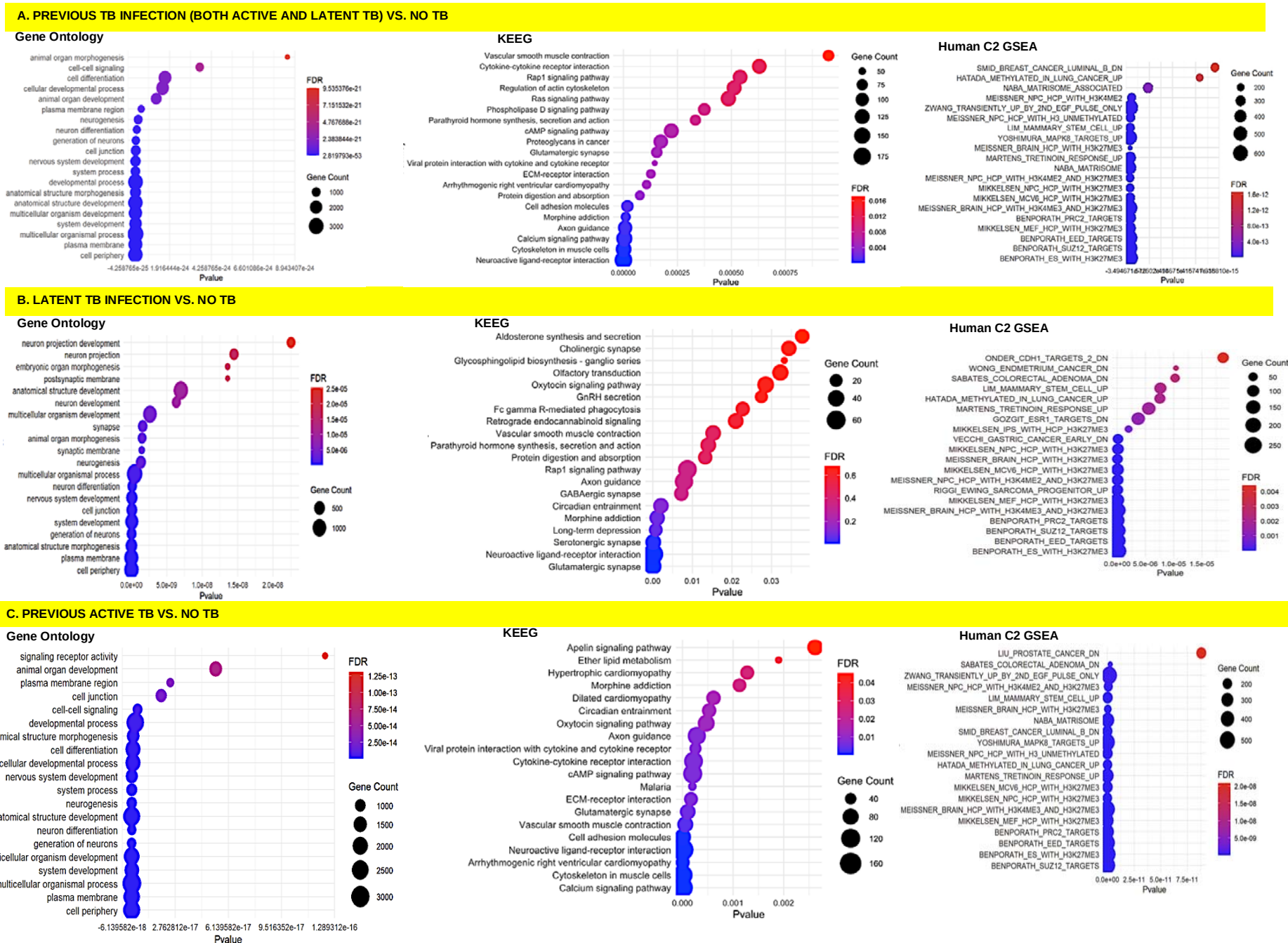


Figure 3: Pathway enrichment analysis for association of prior TB and DNA methylation. In each panel, the x-axis represents the p-value for pathway enrichment (adjusted for false discovery rate [FDR]), while the y-axis lists the enriched pathways. The size of the dots indicates the number of genes involved in each pathway, and the color gradient represents the FDR, with red indicating higher significance (lower FDR)

DISCUSSION

In this study, we investigated DNAm patterns associated with prior TB infection in individuals with HIV. Our findings reveal that both prior active TB and LTBI are associated with distinct DNAm changes, particularly in pathways related to neural function and development, cardiovascular health, and cancer risk. In addition, there was a trend suggesting that prior TB accelerates aging among PWH. These observations suggest potential mechanisms through which TB infection may contribute to long-term complications in individuals with HIV.

Our study identified numerous dmCpGs and DMRs associated with prior TB infection. Notably, both prior active TB and LTBI were associated with DNAm changes in pathways related to neurogenesis and neuron differentiation. LTBI showed additional enrichment in pathways related to glutamatergic and serotonergic synapses, and neuroactive ligand-receptor interactions, potentially indicating an impact on synaptic plasticity and neurotransmission mediated through the Wnt signaling pathway and inflammation pathways as shown by our enrichment analysis from the PANTHER database [33]. Interestingly there was a trend in enrichment of pathways related to long-term depression as well ($p=7.57 \times 10^{-4}$, FDR = 0.068). These findings suggest that prior TB infection, even in the absence of active disease, may have long-term consequences for neurocognitive function in PWH [34].

Furthermore, we observed distinct DNAm changes related to cardiovascular health. Prior active TB was specifically associated with enrichment in pathways related to vascular smooth muscle contraction, arrhythmogenic right ventricular cardiomyopathy, hypertrophic cardiomyopathy, and dilated cardiomyopathy. It is unclear whether this is through TB's modification of traditional CVD risk factors or a direct effect of TB on the myocardium [19,20]. However, our previous work among PWH with prior active TB suggests that prior TB is associated with normal body mass index, lower prevalence of dyslipidemia and no significant effect on hypertension risk [35]. Therefore, more studies are needed to

understand mechanisms by which prior pulmonary TB affects the cardiac rhythm, myocardium and myocardial contractility.

Our study also revealed DNAm changes in pathways associated with various cancers. Both prior active TB and LTBI showed enrichment in gene sets associated with lung, colorectal, gastric, and breast cancers, suggesting a potential link between prior TB infection and an increased risk of these cancers. Additionally, prior active TB demonstrated enrichment for prostate cancer and proteoglycans in cancer, while LTBI showed enrichment for endometrial, esophageal, liver cancers, and Ewing's sarcoma. These findings suggest that prior TB infection may influence cancer risk in individuals with HIV through epigenetic modifications. This is an agreement with epidemiological studies that have demonstrated that TB is associated with higher risk for incident lung, gastrointestinal, and tumors of the reproductive system tumors [15,36]. Moreover, the prominent enrichment in the gene sets associated with embryonic stem cell identity: BENPORATH_ES_WITH_H3K27ME3, BENPORATH_EED_TARGETS, and BENPORATH_SUZ12_TARGETS suggests potential risk for the development of aggressive, poorly differentiated, and estrogen receptor-negative tumors [37]. The association between prior TB and advanced cancer types needs to be demonstrated in epidemiological studies.

The mechanisms underlying the observed DNAm changes and their specific contributions to long-term complications warrant further investigation by larger studies. It is possible that chronic inflammation and immune dysregulation associated with TB infection may lead to persistent epigenetic modifications, even after the resolution of active disease [22].

Additionally, the interaction between TB infection, HIV infection, and antiretroviral therapy may further contribute to epigenetic changes and long-term complications.

Our study has several limitations. The cross-sectional design limits our ability to establish causal relationships between prior TB infection and DNAm changes. The relatively small sample size may have limited our power to detect additional significant associations.

Furthermore, our study focused on peripheral blood DNAm, which may not fully reflect epigenetic changes in other tissues relevant to long-term complications.

CONCLUSION

The findings of this study have several critical implications for clinical care, and public health. First, the identification of DNAm patterns associated with prior TB infection highlights the potential for developing predictive biomarkers. These biomarkers could enable early risk stratification in PWH, allowing targeted interventions to mitigate long-term complications such as CVD, neurocognitive disorders, and cancers. Second, integrating epigenetic profiling into routine TB-HIV care could pave the way for personalized medicine. Tailored follow-up protocols based on DNAm profiles could optimize screening and preventive care, particularly in resource-limited settings where resource constraints demand efficient allocation of healthcare resources. Nonetheless, further research is needed to elucidate the specific mechanisms underlying these epigenetic modifications and to explore potential interventions to mitigate the long-term risks associated with prior TB infection in individuals with HIV.

LIST OF ABBREVIATIONS

ART - Antiretroviral Therapy

CVD - Cardiovascular Disease

DMR - Differentially Methylated Region

DNAm - DNA Methylation

dmCpGs - Differentially Methylated CpG Sites

FDR - False Discovery Rate

HIV - Human Immunodeficiency Virus

KEGG - Kyoto Encyclopedia of Genes and Genomes

LTBI - Latent Tuberculosis Infection

326 **MSigDB** - Molecular Signatures Database

327 **NK** - Natural Killer (cells)

328 **PANTHER** - Protein Analysis Through Evolutionary Relationships

329 **PWH** - People with HIV

330 **QFT** - QuantiFERON

331 **RMSD** - Root Mean Squared Deviation

332 **TB** - Tuberculosis

333 **DECLARATIONS**

334 **Ethics approval and consent to participate**

335 Study participants provided written informed consent before study measurements were
336 undertaken. The study protocol was approved by the Mildmay Uganda Research and Ethics
337 Committee (#REC REF MUREC-107-2022). Further, the Uganda National Council of
338 Science and Technology provided additional approval as required by the guidelines for
339 conducting research in Uganda (HS2328ES). All methods were performed in accordance
340 with the relevant guidelines and regulations.

341 **Consent for publication**

342 Not applicable

343 **Availability of data and materials**

344 Datasets used in this study are available from the corresponding author upon reasonable
345 request.

346 **Competing interests**

347 The authors declare no relevant conflict of interest

348 **Funding**

349 This project was supported by funding from the National Cancer Institute through the Case
350 Comprehensive Cancer Center (Grant number U54CS254566). The funding source had no

role in the study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

Authors' contributions

JBB – conceptualization, methodology, investigation, data accrual, formal analysis, interpretation of results, drafting manuscript, editing manuscript, final approval
 SN - investigation, data accrual, interpretation of results, drafting manuscript, editing manuscript, final approval
 DK - investigation, data accrual, interpretation of results, drafting manuscript, editing manuscript, final approval
 BN - investigation, data accrual, interpretation of results, drafting manuscript, editing manuscript, final approval
 HK – methodology, investigation, data accrual, formal analysis, interpretation of results, drafting manuscript, editing manuscript, final approval
 IJ - interpretation of results, drafting manuscript, editing manuscript, final approval
 WG – methodology, investigation, formal analysis, interpretation of results, drafting manuscript, editing manuscript, final approval
 NN - investigation, interpretation of results, drafting manuscript, editing manuscript, final approval
 NB - investigation, interpretation of results, drafting manuscript, editing manuscript, final approval
 EW - investigation, data accrual, interpretation of results, drafting manuscript, editing manuscript, final approval
 JR - investigation, interpretation of results, drafting manuscript, editing manuscript, final approval
 NJ - investigation, interpretation of results, drafting manuscript, editing manuscript, final approval
 CK - investigation, interpretation of results, drafting manuscript, editing manuscript, final approval
 MS - investigation, interpretation of results, drafting manuscript, editing manuscript, final approval
 AC - investigation, interpretation of results, drafting manuscript, editing manuscript, final approval
 IN – methodology, investigation, data accrual, formal analysis, interpretation of results, drafting manuscript, editing manuscript, final approval
 SM - investigation, interpretation of results, drafting manuscript, editing manuscript, final approval
 SG - investigation, interpretation of results, drafting manuscript, editing manuscript, final approval
 BK - investigation, dinterpretation of results, drafting manuscript, editing manuscript, final approval

Acknowledgements

None

REFERENCES

1. World Health Organization. Global tuberculosis report 2024. World Health Organization; 2024.
2. Shah M, Reed C. Complications of tuberculosis. Current opinion in infectious diseases. 2014;27:403–10.

- 398 3. Baluku JB, Namanda B, Namiro S, Rwabwera DK, Mwesigwa G, Namaara C, et al. Death
399 after cure: Mortality among pulmonary tuberculosis survivors in rural Uganda. *International*
400 *Journal of Infectious Diseases* [Internet]. 2024 [cited 2024 Sep 16];144. Available from:
401 [https://www.ijidonline.com/article/S1201-9712\(24\)00140-1/fulltext](https://www.ijidonline.com/article/S1201-9712(24)00140-1/fulltext)
- 402 4. Romanowski K, Baumann B, Basham CA, Ahmad Khan F, Fox GJ, Johnston JC. Long-
403 term all-cause mortality in people treated for tuberculosis: a systematic review and meta-
404 analysis. *Lancet Infect Dis*. 2019;19:1129–37.
- 405 5. Blöndal K, Rahu K, Altraja A, Viiklepp P, Rahu M. Overall and cause-specific mortality
406 among patients with tuberculosis and multidrug-resistant tuberculosis. *The International*
407 *Journal of Tuberculosis and Lung Disease*. 2013;17:961–8.
- 408 6. Chakaya J, Kirenga B, Getahun H. Long term complications after completion of pulmonary
409 tuberculosis treatment: a quest for a public health approach. 2016;
- 410 7. Visca D, Centis R, Munoz-Torrico M, Pontali E. Post-tuberculosis sequelae: the need to
411 look beyond treatment outcome. *The International Journal of Tuberculosis and Lung*
412 *Disease*. 2020;24:761–2.
- 413 8. Sheu J-J, Chiou H-Y, Kang J-H, Chen Y-H, Lin H-C. Tuberculosis and the risk of ischemic
414 stroke: a 3-year follow-up study. *Stroke*. 2010;41:244–9.
- 415 9. Chung W-S, Lin C-L, Hung C-T, Chu Y-H, Sung F-C, Kao C-H, et al. Tuberculosis
416 increases the subsequent risk of acute coronary syndrome: a nationwide population-based
417 cohort study. *Int J Tuberc Lung Dis*. 2014;18:79–83.
- 418 10. Huaman MA, Kryscio RJ, Fichtenbaum CJ, Henson D, Salt E, Sterling TR, et al.
419 Tuberculosis and risk of acute myocardial infarction: a propensity score-matched analysis.
420 *Epidemiol Infect*. 2017;145:1363–7.
- 421 11. Shen T-C, Huang K-Y, Chao C-H, Wang Y-C, Muo C-H, Wei C-C, et al. The risk of
422 chronic kidney disease in tuberculosis: a population-based cohort study. *QJM: An*
423 *International Journal of Medicine*. 2015;108:397–403.
- 424 12. Peng Y-H, Chen C-Y, Su C-H, Muo C-H, Chen K-F, Liao W-C, et al. Increased risk of
425 dementia among patients with pulmonary tuberculosis: a retrospective population-based
426 cohort study. *Am J Alzheimers Dis Other Demen*. 2015;30:629–34.
- 427 13. Shen C-H, Chou C-H, Liu F-C, Lin T-Y, Huang W-Y, Wang Y-C, et al. Association
428 Between Tuberculosis and Parkinson Disease. *Medicine (Baltimore)* [Internet]. 2016 [cited
429 2019 Sep 9];95. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4779022/>
- 430 14. Sweetland AC, Kritski A, Oquendo MA, Sublette ME, Pala AN, Batista Silva LR, et al.
431 Addressing the tuberculosis–depression syndemic to end the tuberculosis epidemic. *Int J*
432 *Tuberc Lung Dis*. 2017;21:852–61.
- 433 15. Leung CY, Huang H-L, Rahman MM, Nomura S, Krull Abe S, Saito E, et al. Cancer
434 incidence attributable to tuberculosis in 2015: global, regional, and national estimates. *BMC*
435 *Cancer*. 2020;20:1–13.
- 436 16. Su VY-F, Yen Y-F, Pan S-W, Chuang P-H, Feng J-Y, Chou K-T, et al. Latent
437 Tuberculosis Infection and the Risk of Subsequent Cancer. *Medicine (Baltimore)*.
438 2016;95:e2352.

17. Hossain MB, Johnston JC, Cook VJ, Sadatsafavi M, Wong H, Romanowski K, et al. Role of latent tuberculosis infection on elevated risk of cardiovascular disease: a population-based cohort study of immigrants in British Columbia, Canada, 1985–2019. *Epidemiology & Infection*. 2023;151:e68.
18. Wong YJ, Noordin NM, Keshavjee S, Lee SWH. Impact of latent tuberculosis infection on health and wellbeing: a systematic review and meta-analysis. *European Respiratory Review* [Internet]. 2021 [cited 2022 Aug 25];30. Available from: <https://err.ersjournals.com/content/30/159/200260>
19. Yang J, Kim S-H, Sim JK, Gu S, Seok JW, Bae D-H, et al. Tuberculosis survivors and the risk of cardiovascular disease: analysis using a nationwide survey in Korea. *Frontiers in Cardiovascular Medicine*. 2024;11:1364337.
20. Huaman MA, Henson D, Ticona E, Sterling TR, Garvy BA. Tuberculosis and cardiovascular disease: linking the epidemics. *Trop Dis Travel Med Vaccines* [Internet]. 2015 [cited 2021 Jan 3];1. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4729377/>
21. Malik AA, Sheikh JA, Ehtesham NZ, Hira S, Hasnain SE. Can Mycobacterium tuberculosis infection lead to cancer? Call for a paradigm shift in understanding TB and cancer. *International Journal of Medical Microbiology*. 2022;312:151558.
22. Qin Y, Li T, An P, Ren Z, Xi J, Tang B. Important role of DNA methylation hints at significant potential in tuberculosis. *Arch Microbiol*. 2024;206:177.
23. Rodés B, Cadiñanos J, Esteban-Cantos A, Rodríguez-Centeno J, Arribas JR. Ageing with HIV: Challenges and biomarkers. *eBioMedicine*. 2022;77:103896.
24. Baluku JB, Namiiro S, Namanda B, Katusabe S, Namusoke D, Nkonge R, et al. Mycobacterium tuberculosis infection and cytogenetic abnormalities among people with HIV. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*. 2023;888:503640.
25. Qiagen. QuantiFERON-TB Gold Plus (QFT-Plus) ELISA package insert. 2017;
26. Musaazi J, Sekaggya-Wiltshire C, Okoboi S, Zawedde-Muyanja S, Senkoro M, Kalema N, et al. Increased uptake of tuberculosis preventive therapy (TPT) among people living with HIV following the 100-days accelerated campaign: A retrospective review of routinely collected data at six urban public health facilities in Uganda. *PLOS ONE*. 2023;18:e0268935.
27. Aryee MJ, Jaffe AE, Corrada-Bravo H, Ladd-Acosta C, Feinberg AP, Hansen KD, et al. Minfi: a flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays. *Bioinformatics*. 2014;30:1363–9.
28. Horvath S, Raj K. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. *Nat Rev Genet*. 2018;19:371–84.
29. The Gene Ontology Consortium. The Gene Ontology Resource: 20 years and still GOing strong. *Nucleic Acids Research*. 2019;47:D330–8.
30. Kanehisa M, Goto S. KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Research*. 2000;28:27–30.
31. Liberzon A, Subramanian A, Pinchback R, Thorvaldsdóttir H, Tamayo P, Mesirov JP. Molecular signatures database (MSigDB) 3.0. *Bioinformatics*. 2011;27:1739–40.

32. Mi H, Ebert D, Muruganujan A, Mills C, Albou L-P, Mushayamaha T, et al. PANTHER version 16: a revised family classification, tree-based classification tool, enhancer regions and extensive API. *Nucleic Acids Research*. 2021;49:D394–403.
33. Freese JL, Pino D, Pleasure SJ. Wnt signaling in development and disease. *Neurobiology of Disease*. 2010;38:148–53.
34. Hestad KA, Chinyama J, Anitha MJ, Ngoma MS, McCutchan JA, Donald R Franklin J, et al. Cognitive Impairment in Zambians with HIV infection and Pulmonary Tuberculosis. *Journal of acquired immune deficiency syndromes (1999)*. 2019;80:110.
35. Baluku JB. Does tuberculosis alter cardiometabolic profiles of TB survivors? A cross sectional study among people with HIV in Uganda. The Union/WHO late-breaker session on HIV and other comorbidities Oral Abstract No: LB-UnionConf-2024-441. Bali, Indonesia; 2024.
36. Luczynski P, Poulin P, Romanowski K, Johnston JC. Tuberculosis and risk of cancer: A systematic review and meta-analysis. *PLOS ONE*. 2022;17:e0278661.
37. Ben-Porath I, Thomson MW, Carey VJ, Ge R, Bell GW, Regev A, et al. An embryonic stem cell-like gene expression signature in poorly differentiated aggressive human tumors. *Nature genetics*. 2008;40:499.

512 **Table 1: Characteristics of study participants**

Characteristic	Total (N = 30), (%)	Prior active TB (n= 10), (%)	Prior latent TB infection (n = 10), (%)	No prior TB (n = 10), (%)	p-value
Age, median (IQR), (years)	46.5 (40.0-50.0)	47.5 (47.0-51.0)	42.0 (36.0-51.0)	45.5 (40.0-47.0)	0.36
Female sex	17 (57%)	5 (50%)	7 (70%)	5 (50%)	0.58
Duration on ART, median (IQR) (months)	144.0 (117.0-168.0)	144.0 (117.0-197.0)	144.0 (120.0-153.0)	144.0 (117.0-168.0)	0.99
Any history of alcohol use	17 (57%)	5 (50%)	5 (50%)	7 (70%)	0.58
Any history of cigarette smoking	3 (10%)	1 (10%)	1 (10%)	1 (10%)	1.00
Use of biomass fuel for cooking	18 (60%)	7 (70%)	5 (50%)	6 (60%)	0.66
Baseline CD4 Count at HIV diagnosis, median (IQR) (cells/mm ³)	208.0 (47.0-405.0)	217.5 (69.0-420.0)	184.0 (0.0-350.0)	136.0 (47.0-405.0)	0.72
Current CD4 Count at study enrolment, median (IQR) (cells/mm ³)	911.0 (691.0-1374.0)	847.5 (548.0-1357.0)	1141.5 (875.0-1451.0)	875.0 (589.0-1180.0)	0.50
Haemoglobin level, median (IQR), (g/dl)	15.0 (14.3-16.0)	15.2 (14.7-16.3)	14.4 (13.4-15.0)	15.6 (14.3-16.1)	0.15
Aspartate aminotransferase, median (IQR) (IU/L)	26.9 (23.5-33.5)	26.8 (23.4-33.5)	27.4 (24.7-31.4)	27.9 (22.3-35.3)	1.00
Gamma-glutamyl transferase, median (IQR), (IU/L)	46.5 (42.6-58.5)	44.8 (30.1-57.6)	45.6 (42.6-56.9)	51.9 (44.6-61.2)	0.37
Alanine aminotransferase, median (IQR), (IU/L)	22.2 (16.7-31.5)	19.7 (16.0-26.0)	23.6 (16.4-32.7)	23.4 (20.1-32.8)	0.55
Alkaline phosphatase, median (IQR) (IU/L)	114.0 (85.0-152.0)	109.5 (89.0-141.0)	120.0 (81.0-163.0)	119.5 (85.0-152.0)	0.94
Albumin, median (IQR), (g/l)	4.2 (3.4-5.8)	4.5 (3.4-6.3)	3.6 (2.2-4.9)	4.4 (3.6-5.6)	0.50
Total protein, median (IQR)(g/l)	7.7 (7.1-8.3)	7.6 (7.4-8.0)	7.5 (6.9-7.8)	8.3 (7.1-8.6)	0.13
Urea, median (IQR), (mmol/l)	3.7 (2.8-4.7)	3.7 (2.9-4.2)	4.4 (3.1-4.8)	2.9 (2.5-4.6)	0.39
Creatinine , median (IQR), (mmol/l)	113.0 (96.1-126.5)	113.0 (98.1-126.5)	104.7 (92.6-129.2)	118.5 (96.1-124.4)	0.88
Serum Uric acid, median (IQR), (μmol/l)	265.5 (195.0-370.6)	341.2 (261.2-454.8)	222.1 (195.0-287.2)	300.2 (36.1-370.6)	0.14
Serum lactate dehydrogenase, median (IQR), (U/L)	595.5 (490.0-780.0)	636.5 (450.0-882.0)	605.0 (555.0-689.0)	513.0 (430.0-751.0)	0.43

513 **Table 2: DNA methylation patterns among people with HIV with and without prior TB**
 514 **infection**

Chromosome	Start	End	SNP	Gene	Methylation Value	Area
Active TB Vs. No TB						
Chr1	110209635	110209685	rs1280981579	KCNC4-DT	-0.2273238	0.4546476
Chr3	113890077	113890077	rs1026956222	GRAMD1C	0.3387863	0.3387863
Chr19	12230577	12230577	rs1412969463	ZNF44	0.3342976	0.3342976
Chr2	163733762	163733762	rs867364910	FIGN	-0.2206179	0.2206179
Chr1	154867337	154867337	rs1015124254	KCNN3	0.2084786	0.2084786
Chr12	120326012	120326012	rs746790393	PLA2G1B	0.2078026	0.2078026
Chr3	46665072	46665072	rs1309260839	Non-exon region	-0.2070116	0.2070116
Chr11	60251763	60251763	rs112829924	MS4A4A	-0.2055199	0.2055199
Latent TB vs. No TB						
chr21	46161128	46161644	-	SPATC1L	-0.2251591	0.6754773
chr6	29680827	29681124	-	ZFP57	-0.2725237	0.5450474
chr21	46184138	46184252	-	SPATC1L	0.272069	0.544138
chr1	154867337	154867343	-	KCNN3	0.2201583	0.4403155
chr9	127479094	127479094	rs983018719	LRSAM1	0.3328147	0.3328147
chr1	6491645	6491645	rs1042531521	PLEKHG5	-0.3182199	0.3182199
chr21	46185260	46185260	rs1458119335	AP001468.58	0.304427	0.3044274
chr13	113176555	113176555	rs1011009098	Non-exon region	0.2545671	0.2545671
Chr17	16997705	16997705	rs756899690	Non-exon region	0.2430883	0.2430883
chr13	112980664	112980664	rs1040730563	MCF2L	-0.2410457	0.2410457
chr2	127695686	127695686	rs996351088	Non-exon region	0.2223182	0.2223182
chr11	1391915	1391915	rs1298386467	BRSK2	-0.2193803	0.2193803
chr4	183349316	183349316	rs751119789	Non-exon region	0.2173798	0.2173798
chr22	47918625	47918625	rs536912378	CTA-280A3.2	0.2166322	0.2166322
chr9	17612106	17612106	rs750183679	SH3GL2	0.2113893	0.2113893
chr10	132231205	132231205	rs1163463011	STK32C	-0.2069165	0.2069165
chr5	151613206	151613206	rs956799603	Non-exon region	-0.2026999	0.2026999
chr11	1435923	1435923	rs551352724	BRSK2	0.2009539	0.2009539
Previous TB infection (both active and latent TB) Vs. No TB						
chr1	154867337	154867433	rs1015124254	KCNN3	0.2100413	0.4200826
chr1	6491645	6491645	rs1042531521	PLEKHG5	-0.2672408	0.2672408
chr13	113176555	113176555	rs1011009098	Non-exon region	0.2242132	0.2242132
chr1	110209685	110209685	rs188603653	KCNC4-DT	-0.2226747	0.2226747
chr13	112980664	112980664	rs1040730563	MCF2L	-0.2195602	0.2195602
chr10	59654071	59654071	rs761027162	SLC16A9	-0.2140531	0.2140531
chr11	1391915	1391915	rs1298386467	BRSK2	-0.2074399	0.2074399
chr4	183349316	183349316	rs751119789	Non-exon region	0.2006364	0.2006364
Latent TB Vs. active TB						
chr19	50051194	50051194		ZNF473, FLJ26850	-0.2943314	0.2943314

chr2	30446891	30446891	rs1681268169	Non-exon region	-0.2796443	0.2796443
chr2	30446893	30446893	rs1327445118	Non-exon region	-0.2789843	0.2789843
chr21	46185260	46185260	rs1458119335	AP001468.58	-0.2477524	0.2477524
chr16	68070553	68070553	rs959716619	DUS2	-0.2143316	0.2143316
chr2	201666059	201666059	rs918060583	MPP4	0.2120617	0.2120617

Table 3: Pathway enrichment in PWH with and without prior TB from the PANTHER database

Pathway	Prior TB (both active and latent) vs. No TB	Active TB vs. No TB	Latent TB vs. No TB	Active TB vs. Latent TB
Wnt signaling pathway	41 genes	34 genes	17 genes	5 genes
Inflammation mediated by chemokine and cytokine signaling	31 genes	27 genes	7 genes	4 genes
Integrin signaling pathway	25 genes	25 genes	4 genes	2 genes
Angiogenesis	24 genes	20 genes	11 genes	1 gene
Cadherin signaling pathway	22 genes	18 genes	10 genes	5 genes
Heterotrimeric G-protein signaling pathway	26 genes	18 genes	8 genes	2 genes
FGF signaling pathway	14 genes	15 genes	None	None
Nicotinic Acetylcholine Receptor signaling pathway	13 genes	13 genes	5 genes	None
EGF signaling pathway	12 genes	12 genes	None	None
CCKR signaling pathway	16 genes	None	6 genes	1 gene
Interleukin signaling pathway	12 genes	None	5 genes	None
PDGF signaling pathway	10 genes	None	5 genes	1 gene
Hedgehog signaling pathway	None	None	16 genes	None
Apoptosis signaling pathway	None	None	None	2 genes
T-cell Activation	None	None	None	2 genes

520 **Supplementary Table: Proportion of blood Cell counts in the different study samples**
521

Sample IDs	CD8T	CD4T	NK	Bcell	Mono	nRBC
207895920065_R01C01	0.0192	0.119	0.0682	0.0555	0.0242	0.4665
207895920065_R02C01	0.0193	0.1188	0.0683	0.0555	0.0242	0.4665
207895920065_R03C01	0.0193	0.1188	0.0682	0.0555	0.0242	0.4667
207895920065_R04C01	0.0192	0.1189	0.0682	0.0554	0.0243	0.4666
207895920065_R05C01	0.0192	0.1189	0.0682	0.0555	0.0242	0.4666
207895920065_R06C01	0.0192	0.119	0.0683	0.0556	0.0242	0.4666
207895920065_R07C01	0.0192	0.1188	0.0683	0.0555	0.0242	0.4666
207895920065_R08C01	0.0192	0.1193	0.0686	0.0557	0.024	0.4664
207895920099_R01C01	0.0187	0.1201	0.0689	0.0557	0.0237	0.4656
207895920099_R02C01	0.0189	0.1198	0.0688	0.0555	0.0239	0.4662
207895920099_R03C01	0.0192	0.1189	0.0682	0.0555	0.0242	0.4667
207895920099_R04C01	0.0192	0.1188	0.0682	0.0555	0.0242	0.4667
207895920099_R05C01	0.0193	0.1187	0.0682	0.0555	0.0242	0.4667
207895920099_R06C01	0.0193	0.1187	0.0682	0.0554	0.0243	0.4666
207895920099_R07C01	0.0192	0.1187	0.0682	0.0554	0.0243	0.4666
207895920099_R08C01	0.0192	0.1188	0.0683	0.0555	0.0243	0.4664
208553420050_R01C01	0.0193	0.1189	0.0683	0.0555	0.0242	0.4664
208553420050_R02C01	0.0191	0.119	0.0683	0.0555	0.0242	0.4663
208553420050_R03C01	0.0192	0.1189	0.0683	0.0555	0.0242	0.4664
208553420050_R04C01	0.0192	0.1189	0.0684	0.0554	0.0242	0.4664
208553420050_R05C01	0.0192	0.1189	0.0683	0.0555	0.0242	0.4664
208553420050_R06C01	0.0192	0.1188	0.0682	0.0555	0.0243	0.4665
208553420050_R07C01	0.0191	0.119	0.0684	0.0554	0.0243	0.4664
208553420050_R08C01	0.019	0.1192	0.0686	0.0555	0.024	0.4662
208553420085_R01C01	0.0191	0.1193	0.0683	0.0556	0.0242	0.4666
208553420085_R02C01	0.0192	0.1188	0.0682	0.0555	0.0242	0.4665
208553420085_R03C01	0.0193	0.1187	0.0682	0.0554	0.0243	0.4664
208553420085_R04C01	0.0192	0.1188	0.0683	0.0555	0.0242	0.4665
208553420085_R05C01	0.0191	0.1189	0.0683	0.0555	0.0242	0.4664
208553420085_R06C01	0.0191	0.119	0.0683	0.0555	0.0242	0.4664

522