Supplementary Information

SHINE Sub-study Team

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Supplementary Methods

Ethical considerations

The study protocol for the SHINE clinical trial was approved by the individual ethics committees at each of the participating sites in South Africa (Stellenbosch University and University of Cape Town Research Ethics Committees), Uganda (Joint Clinical Research Centre Institutional Review Board), Zambia (University of Zambia Biomedical Research Ethics Committee and National Health Research Ethics Committee), India (BJMC Ethics Committee-Pune, National Institute of Research in Tuberculosis Ethics Committee-Chennai and Health Ministry Screening Committee), and by University College London (UCL Research Ethics Committee). The study also received ethical clearance from regulatory authorities in all the participating countries. The transcriptomic signatures sub-study protocol was reviewed and approved by the University of Cape Town Human Research Ethics Committee (522/2020).

Recruitment to the SHINE trial began in 2016 and trial registration details were as follows; International Standard Randomised Controlled Trials Number: ISRCTN63579542 on 14 October 2014, Pan African Clinical Trials Registry Number: PACTR201505001141379 on 14 May 2015 and Clinical Trial Registry-India, registration number: CTRI/2017/07/009119 on 27 July 2017.

Parents or caregivers provided written informed consent while assent was provided by children whose comprehension was assessed as sufficient. Following ICH-GCP, the confidentiality of all study participants was upheld throughout the entire course of the trial.

Blinding

The laboratory team were blinded to the tuberculosis (TB) disease category and the treatment arm assignment of the participants while samples were being processed. Clinical and demographic data (including TB disease category) were shared by collaborators only after all laboratory work was completed and signature scores had been calculated. A statistical analysis plan was generated, agreed upon, and signed off before any data analysis officially began.

Gene expression data quality control

Gene expression cycle threshold (Ct) data for each 96.96 gene expression chip was pre-processed using a locked-down R script to perform quality control (QC). This included checking that the correct settings on the BioMark HD instrument had been applied, performance of control samples included

on each chip, and performance of each of primer-probe assays. Deviations from pre-determined QC thresholds were used to ascertain whether any chips yielded anomalous results, such that the chip needed to be repeated. The following QC criteria were applied:

1. Detection of amplification within the non-template control (NTC) sample was checked to ensure that no contamination was present. If Ct values for more than 2 probes within the NTC were detected at values less than 35 cycles, the NTC was deemed to have failed.
2. Ct values for each primer-probe assay within the internal positive control (IPC) sample had to be within +/- 4 standard deviations (SD) of the range of Ct values for IPC samples established from 11 historical chip runs, else they were deemed to have failed and were excluded from analysis. If more than 10 of 96 probes were outside of this range, the chip was deemed to have failed.
3. Ct values for the probes in the IPC sample had to have a Spearman correlation coefficient greater than 0.98 and have a concordance correlation coefficient greater than 0.95 compared to Ct values for IPC samples from historical chip runs, else the chip was deemed to have failed. The Penn-Nicholson6 signature (RISK6) score (9) for the IPC was compared to the 11 historical chip runs; a score outside of +/- 2SD of the mean RISK6 scores from historical chip runs was deemed to have failed.
4. If more than 20% of probes were not detected for a particular sample, that sample was excluded from analysis.
5. If more than 20% of samples on a chip failed, then the chip failed.
6. If more than 30% of samples had no detectable amplification for a particular primer-probe assay, that assay failed and was excluded from signature score calculations for that chip.
7. If more than 10% of the primer-probe assays failed on a chip, then the chip failed.

Failed samples and chips were repeated in separate runs. If repeated samples still failed, they were excluded from analyses.

Signature score calculation

Signature scores were calculated from raw Ct measurements using the original algorithms developed for each individual signature, where possible (**Supplementary Table 1**). Signatures discovered using methods other than RT-qPCR, such as RNA-sequencing or microarray, were subsequently re-parameterised to RT-qPCR [1-3]. In cases where predesigned TaqMan primer-probe assays were not available from the original publication, we used either inventoried primer-probes or designed novel

TaqMan assays based on the published Illumina probe, RNA-seq transcript, or primer-probe sequence data. Furthermore, we contacted the authors of relevant publications when the required information was not available. We included three reference primer-probes (*ACTR3*, *TMBIM6*, and *USF2*; **Supplementary Table 2**) for standardisation of gene expression for signatures which require a normalised (delta) Ct.

Signature reparameterization

Eight signatures (da Costa3, Duffy9, Francisco2, Gjøen7, Jacobsen3, Roe3, Sambarey10, and Satproedprai7) developed using gene expression analysis techniques other than RT-qPCR required reparameterization to compute signature scores from microfluidic multiplex RT-qPCR BioMark HD readouts [3]. This was necessary because different platforms each have unique dynamic ranges and quantitative scales for quantifying gene expression levels. The machine learning models, and statistical methods used for computing signature scores therefore needed to be reconstructed (reparametrized) using Ct values generated from RT-qPCR data on the BioMark HD platform. This ensured that there was uniformity in the way scores were calculated for different signatures and how results were interpreted. The model parameters for the eight signatures were reconstructed using the original published methods [4-11] to best differentiate between TB cases and controls when adapted to Ct values, generated using PAXgene RNA samples from a previously described cross-sectional TB cohort (CTBC) [12, 13]. This cohort is completely independent of the RePORT-Brazil study and included both HIV positive and HIV negative TB cases and healthy controls; use of this independent dataset for reparameterization reduced potential bias. The RT-qPCR protocol for the reparameterization experiments was consistent with the methods used for all RT-qPCR in this paper.

Several signatures had not previously been evaluated using the Biomark RT-qPCR instrument, but did not require reparameterization even though they were discovered using approaches other than RT-qPCR. Examples include the Gliddon3/4 [14], Kaforou25 [15], and Rajan5 [16] signatures. Gliddon3/4 and Kaforou25 were discovered using microarray data using a simple Disease Risk Score (DRS), which sums normalized expression values from all upregulated transcripts and subtracts the normalized expression values of all downregulated transcripts, making the signature essentially "self-normalizing". For Gliddon4, the original publication reported GBP6 and PRDM1 as upregulated and TMCC1 and ARG1 as downregulated in TB; however, when measured using the same TaqMan

assays on our microfluidic RT-qPCR platform, ARG1 consistently showed higher expression in TB (lower Ct) while PRDM1 was not consistently differentially expressed across cohorts. Therefore, consistent with the DRS framework, we calculated the Gliddon4 score as ($_{deltaCt}$TMCC1 + $_{deltaCt}$PRDM1) − ($_{deltaCt}$GBP6 + $_{deltaCt}$ARG1). Rajan5 was also discovered based on microarray gene expression data, but developed using the signed and unsigned sums methods, which also allowed the signature to be "self-normalising".

Batch correction

Batch correction was performed to correct for differences in signature score distribution between chip runs using a quantile regression method with the adjust_batch function in the batchtma R package (10) (R package version 0.1.6, 2021). This method unifies lower quantiles (25th percentile) and ranges between the lower and upper quantile (75th percentile) between batches, allowing for differences in both parameters due to confounders. Thereafter, signature scores were winsorized (clipped) to account for outliers (Winsorize function, DescTools R package (11); R package version 0.99.49, 2019), log transformed to normalise score distribution (log function, base R package), converted to z-scores (scale function, base R package), and compared using the cumulative distribution function (pnorm function, stats R package).

Multivariable regression analyses

Data points for variables included in the multivariable linear regression models needed to be available for greater than 90% of study participants. BCG vaccination status was excluded as all children in South Africa were assumed to be immunized in childhood. Highly correlated variables, such as weight and age, were not included together in the regression model. Correction for multiple testing was performed using the Benjamini-Hochberg FDR method. An FDR-adjusted p-value of <0.05 was considered significant for all analyses.

Supplementary Tables

Supplementary Table 1. Characteristics of whole blood transcriptomic signatures evaluated in this study.

Signature name	Reference	Signature model	Discovery cohort age	Discovery cohort country	Discovery cohort application
da Costa3	*Tuberculosis* (2015) [4]	Random forest	Adults	Brazil	Diagnostic; TB vs LTBI, HC, and OD
de Araujo1 (NPC2)	*Front Microbiol* (2016) [17]	Standardised expression of NPC2	Adults	Brazil	Diagnostic; TB vs LTBI and HC
Duffy9 [10]†	*PLoS One* (2019) [5]	Six-class multinomial random forest	Adults	South Africa Malawi (7,40)	Diagnostic, TB vs LTBI and OD
Francisco2	*J Infect* (2017) [6]	Random forest	Adults	China	Diagnostic; TB vs OD and HC
Gjøen7	*Sci Rep* (2017) [7]	LASSO regression	Children	India	Diagnostic; TB vs HC
Gliddon3	*Front Immunol* (2021) [14]	$_{DeltaCT}$ZNF296 – ($_{DeltaCT}$FCGR1A + $_{DeltaCT}$C1QB)	Adults	South Africa, Malawi	Diagnostic; TB vs LTBI
Gliddon4		($_{DeltaCT}$TMCC1 + $_{DeltaCT}$PRDM1) – ($_{DeltaCT}$GBP6 + $_{DeltaCT}$ARG1) §	Adults	South Africa, Malawi	Diagnostic; TB vs OD
Jacobsen3	*J Mol Med* (2007) [8]	Linear discriminant analysis	Adults	Germany	Diagnostic; TB vs LTBI and HC
Kaforou22 [27]‡	*PLoS Med* (2013) [15]	Disease risk score	Adults	South Africa, Malawi	Diagnostic; TB vs LTBI
Maertzdorf4	*EMBO Mol Med* (2016) [18]	$_{RawCT}$ID3 – [($_{RawCT}$GBP1 + $_{RawCT}$IFITM3 + $_{RawCT}$P2RY14) / 3] (adapted from Suliman et al. [19])	Adults	India	Diagnostic; TB vs HC and LTBI
Penn-Nicholson6	*Sci Rep* (2020) [13]	Pair-wise ensemble structure	Adolescents	South Africa	Prognostic; TB progressors vs non-progressors
Rajan5	*Clin Infect Dis* (2019) [16]	Unsigned sums	Adults	Uganda	Diagnostic; HIV+ TB vs HIV+ HC
Roe1 (BATF2)	*JCI Insight* (2016) [20]	Standardised expression of BATF2	Adults	United Kingdom	Diagnostic (TB vs HC and TB patients 2-4 years post recovery)
Roe3	*Clin Infect Dis* (2020) [9]	SVM (linear kernel)	Adults	United Kingdom	Prognostic; TB progressors vs non-progressors
Sambarey10	*EBioMedicine* (2017) [10]	Linear discriminant analysis	Adults	India	Diagnostic; TB vs HC and LTBI
Satproedprai7	*Genes Immun* (2015) [11]	LASSO regression	Adults	Thailand	Diagnostic; TB vs HC and previous TB patients
Suliman2	*Am J Respir Crit Care Med* (2018) [19]	Pair-wise ensemble structure	Adults	The Gambia, South Africa	Prognostic; TB progressors vs non-progressors
Suliman4					
Sweeney3	*Lancet Respir Med* (2016) [21]; *JAMA Netw Open* (2018) [22]	$_{RawCT}$KLF2 – ($_{RawCT}$GBP5 + $_{RawCT}$DUSP3) / 2	Adults	Meta-analysis (France, Malawi, South Africa, United Kingdom, USA)	Diagnostic; TB vs. HC, LTBI and ORD
Thompson5	*Tuberculosis* (2017) [23]	Pair-wise ensemble structure	Adults	South Africa	Monitoring response to TB treatment

LASSO = least absolute shrinkage and selection operator. SVM = support vector machines. HC = healthy controls. ORD = other respiratory diseases. LTBI = latent tuberculosis infection. TB = tuberculosis.

Signature names combine the surname of the first author of the original publication, and the number of constituent transcripts used in the RT-qPCR analyses. **§** See Signature reparameterisation in **Supplementary Methods**. **†**Duffy9 originally had 10 transcripts: One primer-probe assay representing the transcript, CERKL1, failed during panel optimisation and was omitted. Signature scores were calculated based on the model described in the corresponding

publications. ‡Kaforou22 had 27 transcripts in the original signature. Three transcripts, C1QC, GBP6, and C1QB, were excluded from analysis due to high primer-probe assays failure rates, while primer-probe assays for two FCGR1B transcripts in the original signature could not be identified since the microarray probe was a duplicate of the same FCGR1B transcript. LOC728744 was discontinued and had all information withdrawn from both the NCBI and RefSeq databases.

Supplementary Table 2. TaqMan PCR primer-probe assay panel evaluated in this study.

Gene Symbol	TaqMan Assay ID	Assay excluded**	Reference probe	# of signatures	da Costa3	de Araujo1	Duffy9 (10)†	Francisco2	Gjøen7	Gliddon3	Gliddon4	Jacobsen3	Kaforou22 (27)‡	Maertzdorf4	Penn-Nicholson6	Rajan5	Roe1	Roe3	Sambarey10	Satproedprai7	Suliman2	Suliman4	Sweeney3	Thompson5
ACTR3	Hs01029159_g1		X	14	X	X	X	X	X	X	X	X	X			X	X	X	X	X				
TMBIM6	Hs00162661_m1		X	14	X	X	X	X	X	X	X	X	X			X	X	X	X	X				
USF2	Hs01100994_g1		X	14	X	X	X	X	X	X	X	X	X			X	X	X	X	X				
ACTA2	Hs00426835_g1			1												X								
ANKRD22	Hs00944015_m1			2									X								X			
APOL1	Hs01066280_m1			1																X				
ARG1	Hs00968979_m1			1							X													
BATF2	Hs00912737_m1			2													X	X						
BCL6	AREPURD*			1															X					
BLK	Hs01017452_m1			1																		X		
C1QA	Hs00706358_s1	X		0																				
C1QB	Hs00608019_m1			2			X			X			‡											
C1QC	Hs00757779_m1	X		0									‡											
C4ORF18 /FAM198B	Hs00259260_s1			1									X											
C5	Hs00156197_m1			1									X											
CCR6	Hs01890706_s1			1									X											
CD160	Hs00199894_m1			1			X																	
CD1C	Hs00957534_g1			1																		X		
CD36.2	Hs01567186_m1			1			X																	
CD3E	APDJ3NT*			1					X															
CD40L	Hs00163934_m1			1			X																	
CD74-j1	Hs04983808_s1	X		0																				
CD74-j2	AP9HN47*	X		0																				
CD79A	Hs00998120_g1			1									X											
CD79B	Hs01058826_g1			1									X											
CDCA7	Hs00230589_m1	X		0																				
CDKN1C	Hs00175938_m1	X		0																				
CXCR5	Hs00540548_s1			1									X											
CYP4F3	Hs01587860_mH			1															X					
DUSP3	Hs01115776_m1			2									X										X	
F2RL1	Hs00608346_m1	X		0																				
FAM20A	Hs01034066_m1			1									X											
FCGR1A	Hs00174081_m1			5	X					X		X	X						X					
FCGR1A	AP2XDMK*			1																X				
FCGR1B	Hs02341825_m1			3			X						X		X									
FCGR1B_VARIANT1	AP3267H*			1																X				
FCGR1B_VARIANT2	AP47ZTF*			1																X				
FCGR1C	Hs00417598_m1			1									X											
FLVCR2	Hs00900390_m1			1									X											
GAS6	AR47XGZ*			2									X									X		
GAS6	AR323W3*			1									X											
GBP1	Hs00977005_m1			1										X										
GBP2	Hs00894846_g1			1											X									
GBP5	Hs00369472_m1			5	X			X	X									X					X	
GBP6	Hs01584201_m1			3			X				X		‡			X								
GNG7	Hs00192999_m1			1									X											
GYG1	Hs00907542_g1			1												X								
GZMA	Hs00989184_m1			1	X																			
HK3	Hs01092850_m1			1															X					
ID3	Hs00954037_g1			2			X							X										
IFI44L	Hs00915292_m1			1															X					
IFITM3_G	APMF24C*	X		0																				
IFITM3	Hs03057129_s1			2					X					X										

Gene Symbol	TaqMan Assay ID	Assay excluded**	Reference probe	# of signatures	da Costa3	de Araujo1	Duffy9 (10)†	Francisco2	Gjøen7	Gliddon3	Gliddon4	Jacobsen3	Kaforou22 (27)‡	Maertzdorf4	Penn-Nicholson6	Rajan5	Roe1	Roe3	Sambarey10	Satproedprai7	Suliman2	Suliman4	Sweeney3	Thompson5
KAZN	AP7DVDD*			1																X				
KIF1B	Hs01114512_g1			1					X															
KLF2	Hs00360439_g1			2				X															X	
KLF2	ARH6CF4*	X		0																				
KLHDC8B	Hs00293902_m1	X		0																				
LAG3	Hs00958444_g1			1			X																	
LHFPL2	Hs00299613_m1			1									X											
LTF	Hs00158924_m1			1								X												
MAFB	APZTH2N*			1																X				
MAP7D3	Hs00226257_m1			1																				X
MMP9	APEPW9P*			1					X															
MPO	Hs00165162_m1			1									X											
MTRF1L	Hs01097882_g1			1												X								
NOD2	Hs01550759_g1			1					X															
NPC2	Hs01119244_m1			1		X																		
OSBPL10	Hs00215016_m1			1																	X			
P2RY14	Hs01848195_s1			1										X										
PRDM1	Hs00153357_m1			1							X													
RAB13	Hs04400188_g1			1															X					
Rab33A	Hs00191243_m1			1								X												
RABL2A	Hs00255244_m1			1												X								
RBBP8	Hs01090329_m1			1															X					
RP11-295G20.2	Hs01373568_m1			1																				X
S100A8	Hs00374264_g1			1									X											
SCARF1	Hs01092483_m1			1														X						
SDR39U1	Hs01016970_g1			1											X									
SEPT4	Hs00910208_g1			1																		X		
SERPING1	Hs00934329_m1			1											X									
SLPI	Hs00268206_m1			1															X					
SMARCD3	Hs01088251_g1			3									X						X					X
STAT1	Hs01013996_m1			1																X				
STT3A	Hs00967491_m1			1																				X
TIMM10	APT2DEC*			1															X					
TMCC1	Hs01037666_s1			1							X													
TNIP1	AP47ZYZ*			1					X															
TRMT2A	Hs01000041_g1			1											X									
TUBGCP6	Hs00363509_g1			1											X									
UCP2	Hs01075224_g1			1																				X
VAMP5	Hs01105383_g1			1									X											
WARS-j1	Hs00998737_m1	X		0																				
WARS-j2	APAAFHX*	X		0																				
ZDHHC19	Hs00376118_m1			1			X																	
ZNF296	Hs00377132_m1			2						X			X											

Signatures are named by first author and number of transcripts included in the model (e.g. Author11). Numbers in brackets indicate the original number of transcripts in the published model. Some signatures have a reduced number of transcripts when translated to RT-qPCR due to duplicate transcript symbols (IDs), high-primer probe failure rate (**poor amplification efficiency), or where transcript sequences from an original discovery cohorts could not be mapped to a more recent reference transcriptome.

* Custom designed primer-probe assays; available on request to corresponding author.

† The CERKL1 primer-probe assay failed during panel optimisation and was removed from the Duffy10 signature.

‡ Three transcripts (C1QB, C1QC, and GBP6) were excluded from the Kaforou27 signature due to a high primer-probe assay failure rate (**). Unique primer-probe assays could not be designed for both of the FCGR1B transcript variants; only one FCGR1B primer-probe was included in the Kaforou22 model. We were unable to design a primer-probe for LOC728744; all information had been withdrawn from both the NCBI and RefSeq databases.

Supplementary Table 3. Performance of transcriptomic signatures in differentiating between the different TB classification groups at baseline.

Signature	Confirmed TB (N=37) n (%)	Unconfirmed TB (N=129) n (%)	Unlikely TB (N=32) n (%)	Confirmed vs unlikely TB AUC (95% CI)	Unconfirmed vs unlikely TB AUC (95% CI)	Confirmed and unconfirmed vs unlikely TB AUC (95% CI)
da Costa3	37 (100)	128 (99)	30 (94)	0.69 (0.56-0.82)	0.55 (0.44-0.66)	0.58 (0.48-0.69)
de Araujo1	36 (97)	128 (99)	30 (94)	0.59 (0.45-0.73)	0.60 (0.50-0.70)	0.56 (0.46-0.66)
Duffy9	37 (100)	126 (98)	30 (94)	0.79 (0.68-0.90)	0.55 (0.43-0.67)	0.61 (0.50-0.71)
Francisco2	37 (100)	127 (98)	30 (94)	0.64 (0.50-0.77)	0.52 (0.40-0.64)	0.55 (0.43-0.66)
Gjøen7	37 (100)	128 (99)	30 (94)	0.68 (0.55-0.81)	0.49 (0.37-0.61)	0.54 (0.42-0.65)
Gliddon3	37 (100)	128 (99)	30 (94)	0.74 (0.62-0.86)	0.56 (0.44-0.67)	0.60 (0.49-0.71)
Gliddon4	37 (100)	128 (99)	30 (94)	0.81 (0.70-0.92)	0.67 (0.58-0.75)	0.70 (0.62-0.78)
Jacobsen3	37 (100)	128 (99)	29 (91)	0.73 (0.61-0.86)	0.55 (0.44-0.65)	0.59 (0.49-0.69)
Kaforou22	35 (90)	123 (95)	30 (94)	0.80 (0.70-0.91)	0.58 (0.47-0.69)	0.63 (0.53-0.73)
Maertzdorf4	34 (92)	118 (90)	30 (94)	0.70 (0.57-0.83)	0.51 (0.40-0.63)	0.56 (0.45-0.66)
Penn-Nicholson6	37 (100)	128 (99)	30 (94)	0.73 (0.60-0.86)	0.55 (0.44-0.66)	0.59 (0.48-0.70)
Rajan5	37 (100)	128 (99)	30 (94)	0.76 (0.64-0.87)	0.54 (0.43-0.65)	0.59 (0.49-0.69)
Roe1	37 (100)	128 (99)	30 (94)	0.73 (0.61-0.85)	0.53 (0.42-0.64)	0.57 (0.47-0.68)
Roe3	37 (100)	128 (99)	30 (94)	0.71 (0.58-0.84)	0.53 (0.41-0.65)	0.57 (0.46-0.69)
Sambarey10	37 (100)	128 (99)	30 (94)	0.76 (0.64-0.88)	0.56 (0.46-0.67)	0.61 (0.51-0.71)
Satproedprai7	37 (100)	116 (90)	28 (88)	0.76 (0.64-0.88)	0.55 (0.44-0.66)	0.60 (0.49-0.71)
Suliman2	37 (100)	128 (99)	30 (94)	0.74 (0.61-0.86)	0.55 (0.43-0.66)	0.59 (0.48-0.69)
Suliman4	37 (100)	127 (98)	30 (94)	0.75 (0.63-0.87)	0.51 (0.40-0.61)	0.56 (0.47-0.66)
Sweeney3	37 (100)	127 (98)	30 (94)	0.75 (0.63-0.87)	0.54 (0.43-0.64)	0.58 (0.49-0.68)
Thompson5	37 (100)	128 (99)	30 (94)	0.74 (0.62-0.86)	0.61 (0.49-0.72)	0.64 (0.53-0.74)

AUC = area under the curve. CI = confidence interval.

Supplementary Table 4. Multivariable linear regression analysis determining clinical and demographic variables associated with transcriptomic signature scores at baseline.

Variable	**Duffy9** β-coefficient (95% CI); adjusted p-value	**Gliddon4** β-coefficient (95% CI); adjusted p-value	**Jacobsen3** β-coefficient (95% CI); adjusted p-value	**Kaforou22** β-coefficient (95% CI); adjusted p-value
Age				
> 3 years	Reference	Reference	Reference	Reference
< 3 years	0.04 (-0.05, 0.13); 0.62	0.03 (-0.04, 0.10); 0.65	0.00 (-0.09, 0.09); 1.00	0.01 (-0.06, 0.07); 0.94
Sex				
Female	Reference	Reference	Reference	Reference
Male	0.02 (-0.06, 0.11); 0.77	0.02 (-0.05, 0.08); 0.77	0.01 (-0.08, 0.10); 0.94	0.02 (-0.03, 0.08); 0.65
Clinical presentation				
Respiratory TB	Reference	Reference	Reference	Reference
Mixed TB	0.00 (-0.09, 0.08); 0.98	-0.06 (-0.13, 0.01); 0.29	-0.01 (-0.10, 0.09); 0.97	-0.01 (-0.07, 0.06); 0.94
TB classification				
Confirmed TB	Reference	Reference	Reference	Reference
Unconfirmed TB	**-0.23 (-0.34, -0.11); 0.01**	**-0.10 (-0.20, -0.01); 0.16**	**-0.18 (-0.30, -0.06); 0.03**	**-0.15 (-0.24, -0.07); 0.01**
Unlikely TB	**-0.23 (-0.39, -0.07); 0.05**	**-0.18 (-0.31, -0.05); 0.05**	-0.18 (-0.35, -0.01); 0.18	**-0.16 (-0.28, -0.05); 0.05**
CXR severity				
Normal	Reference	Reference	Reference	Reference
Non-typical TB	0.00 (-0.11, 0.11); 1.00	0.04 (-0.05, 0.13); 0.65	0.04 (-0.08, 0.16); 0.70	0.02 (-0.06, 0.10); 0.75
Typical TB, non-severe	0.08 (-0.03, 0.19); 0.35	0.05 (-0.04, 0.14); 0.47	0.09 (-0.02, 0.21); 0.29	0.05 (-0.03, 0.12); 0.47
Typical TB, severe	0.17 (-0.09, 0.44); 0.42	0.18 (-0.04, 0.39); 0.29	0.03 (-0.25, 0.30); 0.94	0.13 (-0.06 0.31); 0.41

CI = confidence interval. CXR = chest X-ray.

Supplementary Table 5. Multivariable linear regression analysis determining clinical and demographic variables associated with transcriptomic signature scores at baseline, including weight-for-age z-scores.

Variable	**Duffy9** β-coefficient (95% CI); adjusted p-value	**Gliddon4** β-coefficient (95% CI); adjusted p-value	**Jacobsen3** β-coefficient (95% CI); adjusted p-value	**Kaforou22** β-coefficient (95% CI); adjusted p-value
Age				
> 3 years	Reference	Reference	Reference	Reference
< 3 years	0.05 (-0.04, 0.14); 0.64	0.03 (-0.04, 0.10); 0.68	0.01 (-0.09, 0.10); 0.96	0.01 (-0.05, 0.08); 0.88
Weight-for-age z-score	0.01 (-0.03, 0.05); 0.84	0.00 (-0.04, 0.03); 0.94	0.00 (-0.04, 0.05); 0.95	-0.01 (-0.04, 0.03); 0.86
Sex				
Female	Reference	Reference	Reference	Reference
Male	0.02 (-0.07, 0.11); 0.84	0.00 (-0.07, 0.07); 0.96	0.01 (-0.09, 0.11); 0.94	0.02 (-0.05, 0.08); 0.82
Clinical presentation				
Respiratory TB	Reference	Reference	Reference	Reference
Mixed TB	0.00 (-0.09, 0.09); 1.00	-0.06 (-0.14, 0.01); 0.42	-0.01 (-0.11, 0.09); 0.94	-0.01 (-0.07, 0.06); 0.94
TB classification				
Confirmed TB	Reference	Reference	Reference	Reference
Unconfirmed TB	-0.19 (-0.31, -0.06); 0.14	-0.08 (-0.18, 0.02); 0.44	-0.16 (-0.29, -0.02); 0.22	-0.14 (-0.23, -0.04); 0.14
Unlikely TB	-0.19 (-0.37, -0.02); 0.24	-0.17 (-0.31, -0.03); 0.22	-0.15 (-0.34, 0.03); 0.43	-0.14 (-0.27, -0.02); 0.23
CXR severity				
Normal	Reference	Reference	Reference	Reference
Non-typical TB	0.01 (-0.11, 0.14); 0.94	0.04 (-0.05, 0.14); 0.67	0.03 (-0.10, 0.16); 0.84	0.03 (-0.06, 0.12); 0.77
Typical TB, non-severe	0.08 (-0.03, 0.20); 0.46	0.05 (-0.04, 0.14); 0.64	0.08 (-0.05, 0.20); 0.54	0.04 (-0.04, 0.13); 0.65
Typical TB, severe	0.17 (-0.12, 0.47); 0.58	0.19 (-0.04, 0.43); 0.43	-0.01 (-0.33, 0.31); 0.98	0.13 (-0.08, 0.34); 0.54

CI = confidence interval. CXR = chest X-ray.

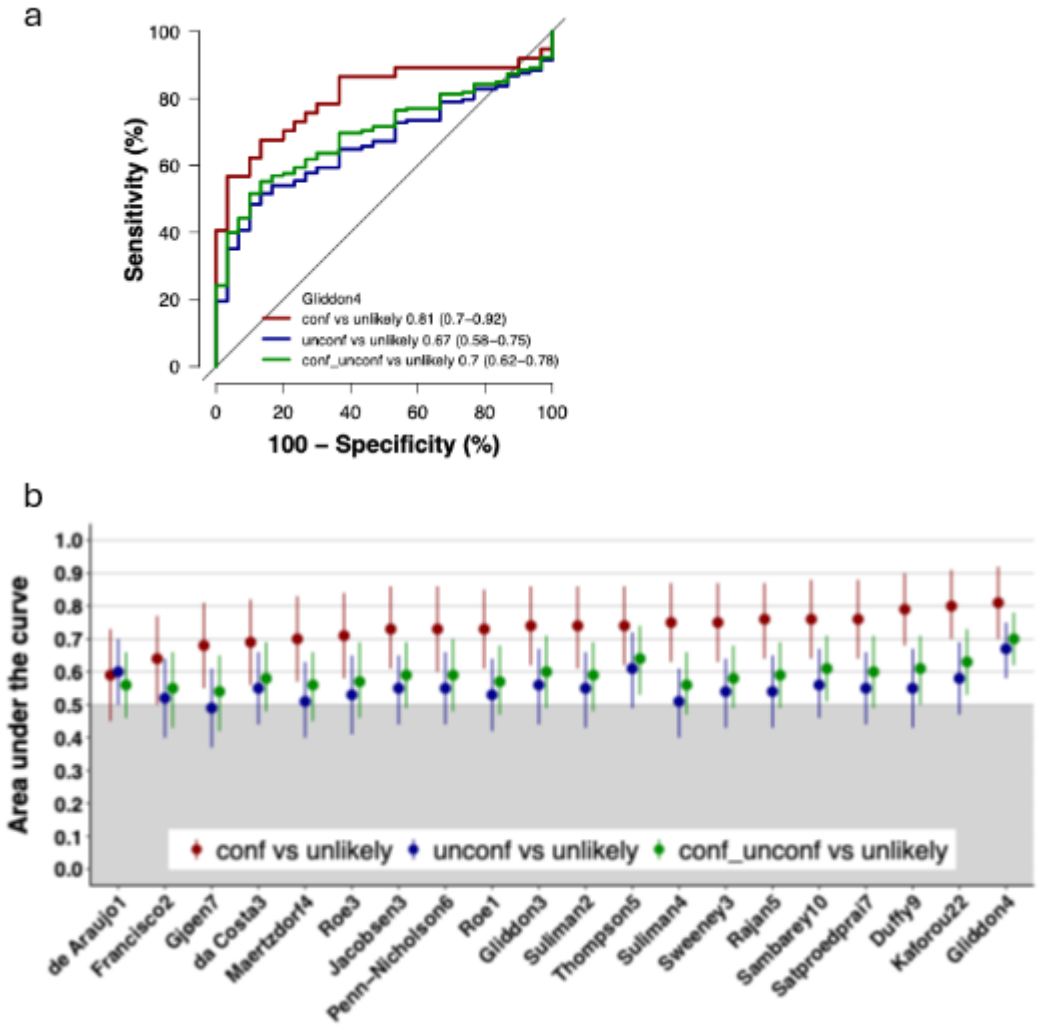
Sixteen children were not included in this analysis as weight-for-age z-scores could not be calculated: The *zscorer* package in R used to generate z-scores excluded children older than ten years.

Supplementary Table 6. Performance of transcriptomic signatures for discriminating between baseline and week 2, week 8, and end of treatment time points among children with confirmed TB.

Signature	Baseline (N=37) n (%)	Week 2 (N=37) n (%)	Week 8 (N=37) n (%)	EoT (N=37) n (%)	Baseline vs week 2 AUC (95% CI)	Baseline vs week 8 AUC (95% CI)	Baseline vs EoT AUC (95% CI)
da Costa3	37 (100)	37 (100)	37 (100)	37 (100)	0.68 (0.55-0.80)	0.70 (0.58-0.83)	0.74 (0.61-0.86)
de Araujo1	37 (100)	36 (97)	36 (97)	36 (97)	0.48 (0.35-0.62)	0.65 (0.52-0.78)	0.64 (0.51-0.77)
Duffy9	35 (95)	37 (100)	37 (100)	37 (100)	0.68 (0.56-0.81)	0.77 (0.66-0.88)	0.78 (0.67-0.89)
Francisco2	37 (100)	37 (100)	37 (100)	37 (100)	0.65 (0.53-0.77)	0.66 (0.53-0.78)	0.66 (0.54-0.79)
Gjøen7	37 (100)	37 (100)	37 (100)	37 (100)	0.67 (0.54-0.79)	0.74 (0.62-0.85)	0.74 (0.62-0.86)
Gliddon3	36 (97)	37 (100)	37 (100)	37 (100)	0.70 (0.57-0.82)	0.77 (0.65-0.88)	0.78 (0.67-0.89)
Gliddon4	36 (97)	37 (100)	37 (100)	37 (100)	0.72 (0.60-0.84)	0.77 (0.66-0.89)	0.77 (0.66-0.88)
Jacobsen3	37 (100)	37 (100)	37 (100)	37 (100)	0.74 (0.62-0.85)	0.77 (0.66-0.88)	0.80 (0.69-0.91)
Kaforou22	36 (97)	35 (95)	35 (95)	35 (95)	0.75 (0.63-0.86)	0.82 (0.72-0.91)	0.85 (0.76-0.94)
Maertzdorf4	36 (97)	34 (92)	34 (92)	34 (92)	0.69 (0.56-0.81)	0.76 (0.64-0.88)	0.76 (0.64-0.88)
Penn-Nicholson6	37 (100)	37 (100)	37 (100)	37 (100)	0.70 (0.58-0.82)	0.74 (0.62-0.85)	0.78 (0.67-0.88)
Rajan5	36 (97)	37 (100)	37 (100)	37 (100)	0.66 (0.54-0.79)	0.75 (0.63-0.86)	0.81 (0.70-0.91)
Roe1	37 (100)	37 (100)	37 (100)	37 (100)	0.70 (0.58-0.83)	0.76 (0.65-0.87)	0.78 (0.67-0.89)
Roe3	37 (100)	37 (100)	37 (100)	37 (100)	0.61 (0.48-0.74)	0.73 (0.61-0.84)	0.74 (0.63-0.85)
Sambarey10	37 (100)	37 (100)	37 (100)	37 (100)	0.75 (0.64-0.87)	0.82 (0.73-0.92)	0.81 (0.70-0.91)
Satproedprai7	36 (97)	37 (100)	37 (100)	37 (100)	0.71 (0.59-0.83)	0.77 (0.66-0.88)	0.81 (0.71-0.91)
Suliman2	37 (100)	37 (100)	37 (100)	37 (100)	0.68 (0.55-0.80)	0.72 (0.60-0.84)	0.75 (0.65-0.86)
Suliman4	37 (100)	37 (100)	37 (100)	37 (100)	0.73 (0.62-0.85)	0.75 (0.64-0.86)	0.77 (0.66-0.88)
Sweeney3	37 (100)	37 (100)	37 (100)	37 (100)	0.62 (0.49-0.75)	0.67 (0.55-0.80)	0.75 (0.64-0.86)
Thompson5	37 (100)	37 (100)	37 (100)	37 (100)	0.73 (0.62-0.85)	0.70 (0.58-0.82)	0.75 (0.64-0.86)

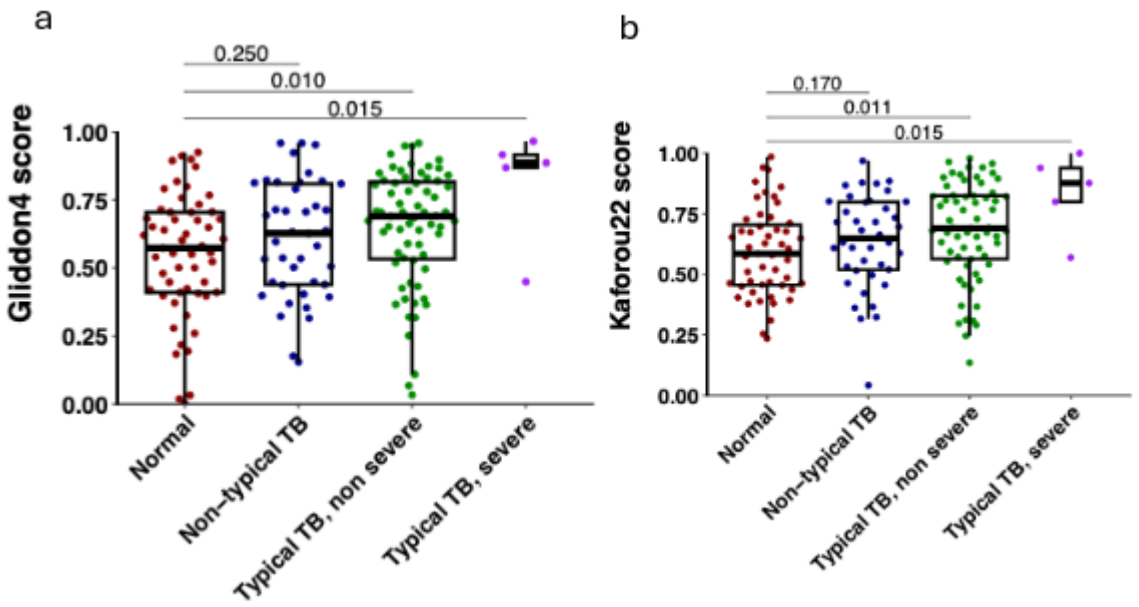
EoT = end of treatment. AUC = area under the curve. CI = confidence interval.

Supplementary Figures

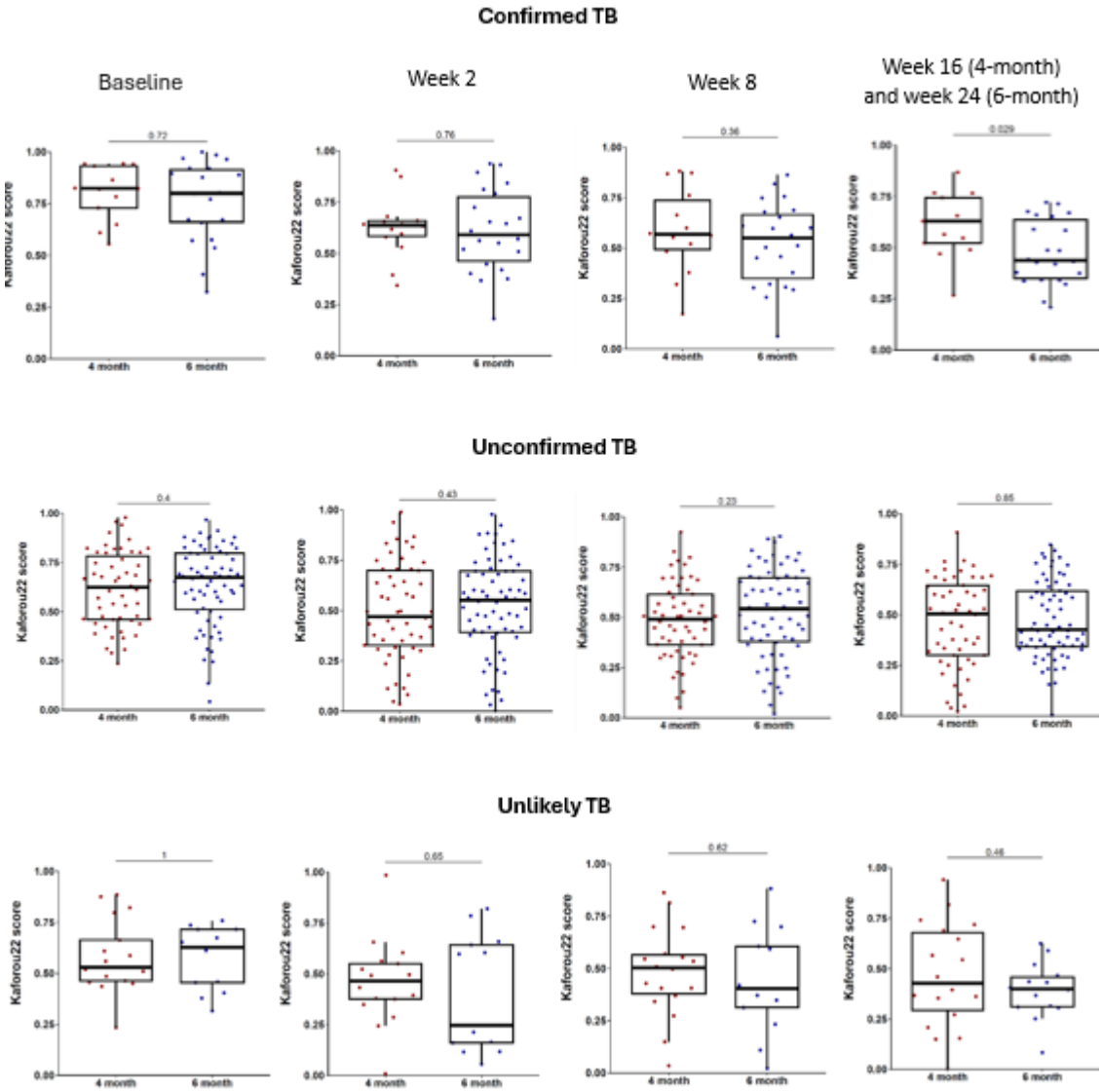


Supplementary Figure 1. Performance of transcriptomic signatures as diagnostic biomarkers at baseline in children with confirmed, unconfirmed, and unlikely TB.

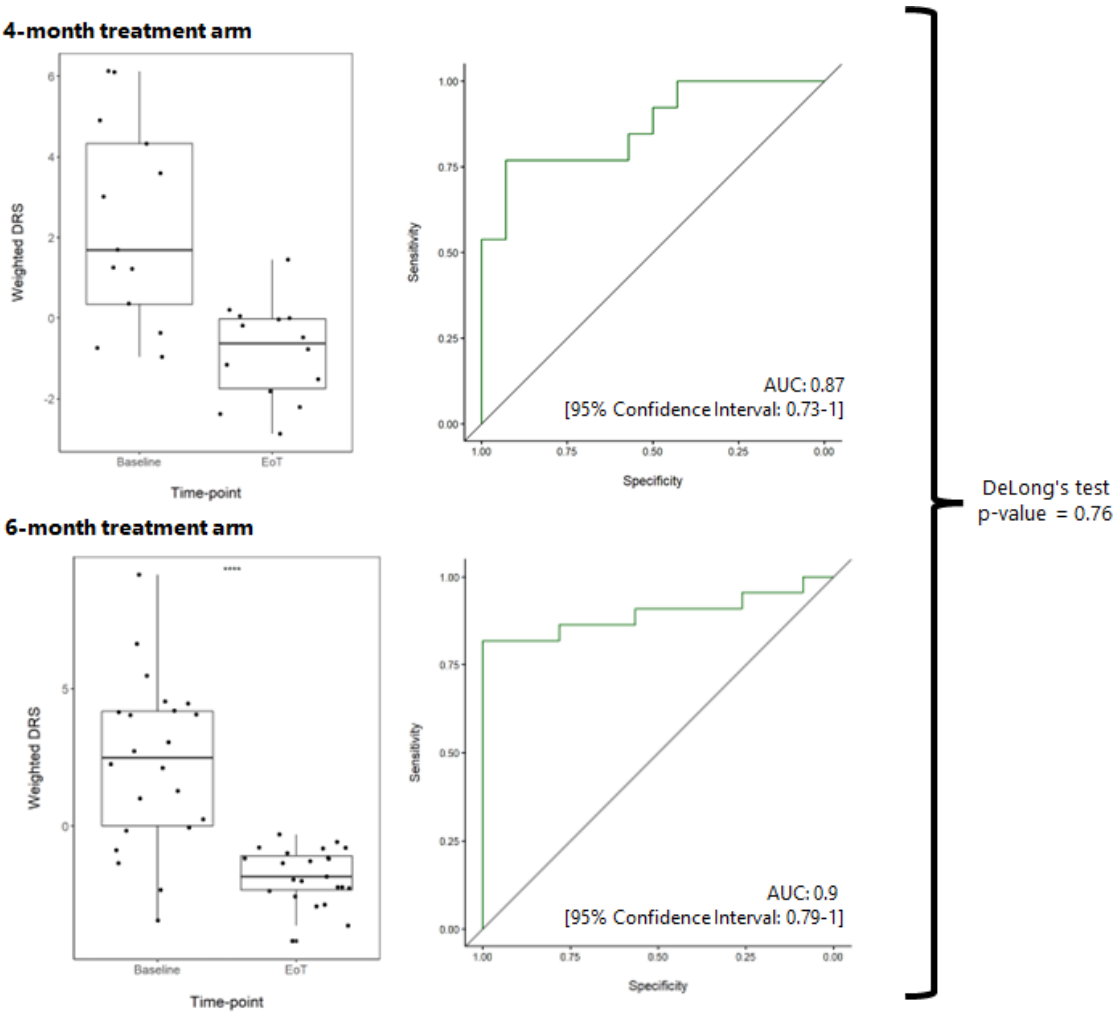
a | Receiver operating characteristic (ROC) curve depicting baseline performance (area under the curve, AUC, with 95% CI) of the Gliddon4 signature in differentiating confirmed (conf) versus unlikely TB, unconfirmed (unconf) versus unlikely TB, and confirmed and unconfirmed TB combined (conf+unconf) versus unlikely TB. **b** | Summary of signature performance, ranked by AUC, for confirmed TB versus unlikely TB (red). Error bars depict the 95% CIs. The AUCs for unconfirmed TB versus unlikely TB (blue), and confirmed and unconfirmed TB combined versus unlikely TB (green) are also shown. AUCs for all signatures are shown in **Supplementary Table 3**.



Supplementary Figure 2. Signature score distributions at baseline stratified by chest X-ray classification. Box-and-whisker plots for (**a**) Gliddon4 and (**b**) Kaforou22 showing signature score distribution (each dot represents a participant) at baseline stratified by disease severity based on chest X-ray. These signatures performed best for distinguishing confirmed from children with unlikely TB at baseline (Gliddon4) and children with confirmed TB at baseline from EoT (Kaforou22). P-values for comparison of signature score distribution between chest X-ray classification groups were calculated using the Mann–Whitney U test. Boxes depict the IQR, the midline represents the median, and whiskers represent 1.5 × IQR.

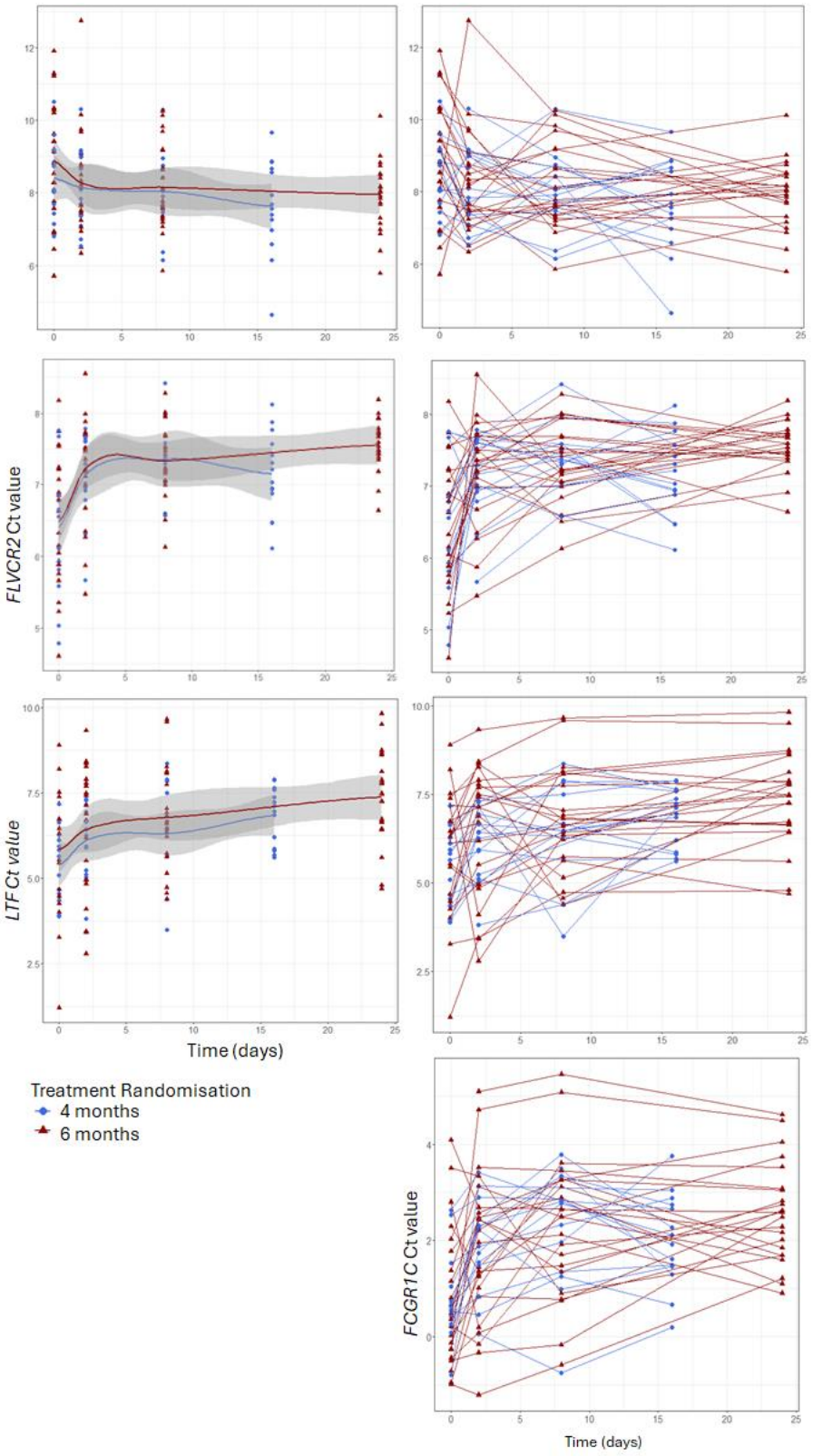


Supplementary Figure 3. Signature score distribution by study arm at each timepoint.
Box-and-whisker plots depicting Kaforou22 signature score distribution (each dot represents a participant) at baseline, week 2, week 8, and the end of treatment (EoT) in children with confirmed, unconfirmed, and unlikely TB. EoT scores are at week 16 (4-month arm) or week 24 (6-month arm). The Kaforou22 signatures performed best for distinguishing children with confirmed TB at baseline from EoT. P-values for comparison of median signature scores between treatment arms were calculated using the Mann–Whitney U test. Boxes depict the IQR, the midline represents the median, and whiskers represent 1.5 × IQR.

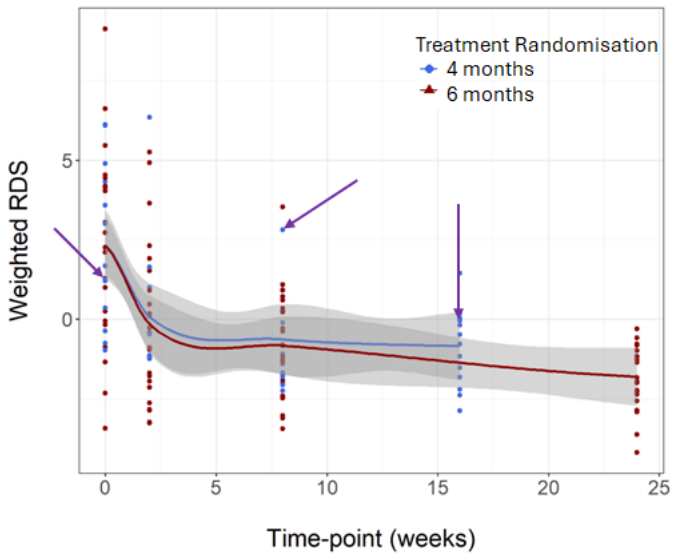


Supplementary Figure 4. Performance of the novel 4-gene monitoring signature at distinguishing enrolment from end-of-treatment samples in the 4-month (n=14 patients) and 6-month (n=23 patients) treatment groups separately.

DRS (Disease Risk Score) boxplots depict the IQR, the midline represents the median, and whiskers represent 1.5 × IQR. AUC: Area Under the Receiver Operating Characteristic curve



Supplementary Figure 5. The temporal expression of the genes in the 4-gene novel monitoring signature in the confirmed TB group (n=37 patients).
The normalised CT values of *CDKN1C*, *FLVCR2*, *LTF* and *FCGR1C* are show with LOESS regression lines (by randomisation group, left, shading represents 95% confidence intervals) and individual patient lines (right). Dots (patient sample timepoints) are coloured and shaped by treatment randomization group (4-month: blue circles, n=14; 6-month: red triangles, n=23). LOESS= Locally Estimated Scatterplot Smoothing



Supplementary Figure 6. The change in DRS over time for the confirmed TB group (n=37)
Dots (patient sample timepoints) are coloured and shaped by treatment randomization group (4-month: blue circles, n=14; 6-month: red triangles, n=23). The lines represent LOESS regression lines with 95% confidence intervals (shading, grouped by treatment randomisation group). Arrows point towards the timepoint samples of an individual whose treatment was extended beyond 16 weeks. DRS=Disease Risk Score, LOESS= Locally Estimated Scatterplot Smoothing

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