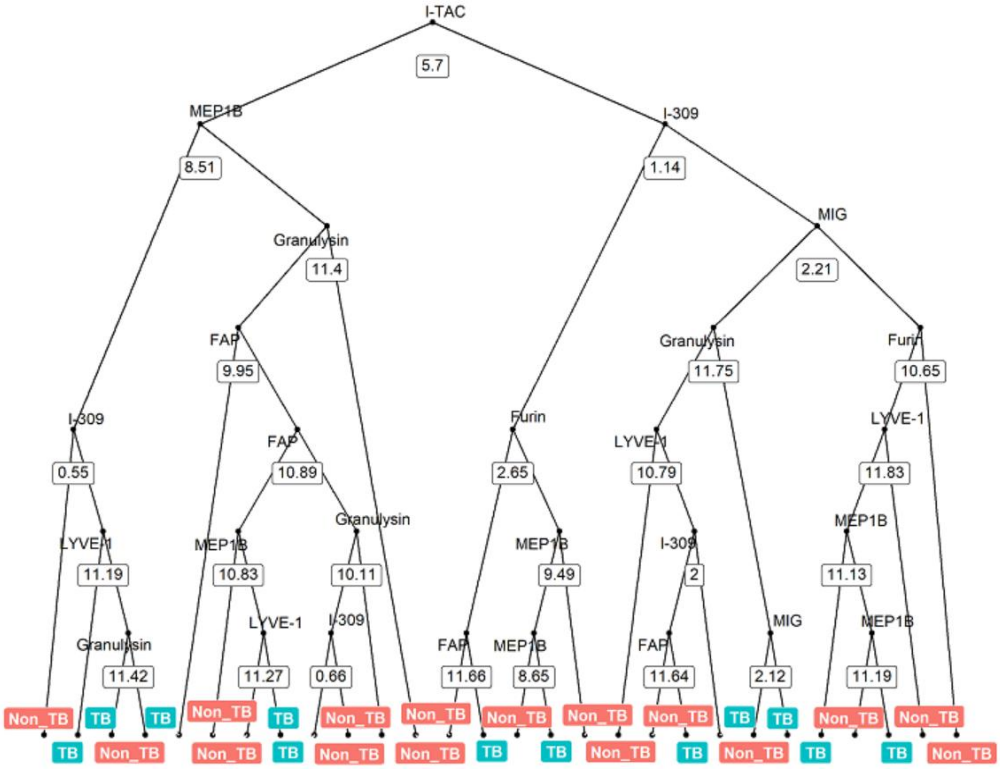

Figure S1. Decision tree of random forest (RF) model with eight proteins. Representative decision tree with a split value in each node and relative protein. Relative protein concentration value in each node was after log2 transformation. A total of 500 trees were made in our RF model.

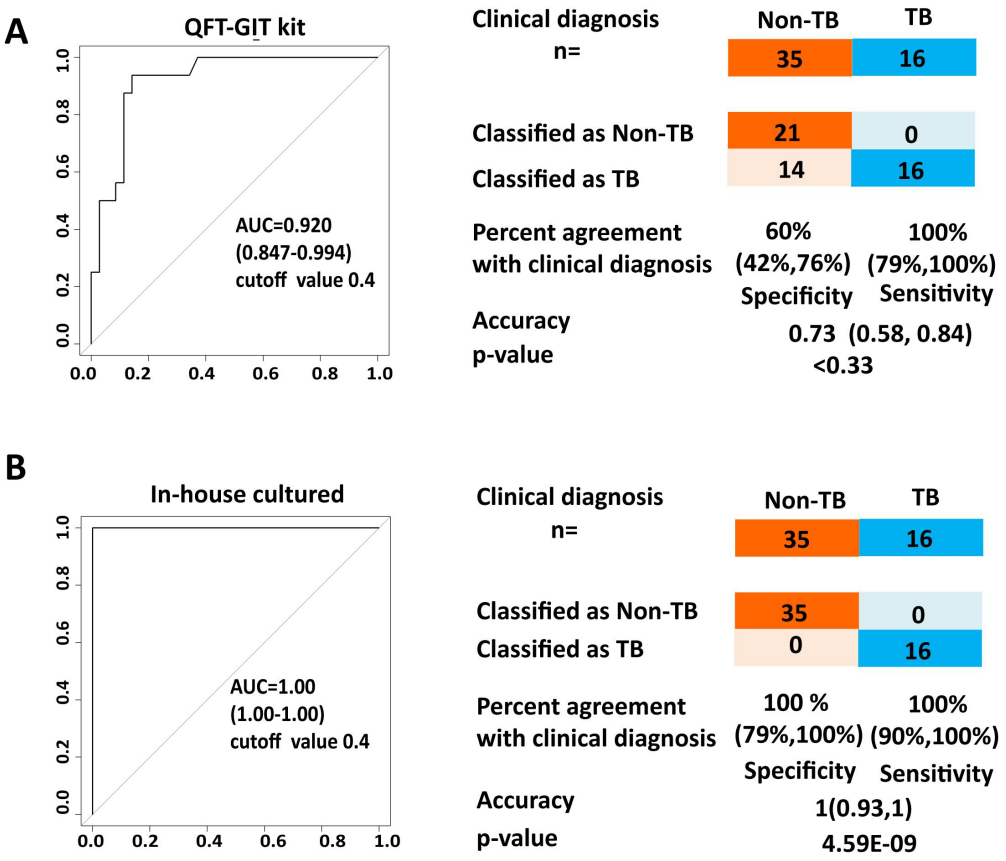

Figure S2. Comparison of data from QFT-GIT assays and in-house cultured blood samples. ROC curves derived from QFT-GIT assay data (A) and the in-house culture data (B) using the RF model. The eight-protein biosignature identified by RF modeling was used for TB (n=16) and non-TB (n=35; 14 HC, 4 PN, 17 LTBI) class prediction. FT-GIT, QuantiFERON-TB Gold in-tube; HC, healthy control; LTBI, latent tuberculosis infection; PN, pneumonia; TB, tuberculosis; RF, Random Forest algorithm.

Table S1. Accuracy of TB diagnosis with a different number of proteins

Variables	AUC	Sensitivity	Specificity	SD of AUC	SD of Sensitivity	SD of Specificity
1	0.633218531	0.73	0.448484848	0.069003856	0.042084102	0.095212402
2	0.715076923	0.865692308	0.534090909	0.133888239	0.080726647	0.19252463
3	0.73602331	0.905230769	0.5	0.114564697	0.071615894	0.173674231
4	0.734264569	0.901230769	0.466666667	0.119427117	0.076607215	0.16548402
5	0.767386364	0.901230769	0.518181818	0.09981402	0.066681655	0.170905688
6	0.786787296	0.913230769	0.516666667	0.100116312	0.067890928	0.171932426
7	0.782061189	0.913076923	0.5	0.099039826	0.079984384	0.14884002
8	0.814589744	0.932615385	0.474242424	0.102847965	0.049649925	0.166980115
9	0.814320513	0.936615385	0.491666667	0.095877879	0.038030404	0.161041038
10	0.808486014	0.936615385	0.518181818	0.102302107	0.046448065	0.129857904
11	0.811429487	0.932769231	0.500757576	0.096763455	0.052438499	0.15140779
12	0.81431993	0.944615385	0.484090909	0.102014394	0.042319346	0.160406219
13	0.811449883	0.932769231	0.490909091	0.094147831	0.064591164	0.164269569
14	0.810729021	0.940615385	0.456818182	0.096784044	0.062829199	0.119945508

AUC, Area Under Curve; SD, standard deviation.

Table S2. Comparison of different mathematical models in diagnosing TB

Training set (n = 276)										
Model	**Group**	**Clinical diagnosis (n)**	**Classified as Non-TB (n)**	**Classified as TB (n)**	**AUC (95% CI)**	**Sensitivity (95% CI)**	**Specificity (95% CI)**	**PPV (95% CI)**	**NPV (95% CI)**	**Accuracy (95% CI)**
SVM	Non-TB	189	162	27	0.908 (0.871,0.958)	78% (68%,86%)	86% (80%, 90%)	90% (84%, 94%)	72% (61%, 80%)	83% (78%,88%)
	TB	87	19	68						
LDA	Non-TB	189	156	33	0.842 (0.783,0.890)	74% (63%,82%)	83% (76%, 88%)	87% (81%, 92%)	66% (56%, 75%)	80% (74%,84%)
	TB	87	23	64						
RF	Non-TB	189	189	0	1.0 (1,1)	100% (96%,100%)	100% (98%, 100%)	100% (98%, 100%)	100% (96%, 100%)	100% (99%,100%)
	TB	87	0	87						
Test set (n = 92)										
Model	**Group**	**Clinical diagnosis (n)**	**Classified as Non-TB (n)**	**Classified as TB (n)**	**AUC (95% CI)**	**Sensitivity (95% CI)**	**Specificity (95% CI)**	**PPV (95% CI)**	**NPV (95% CI)**	**Accuracy (95% CI)**
SVM	Non-TB	63	52	11	0.800 (0.686,0.911)	72% (53%,87%)	83% (71%, 94%)	87% (75%, 94%)	66% (47%, 81%)	79% (70%,87%)
	TB	29	8	21						
LDA	Non-TB	63	52	11	0.833 (0.740,0.927)	72% (53%,87%)	83% (71%, 91%)	87% (75%, 94%)	66% (47%, 81%)	79% (70%,87%)
	TB	29	8	21						
RF	Non-TB	63	52	11	0.802 (0.696,0.909)	76% (56%,90%)	83% (71%, 91%)	88% (77%, 95%)	67% (48%, 82%)	80% (71%,88%)
	TB	29	7	22						

RF,random Forest algorithm ; LDA ,linear discrimination analysis; SVM, support Vector Machine; NPV, negative predictive value; PPV, positive predictive value; AUC, Area Under Curve;.

Table S3 AUC values of eight proteins in different cohorts

Protein	Non-TB vs Definite TB	HC vs Definite TB	LTBI vs Definite TB	PN vs Definite TB
I-TAC	0.78	0.82	0.81	0.72
I-309	0.73	0.75	0.69	0.75
MIG	0.66	0.57	0.63	0.79
FAP	0.43	0.38	0.45	0.46
Granulysin	0.44	0.29	0.41	0.61
MEP1B	0.38	0.31	0.39	0.43
Furin	0.48	0.49	0.49	0.45
LYVE-1	0.65	0.54	0.74	0.67

I-TAC: Interferon-inducible T-cell alpha; chemoattractant (also called CXLC11); I-309: also called CCL1/TCA3; MIG: Monokine induced by gamma interferon; (also called CXCL9); FAP: Fibroblast activation protein; MEP1B: Meprin A subunit beta; LYVE-1: Lymphatic vessel endothelial hyaluronan receptor 1.

Table S4. The effectiveness in diagnosing definite or probable TB using the eight-protein biosignature

Model	Group	Clinical diagnosis (n)	Classified as Non-TB (n)	Classified as TB (n)	AUC (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	NPV (95% CI)	PPV (95% CI)	Accuracy (95% CI)
Training	non-TB	189	189	0	1.0 (1.0, 1.0)	1.0 (0.98, 1.0)	1.0 (0.92, 1.0)	1.0 (0.98, 1.0)	1.0 (0.92, 1.0)	1.0 (0.9842, 1)
	definite TB	42	0	42						
	non-TB	189	189	0	1.0 (1.0, 1.0)	1.0 (0.98, 1.0)	1.0 (0.92, 1.0)	1.0 (0.98, 1.0)	1.0 (0.92, 1.0)	1.0 (0.9844, 1)
	probable TB	45	0	45						
Test	non-TB	63	53	10	0.857 (0.733-0.981)	0.8413 (0.73, 0.92)	0.7857 (0.59, 1.00)	0.9464 (0.90, 1.00)	0.5238 (0.27, 0.73)	0.8312 (0.7286, 0.9069)
	definite TB	11	3	11						
	non-TB	63	53	10	0.752 (0.585-0.919)	0.8413 (0.73, 0.92)	0.7333 (0.41, 0.87)	0.9298 (0.79, 0.96)	0.5238 (0.32, 0.76)	0.8205 (0.7172, 0.8983)
	probable TB	18	4	11						
Prediction	non-TB	70	59	11	0.943 (0.883-1.0)	0.8429 (0.74, 0.92)	0.7857 (0.49, 0.95)	0.9516 (0.87, 0.99)	0.5 (0.28, 0.72)	0.8333 (0.7362, 0.9058)
	definite TB	14	3	11						
	non-TB	70	59	11	0.893 (0.824-0.9616)	0.8429 (0.74, 0.92)	0.7222 (0.47, 0.9)	0.9219 (0.83, 0.97)	0.5417 (0.33, 0.74)	0.8182 (0.7216, 0.8924)
	probable TB	18	5	13						

NPV, negative predictive value; PPV, positive predictive value; AUC, Area Under Curve;.

Table S5. Diagnostic accuracy of the eight-protein biosignature in classifying TB patients from HC, LTBI or PN patients

Model	Group	Clinical diagnosis (n)	Classified as Non-TB (n)	Classified as TB (n)	AUC (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	NPV (95% CI)	PPV (95% CI)	Accuracy (95% CI)
Training	HC	68	68	0	1.0 (1.0, 1.0)	1.0 (0.95, 1.0)	1.0 (0.96, 1.0)	1.0 (0.95, 1.0)	1.0 (0.96, 1.0)	1.0 (0.9765, 1)
	TB	87	0	87						
	LTBI	54	54	0	1.0 (1.0, 1.0)	1.0 (0.93, 1.0)	1.0 (0.96, 1.0)	1.0 (0.93, 1.0)	1.0 (0.96, 1.0)	1.0 (0.9742, 1)
	TB	87	0	87						
	PN	67	67	0	1.0 (1.0, 1.0)	1.0 (0.95, 1.0)	1.0 (0.96, 1.0)	1.0 (0.95, 1.0)	1.0 (0.96, 1.0)	1.0 (0.9763, 1)
	TB	87	0	87						
Test	HC	16	13	3	0.836 (0.7177, 0.9547)	0.8125 (0.54, 0.96)	0.7586 (0.56, 0.90)	0.65 (0.41, 0.85)	0.88 (0.69, 0.97)	0.7778 (0.6291, 0.888)
	TB	29	7	22						
	LTBI	30	24	6	0.774 (0.6478, 0.9005)	0.8 (0.61, 0.92)	0.7586 (0.56, 0.90)	0.7742 (0.59, 0.90)	0.7857 (0.59, 0.92)	0.7797 (0.6527, 0.8771)
	TB	29	7	22						
	PN	17	16	1	0.821 (0.695, 0.948)	0.9412 (0.71, 1.00)	0.7586 (0.56, 0.90)	0.6957 (0.47, 0.87)	0.9565 (0.78, 1.00)	0.8261 (0.6858, 0.9218)
	TB	29	7	22						
Prediction	HC	19	17	2	0.9457 (0.8864, 1)	0.8947 (0.67, 0.99)	0.75 (0.57, 0.89)	0.68 (0.46, 0.85)	0.9231 (0.75, 0.99)	0.8039 (0.6688, 0.9018)
	TB	32	8	24						
	LTBI	27	24	3	0.94965 (0.8956, 1)	0.8889 (0.71, 0.98)	0.75 (0.57, 0.89)	0.75 (0.57, 0.89)	0.8889 (0.71, 0.98)	0.8136 (0.6909, 0.9031)
	TB	32	8	24						
	PN	24	18	6	0.8522 (0.7558, 0.9486)	0.75 (0.53, 0.90)	0.75 (0.57, 0.89)	0.6923 (0.48, 0.86)	0.8 (0.61, 0.92)	0.75 (0.6163, 0.8561)
	TB	32	8	24						

NPV, negative predictive value; PPV, positive predictive value; AUC, Area Under Curve;.

Supplement data text 1: A step-to-step procedure for randomForest (RF)

Step 1: After starting the software Rstudio (version 1.1.383. R language version: 3.5.1), the discovery cohort (368 cases) with eight biomarker expression values were imported by "read.table" function as a data matrix. The data matrix presented with rows as cases and column as eight biomarker values. Case types were marked as "TB" and "Non-TB" (included HC, PN and RxTB).

Packages were loaded including model building package "caret", RF package "randomForest", ROC curve packages "pROC" and "ROCR", Confidence Interval calculation package "epiR".

Step 2: In order to generate reproducible results, we used a random number generation with "set.seed(20)" function. Before model building, the discovery cohort values were processed with log2 transformation, and then divided randomly at a 3:1 ratio by "createDataPartition" function. Data from the larger (3/4) subset were used for modeling (training set), whereas data from the smaller subset (1/4) were used as the test set. Using "trainControl" function, control parameters were set as 5-fold cross validation and a 5-time-repeat with automatically tuning RF parameters ("mtry", tree numbers and node numbers). Finally, the RF was chosen with mtry = 2 trees = 500 and nodes = 109. The pre-processing options (preProcOptions) included a threshold (thresh) = 0.96, ICAcomp = 3, k = 5, freqCut = 9, uniqueCut = 10, and cutoff = 0.9."

Setp 3: Probability of each case ranging from 0 to 1 in training set and test set was obtained by "extractProb" function using the final RF model, and that in validation cohort was using "predict" function. Then ROC curve was generated by "roc" function and 95% CI of AUC by "ci.auc" function in "pROC" package according to probabilities and case types. The optical threshold was determined by "coords" function with Youden's index. Accuracy, sensitivity, specificity and their 95% CI were accessed by "confusionMatrix" and "epi.tests" functions respectively. All results were saved into .rda files.