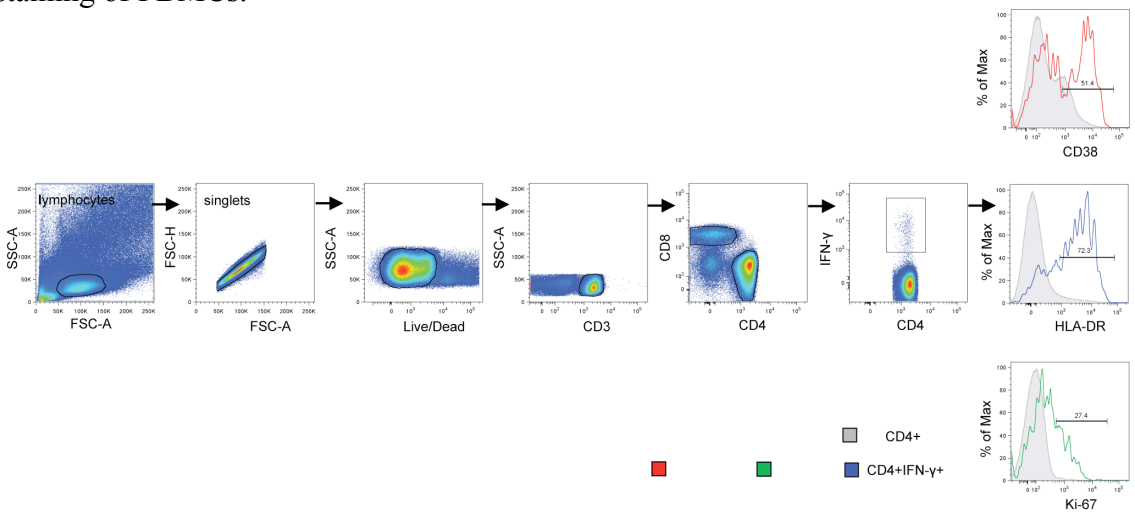
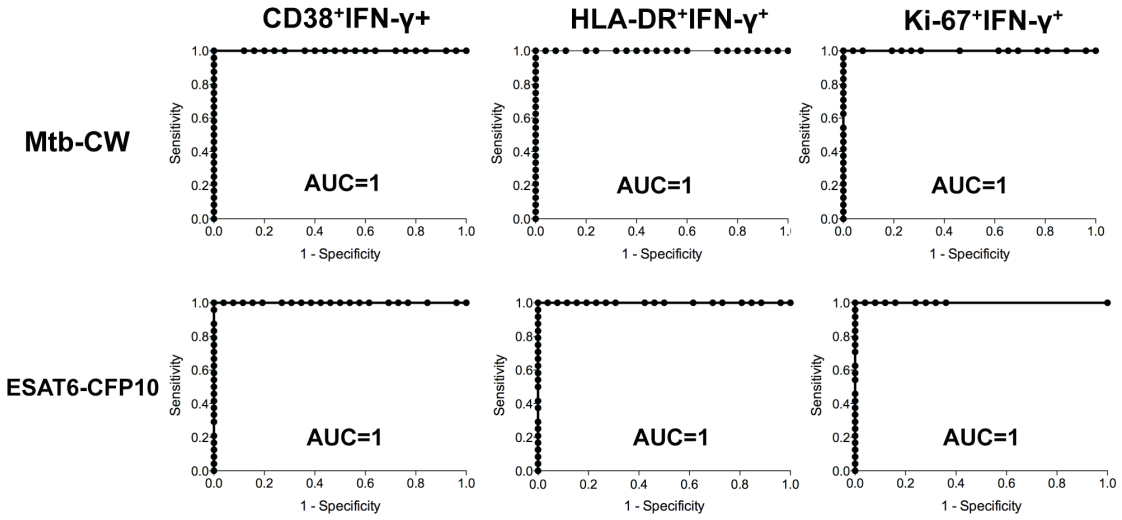
Supplemental Figure 1. Gating strategies for flow cytometry and intracellular cytokine-staining of PBMCs.



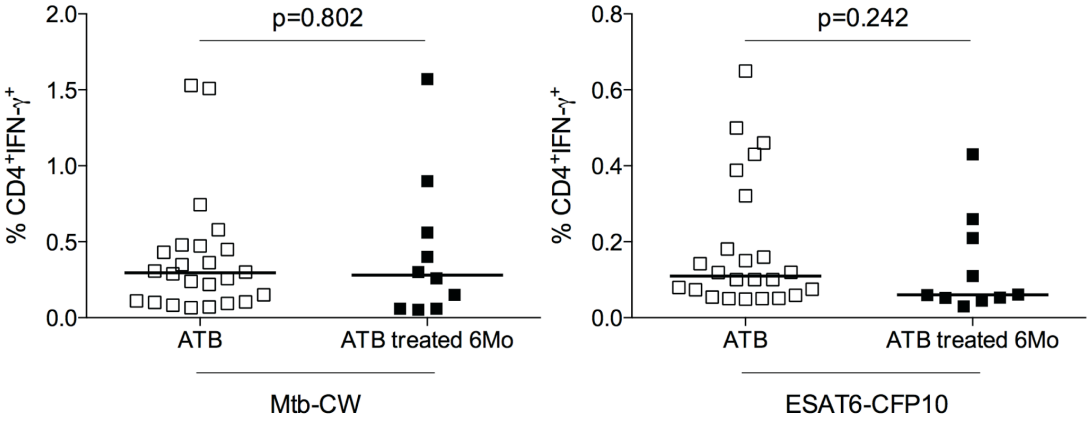
Forward scatter area (FSC-A) versus side scatter area (SSC-A) was used to select lymphocytes followed by FSC-A and forward scatter-height to select singlets. Live lymphocytes were selected using Live-Dead yellow. $CD3^{+}$T cells were selected within the live lymphocytes population, followed by gating on $CD4^{+}$ and $CD8^{+}$T cells. Total population of IFN-γ producing $CD4^{+}$ T cells was selected, followed by CD38, HLA-DR and Ki-67 histograms. The gray filled histograms represent the expression of CD38, HLA-DR and Ki-67 on total $CD4^{+}$T cells. The red, blue and green represent the expression of CD38, HLA-DR and Ki-67 respectively on $CD4^{+}$IFN-γ^{+} T cells.

Supplemental Figure 2. Receiver Operating Characteristic (ROC) curve analysis to evaluate the predictive value of CD38, HLA-DR and Ki-67 in classifying ATB and LTBI.



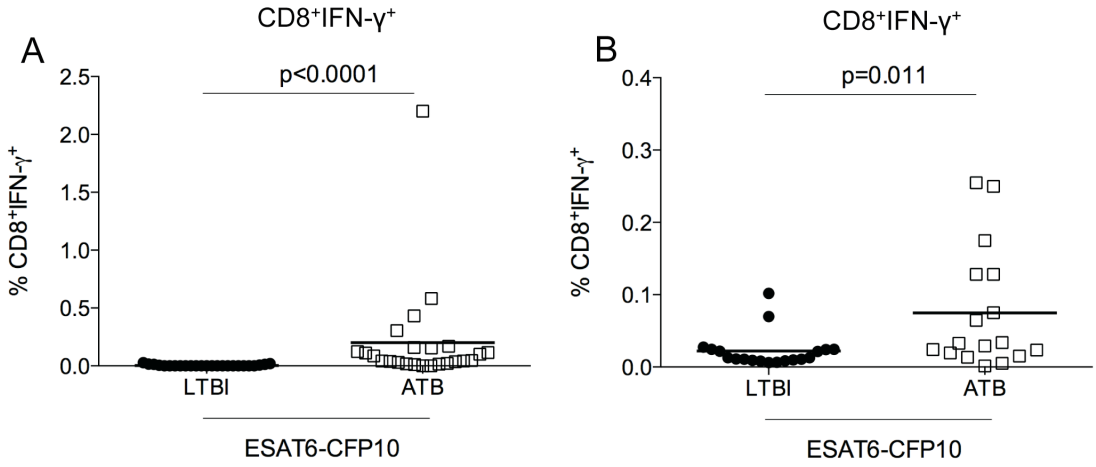
ROC curves which plot sensitivity (true positive rates) versus 1-specificity (false positive rates) for each marker individually are shown for Mtb-CW-specific and ESAT6/CFP10-specific $CD38^{+}IFN\text{-}\gamma^{+}$, $HLA\text{-}DR^{+}IFN\text{-}\gamma^{+}$ and $Ki\text{-}67^{+}IFN\text{-}\gamma^{+}$ $CD4^{+}$T cells. The area under curve (AUC) was determined by numerical integration for each marker to evaluate the predictive value of the marker. The AUC value of 1 obtained for each marker indicates that maximum predictive value was achieved.

Supplemental Figure 3. Flow cytometric analysis of IFN-γ production by $CD4^+$T cells from treatment-naïve ATB (n=24) and individuals who received 6 months of anti-TB treatment (ATB treated-6 mo; n=10).



PBMCs were stimulated with Mtb-CW antigens (left panel) and ESAT6/CFP10 peptides (right panel). Mann-Whitney U test was used to compare the 2 groups. Bars represent medians. *P*-value <0.05 was considered statistically significant.

Supplemental Figure 4. **Analysis of IFN-γ$^+$CD8$^+$ T cells in the test and validation cohorts.**



ESAT6-CFP10 peptides were used to stimulate PBMCs (**A**) from test cohort individuals with LTBI (n=25) and treatment-naïve ATB (n=24) and (**B**) from validation cohort individuals with LTBI (n=21) and treatment-naïve ATB (n=15) after unblinding. The Mann-Whitney U test was used to compare the 2 groups. Bars represent medians. *P*-value <0.05 was considered statistically significant.

Supplemental Table 1: Clinical description of patients diagnosed with active TB from the test cohort

Patient code	Age	Gender	Clinical symptoms	PCR	Baseline smear status (AFB)	Baseline Sputum Culture	Diagnosis	Treatment	Outcome
P1	47	M	cough, shortness of breath, fevers, chills, anorexia, night sweats, chest pain, cavitation in both lung on CT-scan	+	4+	Mtb	Pulmonary TB	Rifampicin Isoniazid Pyrazinamide Ethambutol	resolved
P2	51	M	cough, hemoptsis, fevers, chills, bronchiectasis	+	4+	Mtb	Pulmonary TB	Rifampicin Isoniazid Pyrazinamide Ethambutol	resolved
P3	56	M	cough, night sweats, chills, shortness of breath, weight loss, cavitary lesion in the left upper lobe	+	4+	Mtb	Pulmonary TB	Rifampicin Isoniazid Pyrazinamide Ethambutol	resolved
P4	41	M	cough, shortness of breath, weight loss, weakness, patchy airspace opacities in the left upper lobe	+	4+	Mtb	Pulmonary TB	Rifampicin Isoniazid Pyrazinamide Ethambutol	resolved
P5	56	M	cough, weight loss, bilateral upper lobe cavitary lesions	+	4+	Mtb	Pulmonary TB	Rifampicin Isoniazid Pyrazinamide Ethambutol	resolved
P6	52	M	cough, fever, multifocal pneumonia on X-ray	+	4+	Mtb	Pulmonary TB	Rifampicin Isoniazid Pyrazinamide Ethambutol	resolved
P7	53	M	cough, hemoptsis, weight loss, fevers, poor appetite, cavitary lesion and nodules in lungs on X-ray and CT-scan	+	4+	Mtb	Pulmonary TB	Rifampicin Isoniazid Pyrazinamide Ethambutol	resolved
P8	45	M	Fatigue, night sweats, hemoptysis, left upper lobe nodules and tree-in-bud nodularity in right upper lobe on CT-scan	+	2+	Mtb	Pulmonary TB	Rifampicin Isoniazid Pyrazinamide Ethambutol	resolved
P9	55	M	cough, weight loss, night sweats, chills, biapical airspace opacities in left lower lobe	+	2+	Mtb	Pulmonary TB	Rifampicin Isoniazid Pyrazinamide Ethambutol	resolved
P10	46	M	cough, depressed appetite, shortness of breath, weight loss, reticular pattern in left apex	+	3+	Mtb	Pulmonary TB	Rifampicin Isoniazid Pyrazinamide Ethambutol	resolved
P11	47	M	respiratory distress, thick-walled called in right upper lobe	+	4+	Mtb	Pulmonary TB	Rifampicin Isoniazid Pyrazinamide Ethambutol	resolved
P12	46	M	cough, chest pain, bilateral lung cavitary lesions, pleural fluid and hilar lymph nodes on CT-scan	+	4+	Mtb	Pulmonary TB	Rifampicin Isoniazid Pyrazinamide Ethambutol	resolved
P13	54	M	cough, shortness of breath, bilateral multifocal pneumonia with right upper lobe cavitation	+	1+	Mtb	Pulmonary TB	Rifampicin Isoniazid Pyrazinamide Ethambutol	death (attributed to TB)
P14	23	M	cough, fever, night sweats, weight loss, cavitation lesion in right upper lobe on X-ray and CT-scan	+	4+	Mtb	Pulmonary TB	Rifampicin Isoniazid Pyrazinamide Ethambutol	resolved
P15	71	M	weight loss, poor appetite, fevers, cavitation lesion in right lung apex and pulmonary nodules in the left lung base	+	1+	Mtb	Pulmonary TB	Rifampicin Isoniazid Pyrazinamide Ethambutol	resolved
P16	54	M	cough, hemoptsis, cavitary lesion in the right upper lung	+	1+	Mtb	Pulmonary TB	Rifampicin Isoniazid Pyrazinamide Ethambutol	resolved

P17	28	M	cough, weight loss, fevers	+	4+	Mtb	Pulmonary TB	Rifampicin Isoniazid Pyrazinamide Ethambutol	resolved
P18	83	M	cough, weight loss, cavitation in the right upper lobe and left upper lobe nodular density	+	4+	Mtb	Pulmonary TB	Rifampicin Isoniazid Pyrazinamide Ethambutol	resolved
P19	58	M	cough, shortness of breath, chills, night sweats, anorexia, weight loss, cavitation in the right lung on X-ray and CT-scan	+	3+	Mtb	Pulmonary TB	Rifampicin Isoniazid Pyrazinamide Ethambutol	death (attributed to TB)
P20	55	M	cough, hazy opacities within the right upper lobe	+	1+	Mtb	Pulmonary TB	Rifampin Isoniazid Pyrazinamide Ethambutol	resolved
P21	75	M	productive cough, weight loss, bilateral cavitation	+	4+	Mtb	Pulmonary TB	Rifampin Isoniazid Pyrazinamide Ethambutol	resolved
P22	61	M	abdominal pain, weight loss, cough, bilateral small nodular opacities	+	2+	Mtb	Pulmonary TB	Rifampin Isoniazid Pyrazinamide Ethambutol	death (attributed to traumatic brain injury)
P23	52	M	cough, weight loss, chills, pulmonary infiltrates on Chest-X ray	+	4+	Mtb	Pulmonary TB	Rifampin Isoniazid Pyrazinamide Ethambutol	resolved
P24	55	F	fatigue, weight loss, cough, night sweats, bronchiectasis and pleural effusion on X-ray, lung cavitation on CT-scan	+	2+	Mtb	Pulmonary TB	Rifampin Isoniazid Pyrazinamide Ethambutol	resolved

AFB acid-fast bacilli
In bold: patients followed longitudinally
CT-scan: computed tomography scan

Supplementary Table 2: Patients' demographics and clinical characteristics

	Study population	Pulmonary TB suspects that were screened and excluded	All additional TB patients diagnosed at Grady during the study period who also met the inclusion criteria (but were not enrolled or included in the study data)
	n=26*	n=60	n=39
Male Gender	23/24 (96%)	46 (77%)	27 (69%)
Median Age	53.5 yrs	49 yrs	50 yrs
Black Race	21/24 (88%)	55 (92%)	31 (79%)
Non-Hispanic Ethnicity	23/24 (96%)	57/59 (97%)	34 (87%)
US born	21/24(88%)	51 (85%)	27 (69%)
HIV positive	0 (0%)	39/59 (66%)	0 (0%)
Culture proven-pulmonary	26/26 (100%)	27 (45%)	39 (100%)
Sputum smear positive	26/26 (100%)	43 (72%)	39 (100%)
Culture proven-extrapulmonary only	0 (0%)	1 (2%)	0 (0%)

* age, race, ethnicity and Gender were not known for 2 patients

Supplemental Table 3: Characteristics of the subjects from the validation cohort

Validation cohort (ATB+LTBI)	n=36
Average age, years (range)	35.5 (18-64)
Sex (female)	13 (36)
HIV status	0 (36)
Anti-TB treatment	0 (36)
Clinical signs + microbiological positive tests	15 (36)