Dominant TNF-α^+ *Mycobacterium tuberculosis*-Specific CD4 T Cell Responses Discriminate Between Latent Infection and Active Disease

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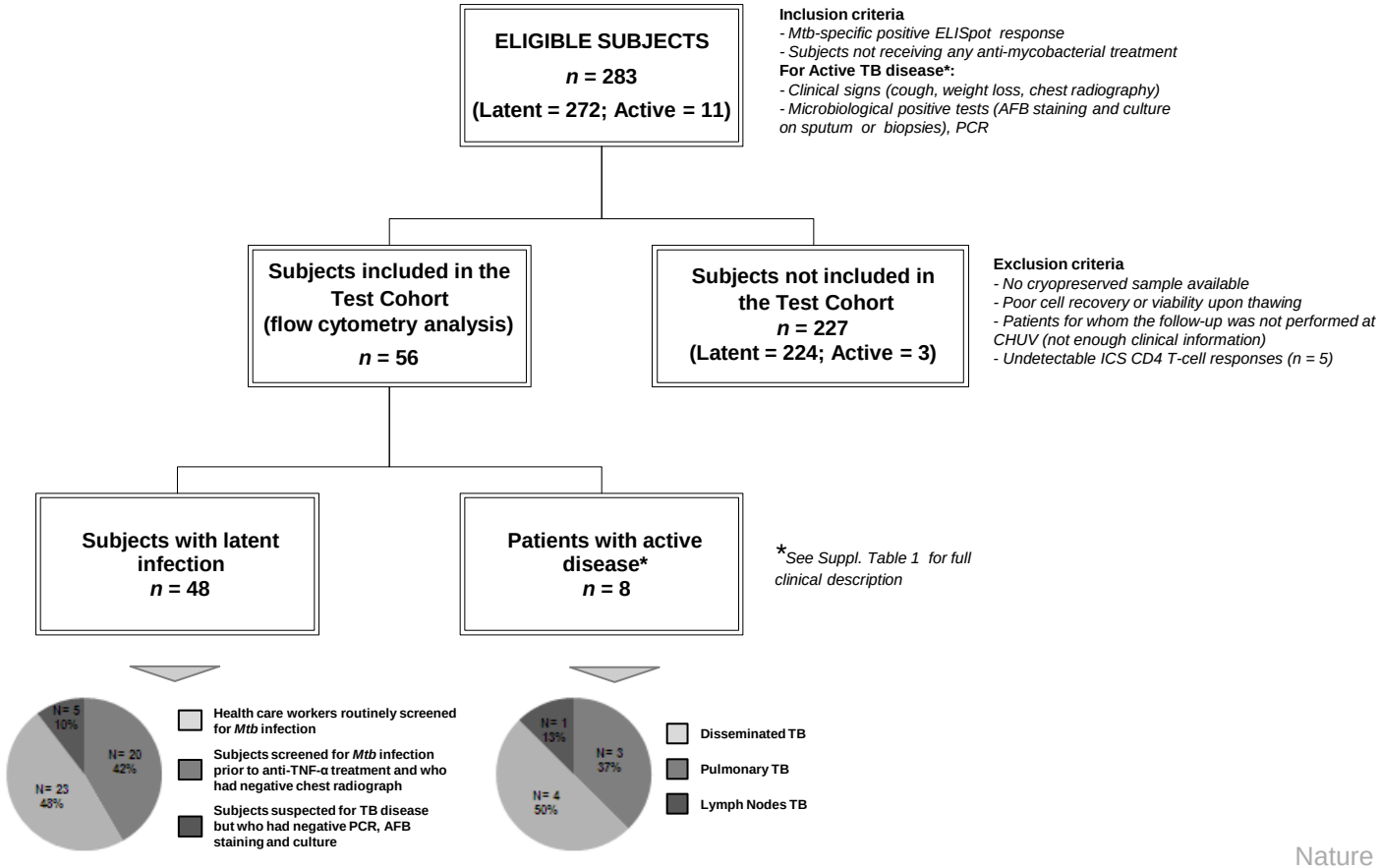
Suppl. Figure 1: Flow chart description of subjects included in the Test Cohort

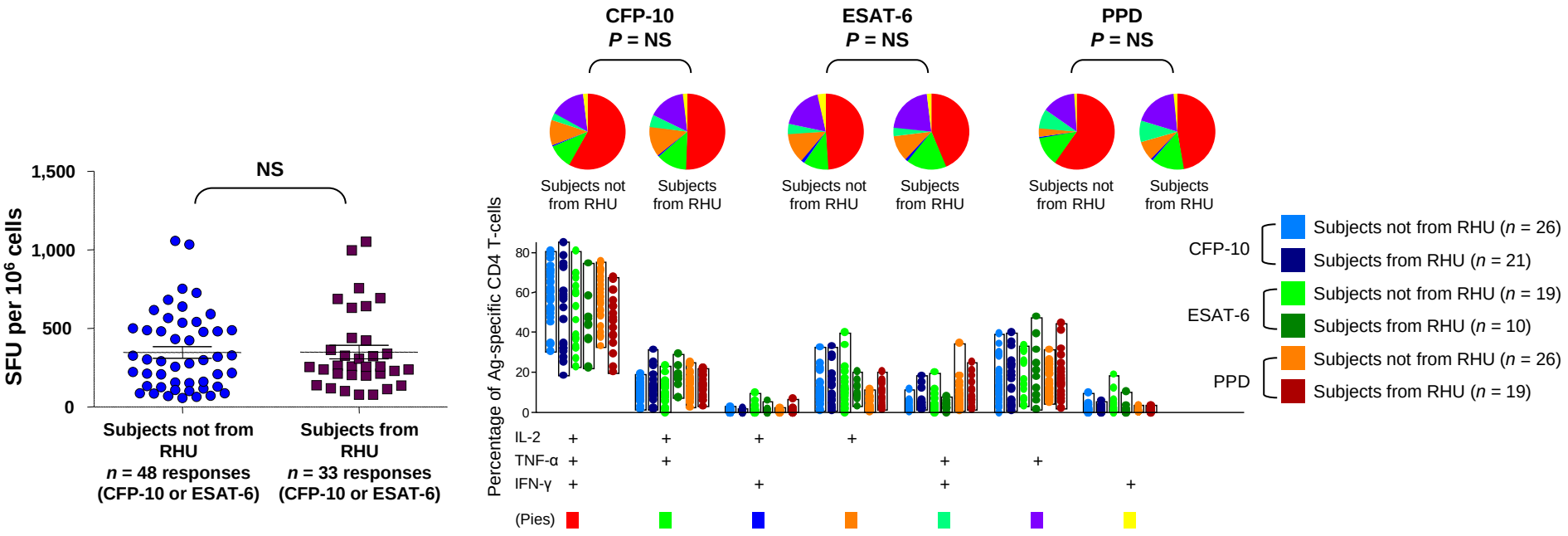


Suppl. Table 1: Clinical description of patients diagnosed with active TB disease from the Test Cohort

Patient code #	Gender	Age	Clinical history	PCR	AFB[§]	Culture[§]	Cytology and Histopathology	Diagnosis	Treatment	Comments
A1	F	31	Typical clinical presentation (weight loss, malaise, productive coughing and hemoptysis, night sweats)	+	+	+	NA[*]	Pulmonary TB	Rifampicine, isoniazide, pyrazinamide and ethambutol	PCR, AFS and culture performed on sputum
A2	F	59	End-stage renal disease related to SLE. APLS. Chronic HCV. Weight loss, fever and liver tests abnormality	+	+	-	Bone marrow aspiration and biopsy	Disseminated TB	Rifampicine, ethambutol and amikacine	Positive AFS and PCR in bronchoalveolar lavage (BAL)
A3	M	27	Fever, pleural effusion, necrotic lymphadenopathy	+	-	-	Cytology of pleural fluid: 96% of small lymphocytes	Disseminated TB	Rifampicine, isoniazide, pyrazinamide and ethambutol	Positive PCR, increase of adenosine deaminase and lymphocytosis in pleural fluid
A4	F	78	Fatigue, weight loss, night sweats. Sterile leucocyturia. CT-scan: micronodules in lungs and spleen	+	+	+	Bronchial biopsy showing caseous necrosis with granulomatous inflammation	Disseminated TB	Rifampicine, isoniazide, pyrazinamide and ethambutol	Rheumatoid arthritis previously treated with methotrexate, leflunomide and steroids. Modification of therapy: isoniazide introduced prior to initiation of adalimumab (ELISpot TB +)
A5	F	35	After delivery: cough, fever, night sweats, chest and abdominal pain. CT-scan: pleural effusion and tubo-ovarian abscesses	+	+	+	Bilateral granulomatous and necrotic salpingitis with AFS resistant bacilli	Disseminated TB	Rifampicine, isoniazide, pyrazinamide and ethambutol	HIV infection treated with didanosine, lamivudine and lopinavir/ritonavir (CD4 count: 152 cells/µL, viremia <40 HIV-1 RNA copies/mL). PCR, AFS and culture positive in peritoneal samples. Increase of adenosine deaminase in pleural fluid
A6	M	8	Immigration routine chest X-ray: lung cavitation	+	+	+	Granulomatous and necrotic inflammation, and calcifications on the lobectomy histopathology	Pulmonary TB	Rifampicine, isoniazide, pyrazinamide and ethambutol	Positive AFS, culture and PCR in sputum, not on the lobectomy tissue. Negative PPD test.
A7	M	18	Fever, fatigue, asthenia, weight loss, night sweats and chest pain. Left hilar and retroperitoneal lymphadenopathies on thoraco-abdominal CT-scan	-	-	-	NA	Lymph nodes TB	Rifampicine, isoniazide and pyrazinamide	PPD skin test positive. Complete remission after TB treatment
A8	F	48	Fever, weight loss, chills, productive cough. Chest X-ray: bilateral pneumonia. Chest CT-scan: right upper and middle lobes hepatisation	+	+	+	NA	Pulmonary TB	Rifampicine, isoniazide, pyrazinamide, ethambutol, moxifloxacine	HIV infection treated with tenofovir, emtricitabine and efavirenz (CD4 count: 265 cells/µL, viremia <40 HIV-1 RNA copies/mL). PCR, AFS and culture performed on sputum
A9	F	72	Cough, severe loss of weight. Confusion. Numerous bilateral lung nodules on chest X-ray and CT-scan	+	+	+	AFS positive bacilli on the bronchoalveolar lavage (BAL) cytology	Pulmonary TB	Rifampicine, isoniazide, pyrazinamide and ethambutol	AFS, culture and PCR positive in bronchoalveolar lavage (BAL). Probable reactivation of latent TB
A10	M	52	Fever, asthenia, chills, large cervical adenopathy. Hepatosplenomegaly on the abdominal CT-scan	+	+	+	Lymph node cytoponction: positive staining and PCR. Epithelioid granulomas on the bone marrow and liver biopsies	Disseminated TB	Rifampicine, isoniazide, pyrazinamide and ethambutol	Recent diagnosis of HIV infection (no treatment). CD4 count: 18 cells/µL, viremia 207'000 HIV-1 RNA copies/mL. Chronic HCV infection (genotype 3a)
A11	M	40	Cough, night sweats, severe weight loss. Pulmonary infiltrates and caverna on the chest X-ray	+	+	+	NA	Pulmonary TB	Rifampicine, isoniazide, pyrazinamide and ethambutol	Positive AFS, culture and PCR in sputum

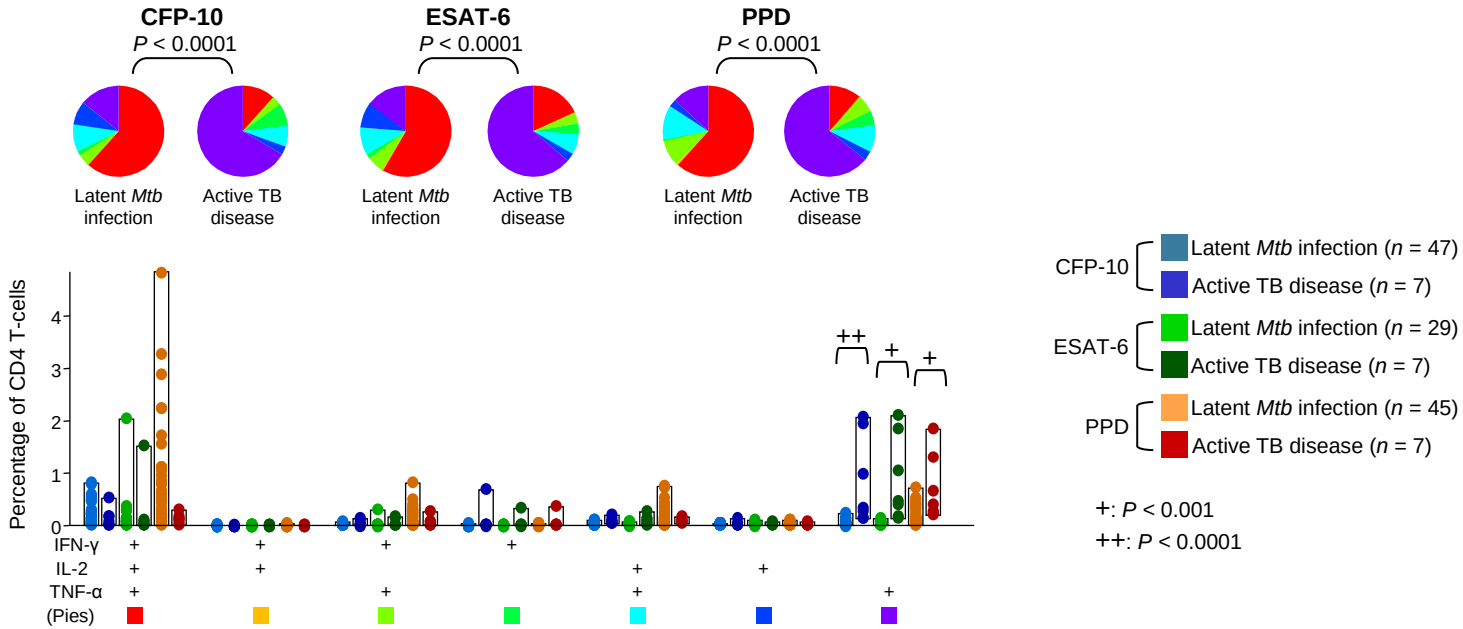
[§]AFB (acid-fast staining) and culture performed according to Murray *et al*, Manual of Clinical Microbiology, American Society of Microbiology; [*]Not applicable

Suppl. Figure 2: Analysis of *Mtb-specific* T-cell responses in subjects from the Test Cohort (*n* = 48) among those screened prior to anti-TNF-α treatment (thereafter referred to as patients from RHU, *n* = 20) and the others (*n* = 28)



Suppl. Figure 2: Analysis of *Mtb*-specific T-cell responses by IFN-γ ELISpot (left panel) and polychromatic flow cytometry (right panel) from subjects screened prior to anti-TNF-α treatment (i.e. patients followed in the department of Rheumatology [RHU]) and the others. All the possible combinations of the different functions are shown on the x axis whereas the percentages of the distinct cytokine-producing cell subsets within *Mtb*-specific CD4 T-cells are shown on the y axis. The pie charts summarize the data, and each slice corresponds to the proportion of *Mtb*-specific CD4 T-cells positive for a certain combination of functions.

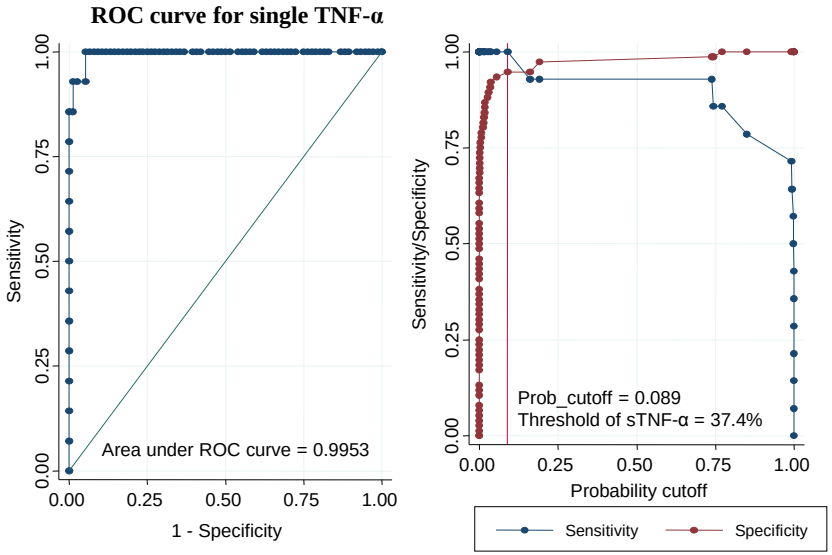
Suppl. Figure 3: Analysis of the functional profile of *Mtb*-specific CD4 T-cells on the basis of IFN-γ, IL-2 or TNF-α production shown in absolute scale



Suppl. Figure 3: Analysis of the functional profile of *Mtb*-specific CD4 T-cells on the basis of IFN-γ, IL-2 or TNF-α production shown in absolute scale. ESAT-6-, CFP-10- and PPD-specific CD4 T-cell responses are shown from 48 and 8 participants with latent *Mtb* infection or active TB disease, respectively, enrolled in the Test Cohort. All the possible combinations of the different functions are shown on the x axis whereas the frequencies of *Mtb*-specific cytokine-producing CD4 T-cells are shown on the y axis. The pie charts summarize the data, and each slice corresponds to the proportion of *Mtb*-specific CD4 T-cells positive for a certain combination of functions.

Suppl. Figure 4

Statistical data on the Test Cohort

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Logistic regression                                   Number of obs   =         56
                                                      LR chi2(1)      =      41.50
                                                      Prob > chi2     =     0.0000
Log likelihood = -2.9512394                           Pseudo R2       =     0.8755
------------------------------------------------------------------------------
       tb01 | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
------------+-----------------------------------------------------------------
      sTNFa |   1.449276   .3601348     1.49   0.135     .8904997    2.358678
------------------------------------------------------------------------------
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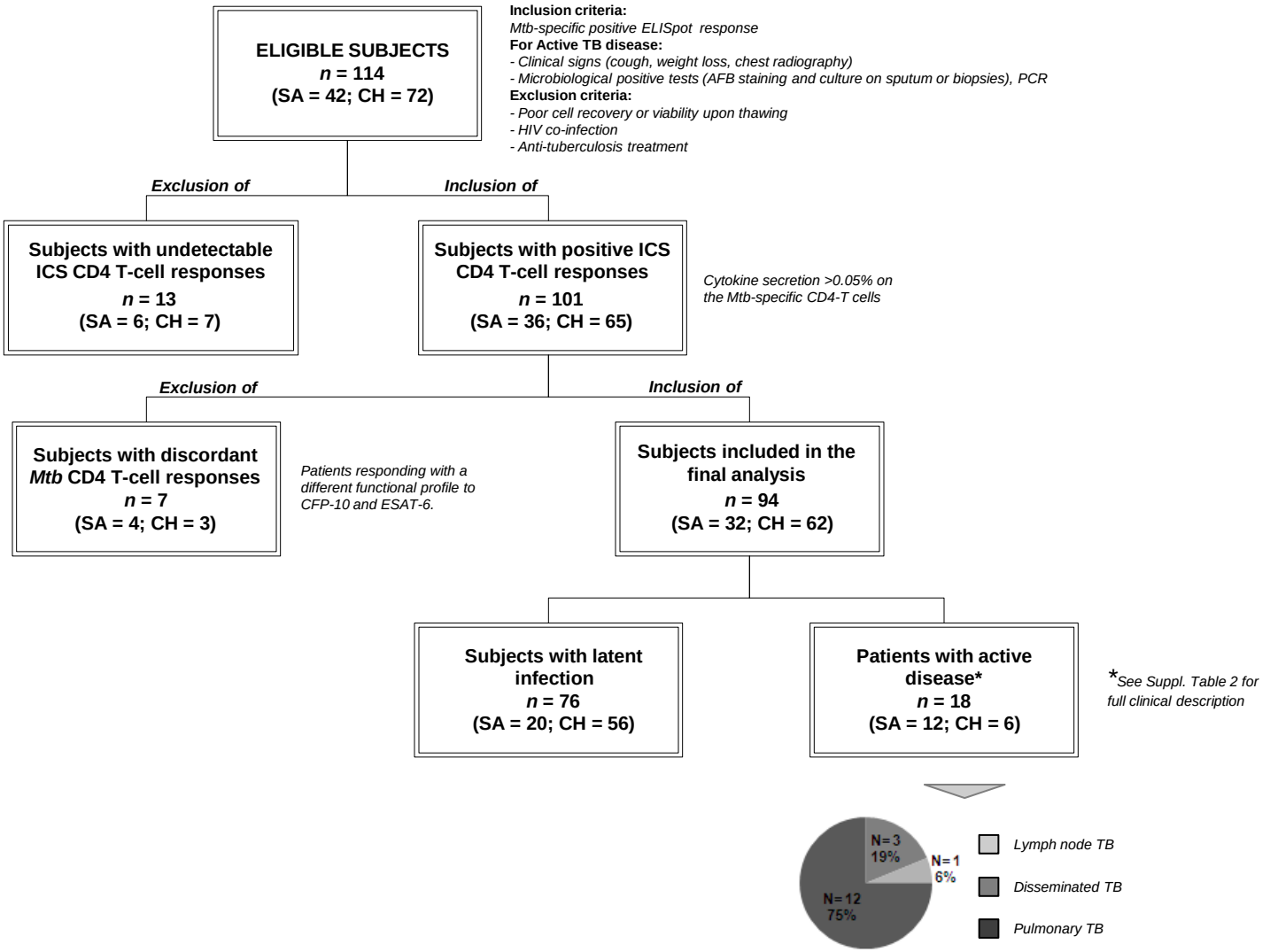
Suppl. Figure 4: Logistic regression analysis (left panel) showing the association between the proportion of single TNF-α with the ability to discriminate between active TB disease and latent *Mtb* infection (AUC = 0.995; [95% confidence interval: 0.984-1]; Odds-Ratio = 1.45) from the Test Cohort. Right panel shows that a cutoff of 37.4% (of single TNF-α-producing CD4 T-cells) was calculated as the value associated with a sensitivity of 100% and specificity of 96%. Specificity and sensitivity analyses apply only to those subjects with detectable ICS responses.

Suppl. Figure 5: Flow chart description of subjects included in the Validation Cohort.



Suppl. Table 2: Clinical description of patients with active TB disease from the Validation Cohort

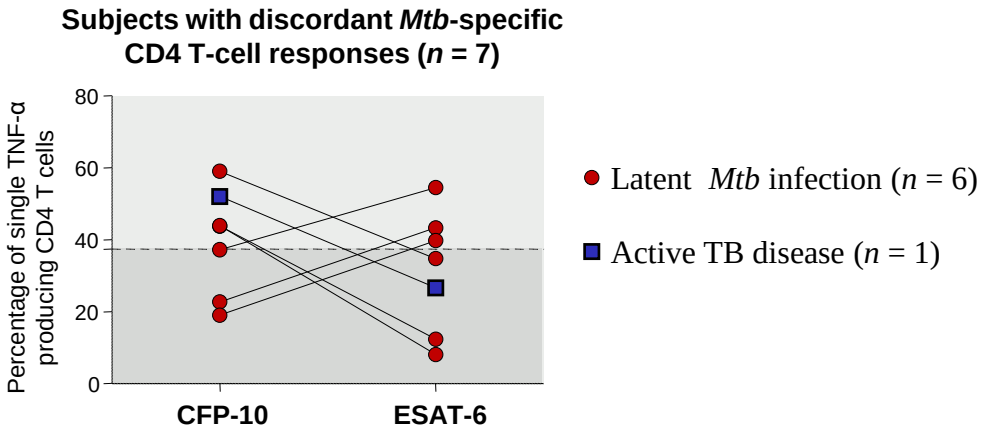
Patient code [#]	Gender	Age	PCR	AFB [§]	Culture [§]	Diagnosis
CH-1	F	63	-	-	-	Disseminated TB
CH-2	F	30	+	-	+	Lymph Node TB
CH-3	M	76	+	+	+	Disseminated TB
CH-4	M	30	-	-	+	Pulmonary TB
CH-5	M	54	+	+	+	Pulmonary TB
CH-6	F	38	+	+	+	Disseminated TB
SA-1	M	41	*	+	+	Pulmonary TB
SA-2	M	59	*	+	+	Pulmonary TB
SA-3	M	31	*	-	+	Pulmonary TB
SA-4	M	31	*	+	+	Pulmonary TB
SA-5	M	47	*	-	+	Pulmonary TB
SA-6	M	37	*	-	+	Pulmonary TB
SA-7	M	54	*	+	+	Pulmonary TB
SA-8	M	50	*	-	+	Pulmonary TB
SA-9	M	38	*	+	+	Pulmonary TB
SA-10	M	51	*	-	+	Pulmonary TB
SA-11	M	23	*	+	+	Pulmonary TB
SA-12	M	45	*	-	+	Pulmonary TB

* Not done

CH: patients from Switzerland; SA: patients from South Africa

[§]AFB (acid-fast bacili) and culture performed according to Murray *et al*, Manual of Clinical Microbiology, American Society of Microbiology

Suppl. Figure 6



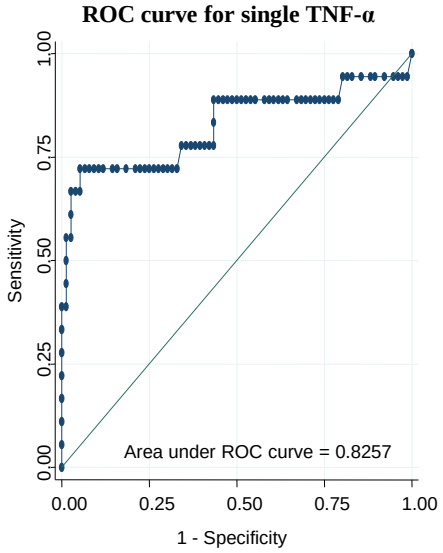
Suppl. Figure 6: Percentages of *Mtb*-specific single TNF-α-producing CD4 T-cells in the 7 subjects (among 101) with discordant responses against ESAT-6 and CFP-10 peptide pools. Dashed line represents the cutoff of 37.4% of single TNF-α. Participants with latent *Mtb* infection (*n* = 6) are represented with red circles whereas the patient with active TB disease (*n* = 1) is shown with blue squares.

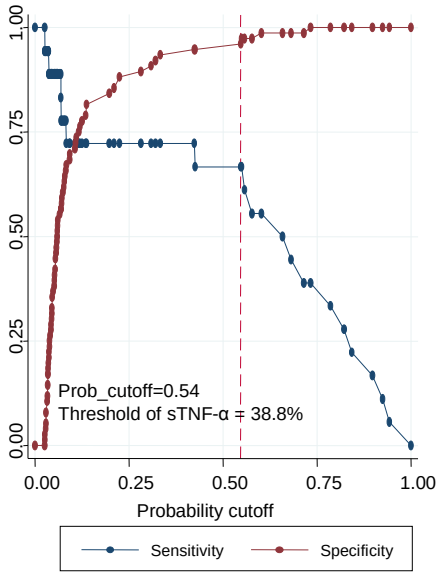
Suppl. Figure 7

Statistical data on the Validation Cohort (Swiss + SA)

	True		Total
Classified	**D**	**Non-D**	
+	**12**	**3**	**15**
-	**6**	**73**	**79**
Total	**18**	**76**	**94**
Sensitivity		Pr(+/D)	**66.67**
Specificity		Pr(-/ND)	**96.05**
Positive predictive value		Pr(D/+)	**80.00**
Negative predictive value		Pr(ND/-)	**92.41**

Suppl. Figure 7: Overall performance of the test showing positive and negative predictive values, sensitivity and specificity (top panel). Logistic regression analysis (left bottom panel) showing the association between the proportion of single TNF-α with the ability to discriminate between active TB disease and latent *Mtb* infection in the Validation Cohort (AUC = 0.825 [95% confidence interval: 0.683-0.968]; Odds-Ratio = 1.10). Right bottom panel shows that a cutoff of 38.8% (of single TNF-α-producing CD4 T cells) was calculated as the optimal threshold. Specificity and sensitivity analyses apply only to those subjects with detectable ICS responses.



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Logistic regression                                   Number of obs   =         94
                                                      LR chi2(1)      =      33.10
                                                      Prob > chi2     =     0.0000
Log likelihood = -29.358109                           Pseudo R2       =     0.3605
------------------------------------------------------------------------------
        tb01 | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       mapgm |   1.106716   .0241542     4.65   0.000     1.060373    1.155084
------------------------------------------------------------------------------
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