Supplementary Figures

Restimulation-induced T cell death through NTB-A/SAP signaling pathway is impaired in tuberculosis patients with depressed immune responses.

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Figure S1.

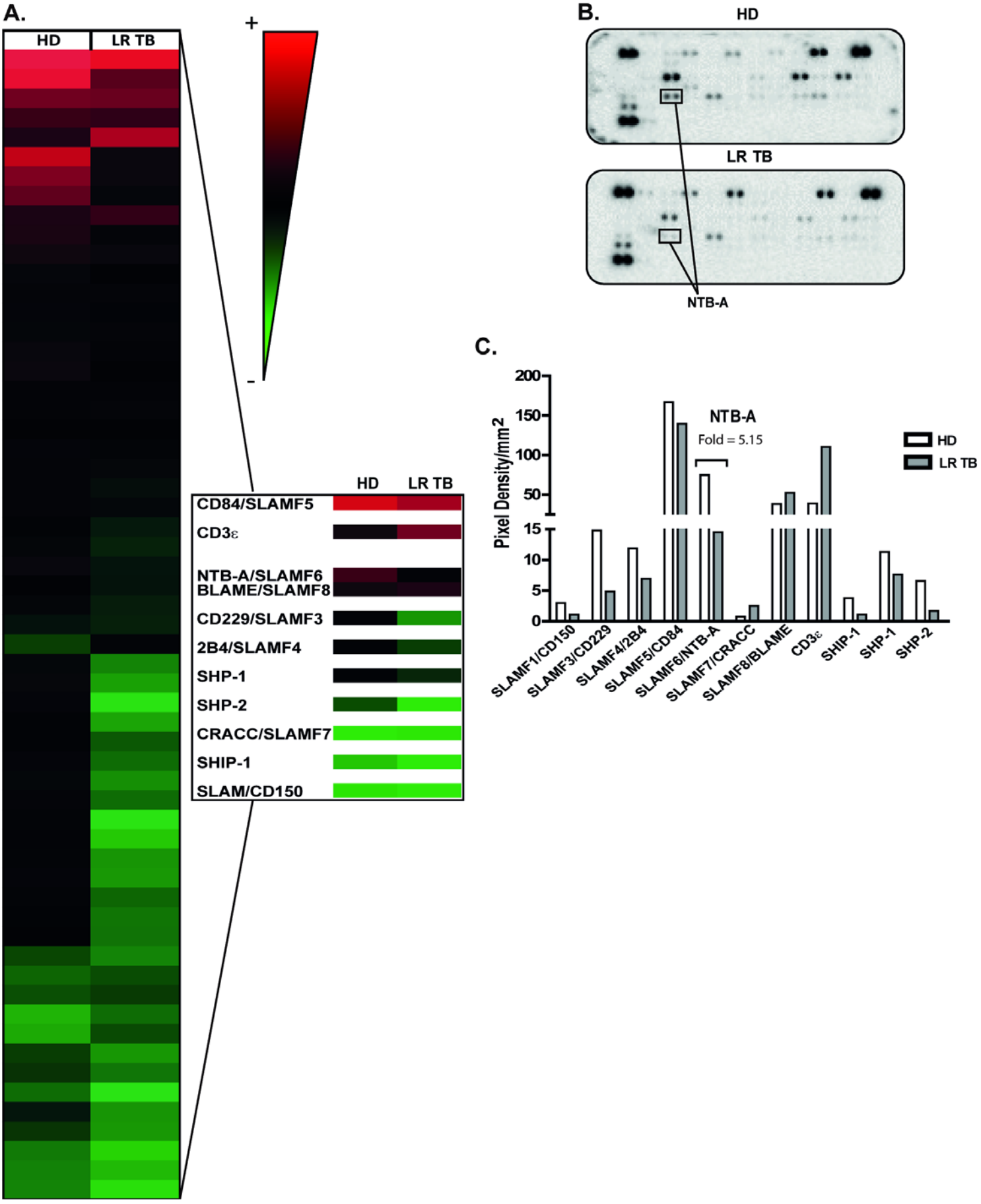

A.
HD
LR TB
+
-
HD
LR TB
CD84/SLAMF5
CD3ε
NTB-A/SLAMF6
BLAME/SLAMF8
CD229/SLAMF3
2B4/SLAMF4
SHP-1
SHP-2
CRACC/SLAMF7
SHIP-1
SLAM/CD150
B.
HD
LR TB
NTB-A
C.
Pixel Density/mm2
200
150
100
50
15
10
5
0
NTB-A
Fold = 5.15
HD
LR TB
SLAMF1/CD150
SLAMF3/CD229
SLAMF4/2B4
SLAMF5/CD84
SLAMF6/NTB-A
SLAMF7/CRACC
SLAMF8/BLAME
CD3ε
SHIP-1
SHP-1
SHP-2

Figure S1. Phosphorylation profile against *M. tuberculosis*. (A-C) Peripheral blood mononuclear cells (PBMCs) from a healthy donor (HD) and a Low Responder patient with active Tuberculosis (LR TB) were stimulated with *Mtb*-Ag for 24h and then, protein extracts were incubated on a phospho-immunoreceptor human array. Phosphorylation levels of individual analytes were determined by calculating an average of the pixel density of the spots in duplicate; values were obtained after subtracting background signals. **(A)** Heat map showing phosphorylation levels of 59 immunoreceptors. Right square show the detailed results of some immunoreceptors of SLAM family and key phosphatases involved in SLAM pathway. **(B)** A blot of NTB-A phosphorylation status in HD and LR TB patient's PBMCs are highlighted. The graph denotes densitometric analysis of the pixel density of the array shown. **(C)** Bar graph denotes densitometric analysis of the array pixel density of other SLAM family receptors and key phosphatases involved in SLAM pathway.

Figure S2.

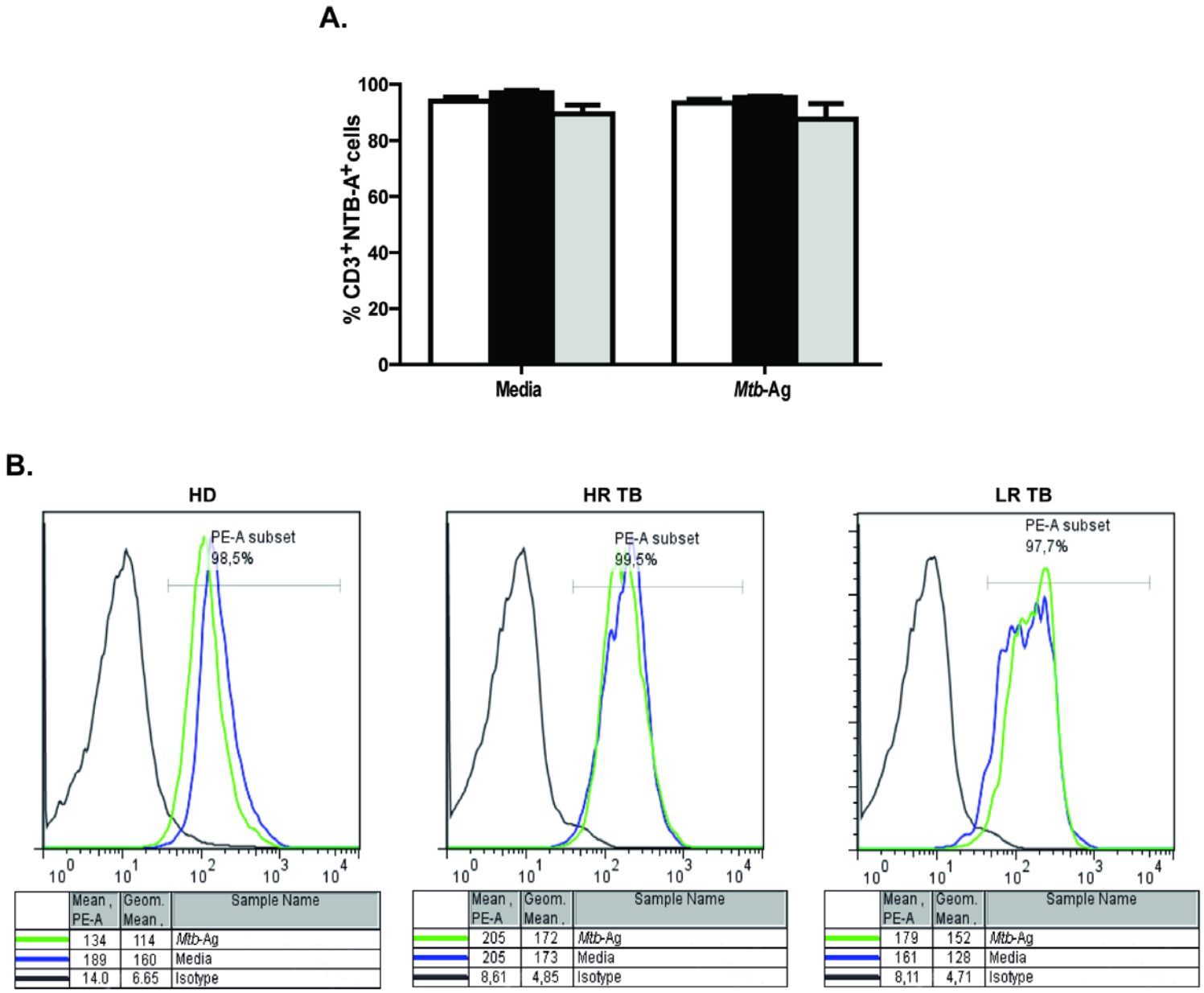


Figure S2. NTB-A expression after *M. tuberculosis* stimulation. (A) PBMCs from HD (N=8), High Responder Tuberculosis patients (HR TB, N=5) and LR (N=4) were stimulated with *Mtb*-Ag for 5 days. Then, NTB-A expression was determined on $CD3^+$ T cells by flow cytometry. Bars represent the percentage ± Standard Error

of the Mean (SEM) of NTB-A^{+} CD3^{+} cells. **(B)** Representative histogram of NTB-A expression on CD3^{+} T cells.

Figure S3.

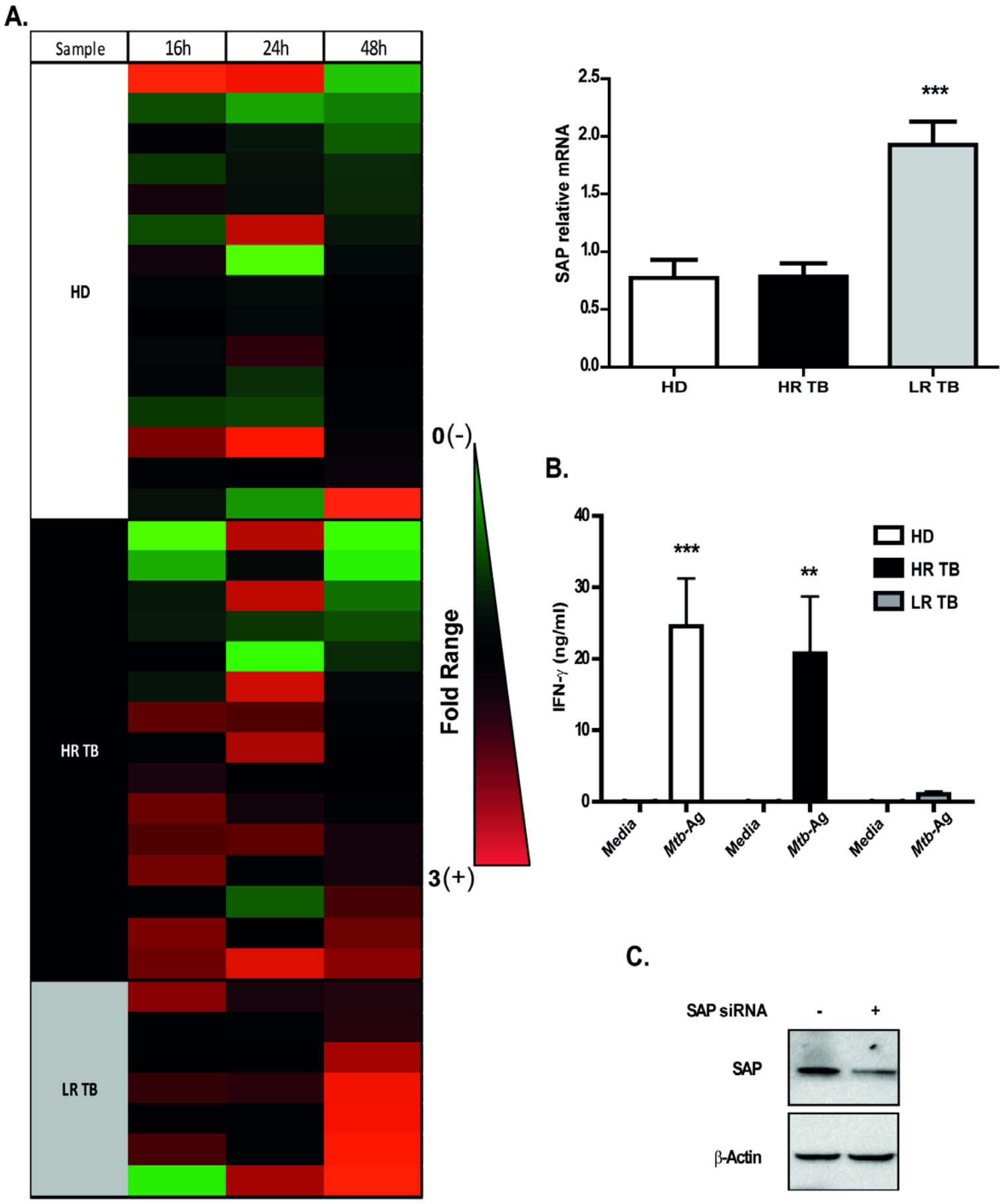

A.
Sample
16h
24h
48h
HD
HR TB
LR TB
0(-)
Fold Range
3(+)
SAP relative mRNA
2.5
2.0
1.5
1.0
0.5
0.0
***
HD
HR TB
LR TB
B.
IFN-γ (ng/ml)
40
30
20
10
0
***
**
HD
HR TB
LR TB
Media
Mtb-Ag
Media
Mtb-Ag
Media
Mtb-Ag
C.
SAP siRNA
-
+
SAP
β-Actin

Figure S3. SAP mRNA and protein levels are increased in patients with low IFN-γ production. (A) PBMCs from Healthy Donors (HD, N=14), High Responder Tuberculosis patients (HR, N=15) and Low Responder Tuberculosis patients (LR, N=7) were stimulated with *Mtb*-Ag for different times. SAP mRNA expression was determined by Real time PCR. Values were calculated as fold of increase using the comparative method for relative quantification after normalization to GAPDH expression. (Left panel) Heat map showing SAP mRNA levels at different time points for the different individuals studied. Heat map was performed with NetWalker 1.6 software. The – and + is a 0 to 3 range of fold values. (Right panel) Bars represent the mean of SAP mRNA Fold Increase after 48h of *Mtb*-Ag stimulation ± Standard Error of the Mean (SEM). **(B)** HD (N=16), HR (N=11) and LR (N=7) tuberculosis patients were stimulated with *Mtb*-Ag for 48h. Cell-free supernatants were then collected and assayed for IFN-γ production by ELISA. Bars represent the mean ± Standard Error of the Mean (SEM) of the values. **(C)** PBMCs from LR tuberculosis patient were incubated in presence of SAP siRNA or unspecific control (GFP) for 48h. To determine siRNA efficiency, SAP expression was determined by Western blot. One representative experiment is shown. The images show cropped lines corresponding to SAP and β-Actin. One way ANOVA-Uncorrected Fisher's LSD. ** p <0.01, *** p <0.001.

Figure S4.

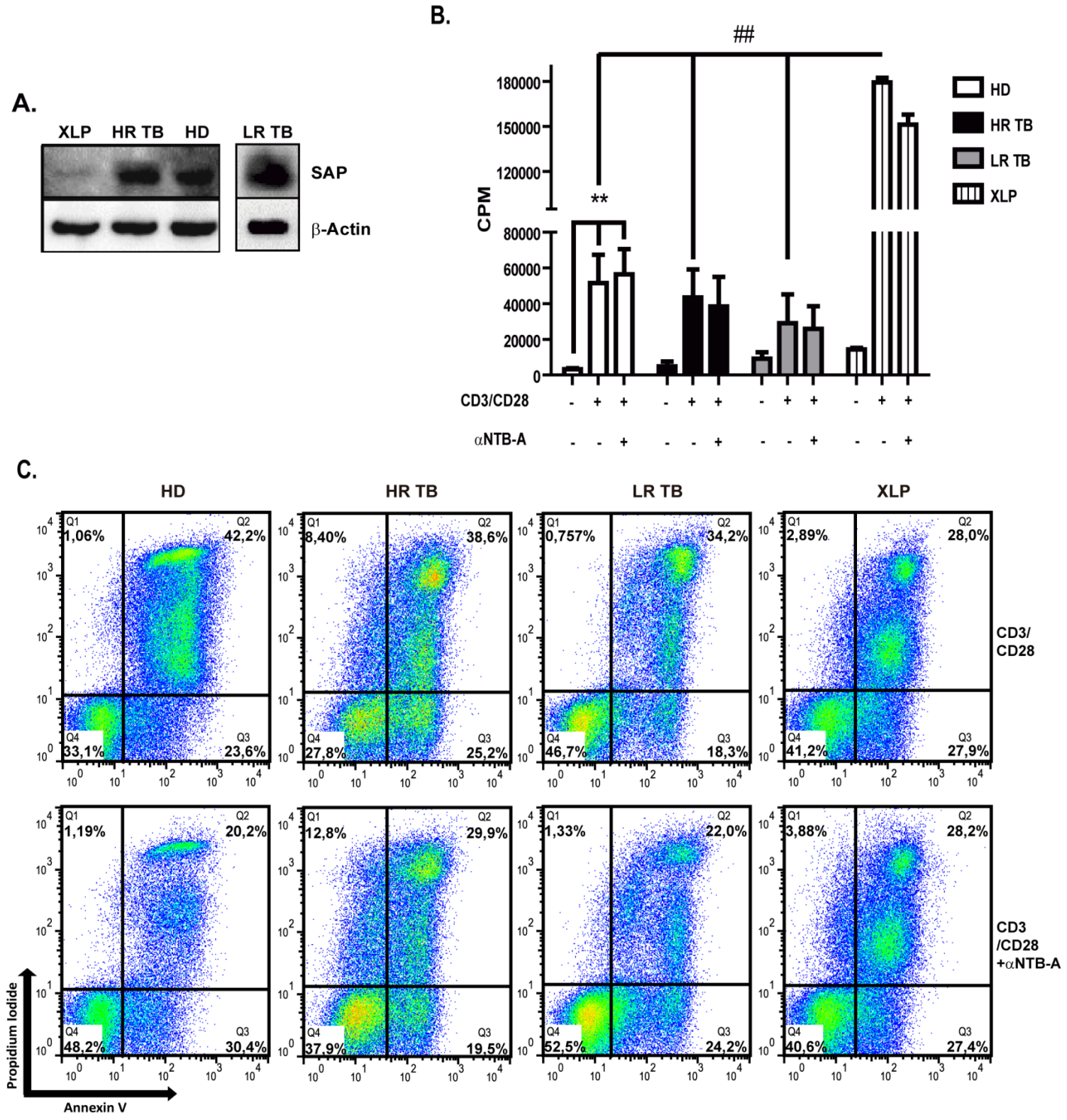
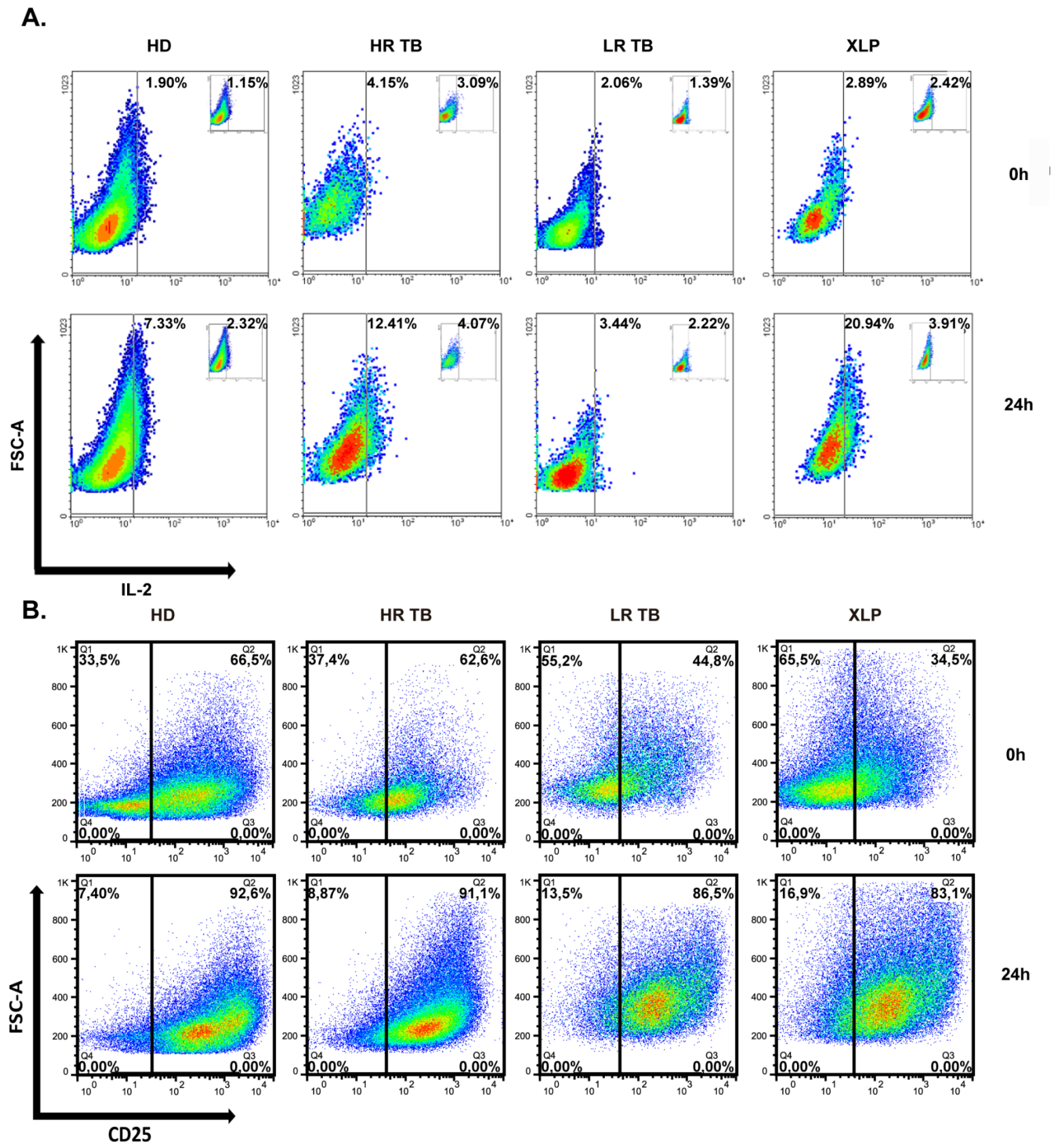

A.
XLP
HR TB
HD
LR TB
SAP
β-Actin
B.
##
**
CPM
180000
150000
120000
80000
60000
40000
20000
0
HD
HR TB
LR TB
XLP
CD3/CD28
αNTB-A
C.
HD
HR TB
LR TB
XLP
Q1 1,06%
Q2 42,2%
Q4 33,1%
Q3 23,6%
Q1 8,40%
Q2 38,6%
Q4 27,8%
Q3 25,2%
Q1 0,757%
Q2 34,2%
Q4 46,7%
Q3 18,3%
Q1 2,89%
Q2 28,0%
Q4 41,2%
Q3 27,9%
CD3/ CD28
Q1 1,19%
Q2 20,2%
Q4 48,2%
Q3 30,4%
Q1 12,8%
Q2 29,9%
Q4 37,9%
Q3 19,5%
Q1 1,33%
Q2 22,0%
Q4 52,5%
Q3 24,2%
Q1 3,88%
Q2 28,2%
Q4 40,6%
Q3 27,4%
CD3 /CD28 +αNTB-A
Propidium Iodide
Annexin V

Figure S4. NTB-A/SAP signaling contributes to mediating apoptosis after TCR restimulation. (A, B) PBMCs from Healthy Donors (HD, N=7), High Responder Tuberculosis patients (HR, N=4), Low Responder Tuberculosis patients (LR, N=4) and SAP deficient (XLP, N=4) patients were stimulated with *Mtb*-Ag for 5 days. Then, the cells were washed and cultured with RPMI plus hrIL-2. After 7 days, lymphocyte enrichment was performed by centrifugation over Ficoll-Hypaque and cells were stimulated with α-CD3 + α-CD28 for 24h. **(A)** Representative blot of SAP protein expression in HD, HR TB, LR TB and XLP patients before restimulation. The images show cropped lines corresponding to SAP and β-Actin. **(B)** Cells were pulsed with [^{3}H] TdR, harvested 16h later and [^{3}H] TdR incorporation was measured in a liquid scintillation counter. Each bar represents the mean ± SEM of the CPM. One way ANOVA-Uncorrected Fisher's LSD. $^{**,\#\#}p <$ 0.01. **(C)** Representative density plot of Annexin V$^+$ and Propidium Iodide$^+$ cells restimulated in the presence or absence of α-NTB-A blocking mAb for 24h.

Figure S5.


A.
HD
HR TB
LR TB
XLP
1.90%
1.15%
4.15%
3.09%
2.06%
1.39%
2.89%
2.42%
0h
7.33%
2.32%
12.41%
4.07%
3.44%
2.22%
20.94%
3.91%
24h
FSC-A
IL-2
B.
HD
HR TB
LR TB
XLP
Q1
Q2
Q3
Q4
33,5%
66,5%
0,00%
0,00%
37,4%
62,6%
0,00%
0,00%
55,2%
44,8%
0,00%
0,00%
65,5%
34,5%
0,00%
0,00%
0h
7,40%
92,6%
0,00%
0,00%
8,87%
91,1%
0,00%
0,00%
13,5%
86,5%
0,00%
0,00%
16,9%
83,1%
0,00%
0,00%
24h
FSC-A
CD25

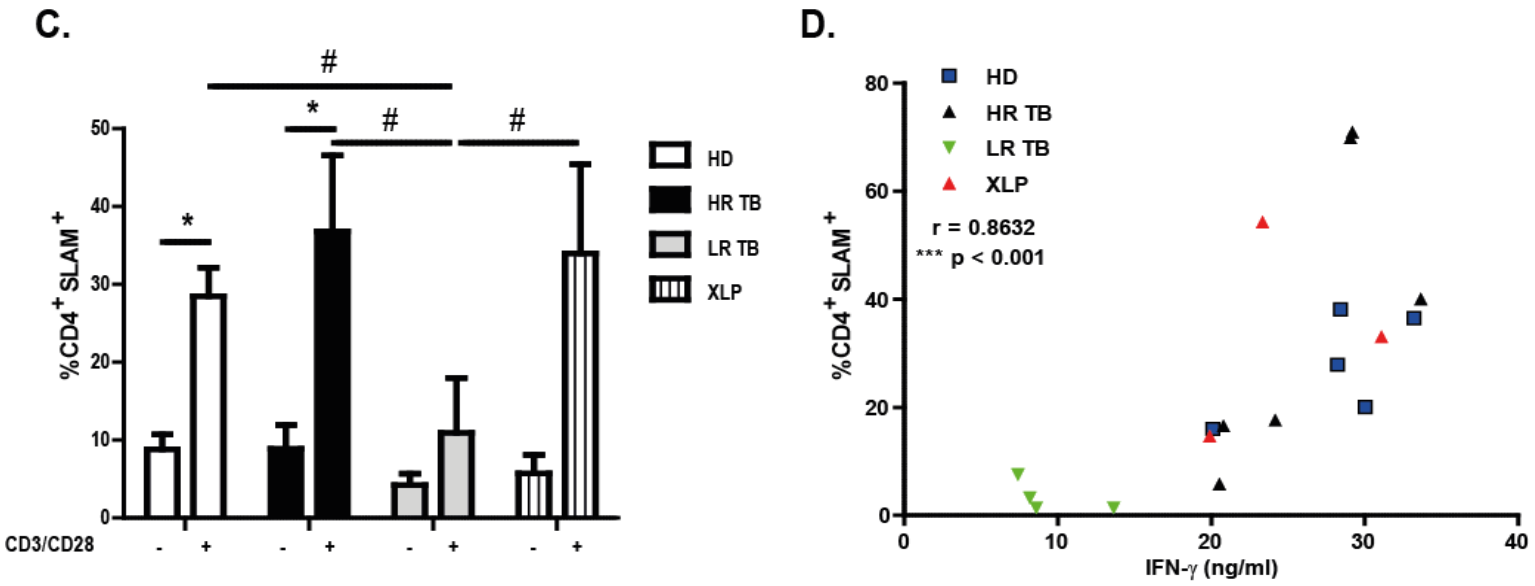


Figure S5. Association between soluble factors and SLAM receptor with RICD sensitivity. PBMCs from Healthy Donors (HD, N=5), High Responder Tuberculosis patients (HR TB, N=6), Low Responder Tuberculosis patients (LR TB, N=4) and SAP deficient (XLP, N=3) patients were stimulated with *Mtb*-Ag for 5 days. Then, the cells were washed and cultured with RPMI plus hrIL-2. After 7 days, a lymphocyte enrichment was performed by centrifugation over Ficoll-Hypaque and cells were stimulated with α-CD3 + α-CD28 for 24h. **(A)** Representative density plot of IL-2 expression on $CD4^+$ T cells. **(B)** Representative density plot of CD25 expression on $CD4^+$ T cells. **(C)** SLAM surface expression was determined by flow cytometry on $CD4^+$ T cells. Bars represent the percentage ± Standard Error of the Mean (SEM) of SLAM. **(D) Correlation between IFN-γ production and SLAM expression.** Cells from HD, HR TB, LR TB and XLP patients were restimulated for 24h. Cell-free supernatants were then collected and assayed for IFN-γ by ELISA (X axis). SLAM expression was determined on $CD4^+$ T cells by flow cytometry (Y axis). One way ANOVA-Uncorrected Fisher's LSD. $^{*,\#}p <$

0.05. Correlation factor (r) and *p* were calculated by the non-parametric Spearman correlation test.

FULL BLOTS

Figure 2E.

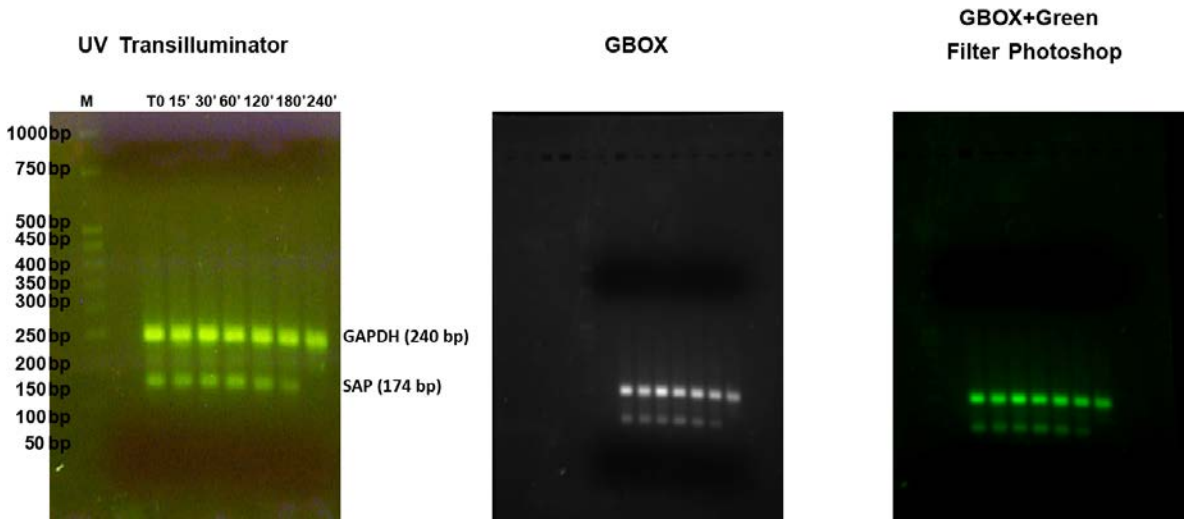
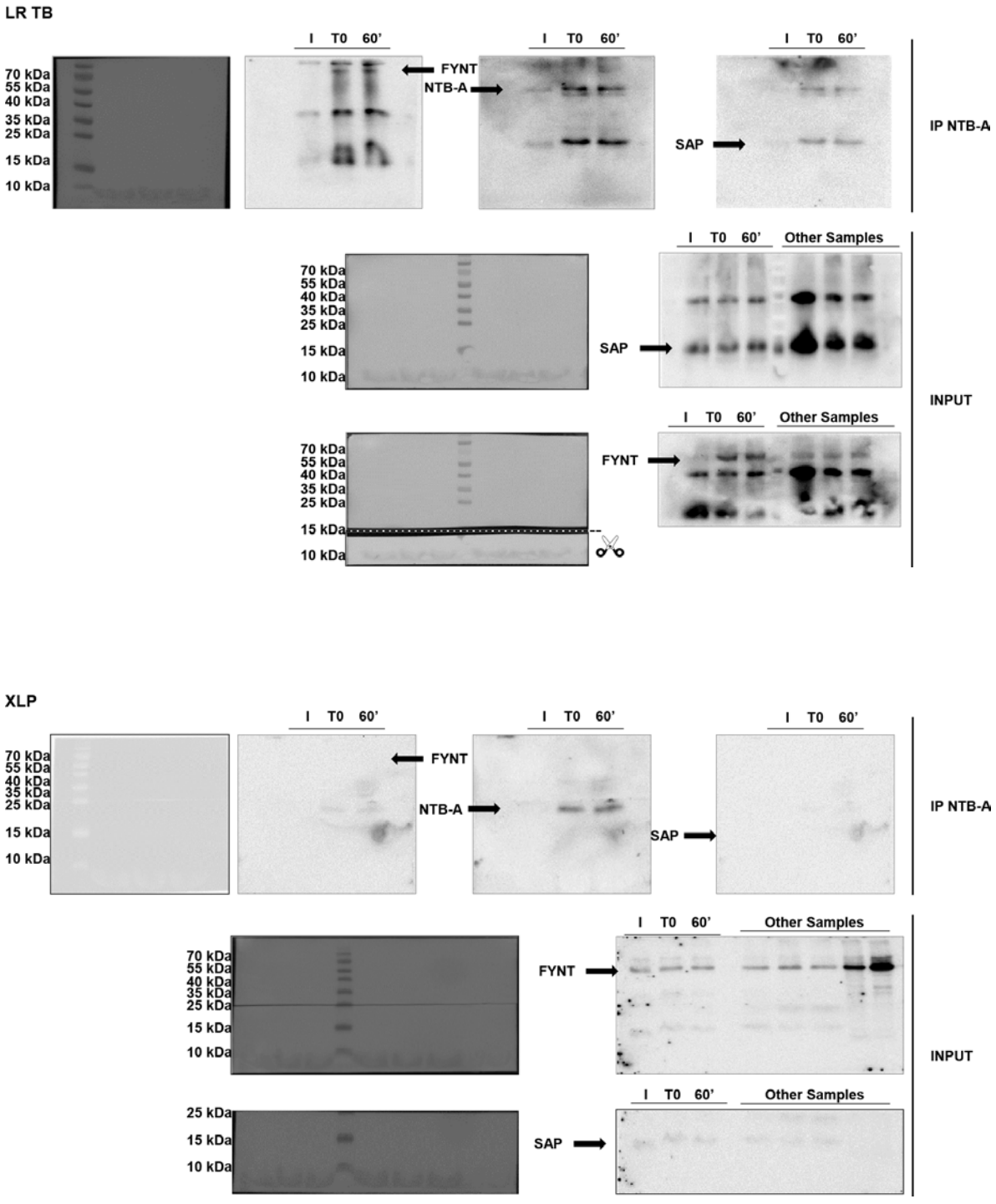


Figure 2. **SAP mRNA and protein levels are increased in patients with low IFN-γ production. (D)** PBMCs from HD (N=5), HR (N=5) and LR (N=4) tuberculosis patients were stimulated with *Mtb*-Ag for 48 hours. Actinomycin D (actD) was added and cells were collected at different time points. SAP mRNA levels were determined by Real Time PCR. Values are expressed as the mean ± SEM of the Fold increase relative to time zero. The images show full-length gels corresponding to GAPDH and SAP mRNA decay.

Figure 5A.


LR TB
I T0 60'
I T0 60'
I T0 60'
70 kDa
55 kDa
40 kDa
35 kDa
25 kDa
15 kDa
10 kDa
FYNT
NTB-A
SAP
IP NTB-A
I T0 60'
Other Samples
SAP
INPUT
I T0 60'
Other Samples
FYNT
XLP
I T0 60'
I T0 60'
I T0 60'
FYNT
NTB-A
SAP
IP NTB-A
I T0 60'
Other Samples
FYNT
INPUT
I T0 60'
Other Samples
SAP

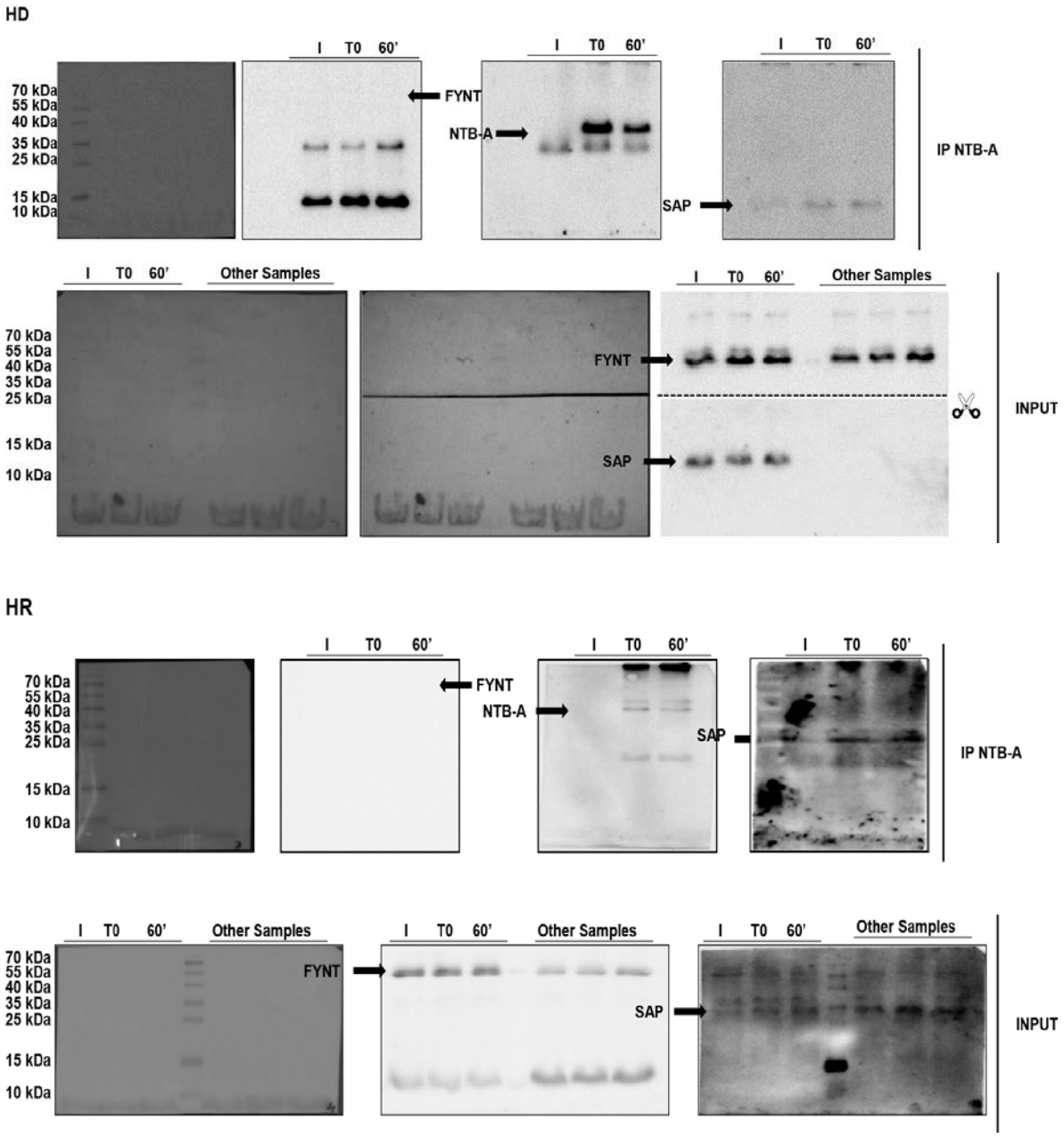


Figure 5. The NTB-A/SAP pathway impairs RICD in LR TB patients. PBMCs from Healthy Donors (HD, N=3), High Responder Tuberculosis patients (HR TB, N=3), Low Responder Tuberculosis patients (LR TB, N=3) and SAP deficient (XLP, N=1) patients were stimulated with *Mtb*-Ag for 5 days. Then, the cells were washed and cultured with RPMI plus hrIL-2. After 7 days, lymphocyte enrichment was performed by centrifugation over Ficoll-Hypaque and afterwards cells were

stimulated with α-CD3 + α-CD28 for 1h. **(A)** Immunoprecipitation of NTB-A. The immunoprecipitates were separated by SDS-PAGE and immunoblotted for the presence of SAP, NTB-A and FYNT. SAP and FYNT expression in input lysates are shown for comparison (bottom) (I = Isotype, T0 = unstimulated cells and 60' = stimulated cells with α-CD3 + α-CD28 for 1h; (--) = manually cut blots). The images show full-length blots corresponding to NTB-A, SAP and FYNT from Figure 5A.