

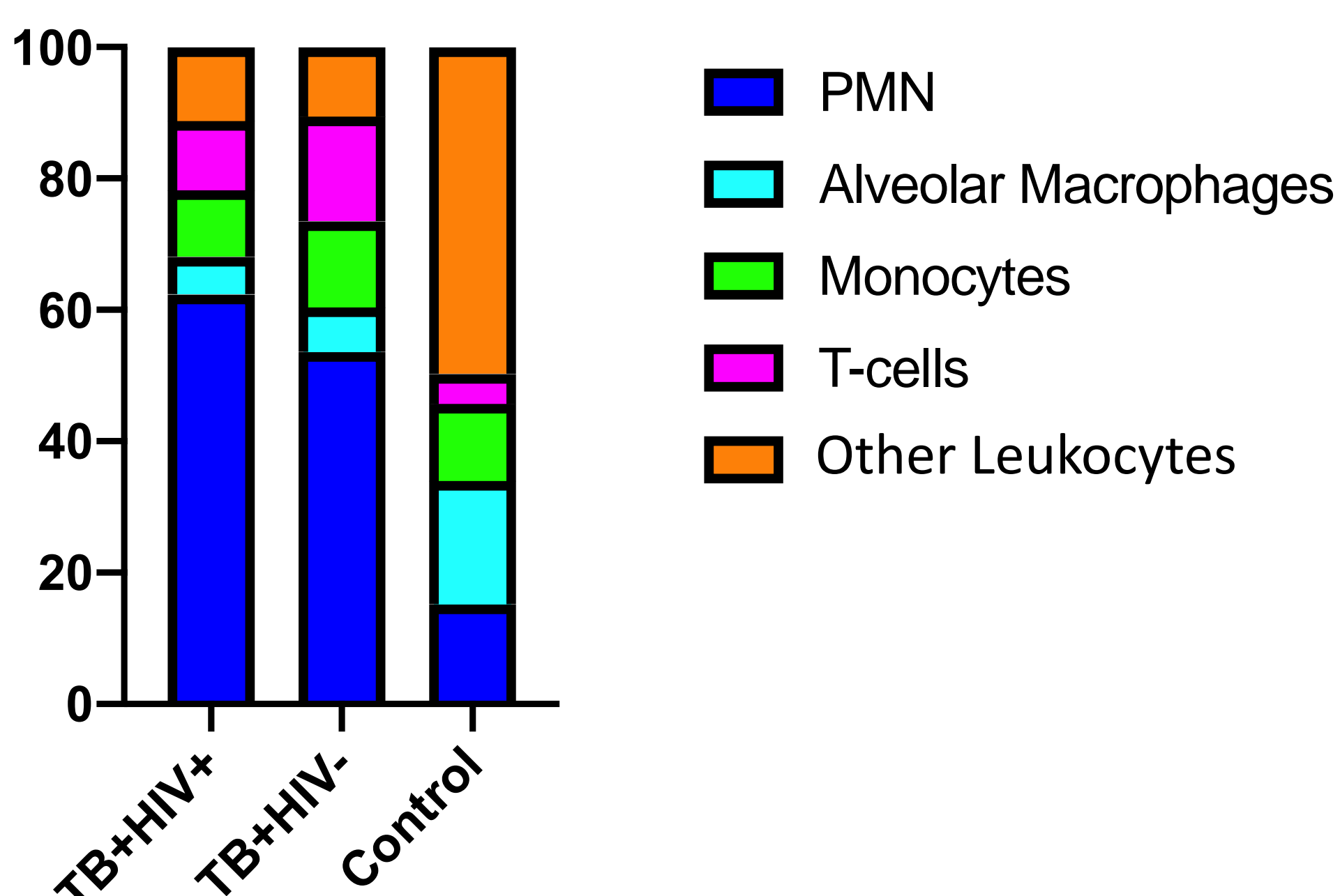
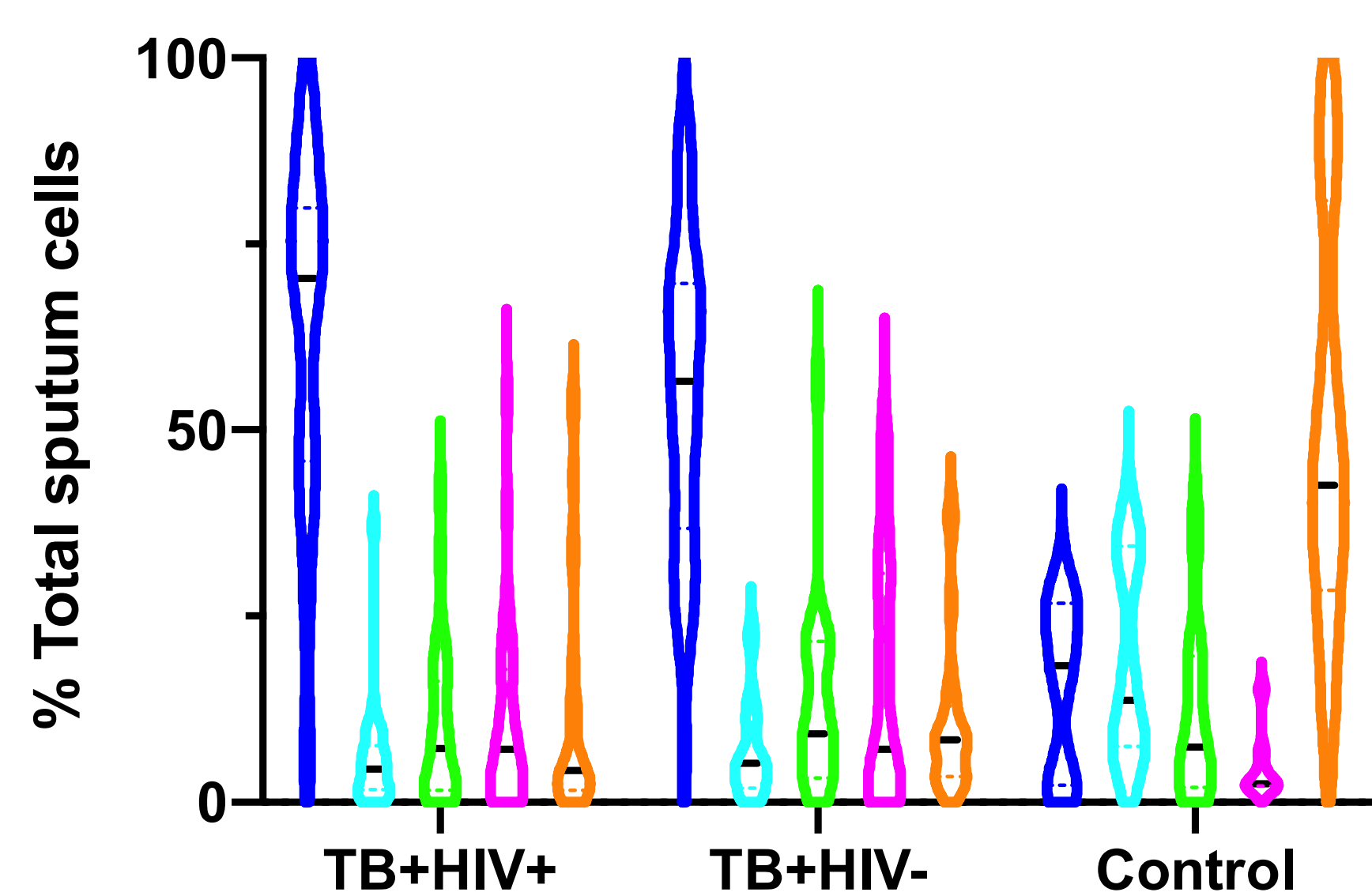
# Quantitation of neutrophils and neutrophil-associated proteins in sputum to aid diagnosis of pulmonary TB

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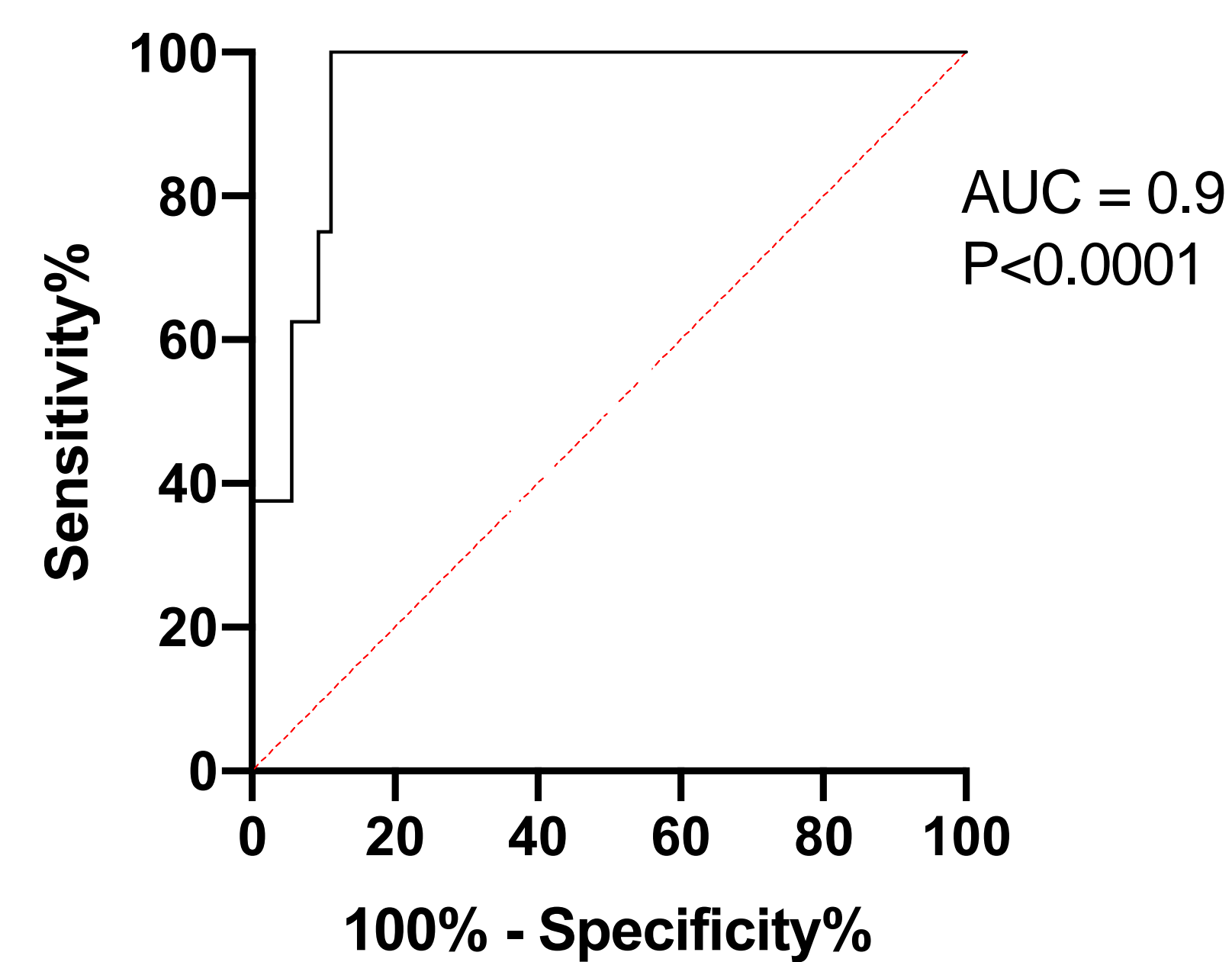
**Background:** Sputum remains the primary biological sample used to diagnose pulmonary TB. However, detecting the presence of Mtb by culture or nucleic acid in this sample can be challenging, especially in certain groups, such as people living with HIV (PLWH). Host immune biomarkers are attractive as they may provide a more reliable signal. We, therefore, examined the potential of using immune features of sputum from individuals with pulmonary TB to aid diagnosis.

**Methods:** Cells were purified from fresh sputum samples collected from RePORT South Africa participants at enrolment with active TB infection (prior to treatment) and respiratory symptomatic non-TB controls. Immune cell populations were extracted, quantified, and phenotyped by flow cytometry. In additional sputum samples were diluted with N-acetyl cysteine to reduce viscosity and neutrophil-specific proteins were quantified by ELISA using commercial kits.

## 1. Neutrophils are the dominant immune cell type in TB+ sputum irrespective of HIV infection and can distinguish pulmonary TB from symptomatic non-TB controls

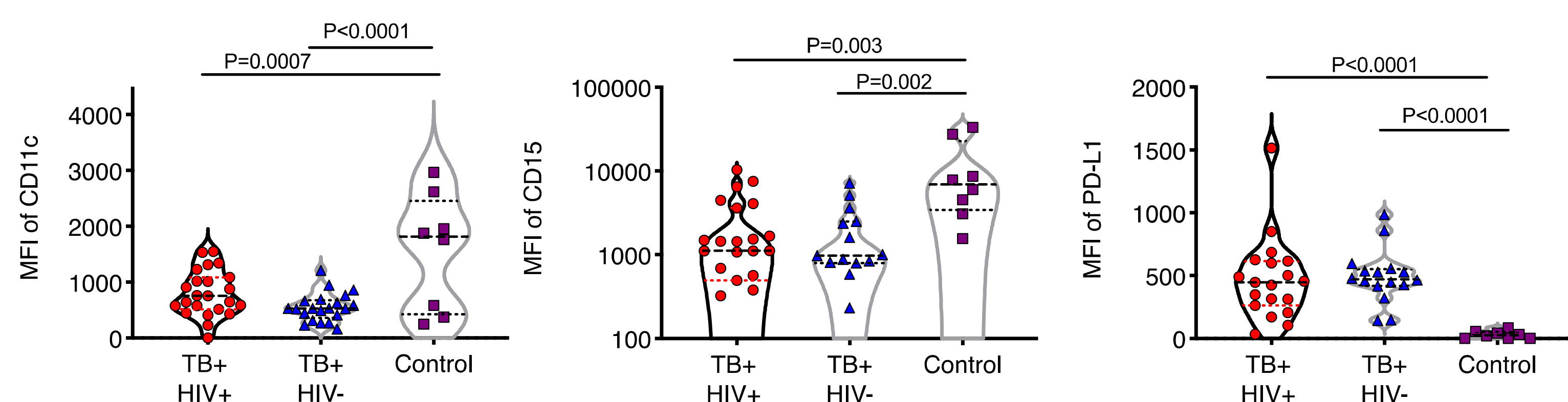


ROC curve: Sputum PMN %



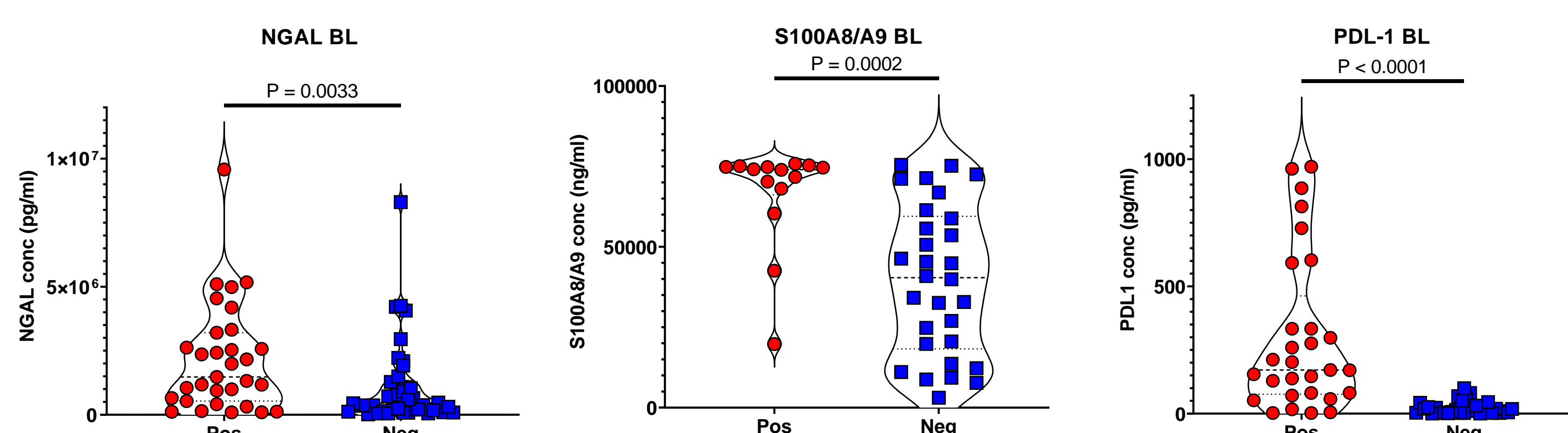
- Neutrophils are the most abundant cell type in sputum of individuals with microbiologically confirmed TB
- There is no significant difference between PLWH and those without HIV infection
- In this cohort, neutrophil count successfully distinguished pulmonary TB from respiratory symptomatic non-TB controls

## 2. Sputum neutrophils are highly activated



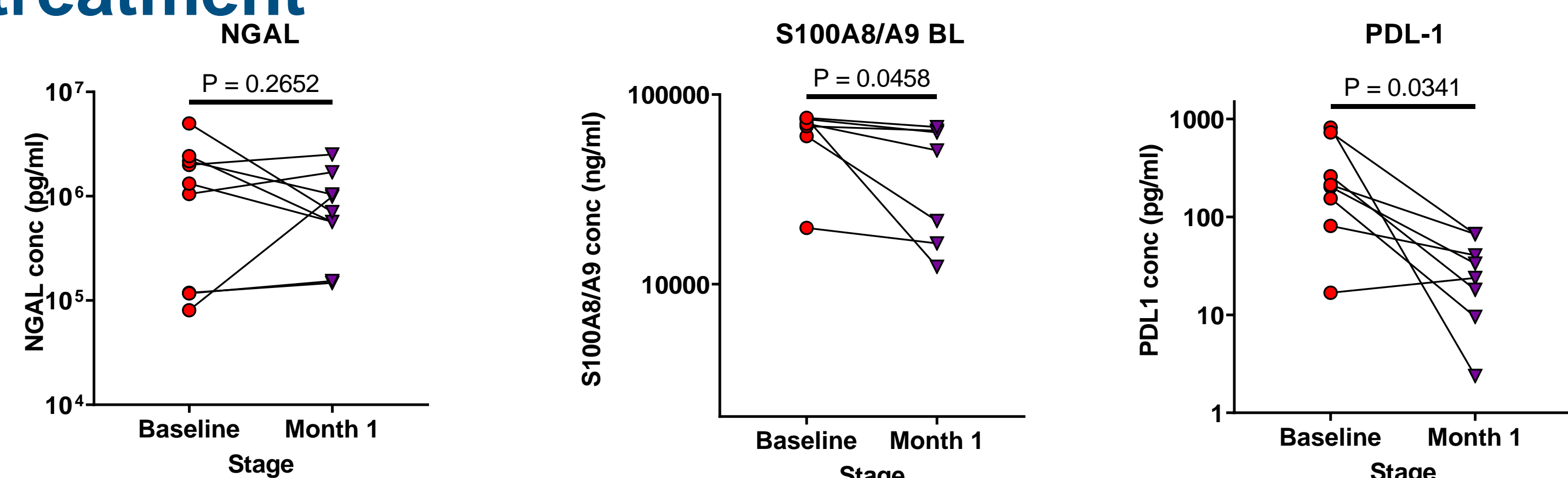
- Sputum neutrophils from TB-infected subjects are highly activated as shown by the down-regulation of CD11c and CD15 and upregulation of PDL-1
- Unaffected by HIV Co-infection

## 3. Upregulation of neutrophil proteins



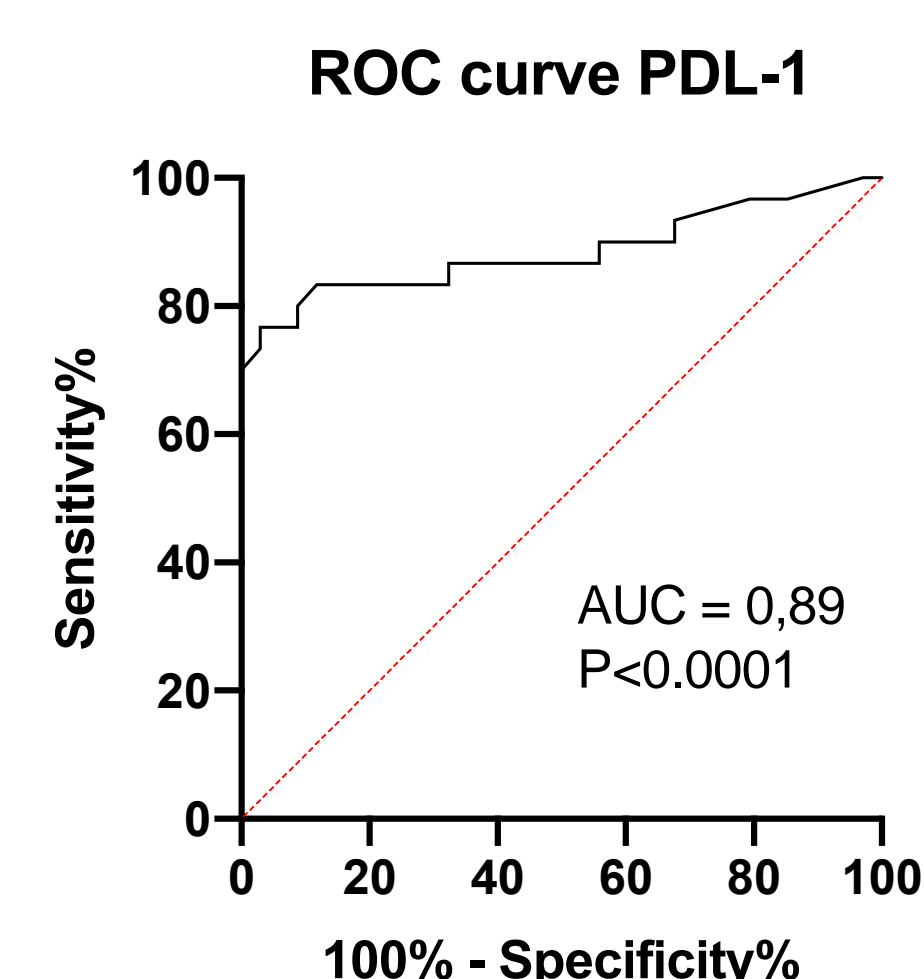
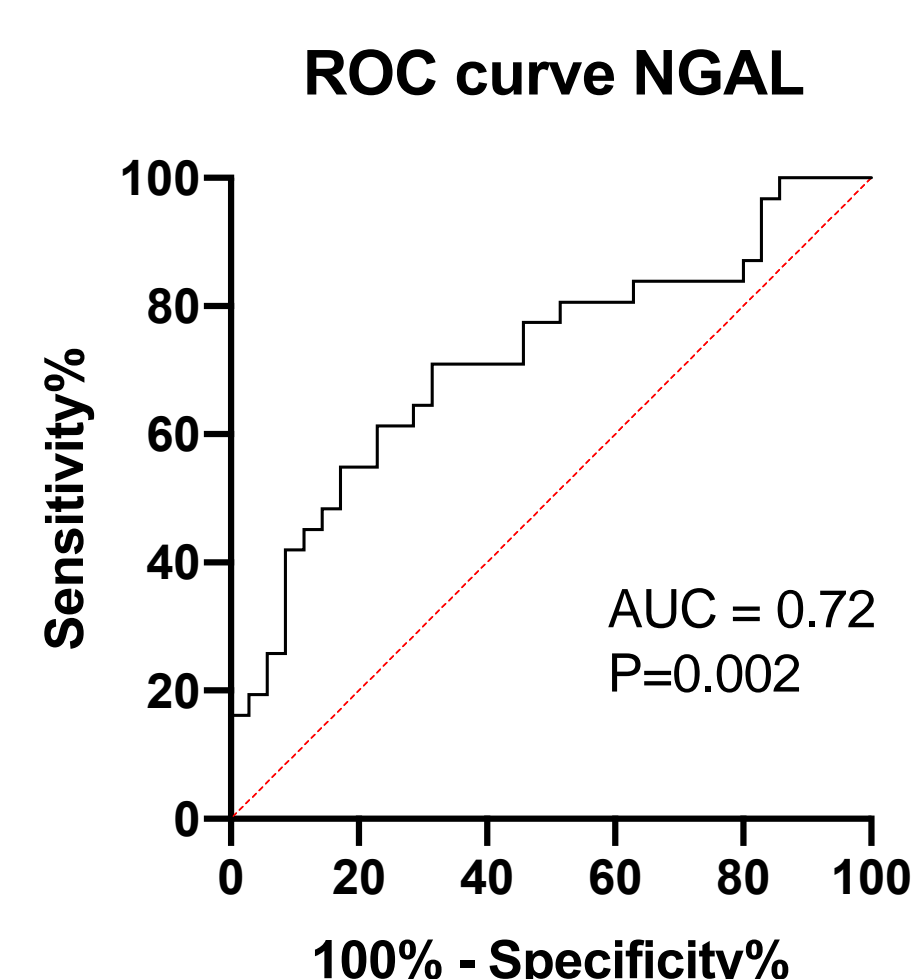
- Immunomodulatory molecule PDL-1 is highly upregulated
- Sputum-specific neutrophil proteins can be readily measured by ELISA with minimal processing.
- Constitutively expressed neutrophil proteins NGAL and S100A8/9 are also upregulated

## 5. Elevated sputum PDL-1 resolves rapidly with TB treatment



- Serial sampling of individuals with Drug sensitive TB showed a significant reduction in sputum PDL-1 and S100A8/9 levels after 1 month of treatment
- NGAL levels did not significantly reduce in the same time frame

## 4. High sputum PDL-1 and S100A8/9 identifies pulmonary TB

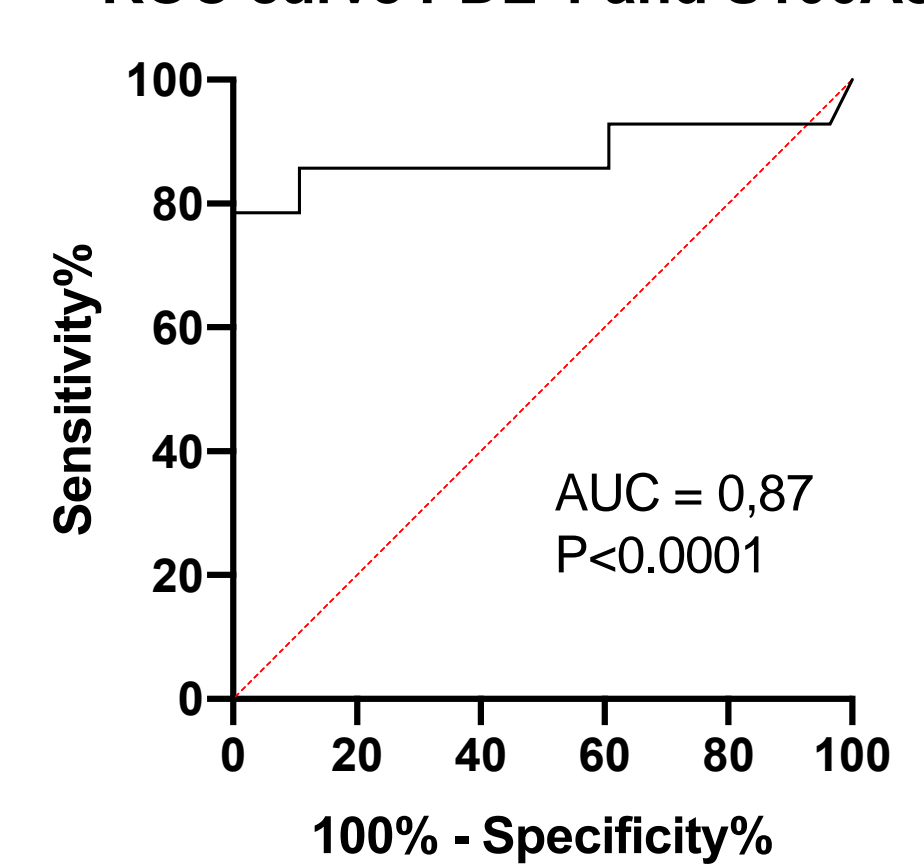
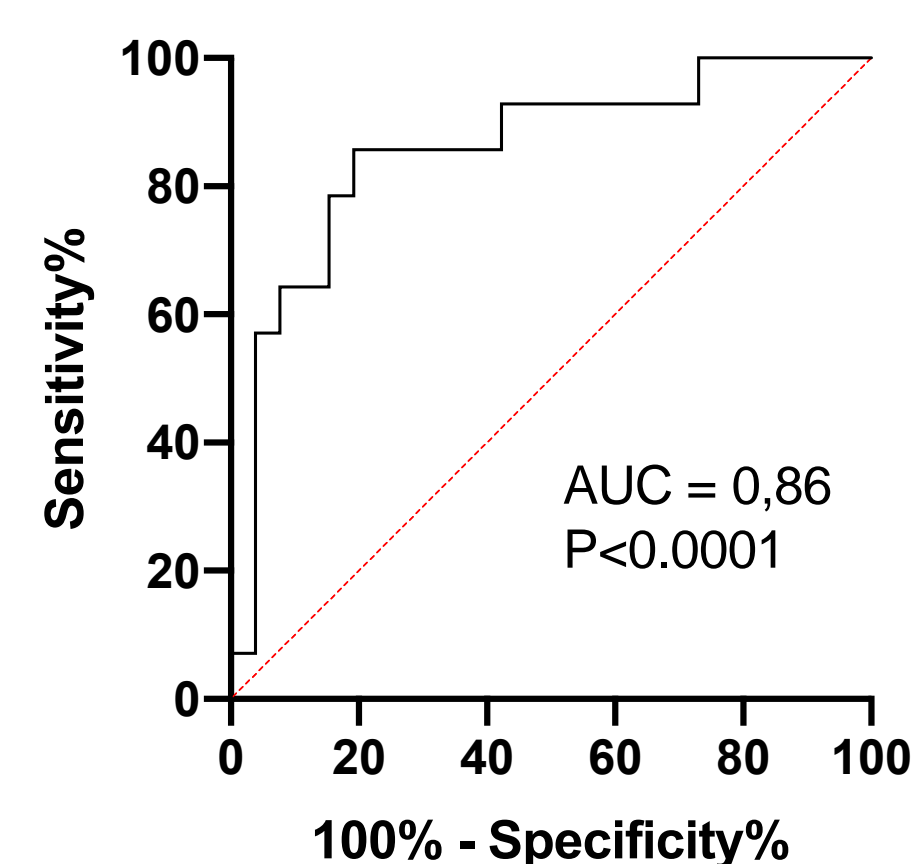


Sensitivity 64%, Specificity 71%

Sensitivity 83%, Specificity 88%

ROC curve S100A8/9

ROC curve PDL-1 and S100A8/9



Sensitivity 86%, Specificity 81%

Sensitivity 86%, Specificity 89%

## Summary

- Measuring sputum neutrophils or neutrophil related proteins PDL-1 and/or S100A8/9 is able to distinguish active pulmonary TB from symptomatic TB uninfected controls
- PDL-1 and S100A8/9 can be easily measured in sputum with minimal processing
- Sputum PDL-1 levels rapidly reduce after initiation of drug therapy
- Sensitivity and specificity for these proteins approach the WHO target product profile for a TB triage test
- Testing of this approach to identify subclinical TB is underway