

# Validation of Novel Biomarker Candidates for Systemic Sclerosis

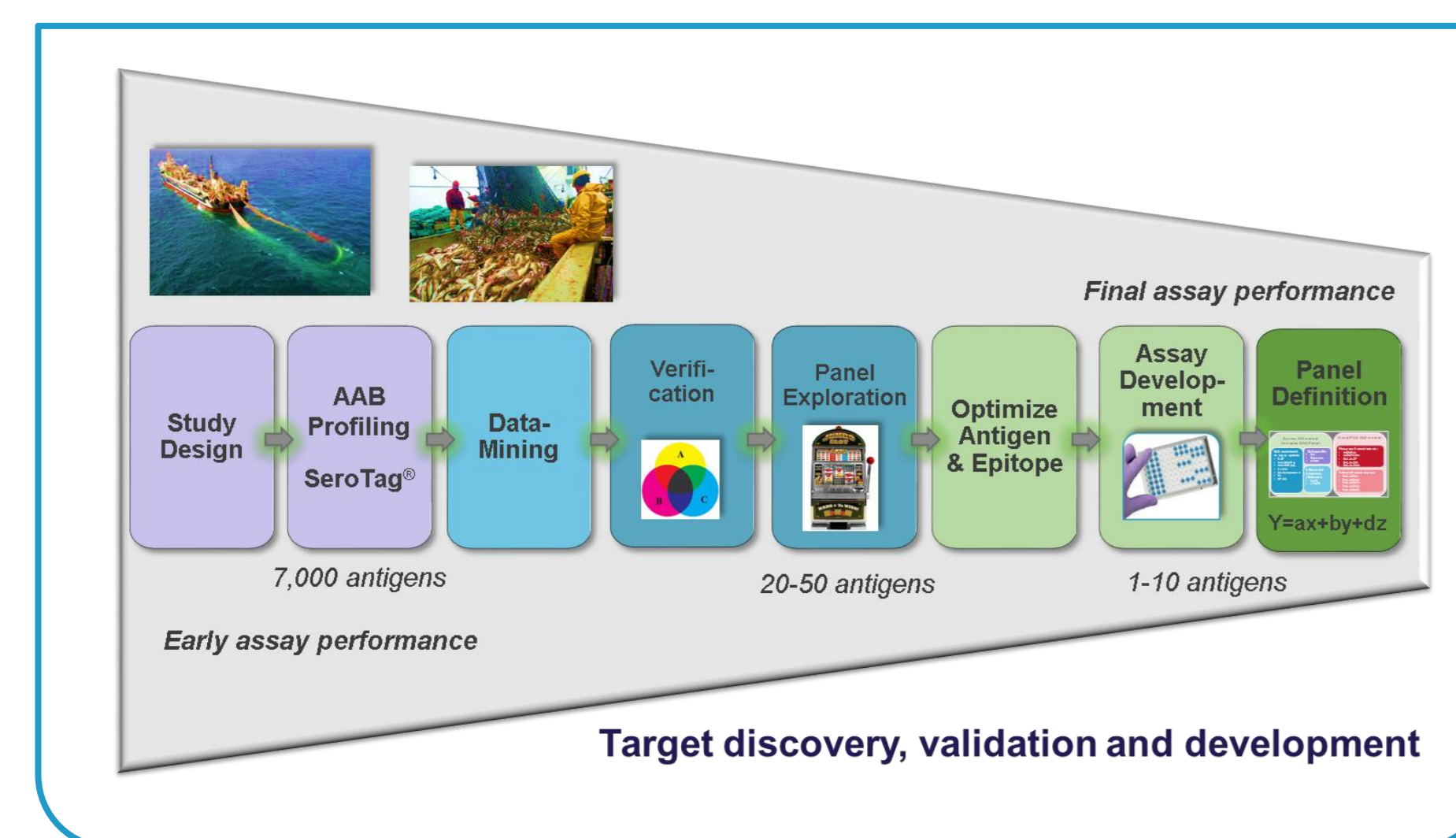
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## Introduction

Systemic sclerosis (SSc) is a systemic autoimmune disease that manifests as progressive fibrosis of the skin and internal organs. SSc is associated with the presence of several autoantibodies (aab) to intracellular targets, with the three most important SSc-specific being anti-centromere antibodies, anti-Scl70 antibodies and anti-RNA polymerase III antibodies, which occur in over 50% of SSc patients. Autoantibody specificities are strongly associated with pattern of organ involvement and disease outcome, making autoantibodies an essential tool in the clinical management of SSc. This highlights the need for additional specific and sensitive diagnostic and prognostic biomarkers in SSc. We have recently conducted high-content autoantibody profiling studies of SSc, systemic autoimmune diseases (AID), and healthy controls and found novel SSc-associated autoantibodies. These novel SSc-associated autoantibody biomarker candidates and their diagnostic value were evaluated by testing samples derived from 2 different SSc cohorts.



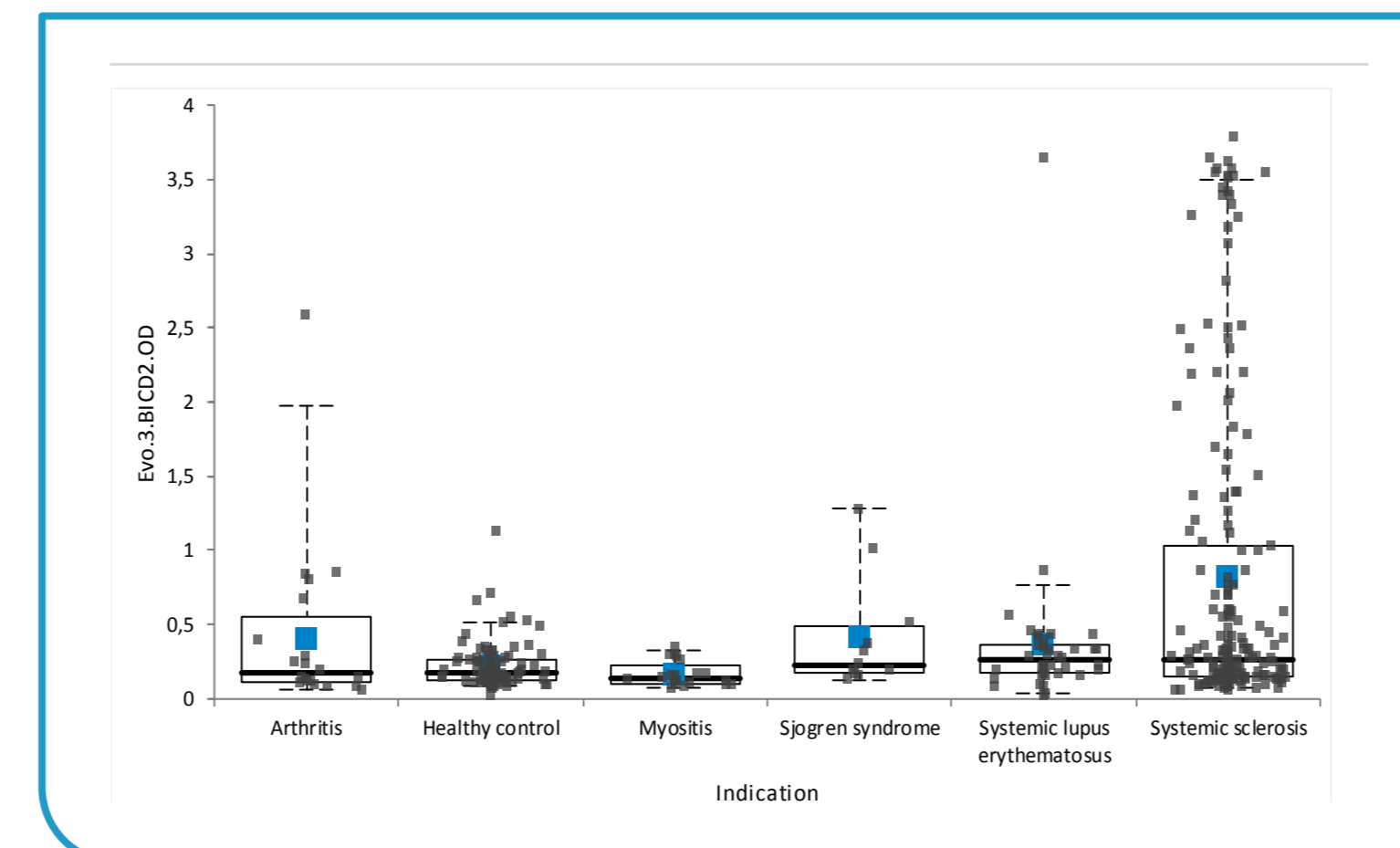
The Protagen Biomarker Discovery workflow for the development of novel biomarker targets. SeroTag<sup>®</sup> autoantibody discovery process is based on a bead-based array screening of recombinant human autoantigens. Assay development is based on ELISA technology

## Methods

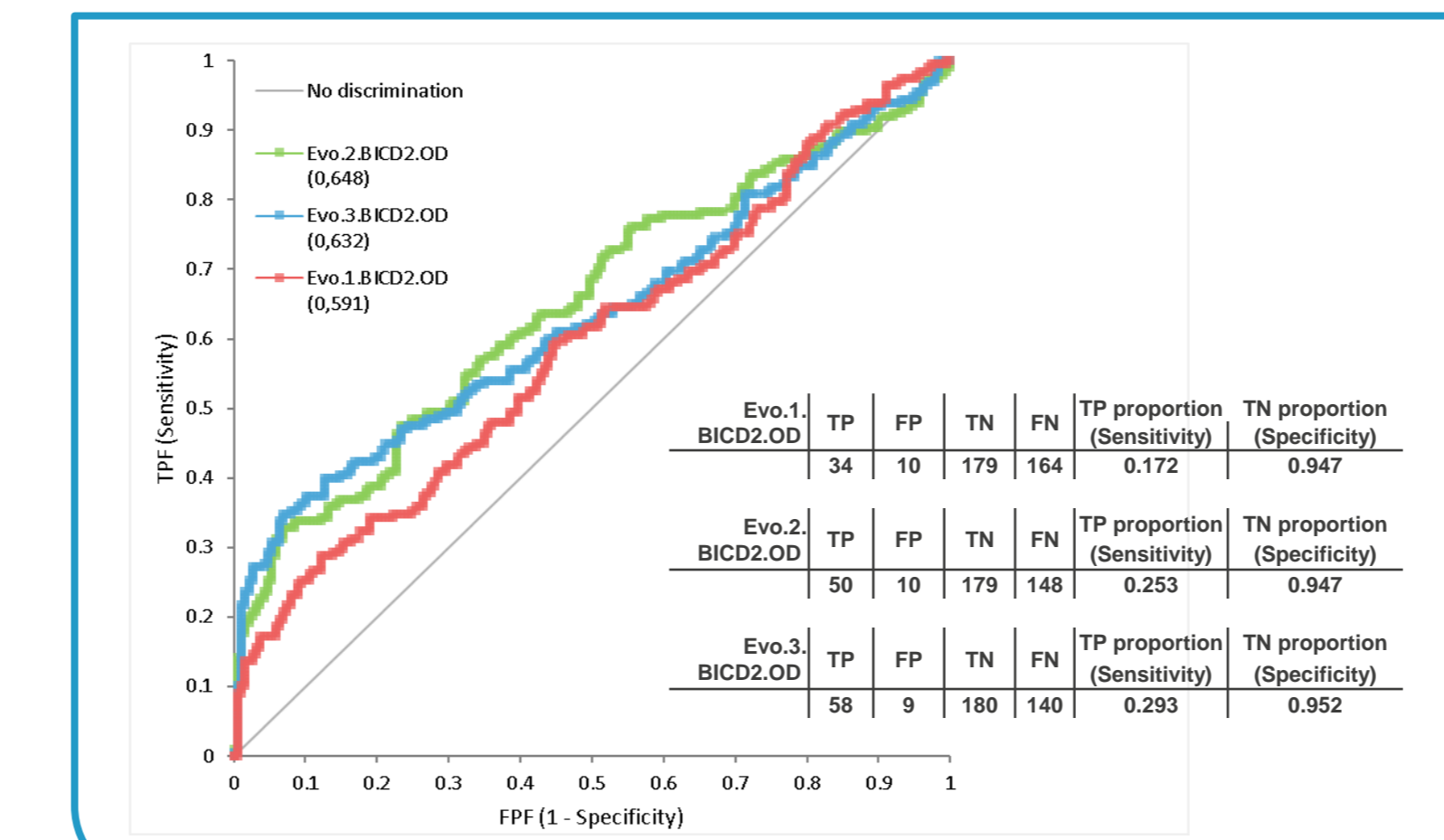
Three novel identified biomarker candidates, namely Lysine (K)-specific demethylase 6B (*KDM6B*), Protein Phosphatase Inhibitor 2 (PPP1R2) and Bicaudal Drosophila Homolog 2 (BICD2) were developed into research ELISA Kits using highly purified recombinant antigen and evaluated. Autoantibody specificities were analyzed using an independent and well characterized cohort consisting with sera of patients suffering from SSc (n=198), myositis (n=20), RA (n=20), SLE (n=40) SjS (n=10) and healthy blood donors (n=100). Assay threshold levels were calculated using receiver operation characteristic analyses. Assay specificities were set to 95% for PPP1R2 and BICD2, respectively.

## Results

Using the respective cut-off value for the ELISA tests we found autoantibody reactivity against BICD2 in 58 of 198 (29.2 %) of SSc patients and 9 of 189 (4.8%) of control samples, against PPP1R2 in 21 of 198 (10.6%) SSc and 9 of 189 controls (4.8%) respectively. Anti-BICD2 and anti-PPP1R2 aab positivity coincides with anti-Centromere aab, but was also found in anti-Centromere and anti-Scl 70 negative samples. Anti-KDM6B reactivity showed high prevalence in ISSc samples (Luminex data). The ELISA is currently being developed.

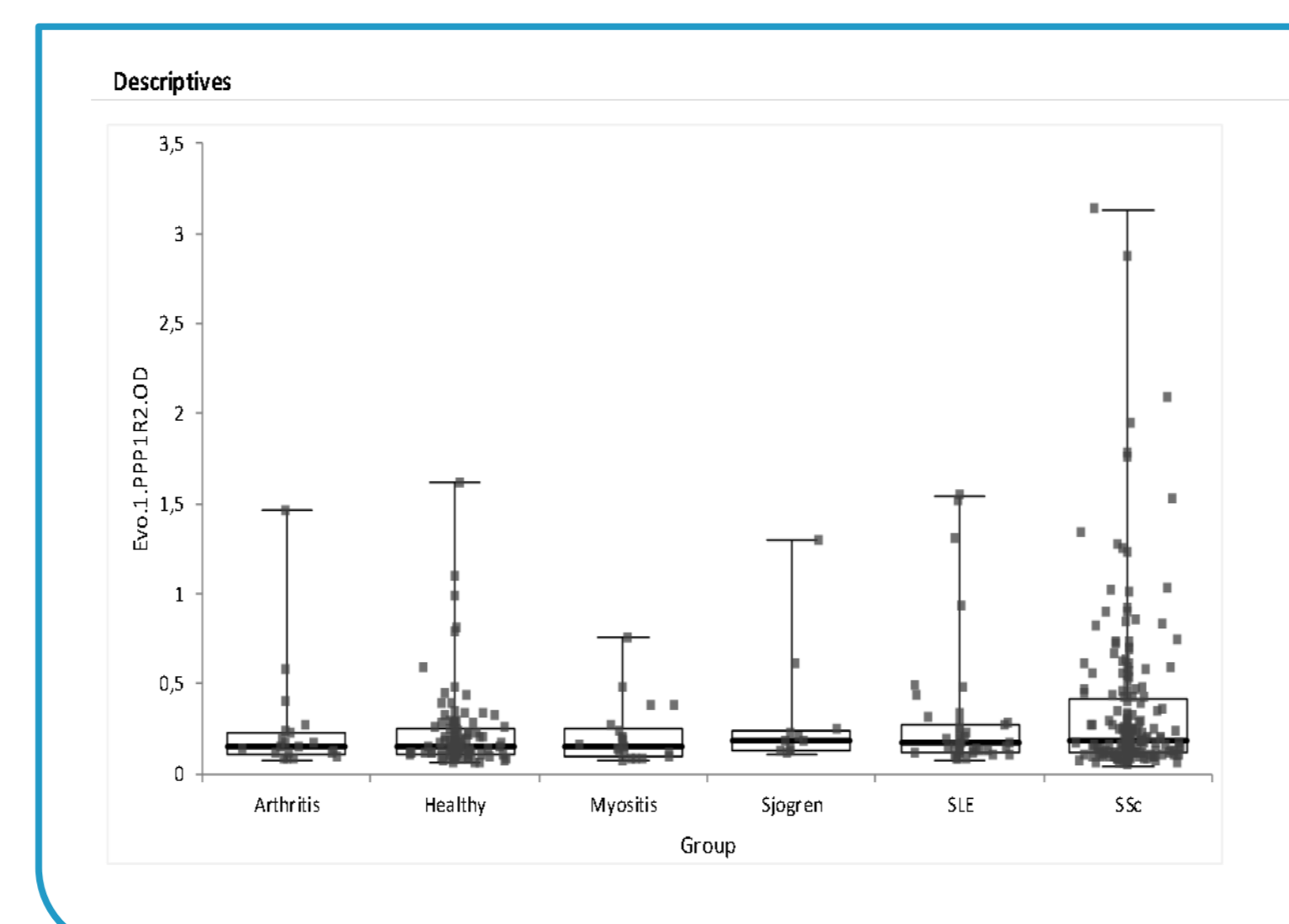


Box-and-whisker plot of anti-BICD2 autoantibody reactivity in the SSc, AID, and healthy control serum samples. Elevated reactivity against BICD2 was observed in the group of the SSc patients.

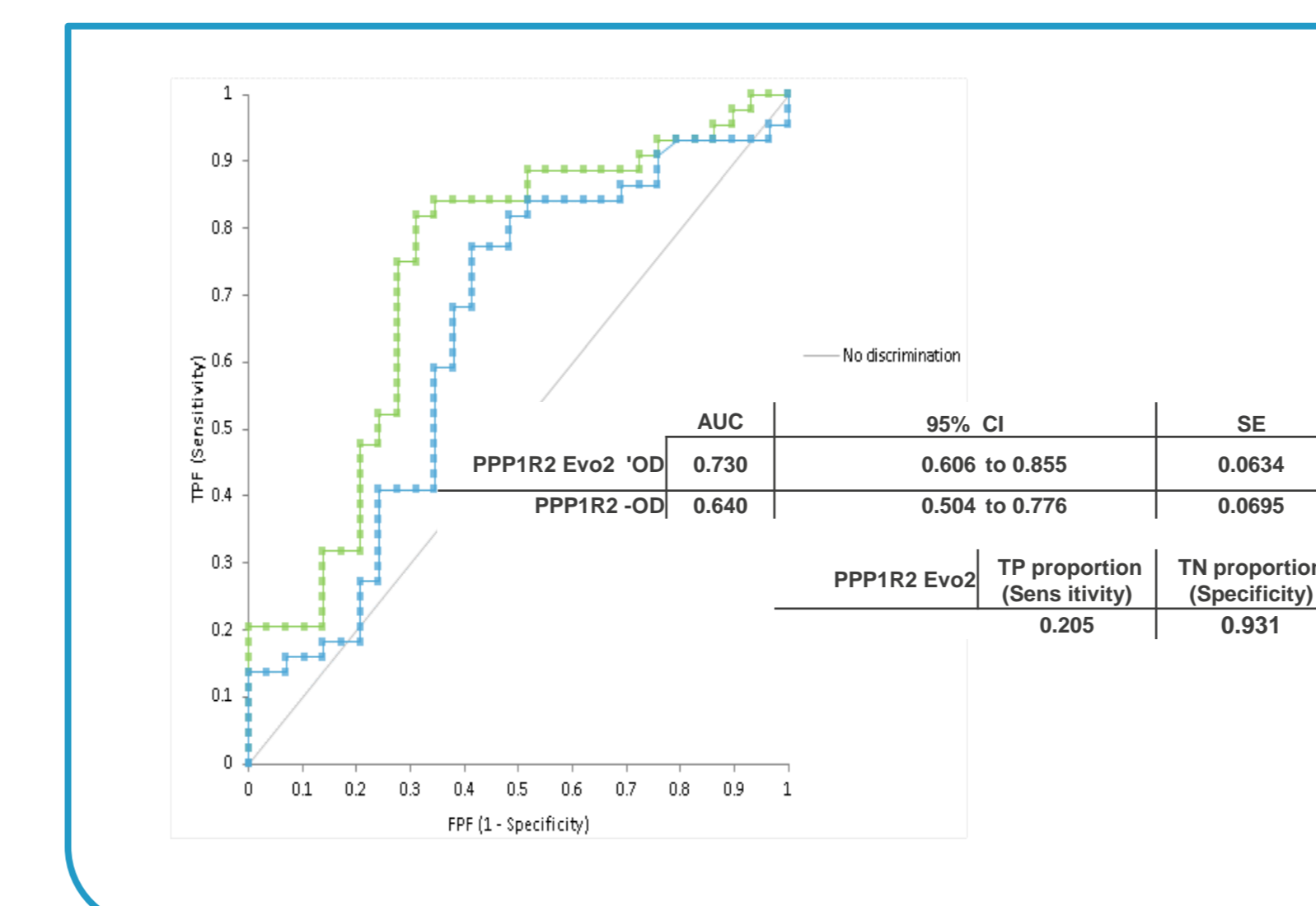


ROC analyses of different evolution steps of the Multilisa BICD2 tested in the same cohort. Interestingly, sensitivity of the Multilisa BICD2 increased from 17.2% to nearly 30% while maintaining the same specificity

Optimizing the solid and liquid phase of the Multilisa PPP1R2 led to a significantly increased sensitivity at maintained high specificity. In a reduced study cohort the second evolution design reached a sensitivity and specificity of 20.6% and 93.1% respectively.



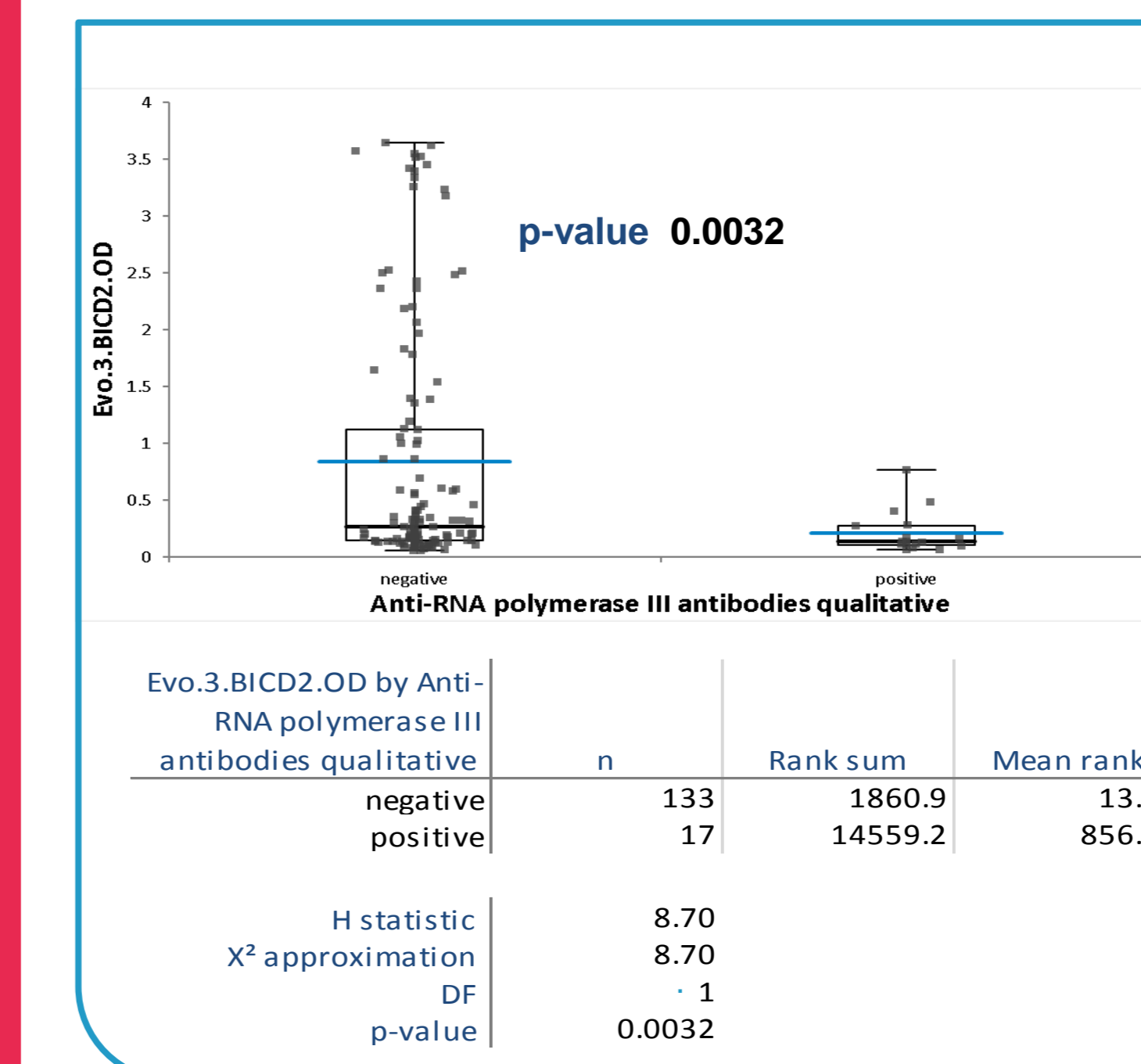
Scatter plot of anti-PPP1R2 autoantibody reactivity in the context of different Systemic autoimmune diseases and healthy control serum samples. High reactivity against PPP1R2 was observed in the group of the SSc patients



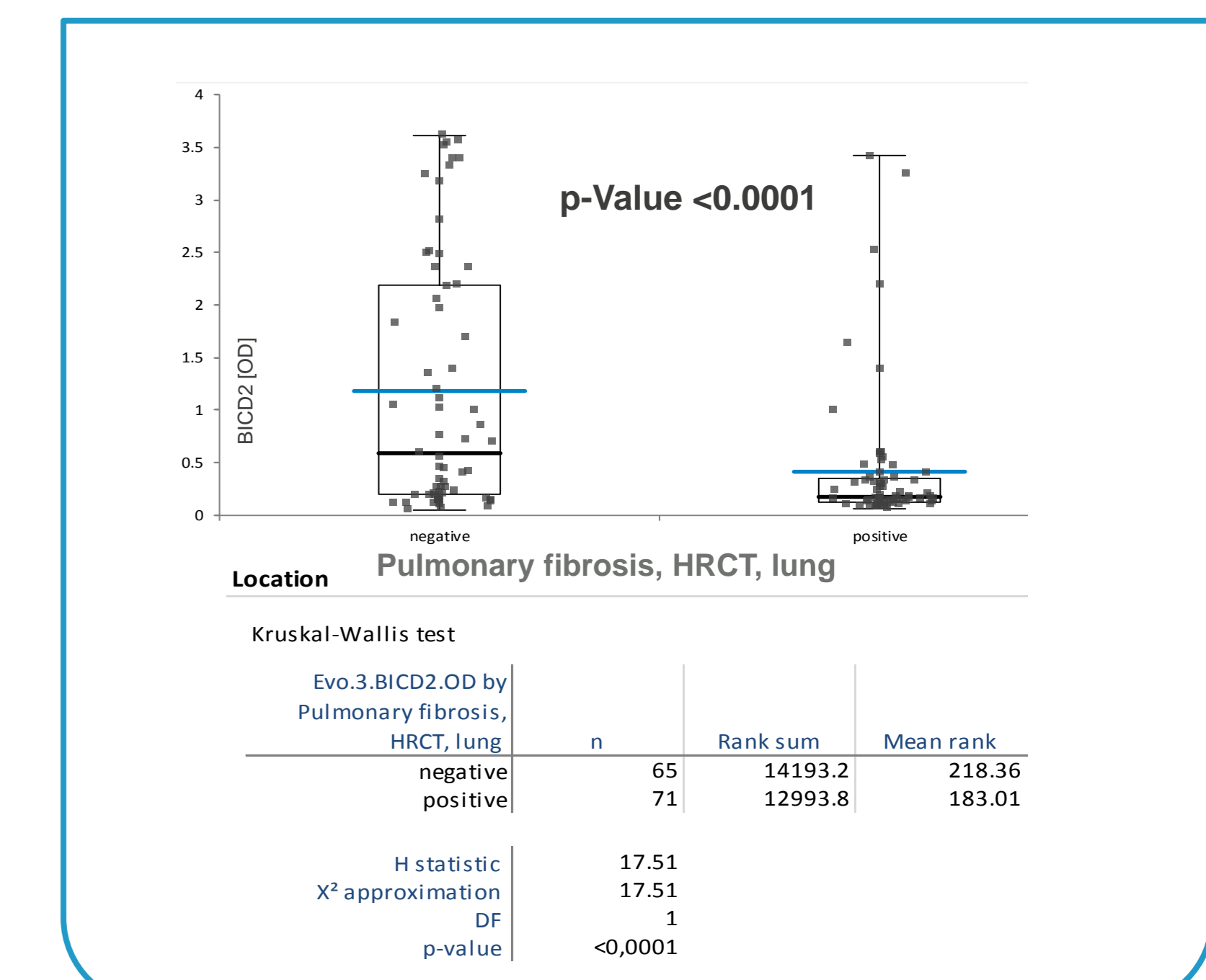
PPP1R2 prototype assay yields 20% sensitivity at >95% specificity for SSc patients vs. HV & SARD controls. Assay optimization allowed for significant increase in sensitivity at maintained specificity of the assay

Interestingly, both anti-BICD2 and anti-PPP1R2 aab were found in SSc samples tested negative for anti-RNA Polymerase III auto-reactivity.

Anti-BICD2 aab showed significant inverse correlation with pulmonary fibrosis (high resolution CT) scan. Thus, anti-BICD2 aab may be associated with a decreased incidence of severe lung disease.

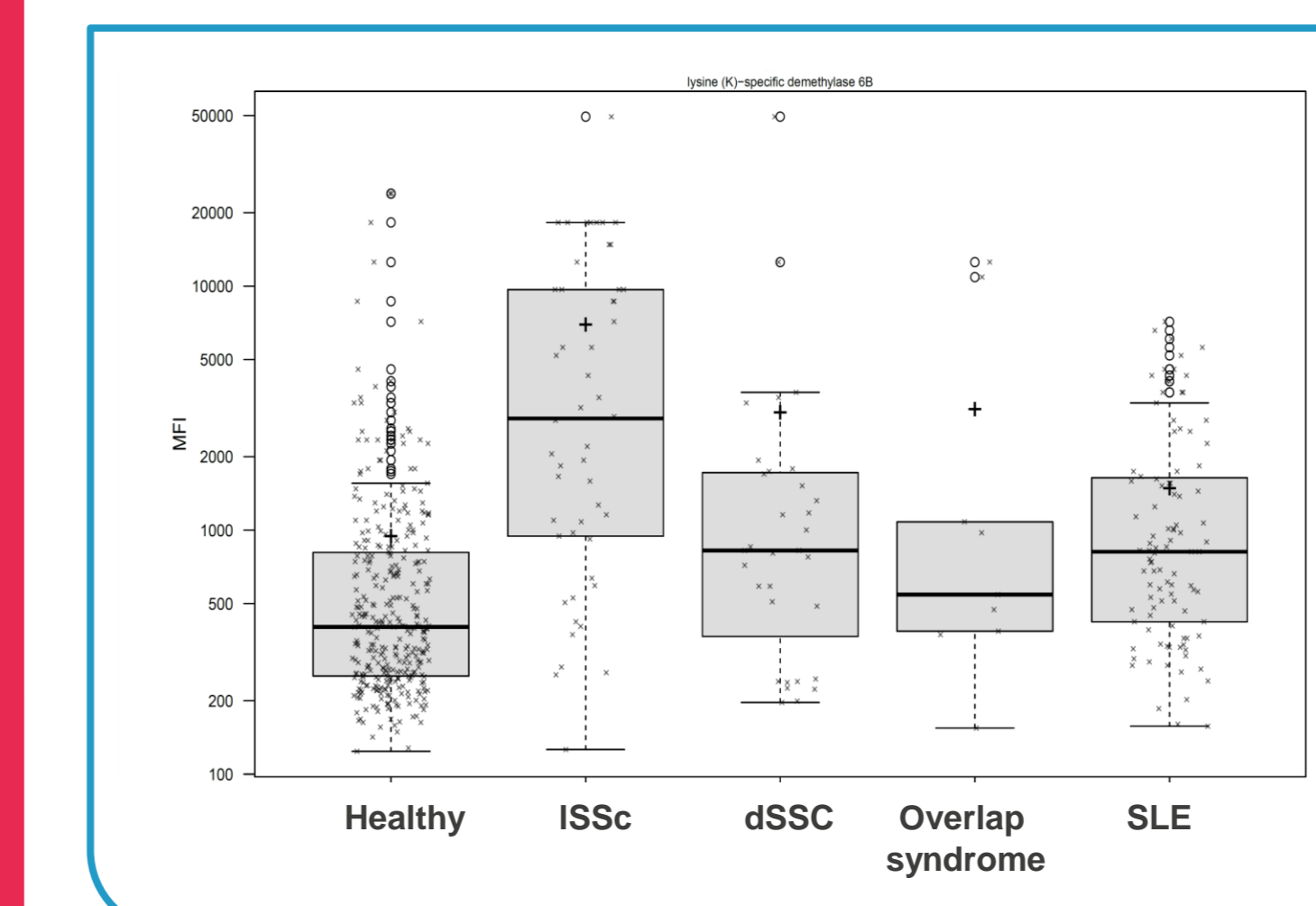


Scatter plot of reactivity of the novel antigen BICD2 compared to anti-RNA-Polymerase III antibody levels in SSc patients. Autoantibodies against BICD2 are elevated in anti-RNA Pol III negative SSc patients

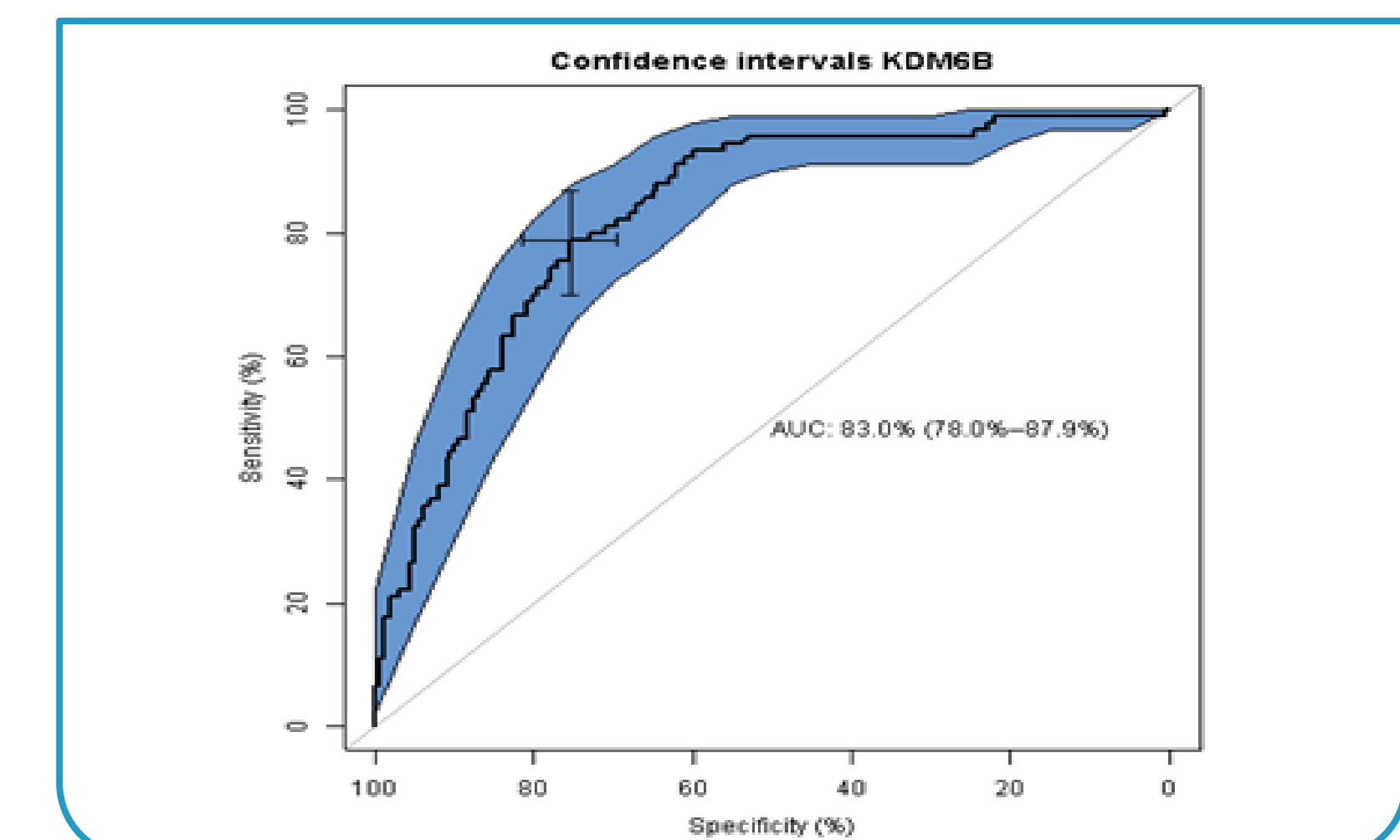


Scatter plot of reactivity of the novel antigen BICD2 compared to High resolution CT results for lung fibrosis in SSc patients. Anti-BICD2 autoantibodies significantly correlate with lowered risk of pulmonary fibrosis

Epitope mapping of KDM6B revealed reactivity against multiple epitopes. The amino acid sequence of KMD6B also includes an EBNA-2 like polyproline-stretch. Aab reactivity to KDM6B could in part be suppressed by polyproline in SSc and AID samples (data not shown).



Anti-KDM6B reactivity is elevated in ISSc patient samples (Luminex data)



ROC Analysis of Luminex data for 100 patients versus 100 healthy controls revealed an AUC value of 0.83.

## Conclusion

In this study we confirmed the diagnostic value and high specificity of the newly discovered autoantigens using ELISA. While anti-PPP1R2 and anti-BICD2 aab were found to be elevated in patients suffering from SSc, we found that autoantibodies against KDM6B are directed against multiple epitopes, which may include an EBNA-2 like polyproline region. These findings suggest a possible link between EBNA infection and autoimmune connective tissue diseases.



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