



Affinity Proteomic Plasma Analysis of Small Intestinal Neuroendocrine Carcinomas and Inflammatory Bowel Diseases

Conclusions

Four novel targets, insulin-like growth factor 1 (IGF1), interleukin-1 alpha (IL1a), mastermind-like protein 3 (MAML3) and SH3KBP1-binding protein 1 (SHKBP1) distinguish healthy controls (HC) and small intestine neuroendocrine carcinoma (SI-NEC) patients at different stage of disease. Moreover, these markers may have a similar role in the inflammatory bowel disease, mainly in the patients with Crohn's disease.

Background

SI-NEC patients receive diagnosis at the metastatic stage, which lacks of curative treatment. Indeed, a relevant lack of proper clinical markers it is well known. This major problem is shared by inflammatory disease (IBD) patients. Although, the pathologies are very different they have in common the pivotal roles of enterochromaffin cells and serotonin in their physiology.

Aims

We aimed at using novel proteomics by Dr Schwenk J. in 2011 to profile antigen signatures in a very limited amount of patients' blood. Our final goal was to utilize the findings as diagnostics and prognostics signatures for SI-NECs and IBDs.

1. We first profiled SI-NEC patients and healthy individuals by serum proteomics
2. We then applied the same method on healthy individuals and IBD patients

Material & Methods

The antibody suspension bead array (Schwenk JM, Nilsson P., Methods Mol Biol. 2011;723:29-36) relies on the antibodies developed by the Human Protein Atlas (HPA) was used to patients blood from SI-NEC and IBD patients to identify proteomic signatures by advanced bioinformatics analyses. We screened 184 different HPA antibodies for potential biomarkers recognition in an initial patient cohort. We restricted to 20 antigens of interest for further validation in a second independent cohort. Selection was based on statistical significance and consistency between experiments. The second cohort comprise 36 healthy controls (age matched), 30 SI-NEC primary tumors, 42 lymph node- and 42 liver-metastases. In addition, 34 healthy controls (age matched) and 31 samples from IBD patients were included.

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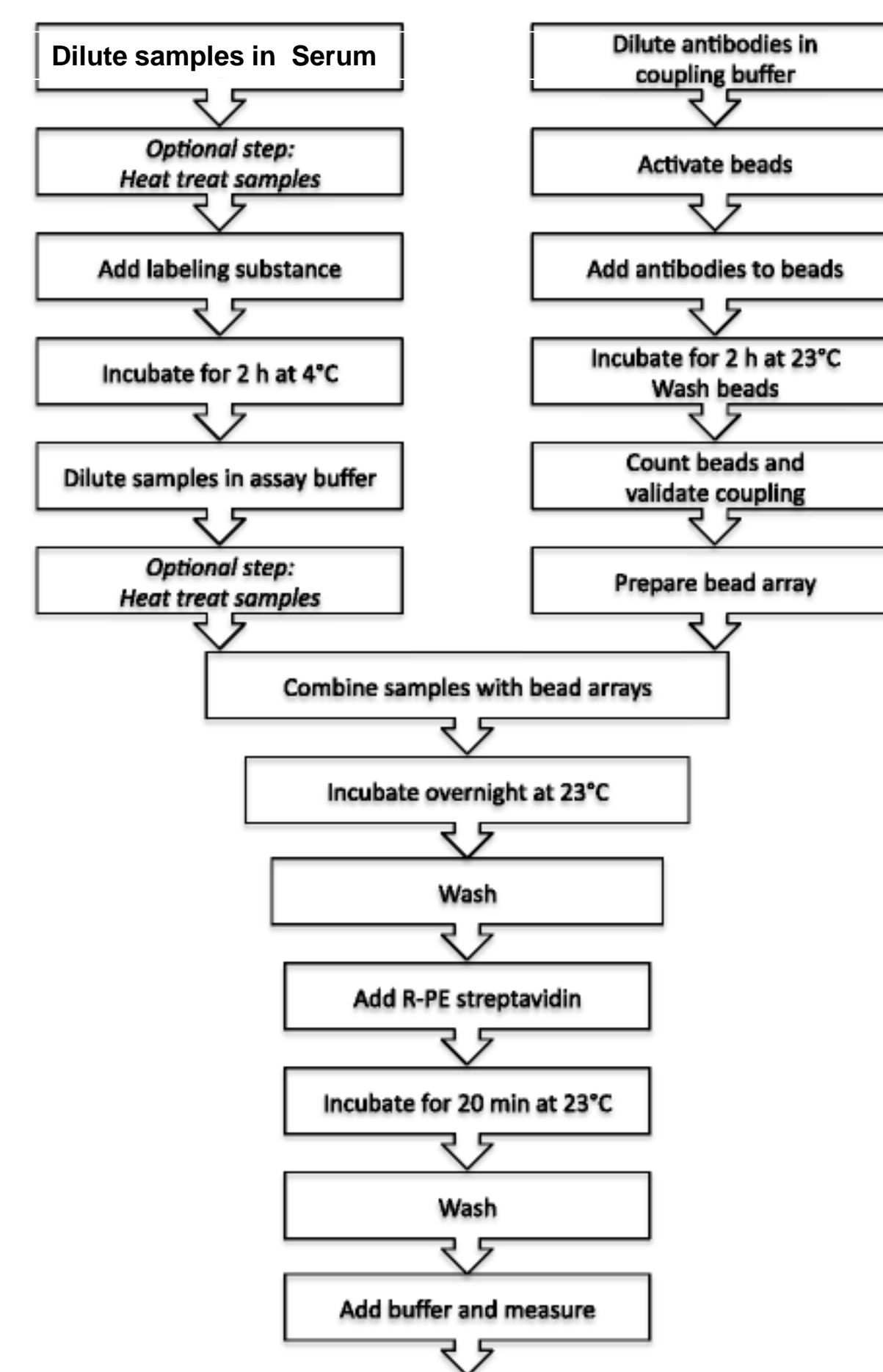


Figure 1 Modified summary of the antibody suspension bead array method by Schenk & Nilsson, 2011

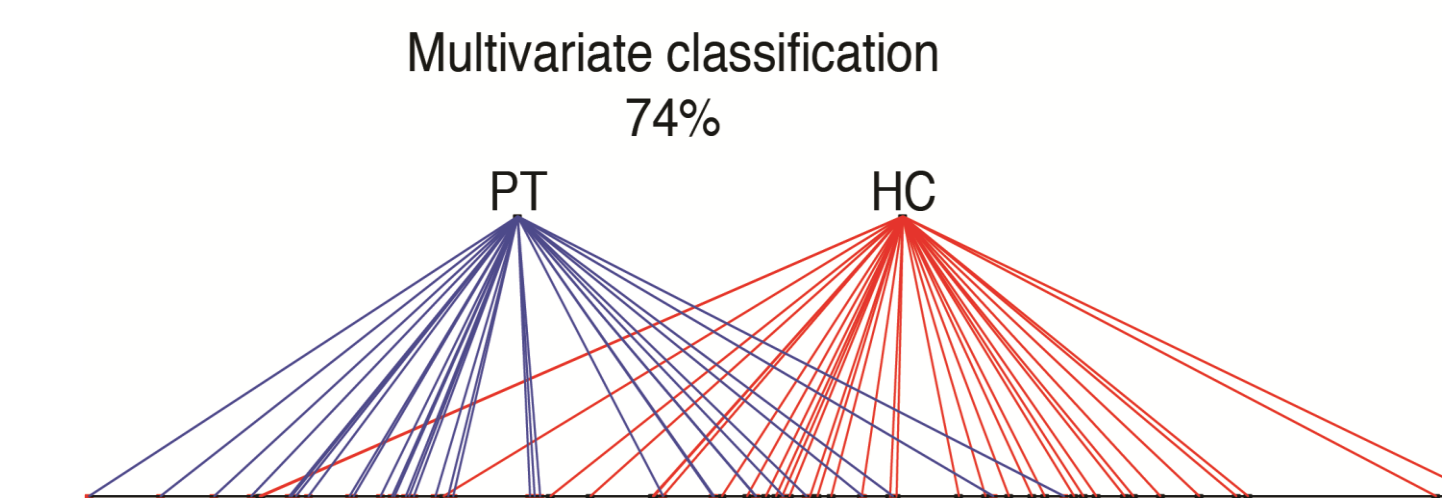
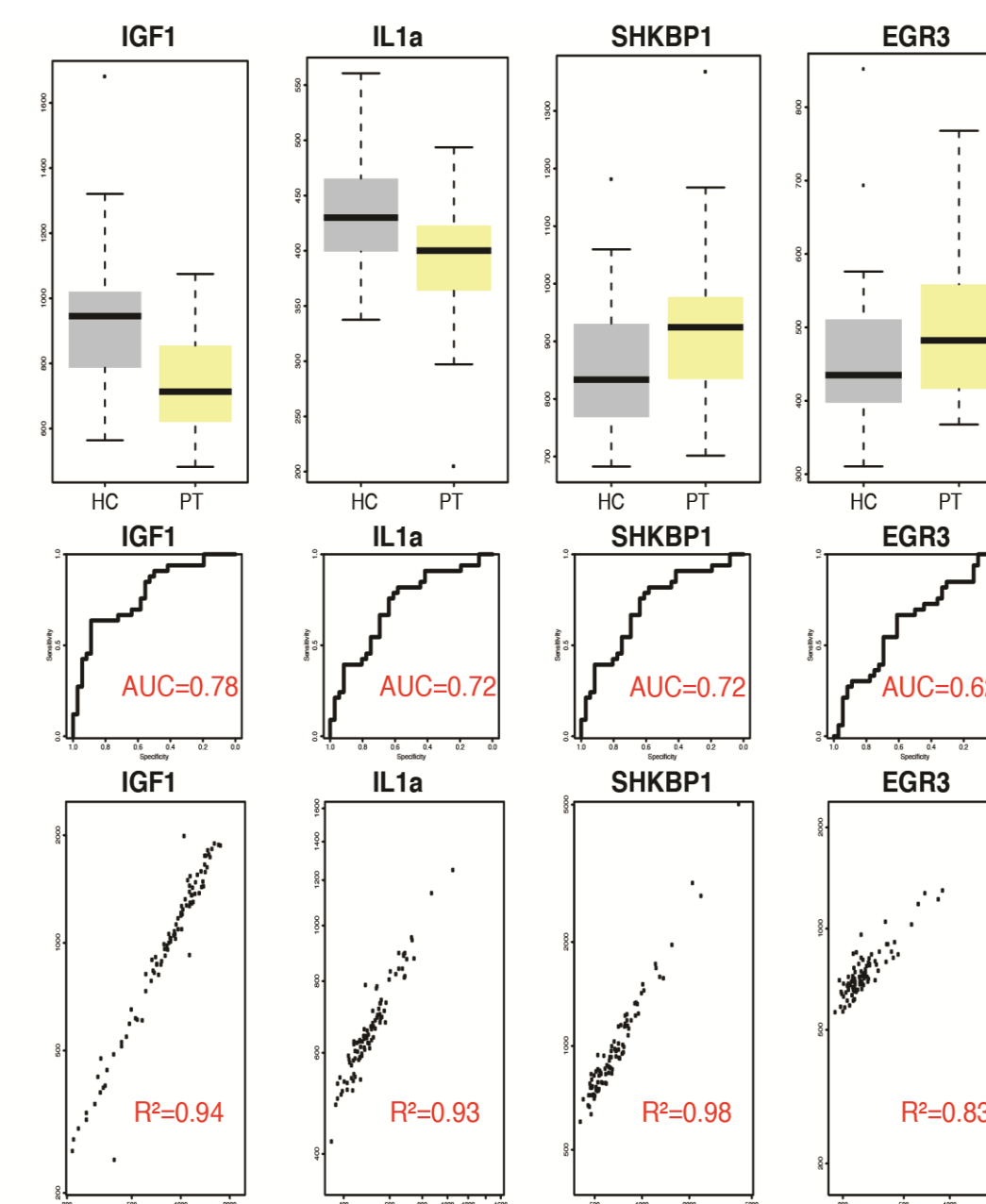


Figure 2 Vertical reading
1st boxplots showing levels of the common significant proteins (cohort 1 and 2) for the classification of healthy controls (HC), in gray. Primary tumors patients. (PT), in yellow. 2nd ROC curves. 3rd the correlation between 2 independent runs. 4th is a ball grid array (BGA) analysis using the markers (classification accuracy is 74%).

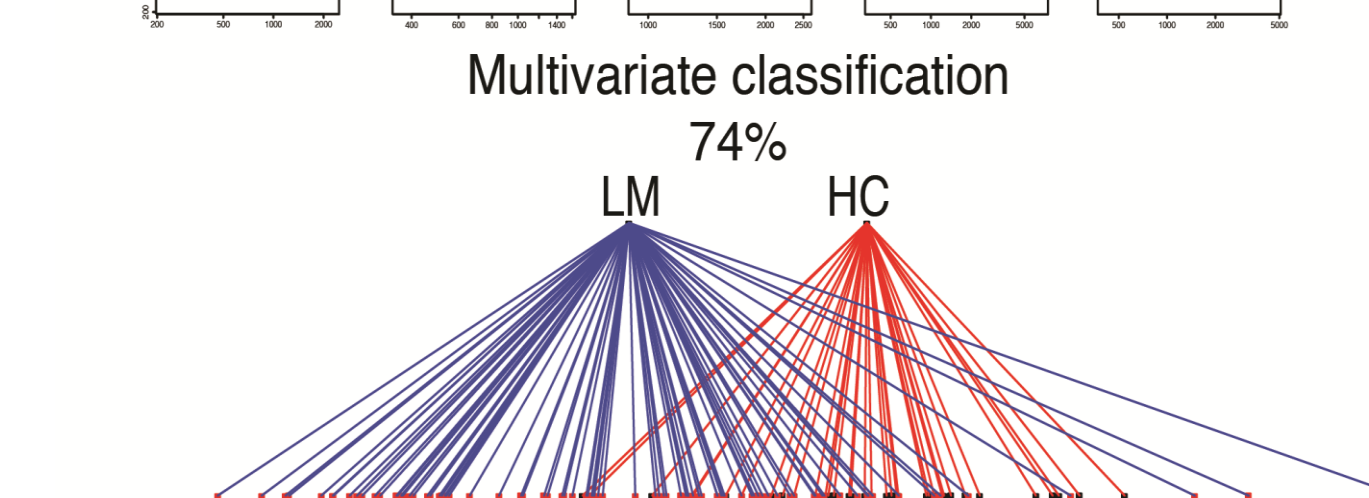
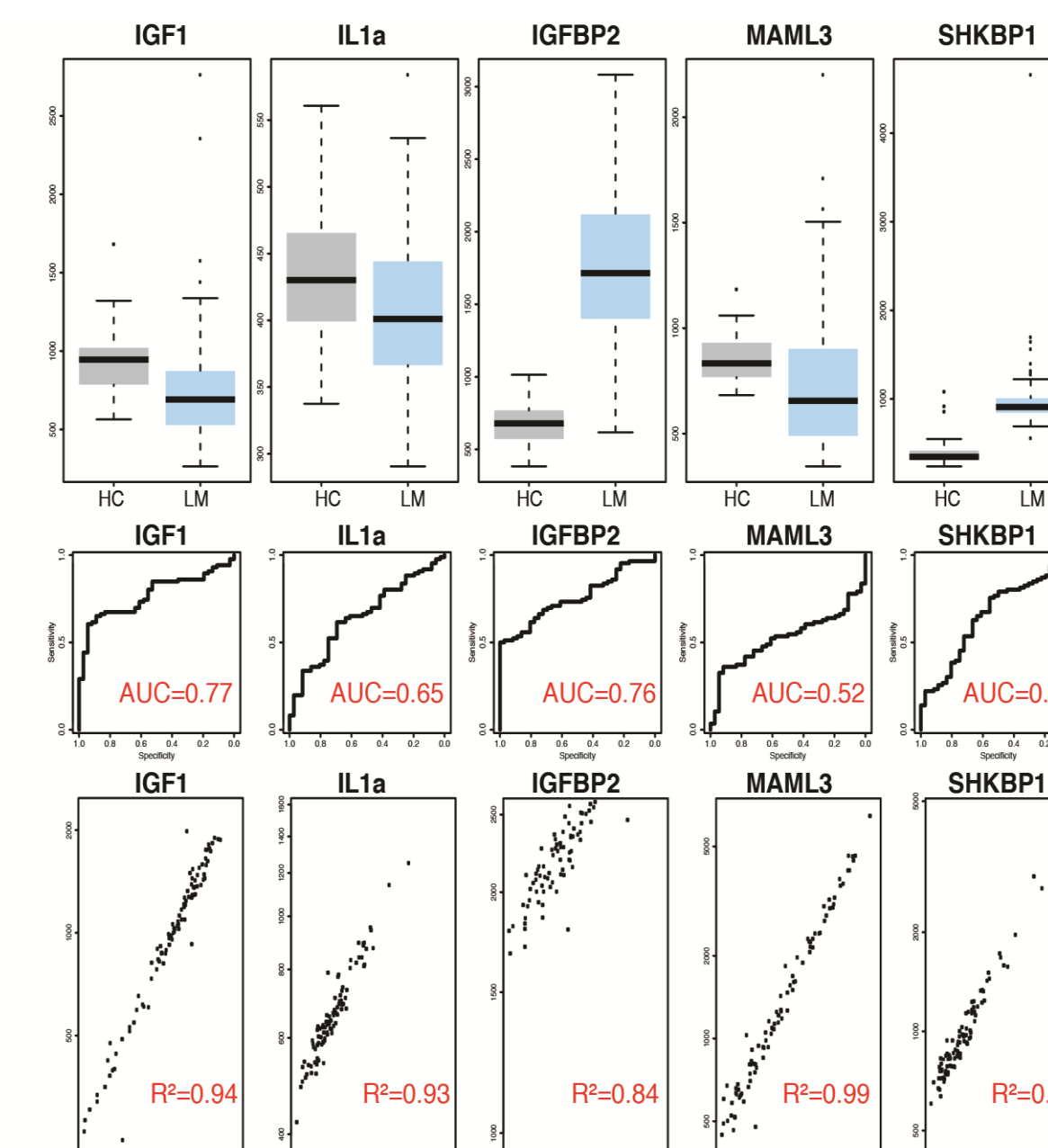


Figure 3 Vertical reading
1st boxplots showing levels of the common significant proteins (cohort 1 and 2) for the classification of healthy controls (HC), in gray. Liver metastases patients (LM), in yellow. 2nd ROC curves. 3rd the correlation between 2 independent runs. 4th is a ball grid array (BGA) analysis using the markers (classification accuracy is 74%).

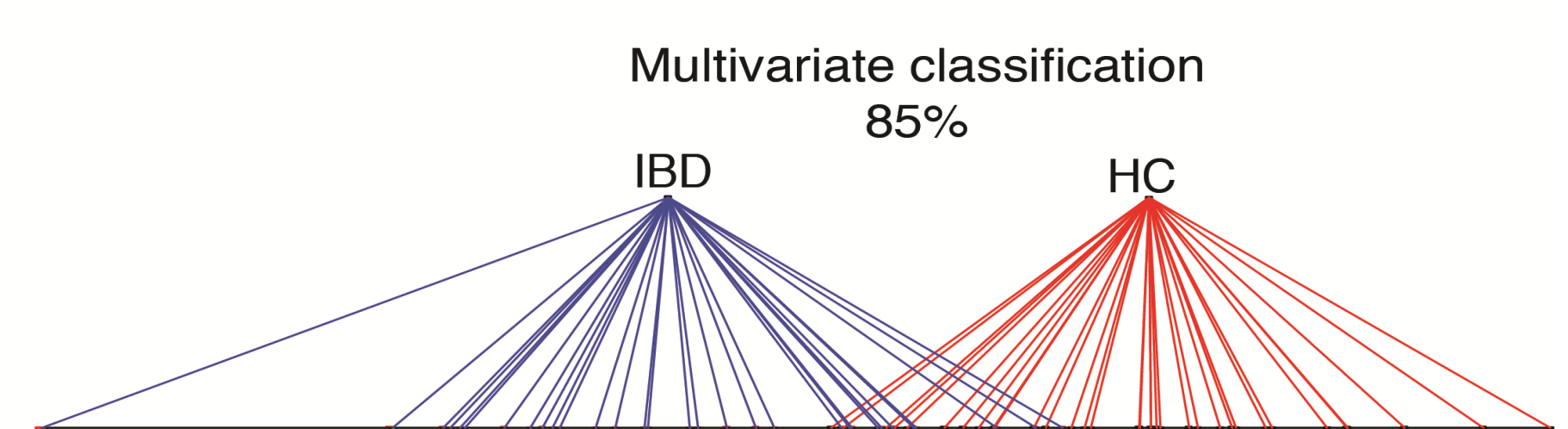
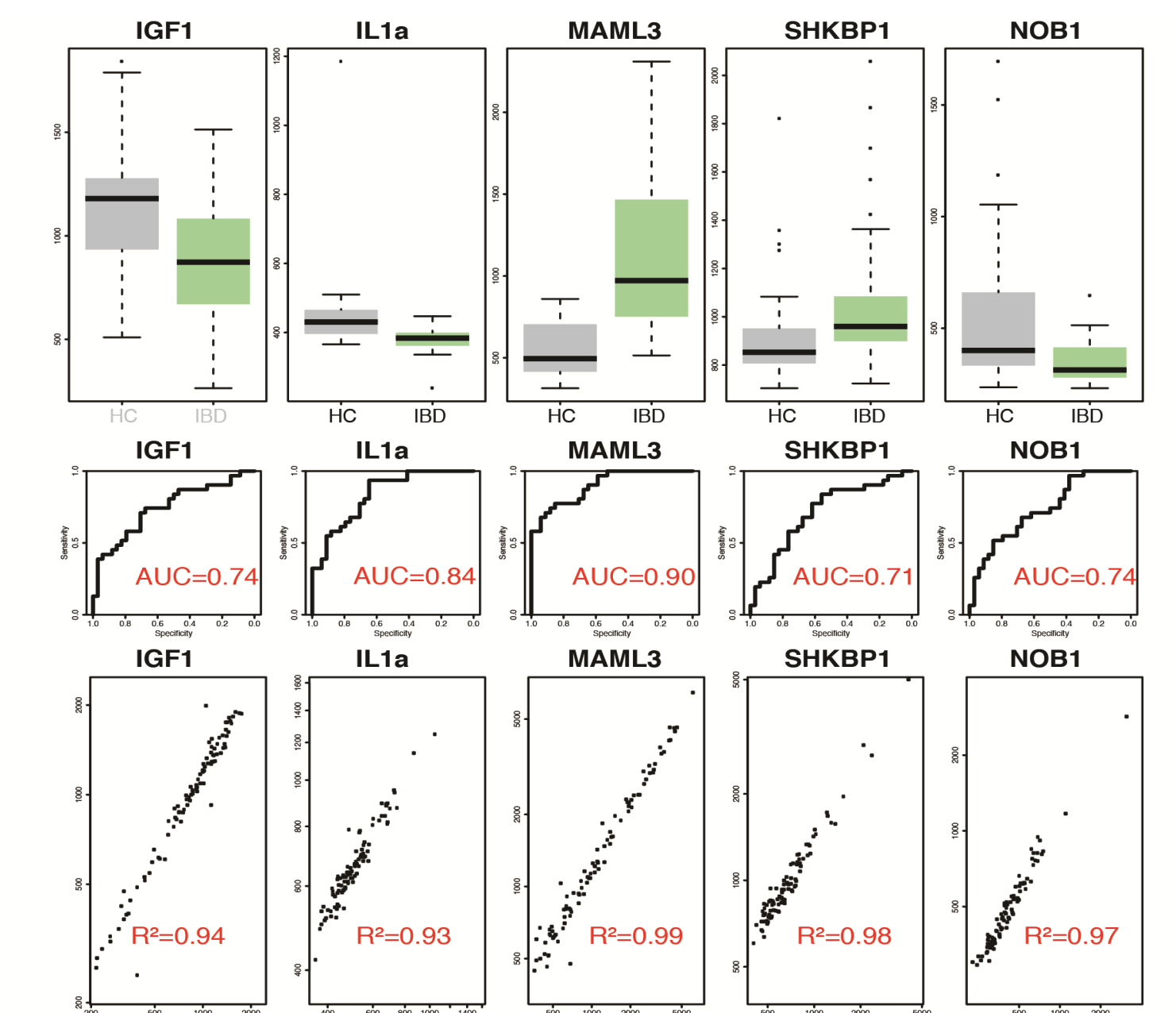


Figure 4 Vertical reading
1st boxplots showing levels of the common significant proteins (cohort 1 and 2) for the classification of healthy controls (HC), in gray. IBD (CD) patients (IBD), in yellow. 2nd ROC curves. 3rd the correlation between 2 independent runs. 4th is a ball grid array (BGA) analysis using the markers (classification accuracy is 85%).

Results

The first cohort was assayed thrice using structure-based assignment targeting of 184 antigens. Proteins that may distinguish healthy individuals and cancer patients using Mann-Whitney U tests as well as Between Group and Random Forest Analysis were selected. Then, an independent sample cohort was assayed for 20 different proteins, which emerged as significant ones. Our findings demonstrated that four targets, insulin-like growth factor 1 (IGF1), interleukin-1 alpha (IL1a), mastermind-like protein 3 (MAML3) and SH3KBP1-binding protein 1 (SHKBP1) were able to distinguish between controls and cancer patients of different stages as well as IBD patients. When proteins were combined in a multivariate classification model they were able to perform with 85% classification accuracy. Figure 1 summarize the proteomic array. Relevant results are shown in Figure 2, 3 and 4.

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