# 3923 | UBIQUITIN LIGASES IMPLICATED AS PREDICTIVE BIOMARKERS FOR POOR OUTCOME TO IMMUNOTHERAPY IN MELANOMA PATIENTS

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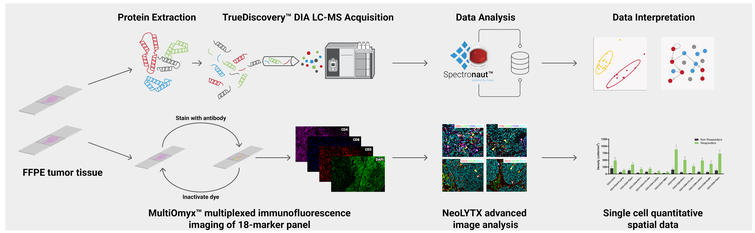
## INTRODUCTION

Melanoma is considered the most serious form of skin cancer. Immune checkpoint inhibitor (ICI) treatment regimes frequently target the PD1 pathway and can be highly effective. Despite successes some patients remain unresponsive and a better understanding of what underlies these non-responders could enable better patient treatment stratification and an overall higher positive response rate. Here we present a multi-modality approach using unbiased mass spectrometry (LC-MS) proteomics profiling in combination with multiplexed immunofluorescence (mIF) spatial tissue analysis, in order to identify protein biomarker signatures in patients with metastatic melanoma that correlate with the patients' intervention responses. The resulting panel of protein biomarkers correlating with improved treatment response after 3 months could serve as a panel for patient stratification prior to commencement of immunotherapy regimes.




## CONCLUSIONS

- **Biognosys and NeoGenomics have developed a joint multi-modality approach that enables deep proteomic analysis together with muliplexed immunofluorescence for a comprehensive characterization of protein markers**
- **Proteome profiling of patient FFPE melanoma tumor samples resulted in quantification of close to 9'280 proteins**
- **A panel of 72 marker proteins allows classification of patients for response to ICI treatment**
- **E1 and E2 ubiquitin ligases are down-regulated in responder patients compared to patients not responding to ICI treatment.**
- **MultiOmyx mIF single-cell quantitative analysis using an 18-marker panel identified 11 proteins differentially regulated in responder patients.**

## RESULTS

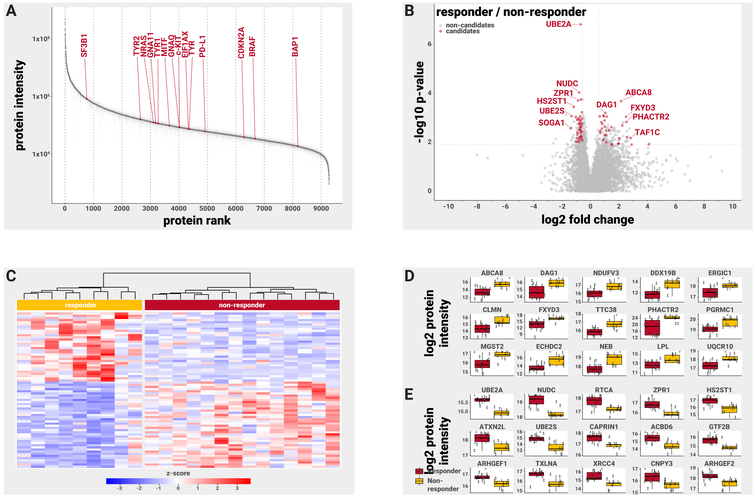



**Figure 1: TrueDiscovery™ whole proteome profiling of FFPE tissue. (a)** 9,280 proteins were quantified across 23 FFPE samples from melanoma patients and ranked according to median intensity. Known melanoma marker proteins are annotated with gene names.
**(b)** 237 proteins were significantly changed (unpaired t-test; $p < 0.01$; log2 fold change > 1.5) between 9 responder and 14 non-responder patient samples. Machine learning (PLS-DA) was used to further identify 72 candidate proteins (red) that separate responder and non-responder patient samples. **(c)** Marker panel of 72 proteins robustly separates responder from non-responder patient samples based on scaled protein intensity. Heatmap was drawn using maximum distance and Ward's clustering method **(d)** Top 15 out of 32 proteins up-regulated in responder (yellow) compared to non-responder patients (red). **(e)** Top 15 out of 40 proteins down-regulated in responder patients.

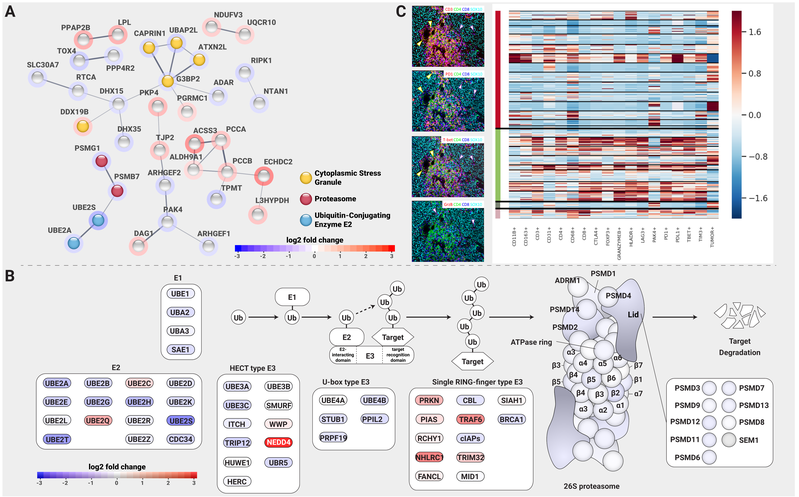



**Figure 2: (a) Protein-protein interaction analysis.** PPI analysis of marker proteins show cytoplasmic stress granule, proteasome, and ubiquitin-conjugating enzyme E2 protein classes as over-represented functional classes. Analysis was performed using String-db (version 11.5), showing only connected nodes. Protein halo is representing log2 fold change (responder/non-responder). **(b) Protein ubiquitination pathway.** Subunits of ubiquitin conjugating enzymes E1 and E2 are expressed at lower levels in patients responsive to ICI treatment. Two E2 subunits with significant change include UBE2A and UBE2S. E3 enzymes covered by this analysis did not show consistent up- or down-regulation. Most proteasome complex subunits are slighly down-regulated in responder patients. **(c) MultiOmyx analysis.** Representative color overlay images of a tumor from a responding patient. Heatmap showing density of the 18 biomarkers analyzed by MultiOmyx.