

Background & Objectives

- Mismatch Repair-deficient (MMRd) status is a robust predictive biomarker for immune checkpoint inhibitors (ICIs) in Endometrial cancer (EC), however half of these patients (pts) do not respond (Oaknin et al, 2023; Makker et al, 2022; O'Malley et al, 2022).
- Aim:** To explore the tumor microenvironment (TME) in ICI-Responder (R) versus Non-Responder (NR) MMRd EC pts to identify new predictive biomarkers of response/resistance.

Methods

- Clinical data and outcomes of recurrent or metastatic MMRd EC patients, treated with ICIs at Gustave Roussy Institute (2016-2022), were retrospectively collected.
- Pts were classified as ICI-R (CR, PR, or SD ≥ 12 months) and NR (PD or SD < 12 months).
- Pre-ICI FFPE tumor samples were subjected to Biognosys **UltraDeep TrueDiscovery™ Mass Spectrometry (MS)**.
- Differential protein expression between Rs and NRs was assessed using Student's t-test. **Criteria for protein candidates' selection were p-value < 0.01 and average log2 fold-change > 0.58 .**
- Hierarchical unsupervised clustering and hypergeometric functional enrichment analyses** were conducted on differentially expressed protein candidates between Rs and NRs.
- Intraepithelial (ie) CD8⁺ density were quantified by Qupath, and correlated with protein expression** (Spearman's rank coefficient)

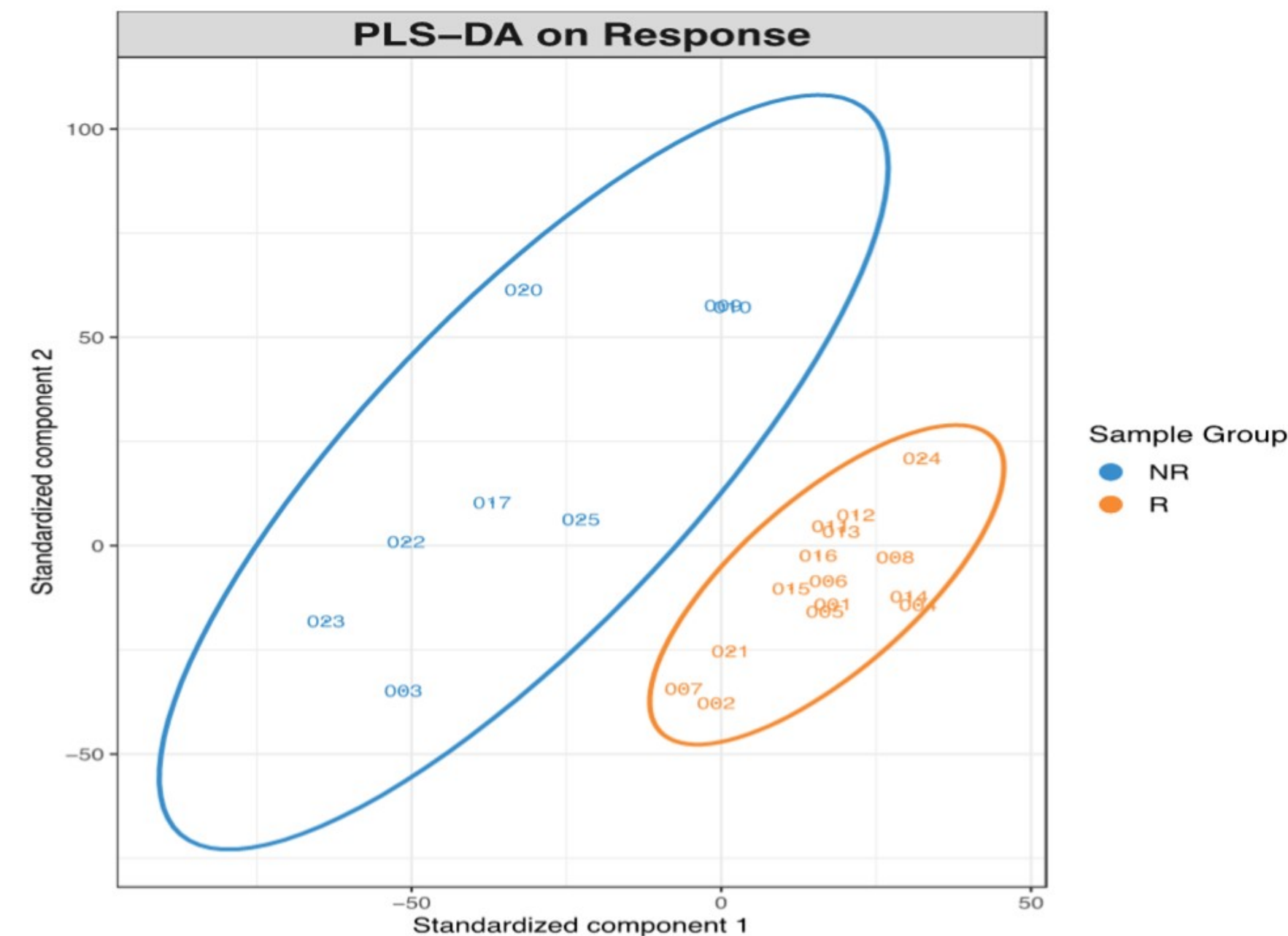
Results

Table 1. Study Population (n=25). Clinicopathological features.

	ICI-Rs n=15 (%)	ICI-NRs n=8 (%)
Age (median, years)	60	61
Histology (%) (Endometrioid/others*)	73 / 27	88 / 12
Histopathologic grade (Low/High grade)	73 / 27	62 / 38
Methylated MLH1 vs. Non-methylated	80 vs. 20	100 vs. 0
Prior lines for recurrent/metastatic disease (median, range)	1 (0 – 5)	1 (0 – 2)
ICI Regimen Single-agent anti-PD(L)1 Combo**	80 / 20	88 / 12

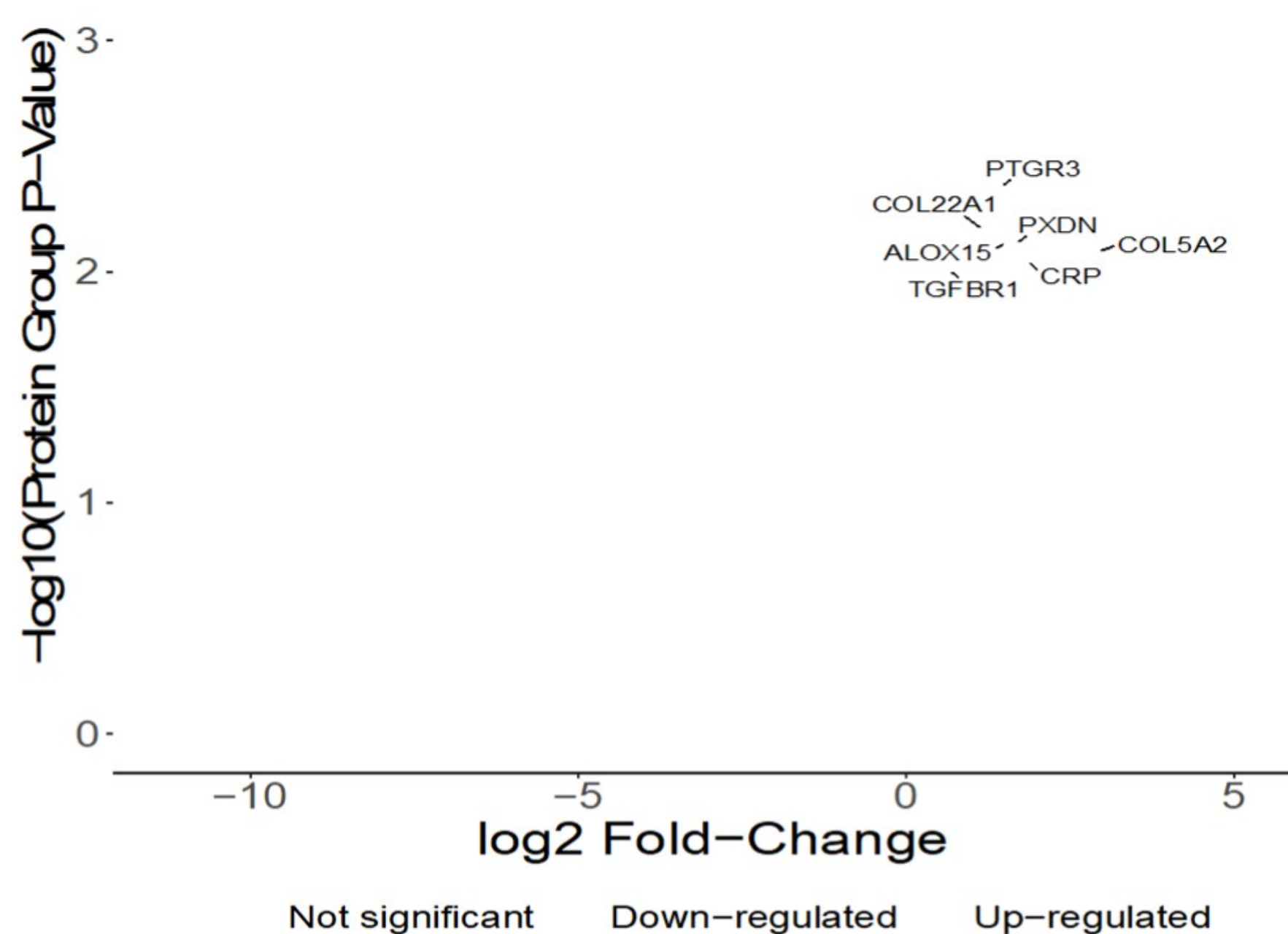
Proteome-Wide Data Set Variance Analyzed by Partial Least Squares Discriminant Analysis (PLS-DA)

- 11,187 proteins, were quantified across the 23 samples of FFPE sections from EC tissue.**
- PLS-DA was performed by fitting a model onto Response Status, allowing a separation between Rs and NRs**



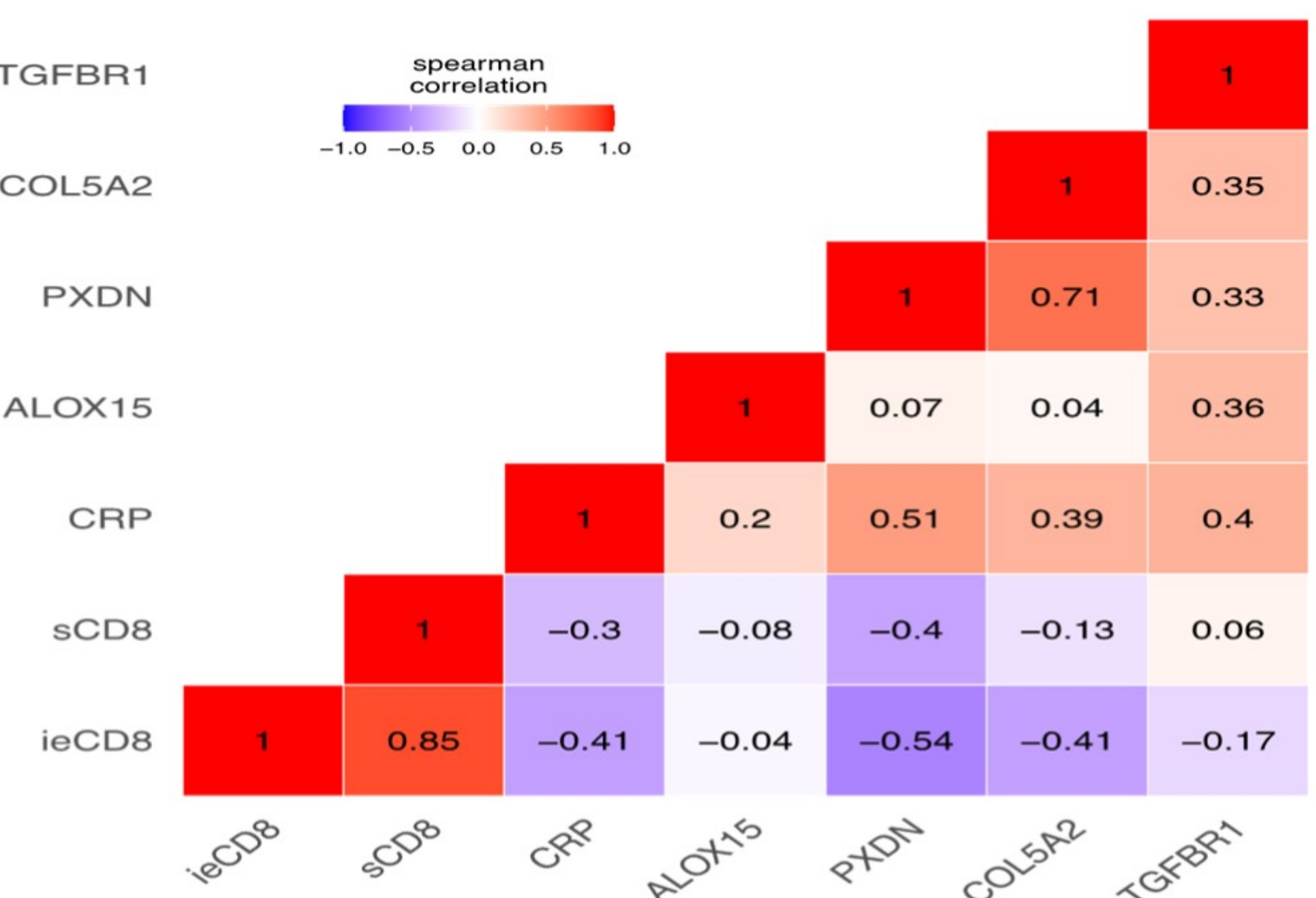
Differentially Regulated Proteins: ICI-R vs. NR MMRd EC

- 72 proteins were found to be differentially abundant between Rs and NRs**
- NRs showed significant Up-regulation of the proteins related with nonspecific Inflammation (CRP), Arachidonic Acid Metabolism (LOX15, PTGR3), Extracellular Matrix (ECM) Synthesis (COL5A2, COL22A1, PXDN), and TGF- β (TFBR1)**

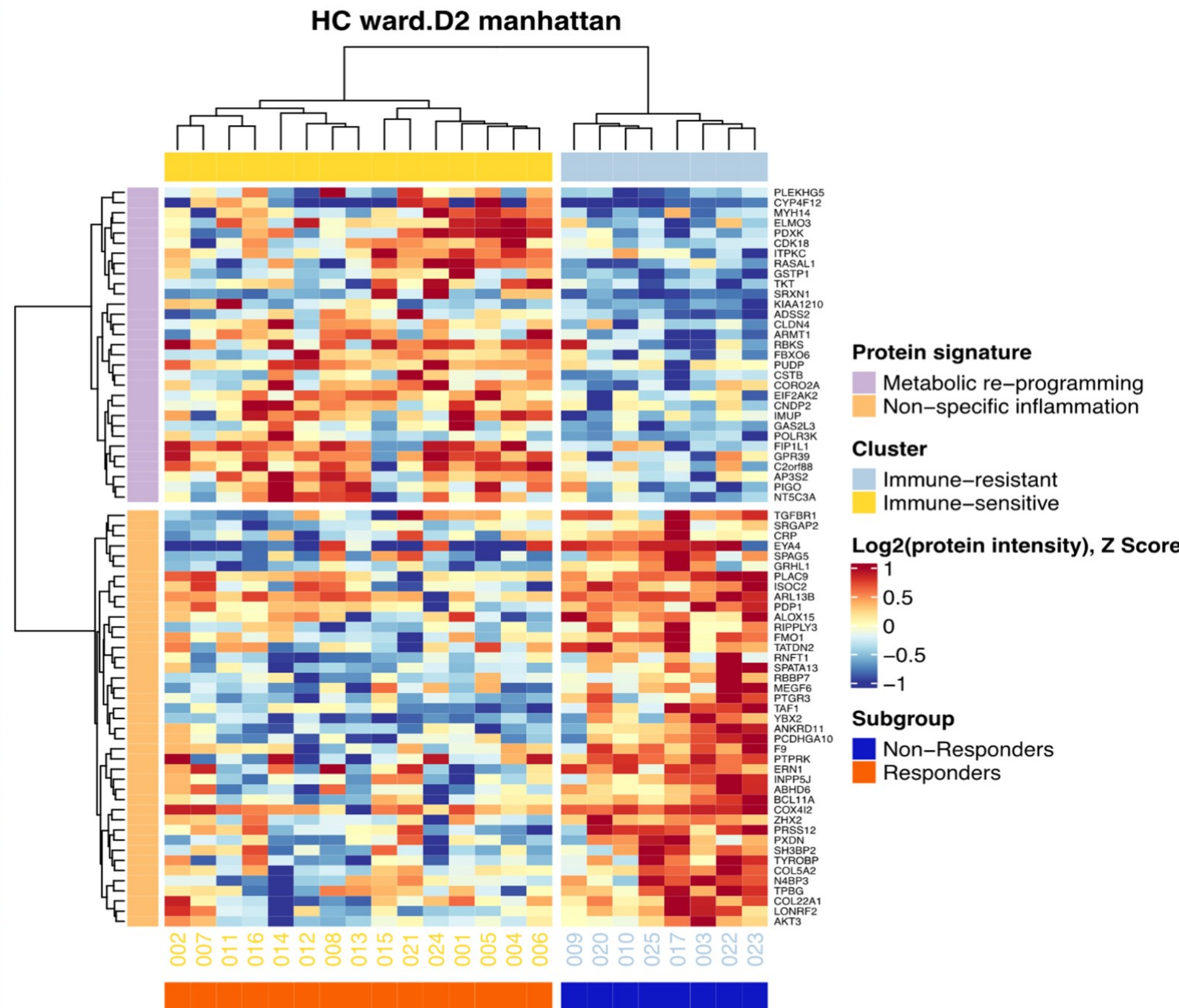


Correlation between protein expression and ie-/s-CD8⁺ intratumoral densities

- CRP, ALOX15, PXDN, COL5A2, and TGFBR1 expressions were negatively correlated with ieCD8⁺ T cell infiltration**
- Up-regulation of nonspecific Inflammation and ECM Synthesis was highly associated with an immunosuppressive TME**



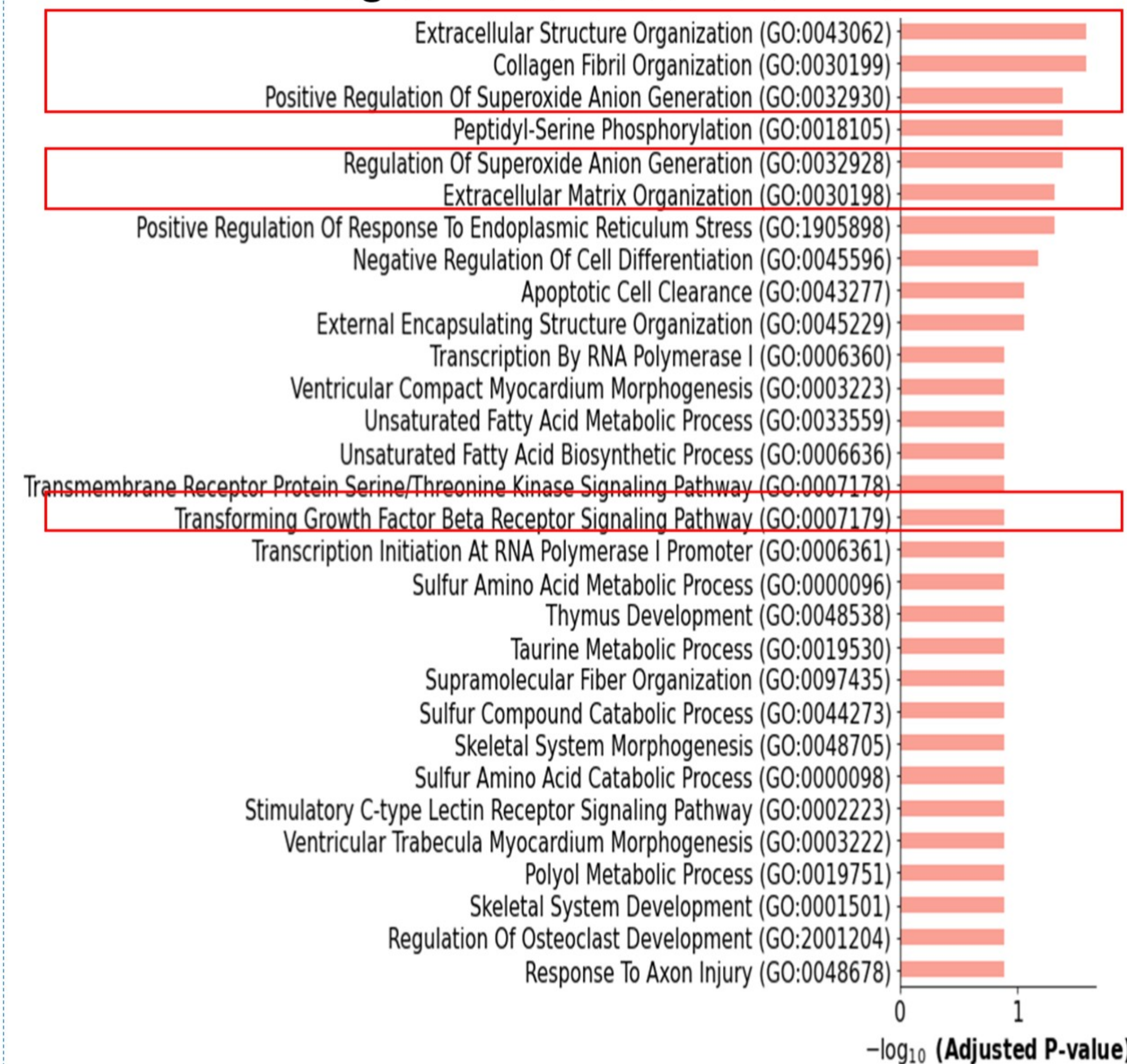
Unsupervised Hierarchical Clustering Analysis of Differentially Regulated Proteins: ICI-R vs. NR MMRd EC



Immune-Resistant Cluster (100% ICI-NRs) is characterized by an enrichment of a signature related to nonspecific inflammation and a reduction in the expression of a metabolic programming signature

Functional Enrichment Analysis of Differentially Regulated Proteins: ICI-R vs. NR MMRd EC

GO_Biological Process: Top 30 Up-Regulated Biological Processes in ICI-NRs



ICI-NRs showed **Up-regulation** of Collagen fibril organization, ECM organization, Regulation of Superoxide Anion Generation, and TGFBR signaling pathway

Conclusions

- Preliminary data on High Throughput Proteomic Analysis identified potential mediators of ICI resistance in MMRd EC pts:**
 - Non-specific acute inflammation**
 - ECM synthesis and structural organization**
 - Unfavorable TME metabolic programming**
- Mechanistic studies are needed to further confirm the actionability of these pathways in order to enhance ICI sensitivity**

Acknowledgments:

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