Deciphering single-cell resolved tumour microenvironment profiles using spatial metabolic mapping

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There's a Growing Need to Improve Prediction of Response

Tumor Mutational Burden

Limited predictive power

Response to immunotherapy in solid tumors[2]

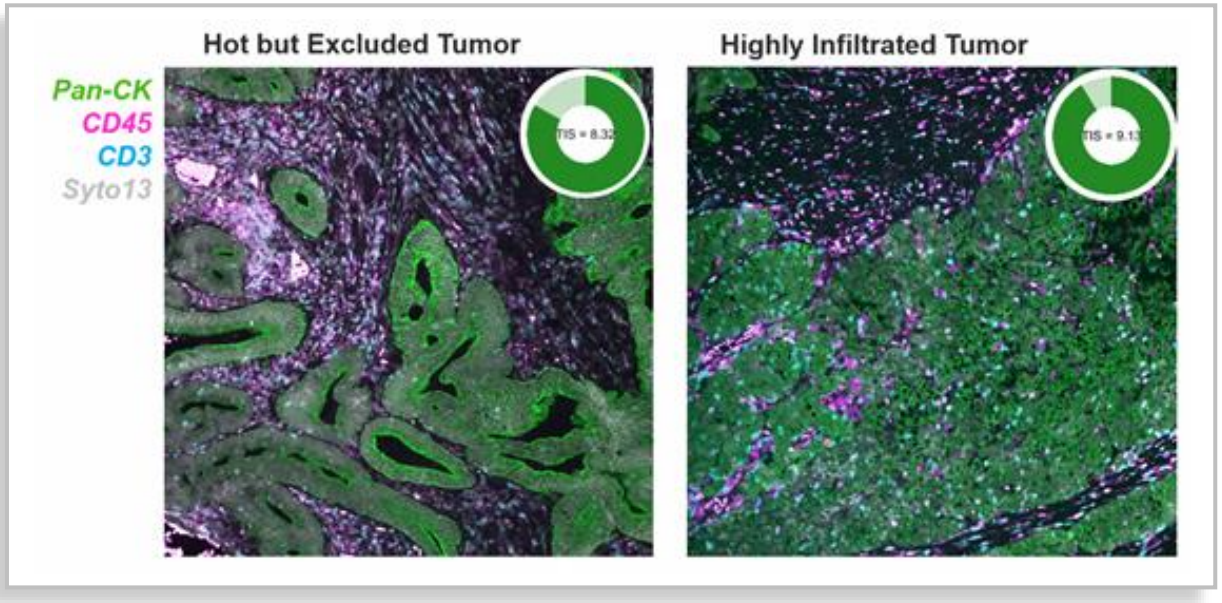
High TMB	Low TMB
~40%	~20%

PDL1 IHC

Limited predictive power

Response to immunotherapy in solid tumors[1]

PDL1 +	PDL1 –
~40%	~13%



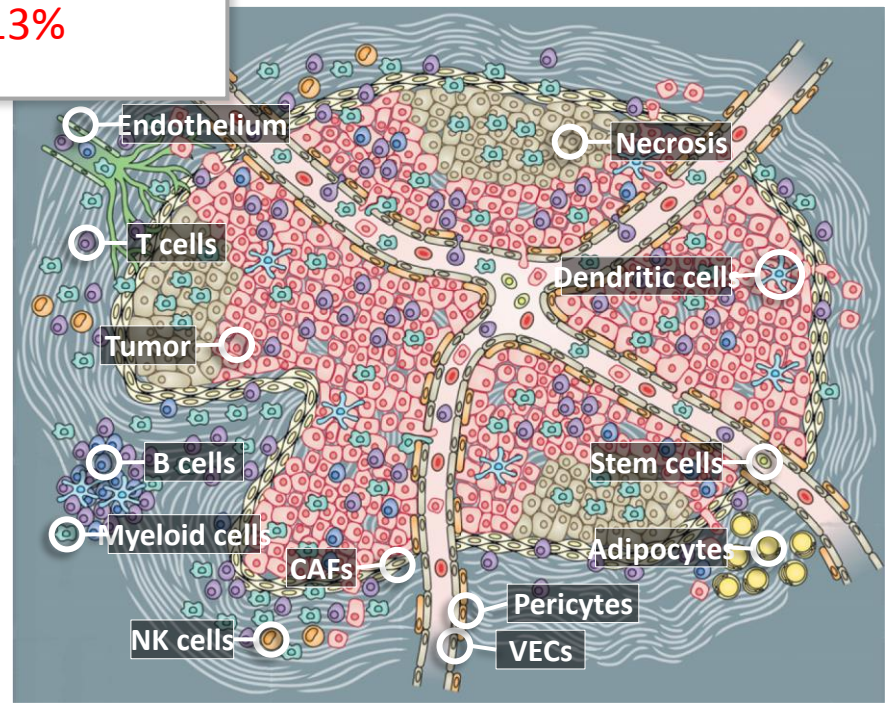
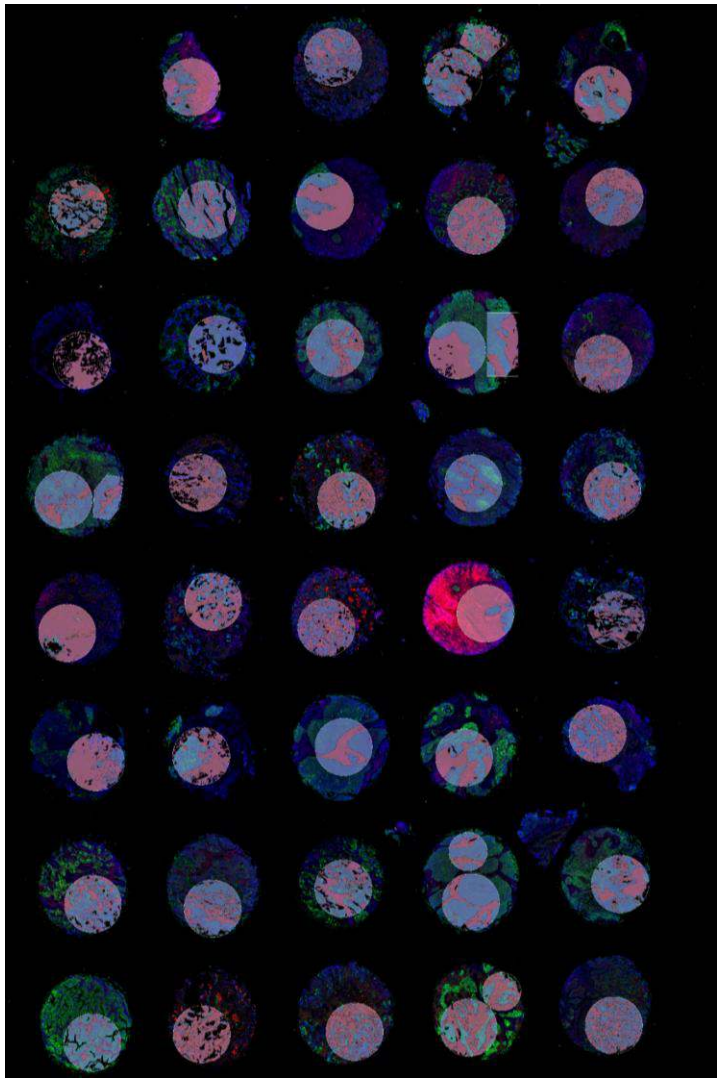
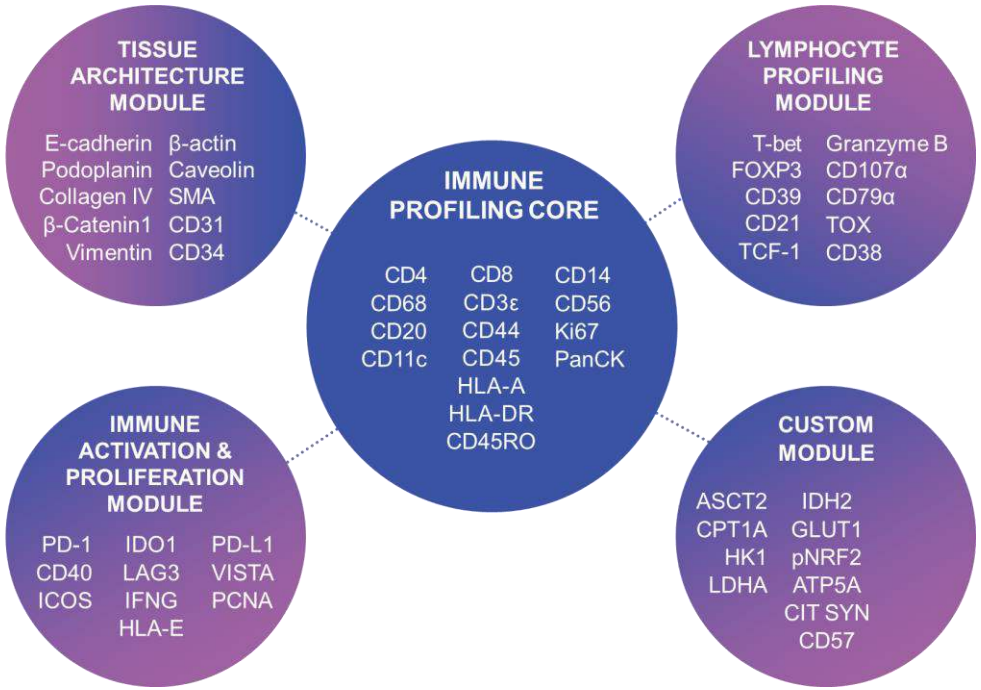


Image modified from: Journal of Cell Science 125, 5591–5596 2012

Multimodal Approach to Discover Response Signatures in NSCLC-ICI Patients (n=43 patients)



- 45-plex Phenocycler

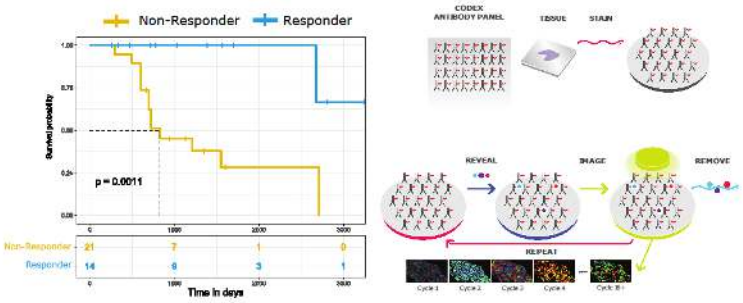


IO Cohort	Non-responder, N = 25	Responder, N = 16
Gender		
F	9 (36%)	5 (31%)
M	16 (64%)	11 (69%)
Age	66 (59, 69)	58 (58, 63)
ICI Treatment		
Durvalumab	0 (0%)	1 (6.2%)
Nivolumab	22 (88%)	11 (69%)
Pembrolizumab	3 (12%)	4 (25%)
Current Status		
Alive	8 (32%)	15 (94%)
Deceased	17 (68%)	1 (6.2%)
Histology		
Adenocarcinoma	12 (48%)	13 (81%)
Squamous Cell Carcinoma	13 (52%)	3 (19%)

Modularised Spatial Analysis

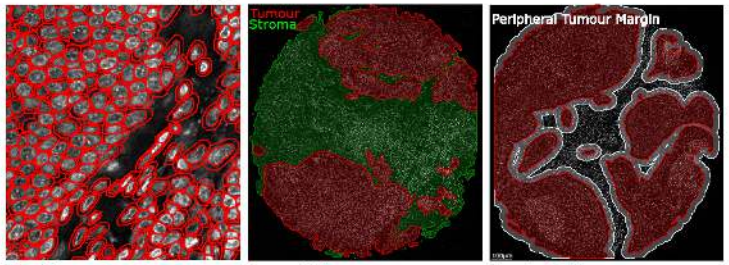
A Multiplex Staining

Tumour tissue from 42 advanced/ metastatic immunotherapy treated patients was stained with 36 protein markers and imaged in cycles by CODEX



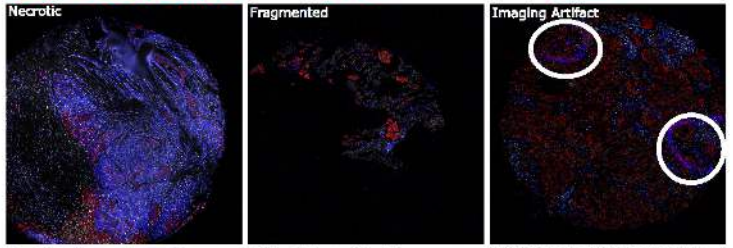
B Image Processing

Images were imported into Qupath for visual inspection. Cells were segmented with Cellpose plug-in using 'cyto2' pre-trained model. Tissues were segmented into tumour/stroma by ANN pixel classifier. Tumour annotation was expanded by 30um for peripheral tumour margin.



C Tissue QC

Tissues that were necrotic, fragmented or contained autofluorescent imaging artifacts were removed from analysis

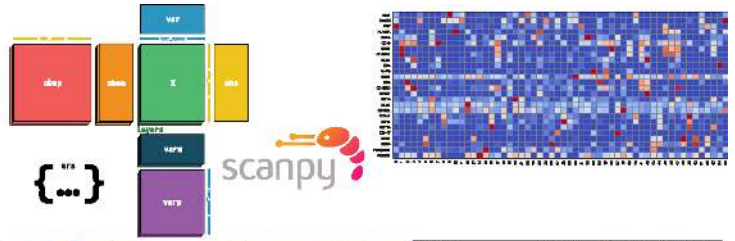


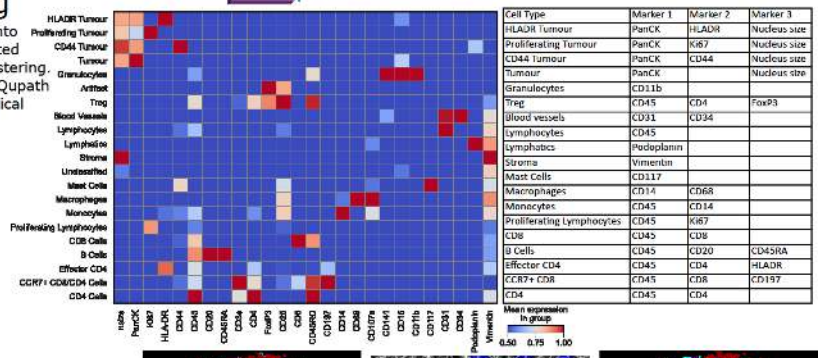
D Staining QC

Protein markers with high non-specific background signal or artifactual staining were removed from analysis

E Cell Phenotyping

Expression matrices were imported into Anndata, preprocessed, batch corrected and phenotyped by unsupervised clustering. Clusters were manually inspected in Qupath and annotated as cell types by canonical marker expression.

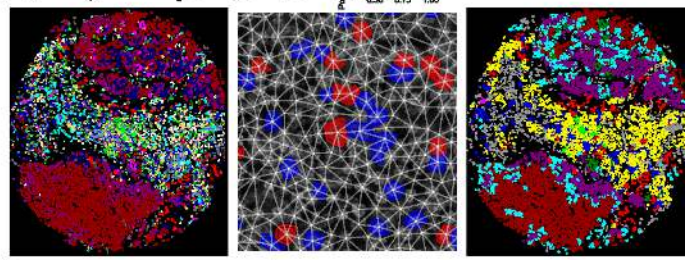




Cell Type	Marker 1	Marker 2	Marker 3
HLADR Tumour	PanCK	HLADR	Nucleus size
Proliferating Tumour	PanCK	Ki67	Nucleus size
CD44 Tumour	PanCK	CD44	Nucleus size
Tumour	PanCK		Nucleus size
Granulocytes	CD11b		
Treg	CD45	CD4	FoxP3
Blood vessels	CD31	CD34	
Lymphocytes	CD45		
Lymphatics	Podoplamin		
Stroma	Vimentin		
Mast Cells	CD117		
Macrophages	CD14	CD68	
Monocytes	CD45	CD14	
Proliferating Lymphocytes	CD45	Ki67	
CD8	CD45	CD8	
B Cells	CD45	CD20	CD45RA
Effector CD4	CD45	CD4	HLADR
CCR7+ CD8	CD45	CD8	CD197
CD4	CD45	CD4	

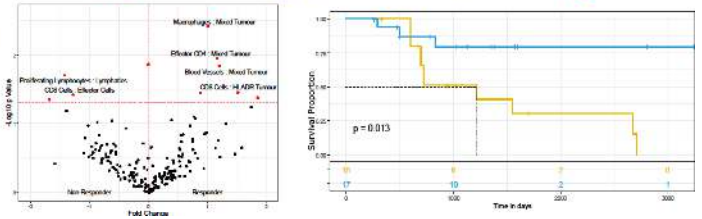
F Spatial Feature Analysis

Cell Proportions were calculated in tumour, stroma and tumour periphery. Interaction frequencies were calculated using Scimap proximity density score. Spatial neighbourhoods were generated by KNN clustering of cell types and cell type enrichment within neighbourhoodscalculated by a published method. 3-way interaction frequencies were calculated by published SpatialScore method.

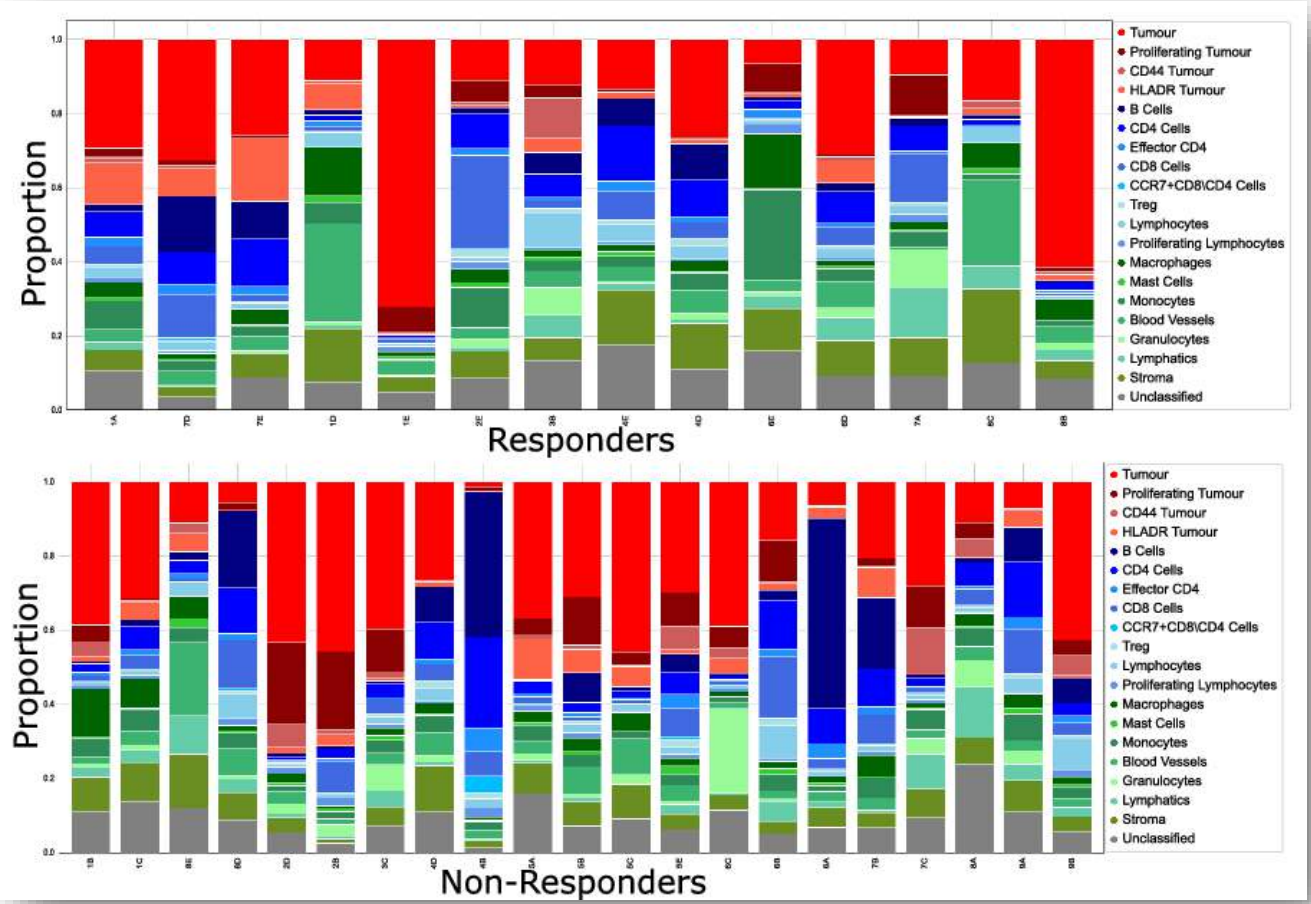


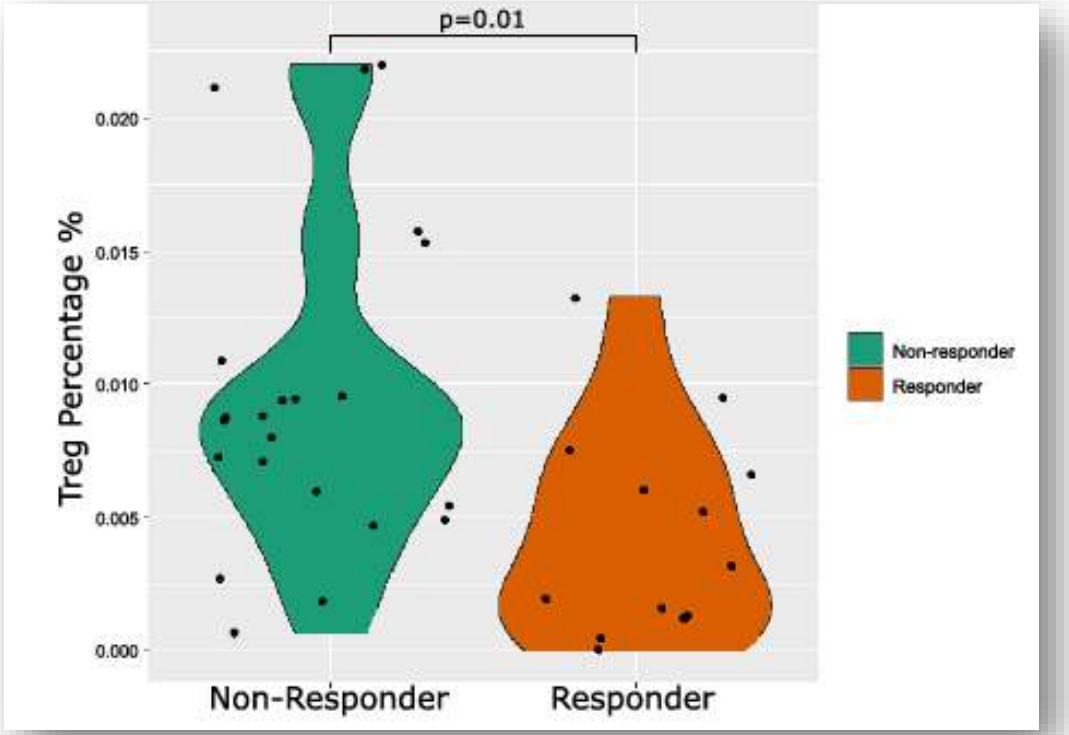
G Comparative Statistics and Survival Analysis

Spatial features were compared by t-test for association with immunotherapy response. Features were also analysed for survival associations by cox regresssion and Kaplan-Meier.

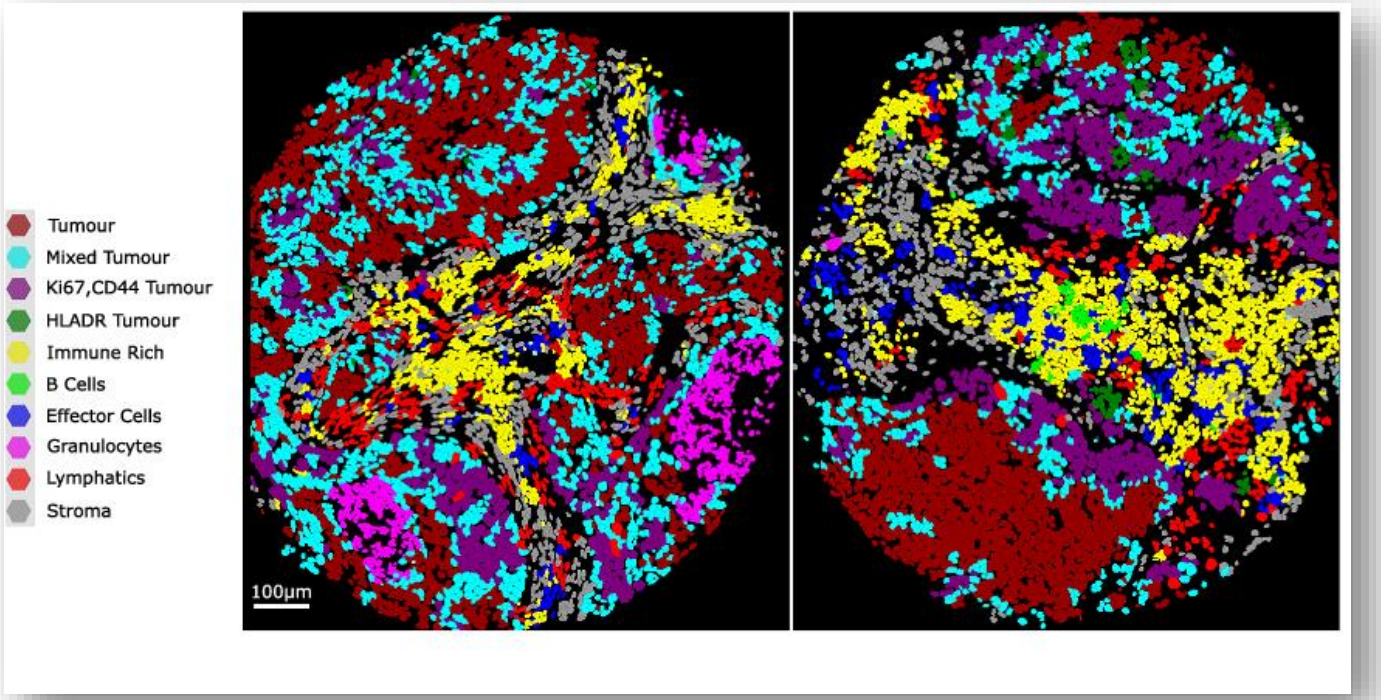


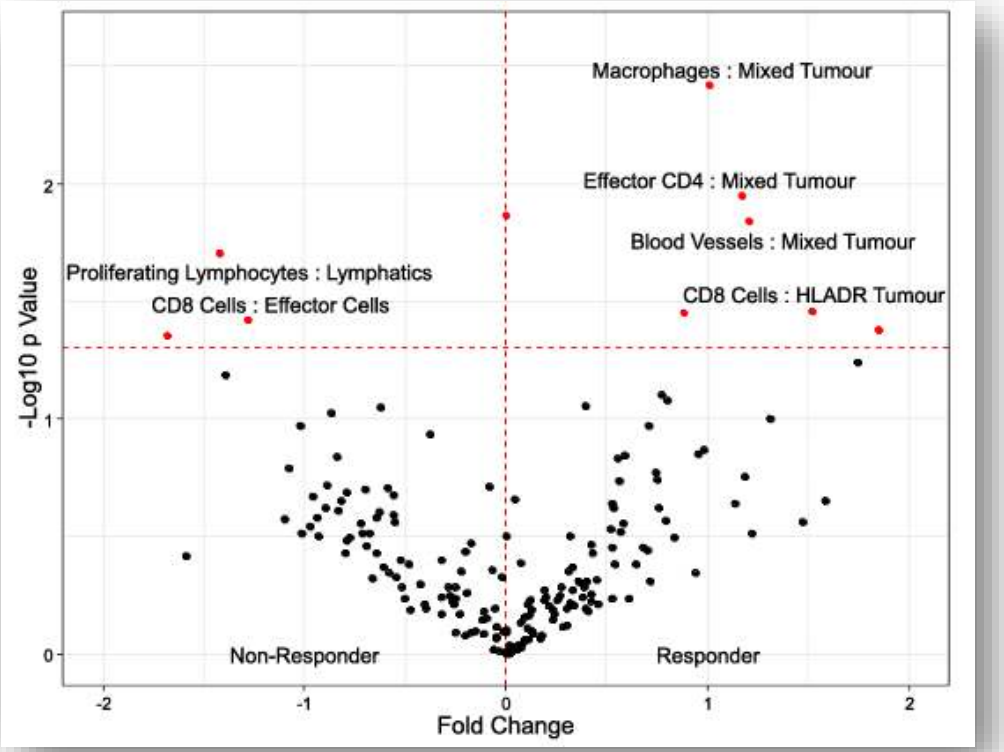
Cellular Proportions per Tissue Core and Tregs Associated with a Non-responsive Phenotype



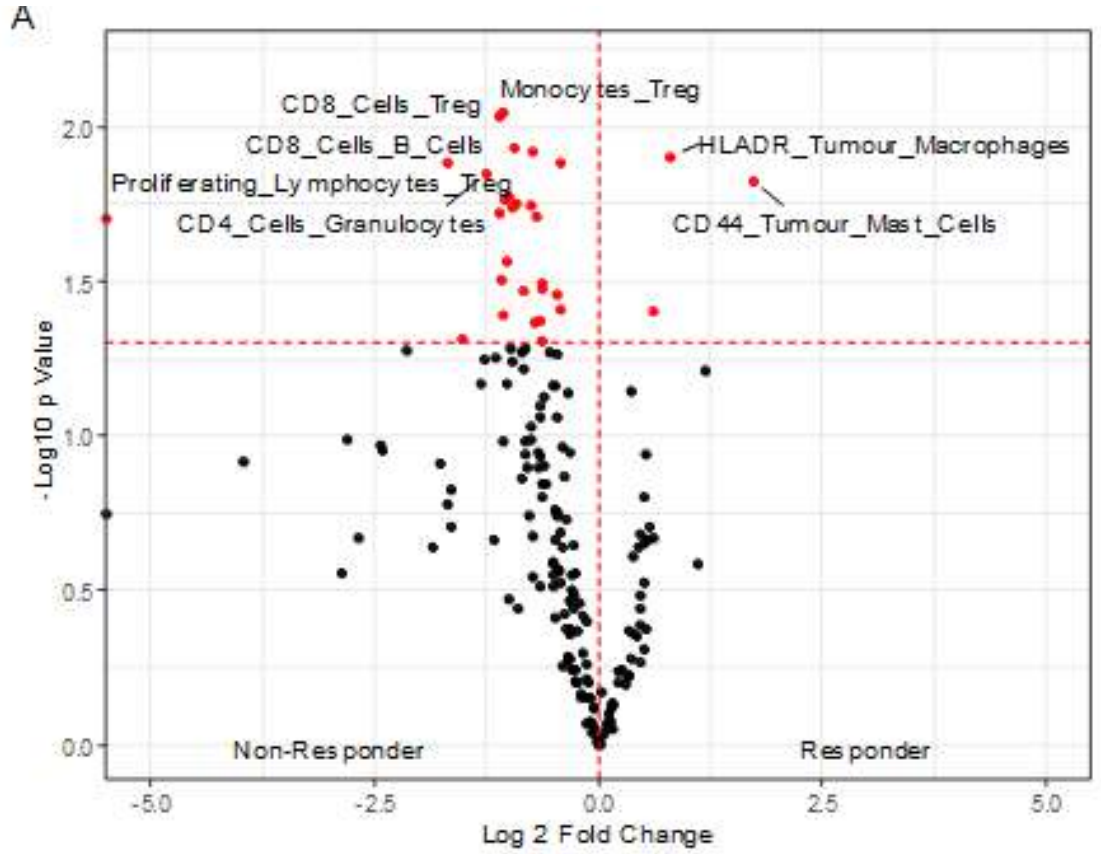


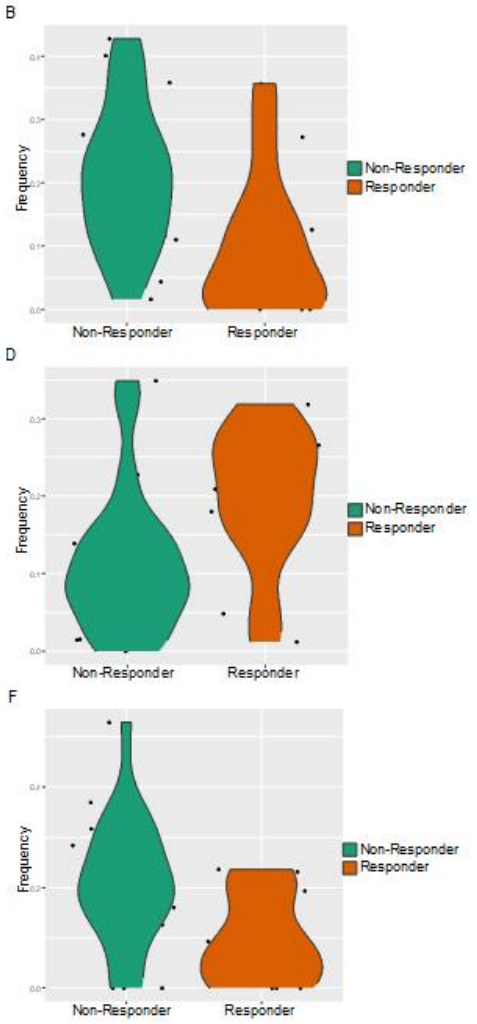
Cellular Neighbourhood Analysis Identified Macrophage-mixed Tumour Phenotype Associated with Response to Therapy

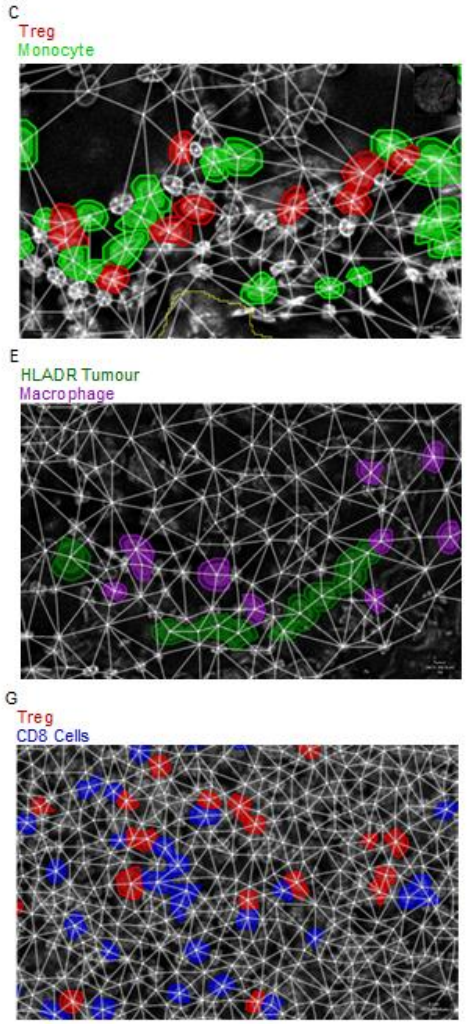




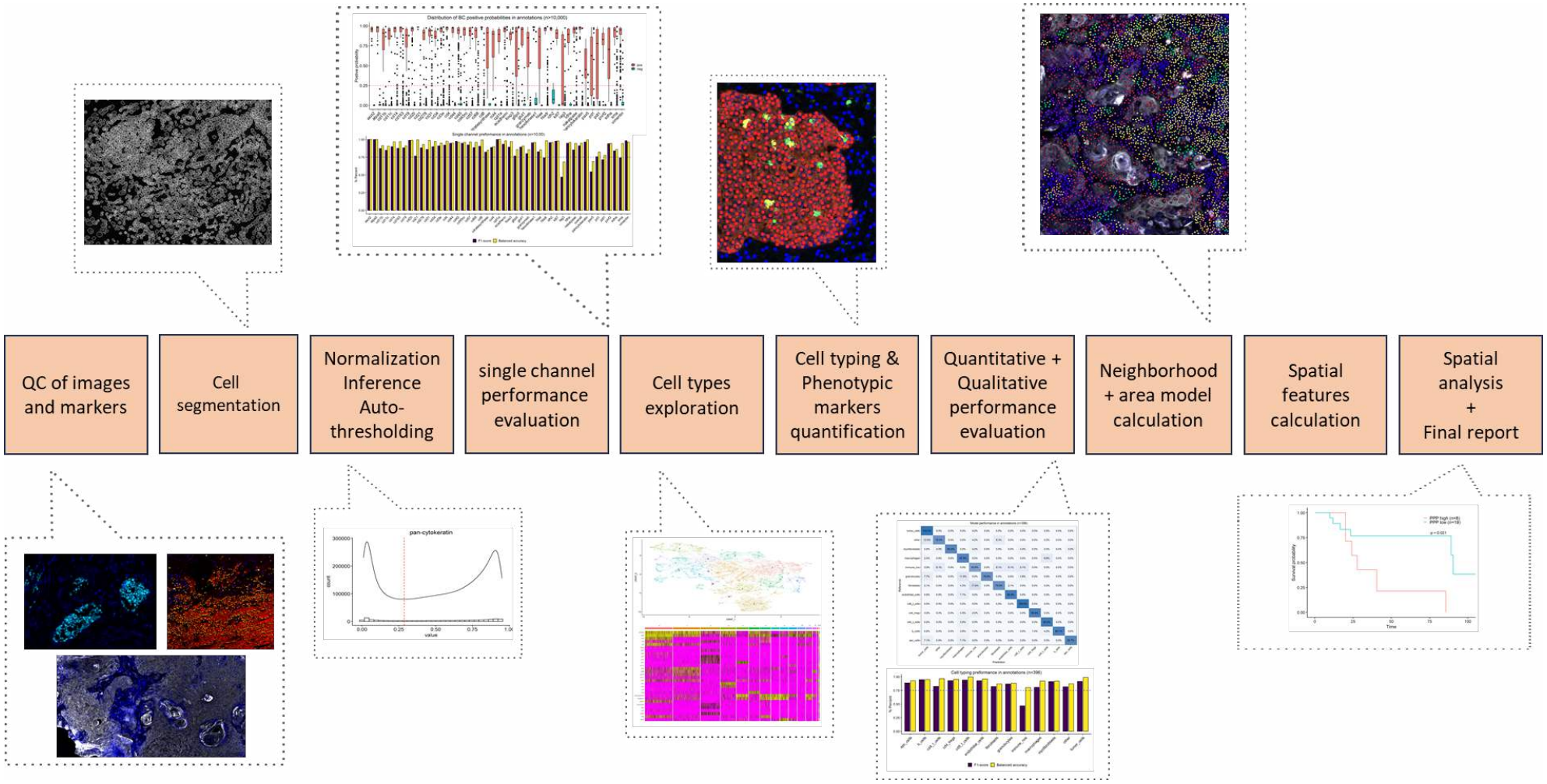
Cellular interaction analysis







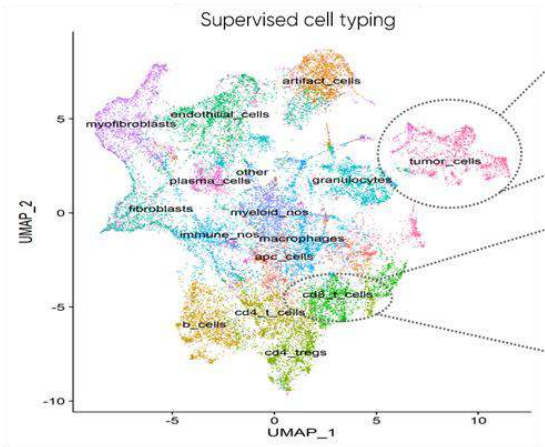
mIF Workflow (deep learning pipeline)

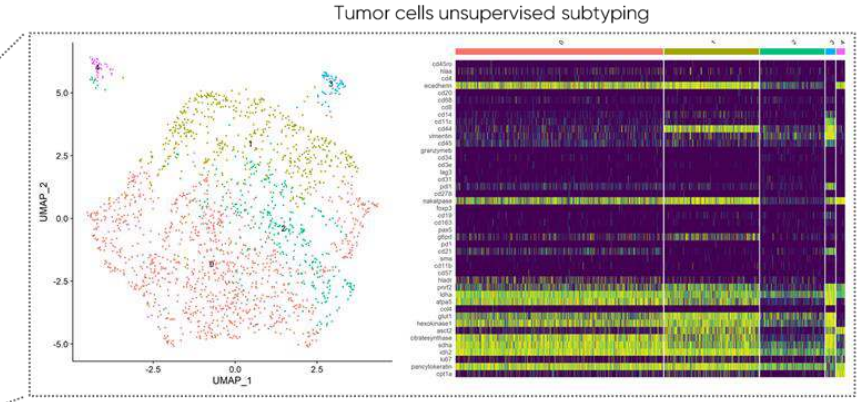


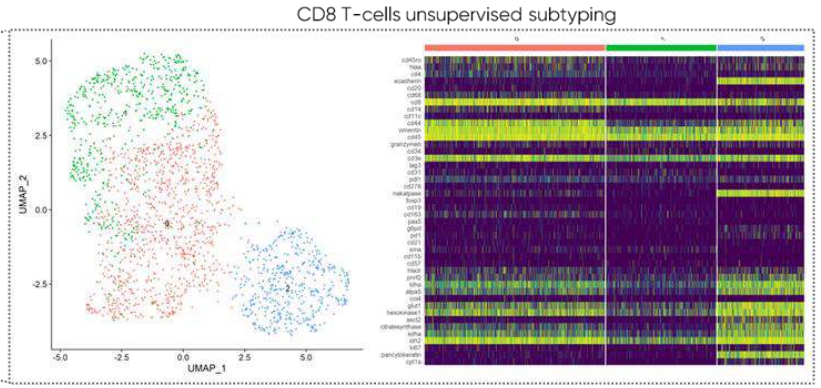
Cell Subtyping by Unsupervised Clustering

QC of images and markers	Cell segmentation	Normalization Inference Auto-thresholding	single channel performance evaluation	**Cell types exploration**	Cell typing & Phenotypic markers quantification	Quantitative + Qualitative performance evaluation	Neighborhood + area model calculation	Spatial features calculation	Spatial analysis + Final report

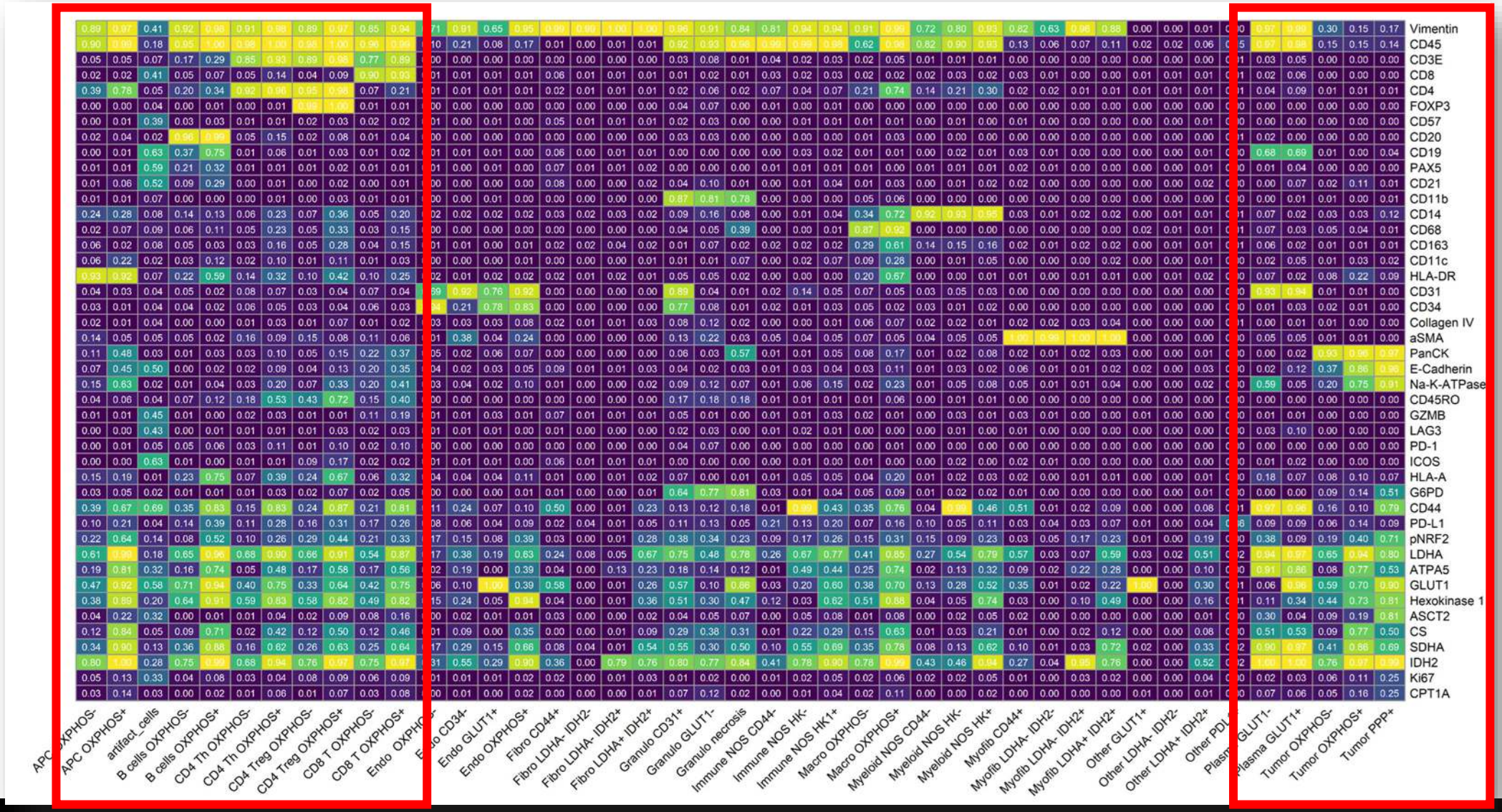
- To identify functional states, unsupervised clustering of positive prediction probabilities within each cell type was performed
- Unsupervised cell subtyping of the 15 cell types identified 43 cell subsets, which were mostly segregated by their metabolic state





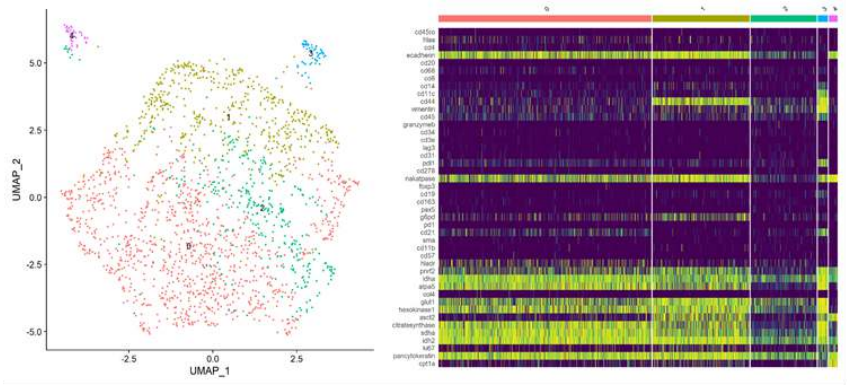


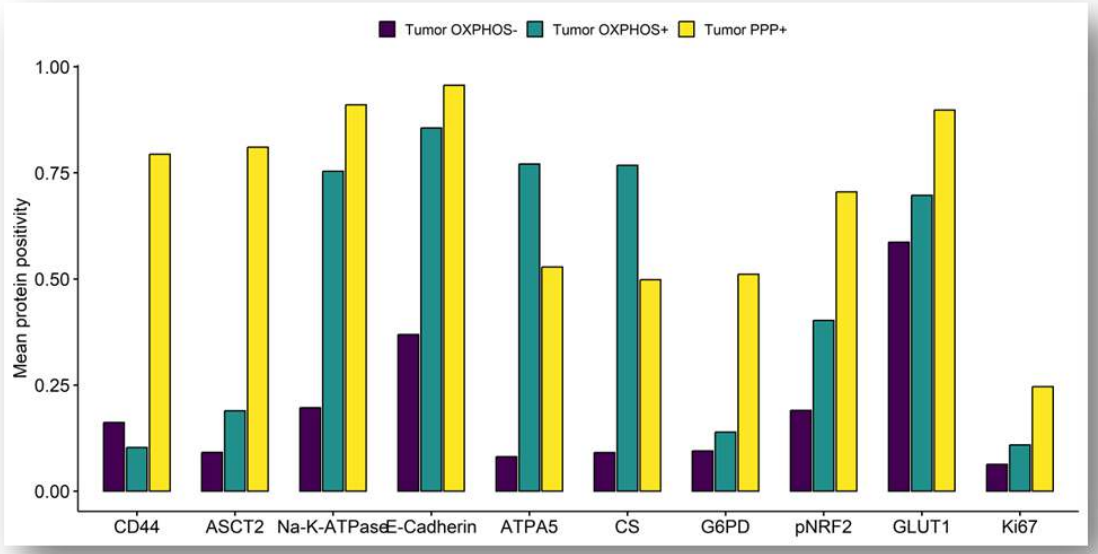
Unsupervised Cell Subtyping Identified 43 Cell Subsets, Mostly Segregated by their Metabolic state

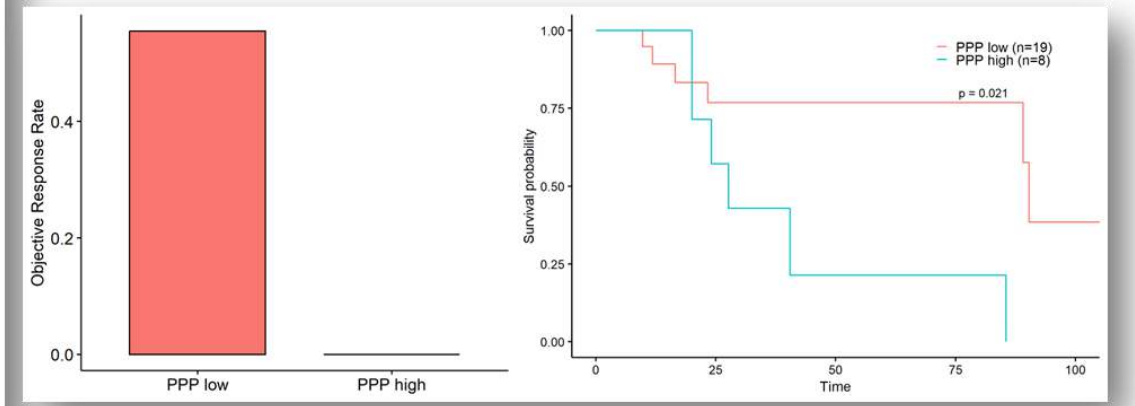


Unsupervised Clustering Identified a Tumor Metabolic State which is Associated with Immunotherapy Resistance

- Tumor cells clustered to three main metabolic states - OXPHOS+, OXPHOS- and a third cluster (PPP+), which was characterized by upregulation of ASCT2, a glutamine transporter, as well as pNRF2 and G6PD, regulators of the pentose phosphate pathway.
- These cells exhibited higher proliferation rate and upregulation of CD44, a tumor stemness marker
- Tumors with high content of PPP+ tumor cells (>40%) were resistant to PD-1 blockade and showed reduced overall survival (OS) rates







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