The Proteomic Atlas of the tumor niche architecture of HER2+ breast cancer in response to neoadjuvant TCHP treatment

MAYO CLINIC

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ABSTRACT

BACKGROUND
The standard of care for early stage HER2+ breast cancer is neoadjuvant TCHP (Taxane, cyclophosphamide, Herceptin, plus Pertuzumab). Approximately 1/3 of such patients derived clinical benefit, as evidenced by pathological complete response (PCR). There is a compelling need to understand the biology that underlies response or failure to respond. Such an understanding is central to the development of alternative therapeutic strategies. To this end we propose to carry out proteomic investigation of the stromal and intraepithelial (tumor niche) compartments of early stage HER2+/ER-tumors using the GeoMx® Digital Spatial Profiling (DSP) and the novel human Immuno Oncology Proteome Atlas (IPA) 500, featuring >500 antibodies coupled to photocleavable DNA barcodes with NGS sequencing readout.

METHODS
We will use the human GeoMx IPA 500 panel to evaluate the proteomic architecture in the tumor niche and the stroma niche in early stage HER2+(IHC3+)/ER- tumors. We will analyze 6 samples, 3 that underwent PCR in response to TCHP and 3 that did not undergo PCR. Differential expression of proteins will be evaluated using the linear mixed model to identify features that are associated with response to TCHP and to evaluate the immune microenvironment of those samples that retained residual cancer burden after therapy.

RESULTS
The 570-plex Immuno Oncology Proteome Atlas (IPA) provides the ability to interrogate the spatial distribution of protein abundance at a depth that has heretofore been unobtainable. This pilot analysis provides novel insight into proteomic features that are associated with favorable response to standard of care therapy in HER2+ breast cancer. Such features may inform prognostic or predictive indicators of outcome and may identify potential therapeutic targets that can be exploited to maximize benefit from neoadjuvant TCHP.

CONCLUSIONS
Analysis of the spatial distribution of protein expression at a depth that has been heretofore unobtainable provides novel insight into the baseline biology of early stage HER2+ cancer. We further demonstrate the power of the combination of the GeoMx DSP and curated human IPA 500to enable more holistic discovery biology by rapidly evaluating 100s of critical potential therapeutic targets on individual tumor and stromal niches

EXPERIMENTAL DESIGN

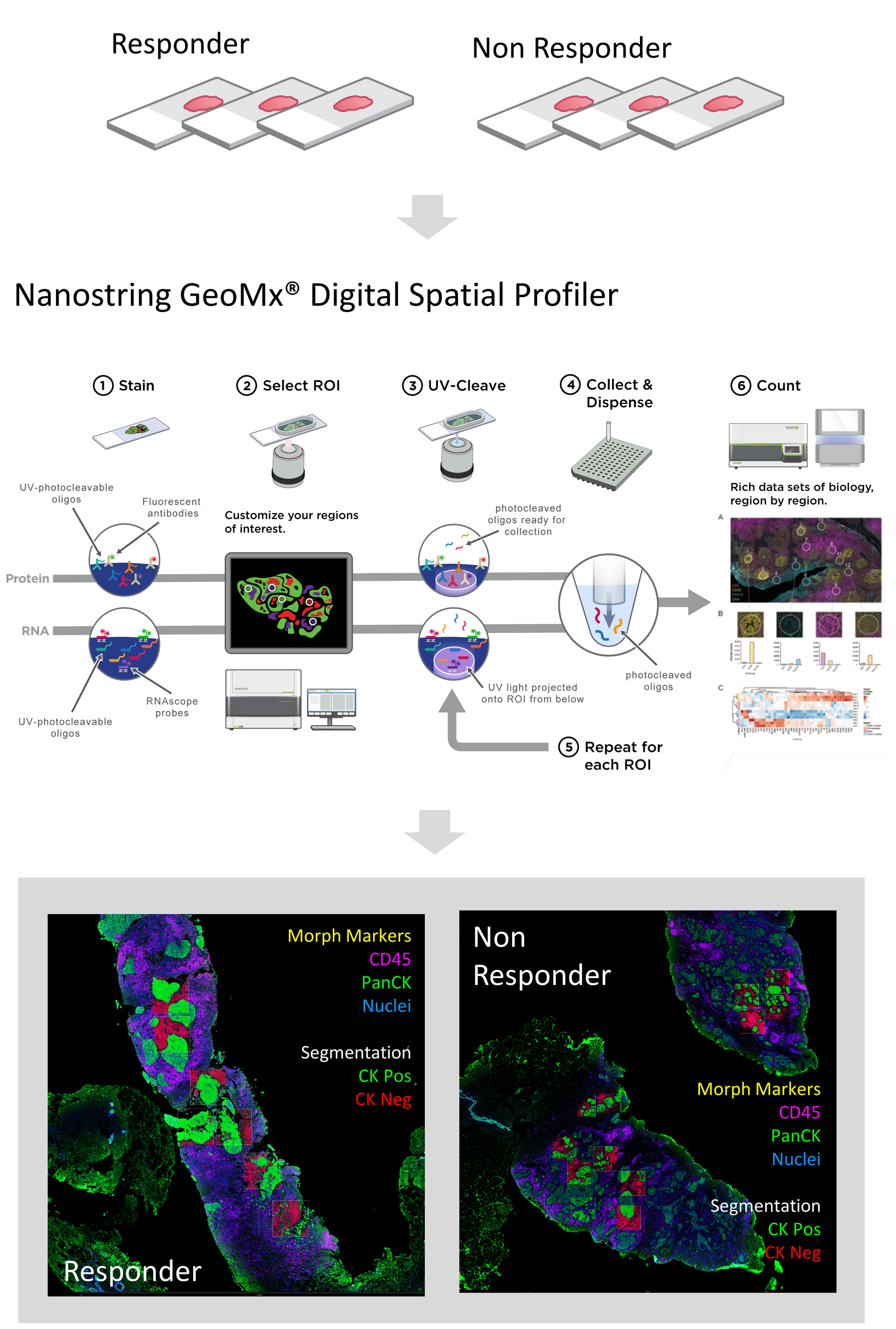

Figure 1: Samples were prepared using the standard GeoMx workflow with the GeoMx Human Immuno Oncology Proteome Atlas and Universal Core and fluorescent morphology markers for CD45 (magenta), PanCK (green) and nuclei (blue). Samples were segmented on the GeoMx DSP for PanCK positive (green) and PanCK negative (red) areas of interest (AOI).

REAGENTS

Nanostring GeoMx® Immuno Oncology Proteome Atlas

570+ IO PROTEOME ATLAS GeoMx DSP

Pathway	# Proteins
Activating Invasion and Metastasis	228
Avoiding immune Destruction	234
Deregulating Cellular Energetics	199
Enabling Replicative Immortality	121
Evading Growth Suppressors	92
Genome Instability & Mutation	139
Inducing Angiogenesis	90
Resisting Cell Death	83
Sustaining Proliferative Signaling	270
Tumor-promoting Inflammation	218

Oct-2	CD66b	Granzyme B	Mannose Receptor	Rho
Oct4	CD68	Granzyme K	M-CSF	Rho A + B + C
15 Lipoxygenase 1	CD70	GRP78 BiP	MDM2 (phospho S166)	ROCK1
15-PGDH	CD73	GSK3 beta	MEK1	ROCK2 + ROCK1
2B4	CD74	GSK3 beta (phospho S9)	MEK1 (phospho S298)	ROS1
5 Lipoxygenase/5-LO	CD79a	HDAC1	MEK2	RPS6 (phospho S235 + S236)
ACE2	CD79b	HDAC2	MEK4/MKK4 (phospho S80)	RPS6 (phospho S240 + S244)
Actin	CD8 alpha	HDAC3	MEKK3	RSK1 p90
active YAP1	CD83	HDAC4 + 5 + 9	MelanA	RSK1 p90 (phospho T359)
Adenosine Receptor A2a	CD86	HDAC6	Melanoma gp100_1	RUNX1 / AML1 + RUNX3 + RUNX2
AIF	CD90 / Thy1	HDAC9	Met (c-Met)	S100 beta
AKR1B10	CD94	Heme Oxygenase 1	Met (c-Met) (phospho Y1349)	S100A12/CGRP
AKT1 (phospho S473)	CD99	Hexokinase 1	MICA + MICB	S100A4
AKT1 (phospho T450)	CDK1 (phospho T161) + CDK2 / CDK3 (phospho T160)	Hexokinase II	MLH1	S100A8 + S100A9
AKT3 + AKT2 + AKT1	CDK1 + Cdk2 + Cdk3 (phospho T14)	HIF-1 alpha	MMP1	S100A9
ALDH1A1	CDK1 + CDK2 + CDK3 + CDK5 (phospho Y15)	Histone H2A (acetyl K5)	MMP14	S100P
Aldolase	CDK1_1	Histone H2A (phospho T120)	MMP3	S6K1
Aldolase B+Aldolase C	Cdk2	Histone H2A.X	MMP7	Scavenging Receptor SR-BI
ALK	Cdk4	Histone H2B (acetyl K5)	MMP8	SCF
alpha smooth muscle Actin	Cdk6	Histone H3 (acetyl K14)	MMP9	SDHA
AMPK alpha 1	CDKN2A/p14ARF	Histone H3 (acetyl K18)	Monoamine Oxidase A/MAO-A	SFRP1
Androgen Receptor	CDKN2A/p16INK4a	Histone H3 (acetyl K27)	MRP8	SFRP4
APC	c-Fos	Histone H3 (acetyl K9)	MS4A2	SHP1
APG5L/ATG5	Chk1	Histone H3 (di methyl K4)	MSH2	SHP2
Argonaute-2	Chk2	Histone H3 (di methyl K79)	MSH6	SIGLEC5
ARID1A	cIAP2	Histone H3 (mono methyl K4)	mTOR	SIRT1
ARID3A	c-Jun	Histone H3 (phospho S10)	mTOR (phospho S2448)	SIRT1 (phospho S47)
ASS1	c-Jun (phospho S63)	Histone H3 (phospho S28)	MUC1	SIRT2
ATF2	c-Kit	Histone H3 (tri methyl K27)	Mucin 5AC	SIRT3
ATF3	Cleaved PARP1	Histone H3 (tri methyl K36)	Musashi 1 / Msi1	SLC7A5/LAT1
ATG12	c-Maf	Histone H3 (tri methyl K9)	MX1	Smac/Diablo
ATG7	c-Myb (phospho S11)	HLA A	MyD88	SMAD1 + SMAD5 + SMAD9 (phospho S463 + S465 + S467)
ATM	c-Myc	HLA B7	Myeloperoxidase	Smad2
ATM (phospho S1981)	c-Myc (phospho S62)	HLA E	N Cadherin	Smad2 (phospho S255)
ATR (phospho S428)	Collagen I	HLA F	NAK/TBK1	Smad2 (phospho S467)
Aurora A	Collagen IV	HLA G	Nanog	Smad3
Axl	COX2 / Cyclooxygenase 2	HLA-C	NCAM1	Smad3 (phospho S423 + S425)
Bad	CPT1A	HLA-DPB1	NCR1	Smad4
Bak	CREB	HLA-DR	Nectin 2	SMAD5
Bax	CREB (phospho S133)	HMGB1	Nectin-4	SOCS3
Bcl-2	CREBBP	HMGB2	Neutrophil Elastase	SOD2/MnSOD
Bcl6	CSF-1-R	Hsp27	NFAT2	SOD2/MnSOD (acetyl K68)
Bcl-XL	CTLA4	Hsp27 (phospho S78)	NFkB p105 / p50	Somatostatin Receptor 2
Beclin 1	Cullin 1/CUL-1	Hsp70	NF-kB p65	Sonic Hedgehog
beta 2 Microglobulin	CXCL10/IP-10	Hsp90	NKG2A + NKG2E + NKG2C	SOX9
beta Actin	CXCL5 + CXCL6	HuR / ELAVL1	NKG2D	SQSTM1 / p62
beta Arrestin 1	CXCR3	ICAM1	Notch1	Src
beta Catenin	CXCR4	ICOS	NQO1	ST2
beta glucuronidase (GUSB)	CXCR5	IDH1	NRF1	STAT1
beta Tubulin	CXCR6	IDH2	NSD3	STAT1 (phospho S727)
BFL-1/GRS	Cyclin A2	IFIT1	Occludin	STAT2
Bid	Cyclin B1	IFNGR1	Osteopontin	STAT3
Bim	Cyclin D1	IGF1 Receptor	p27 KIP 1	STAT3 (phospho Y705)
BRAF	Cyclin E1	IGF2BP1/IMP1	p27 KIP 1 (phospho S10)	STAT5 (phospho Y694)
BRCA1	Cyclin E2	Ikaros	p38 (phospho T180)	STAT5a
Brd4	Cyclophilin A	IKB alpha	p38 beta/MAPK11 + p38 alpha/MAPK14	STAT5b
BRG1	Cytokeratin 1	IKK alpha	p40 - DeltaNp63	STAT6
BTK	Cytokeratin 14	IKK beta	p53	STAT6 (phospho Y641)
C Reactive Protein	Cytokeratin 17	IKK gamma/NEMO	p53 (acetyl K373)	STING
C4a	Cytokeratin 19	IKKi/IKKe	p53 (phospho S392)_2	STK3/MST-2
C5 / C5b	Cytokeratin 5	IL-1 alpha	p53 (phospho S46)	Sumo 1
Carbonic Anhydrase 3/CA3	Cytokeratin 8	IL-1 beta	p57 Kip2	Survivin
Carbonic Anhydrase 9/CA9	Cytokeratin 8 (phospho S431)	IL-12A	p63	Syk
Caspase-10/CASP-10	Dihydrofolate reductase (DHFR)	IL-12RB1	p73	Syk (phospho Y323)
Caspase-3	Dnmt1	IL-15RA	PAK1	Syk (phospho Y352) +ZAP70 (phospho Y319)
Caspase-3 p12	DR5	IL-18	PAK1+PAK2+PAK3 (phospho S141 + S144 + S154)	Syndecan-1
Catalase	DUSP6	IL-1RA	pan Cytokeratin	TAK1
Cathepsin G	E Cadherin	IL-2 Receptor alpha	Pan Trk	TAP2
Cathepsin K	EGFR	IL22 RA2/IL-22BP	PARP1	T-bet / Tbx21
Cathepsin S	EGFR (phospho Y1068)	IL-22RA1	PCNA	TBR2 / Eomes
Caveolin-1	EMR1/ADGRE1	IL-2RG	PD1	TEF1/TEAD-1
CCL18	ENO1 + ENO2 + ENO3	IL-33	PDGF B	TGF beta 1
CCR3	eNOS	IL3RA/CD123	PDGFR alpha	Thymidylate Synthase
CCR5	EpCAM	IL4	PDGFR alpha + PDGFR beta	TIM 3
CCR6	ErbB2 / HER2	IL-5RA	PD-L1	Tissue Factor
CCR7	ErbB2 / HER2 (phospho Y877)	IL-6R	PD-L2	TLR7
CD103	ErbB3 / HER3	IMP3	Periostin	TLR8
CD11a	ErbB4 / HER4 (phospho Y1162)	Indoleamine 2 3-dioxygenase	PF4	TLR9
CD11b	ErbB4 / HER4+ErbB2 / HER2	iNOS	PI 3 Kinase catalytic subunit alpha/PIK3CA	TMPRSS2
CD11c	ERG	Insulin	PI 3 Kinase catalytic subunit gamma/PI3K-gamma	TMS1/ASC
CD127	ERK1	Insulin Receptor alpha	PI 3 Kinase p85 alpha	TNF alpha
CD133	ERK1 (phospho T202 + Y204) + ERK2 (phospho T185 + Y187)	Insulin Receptor beta	PI 3 Kinase p85 beta	TNFAIP3
CD134 / OX40L receptor	ERK1 (phospho T202) + ERK2 (phospho T185)	Integrin alpha 5	PKC beta 1	Topoisomerase I
CD14	ERK2	Integrin alpha 6	PLCG 2	Topoisomerase II alpha
CD15	ETS1	Integrin beta 1	Podoplanin / gp36	Topoisomerase II alpha (phospho S1106)
CD16_1	FABP4	Interferon gamma	Poliovirus Receptor/PVR	Topoisomerase II alpha (phospho T1343)
CD163	FADD	IRF3	PP2A alpha + beta	TRAF1
CD177	FAK	IRF4	PRAME	TRAF2
CD18	FANCI	IRF5	PRAS40	TRAF6
CD20	Fas	ISG15	PRDM1/Blimp1	Transferrin Receptor
CD22	Fatty Acid Synthase	JAK3	Pro Caspase-8	TROP2
CD27	FBP2	JNK1 + JNK2 + JNK3 (phospho T183+T183+T221)	Progesterone Receptor	Tuberin
CD272/BTLA	FGF2	JunD	Progesterone Receptor (phospho S190)	Tuberin (phospho S1254)
CD276	FGFR2	KDM3A / JHDM2A	Prostate Specific Antigen	Tuberin (phospho T1462)
CD28	Fibronectin	Ki67	PSMA	UBE2C
CD3 epsilon	Fos B	KIR2DL1 + KIR2DL2	PTCH2	Ubiquitin
CD31	FOXO1A	KIR2DL3	PTEN	uPA Receptor/U-PAR
CD33	FOXO4/AFX	KIR3DL1 + KIR3DS1	PTEN (phospho T366)	Urokinase
CD34	FOXP3	KMT6 / EZH2	PTGES2/Gbf1	VCAM1
CD36	Galectin 3	KMT6/EZH2 (phospho T487)	PU.1/Spi1	VEGF Receptor 1
CD38	gamma H2A.X (phospho S139)	Lactate Dehydrogenase_1	Rad51	VEGF Receptor 2
CD39	GATA3	LAG-3	RAGE	VEGFA
CD3D	GGT1/GGT	Lamin A + Lamin B1 + Lamin C	Raptor	VEGFD
CD3G	GITR	LAMP1	Ras	Vimentin
CD4	GLB1/Beta-galactosidase	LAMP2A	Rb	VISTA
CD40	GLP-1	LC3B	Rb (phospho S608)	Von Willebrand Factor
CD40 Ligand	GLP-1R	Lck	Rb (phospho S807)	Wee1
CD44	Glucocorticoid Receptor (phospho S226)	LDL Receptor	Rb (phospho T252)	Wee1 (phospho S642)
CD44v6	Glucose 6 Phosphate Dehydrogenase	LEF1	Rb (phospho T356)	Wilms Tumor Protein
CD45	Glucose Transporter GLUT1	LILRB3	Rb (phospho T373)	Wnt3a
CD45RA	Glutaminase	Liver Arginase	Rb (phospho T780)	YAP1
CD47	Glutathione Peroxidase 4	LYVE1	Rel B	YAP1 (phospho S127)
CD63	GNLY/Granulysin	Macrophage Inflammatory Protein 1 alpha / CCL3	Ret	YKL-40/CHI3L1
CD64	Granzyme A	Macrophage Inflammatory Protein 3 alpha	Retinoic Acid Receptor beta	ZAP70

FUNDING STATEMENT

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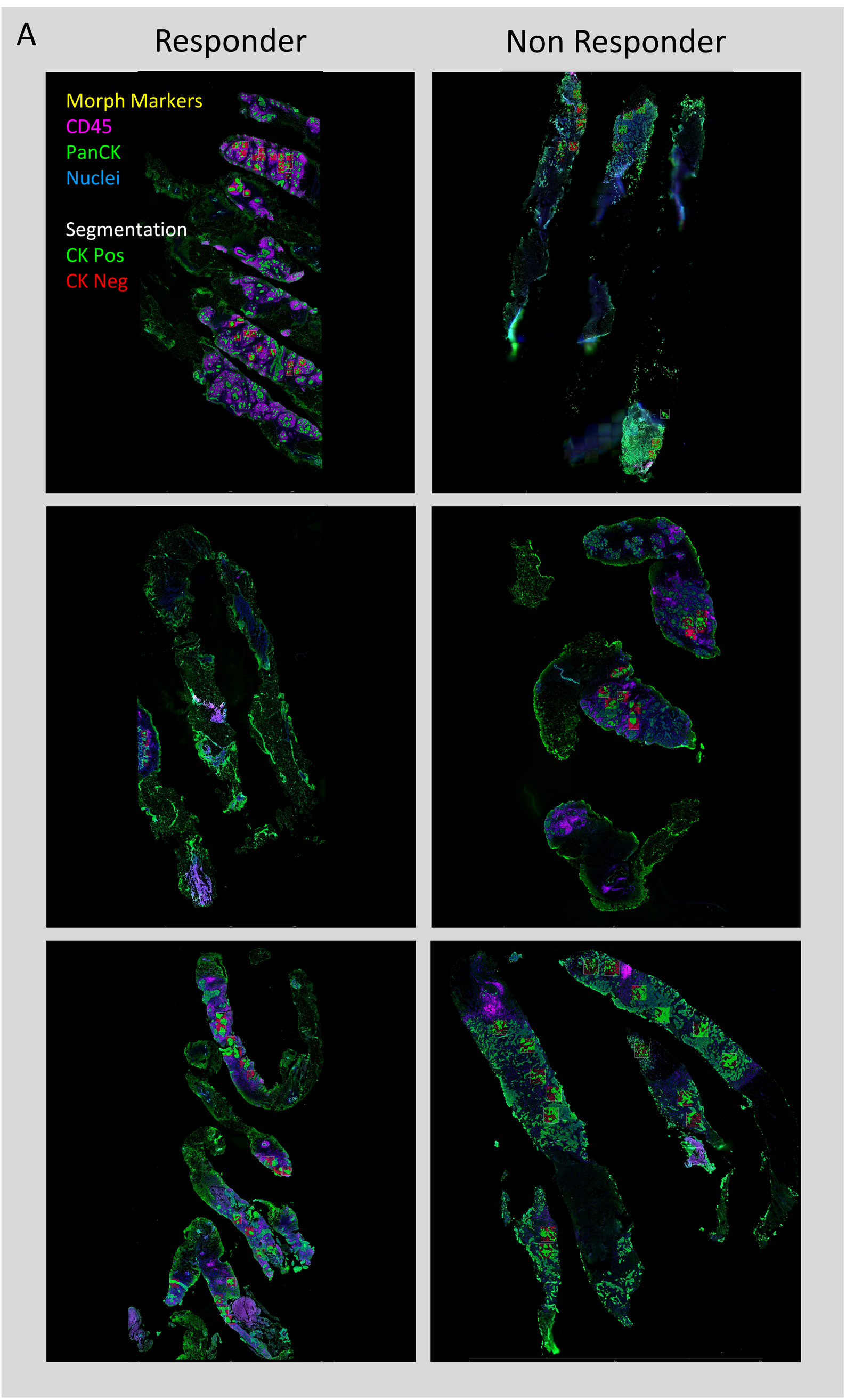
REFERENCES

1. Carter JM, et al. Clin Cancer Res. 2021 Oct 15;27(20):5628-5637. PMID: 34108182
2. Carter JM, et al. Nat Commun. 2023 Apr 18;14(1):2215. PMID: 37072398

IO PROTEOME ATLAS SURVEY OF TUMOR & STROMA NICHES ACROSS THERAPEUTIC RESPONSES



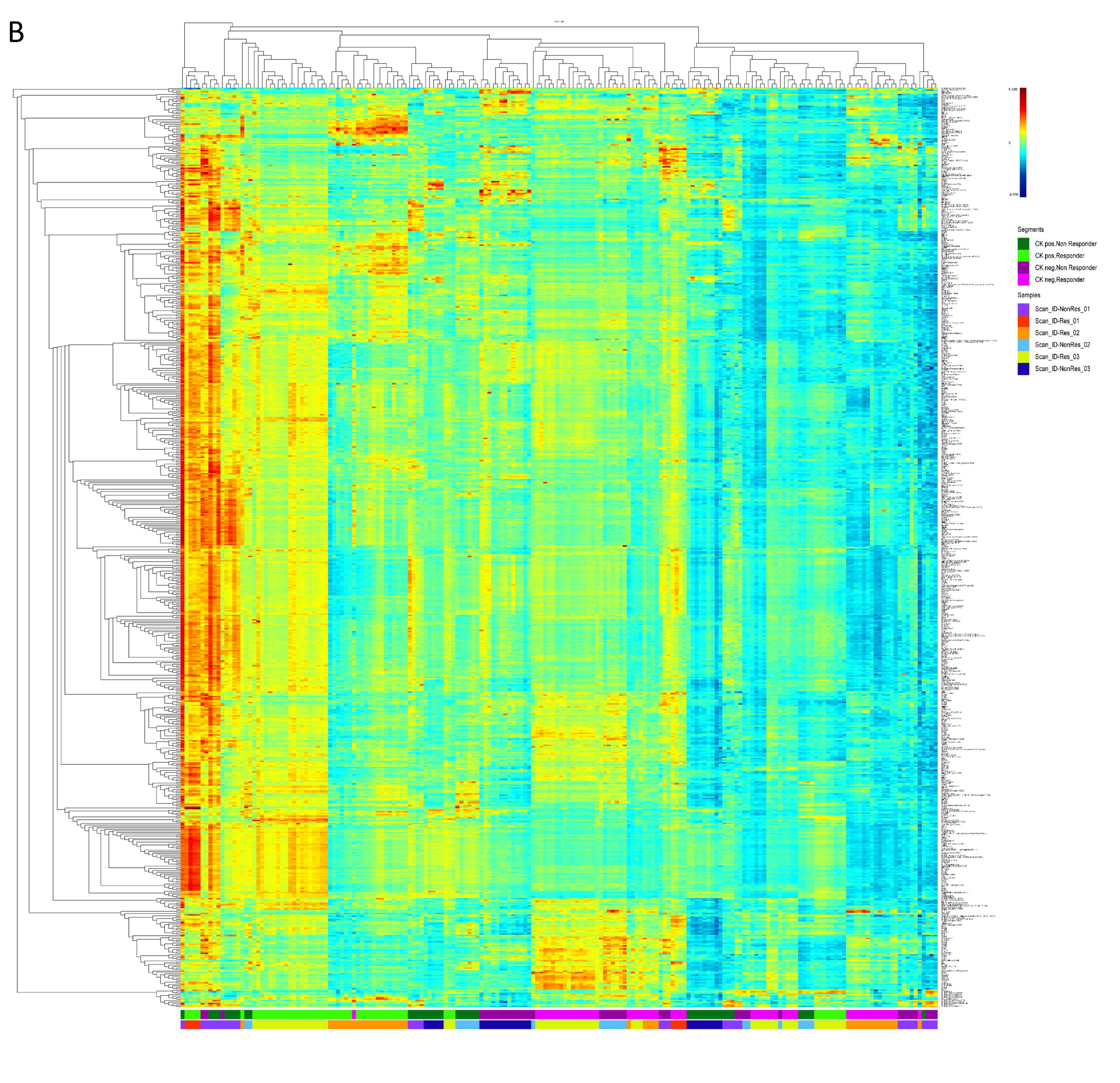
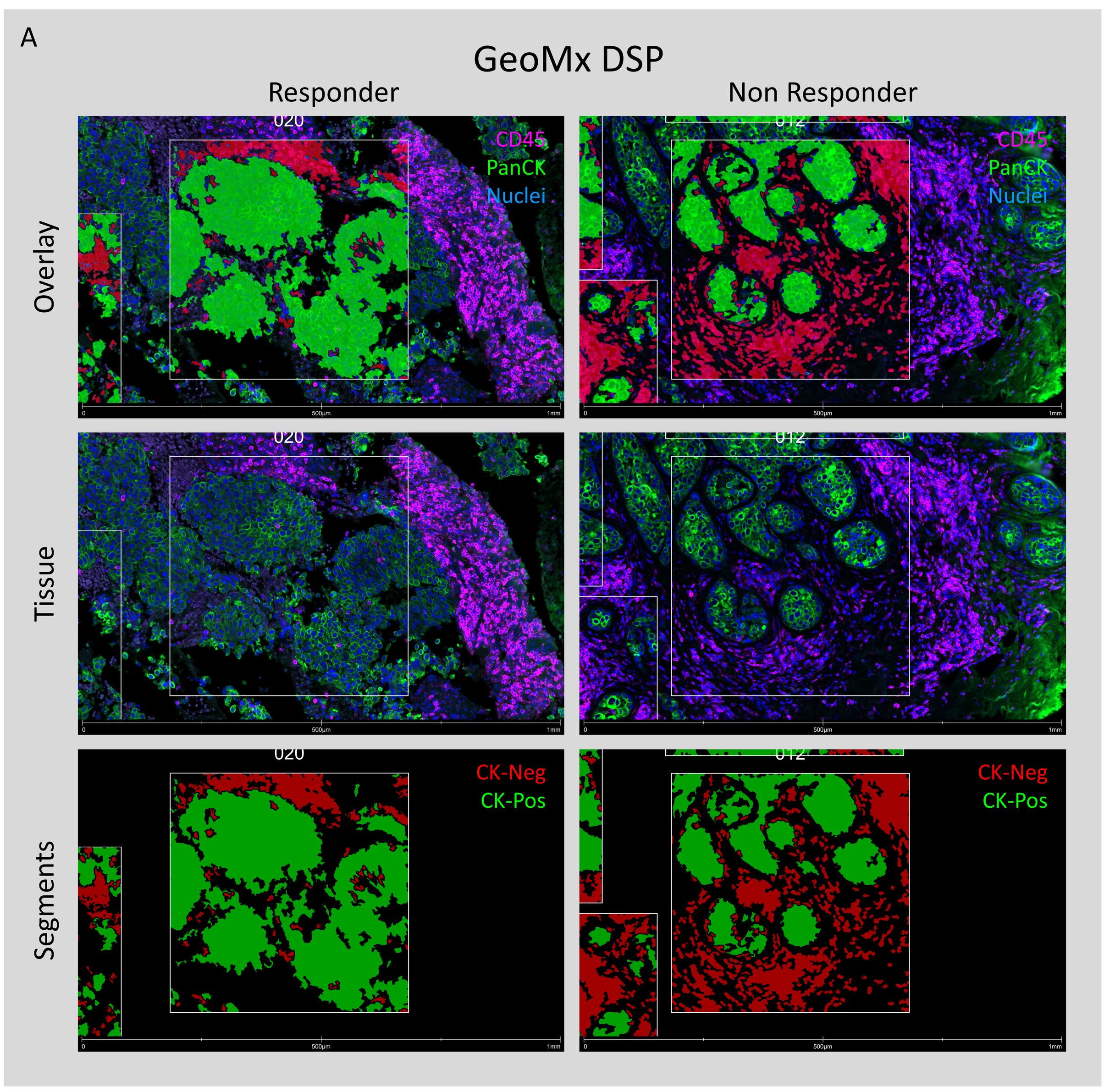


Figure 2: A) Total study ROI/AOI selection on GeoMx images with fluorescent morphology markers for CD45 (magenta), PanCK (green) and nuclei (blue). Samples were segmented for PanCK positive (green) and PanCK negative (red) areas of interest (AOI). **B)** Samples were QC'd and housekeeper normalized as previously described (1,2) prior to B) unsupervised hierarchical clustering of all sample AOIs.

SPATIAL PROTEOMICS OF DEMONSTRATES DISTINCT ERBB SIGNALING PATTERNS IN SAMPLES THAT EXHIBIT PCR IN RESPONSE TO TCHP



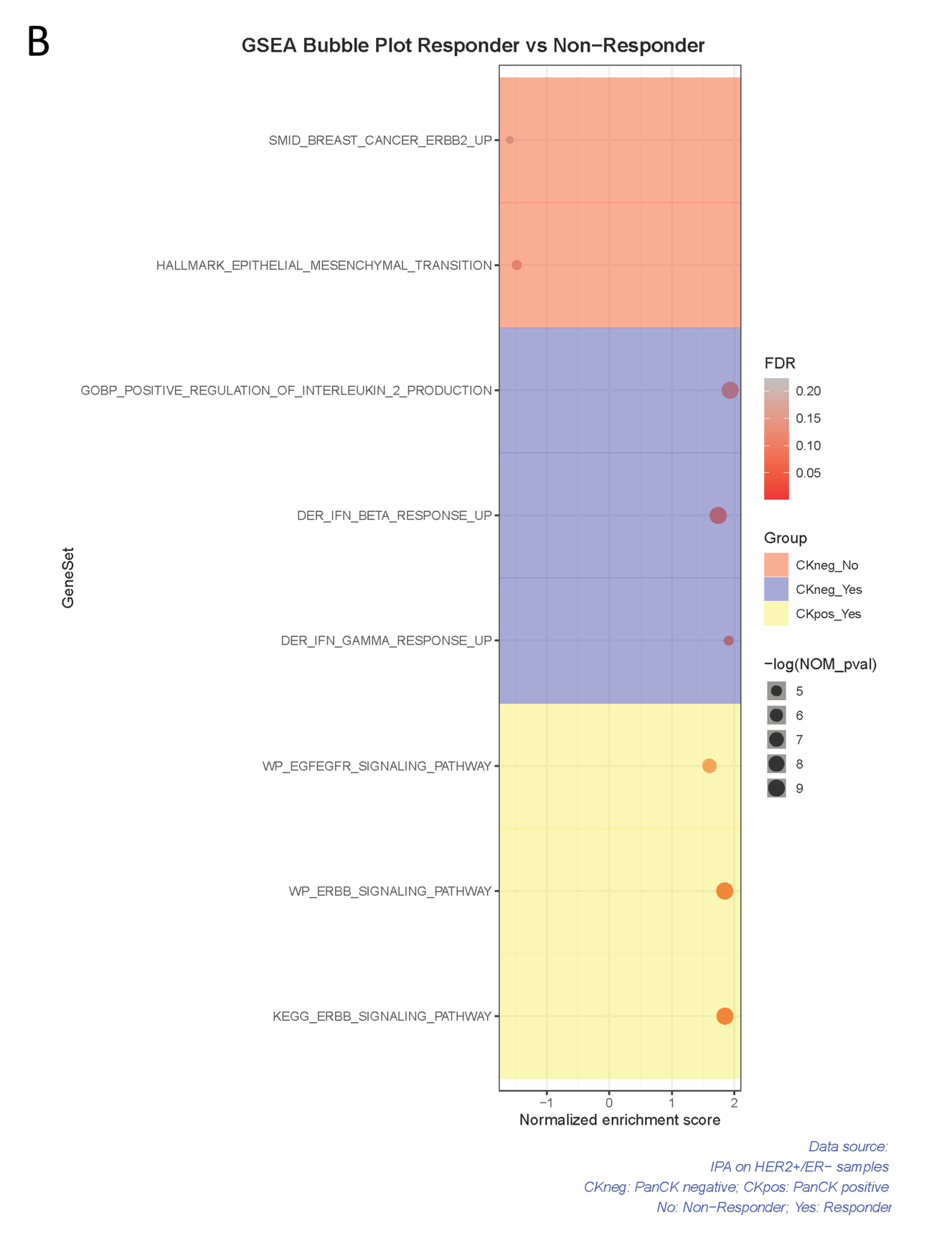
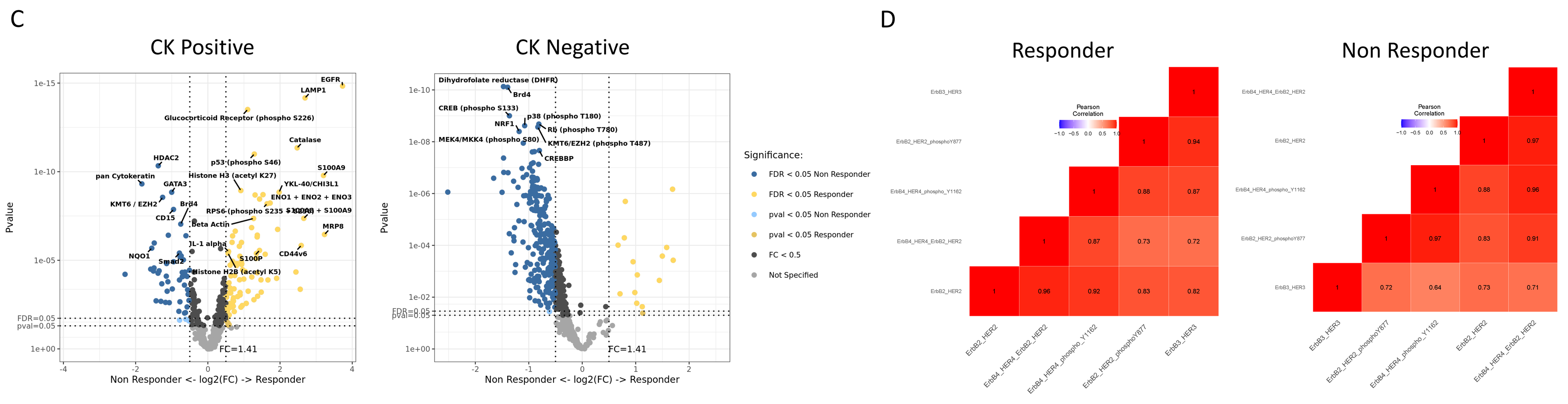
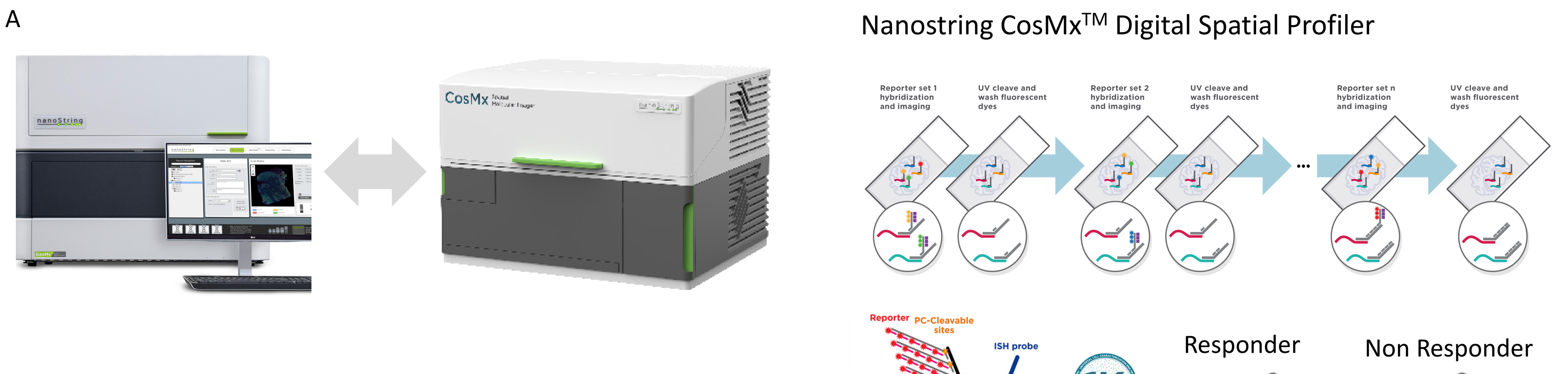
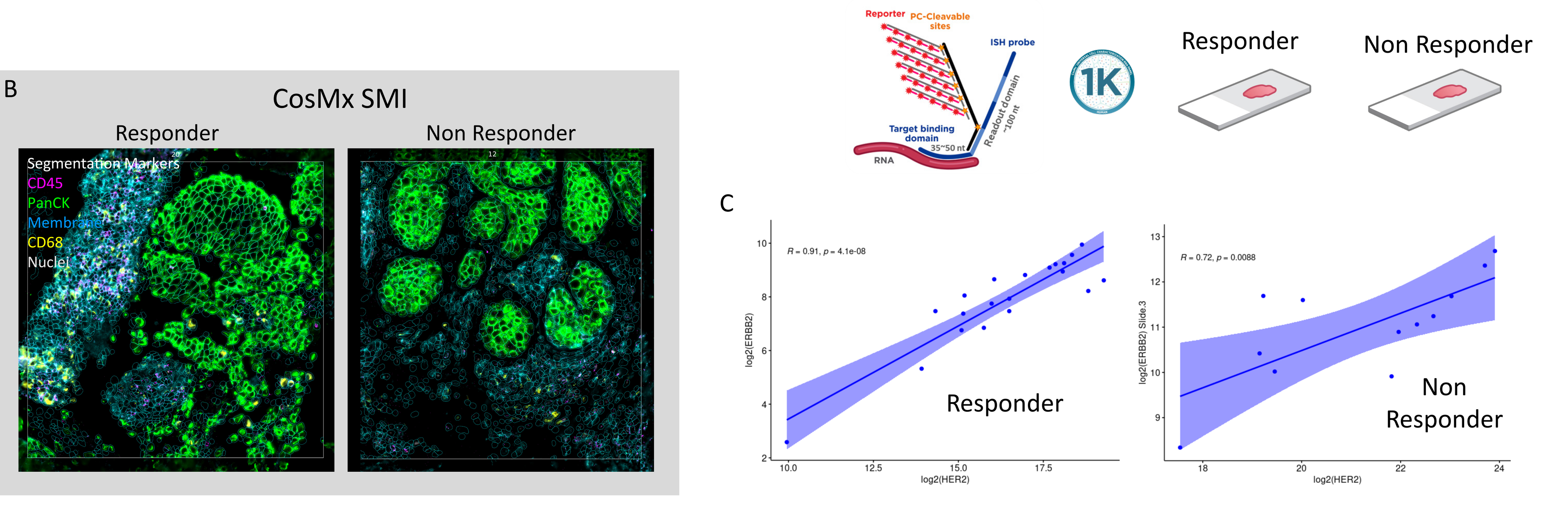


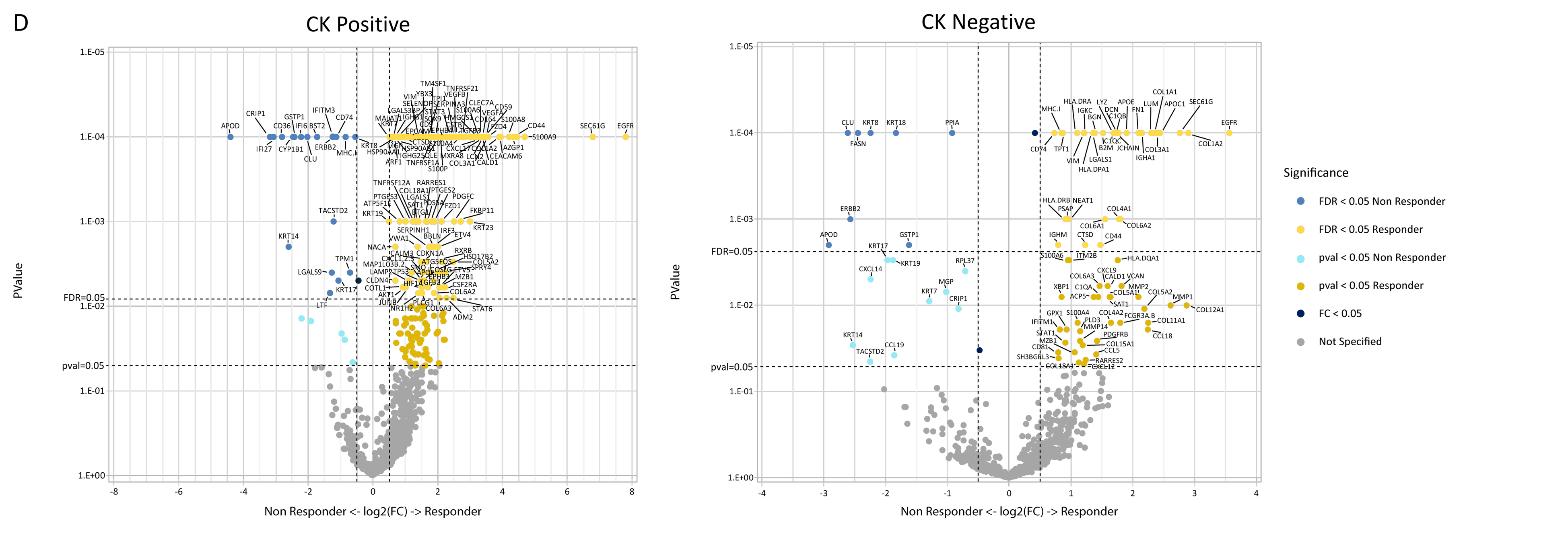
Figure 3: A) GeoMx images with fluorescent morphology markers for CD45 (magenta), PanCK (green) and nuclei (blue). Samples were segmented on the GeoMx DSP for PanCK positive (green) and PanCK negative (red) areas of interest (AOI). **B)** GSEA reveals enrichment of ERBB signaling features in samples that exhibited pCR in response to TCHP. **C)** Differential expression as a function of response (pCR) in the CK+ and CK- niches. **D)** ERBB signaling features are highly correlated in both responders and non-responders.



SPATIAL NICHE PROTEOMICS CORRESPONDS WITH SINGLE CELL TRANSCRIPTOMICS







CONCLUSIONS

The IPA panel allows for high sensitivity quantification of 570 protein targets with spatial resolution in a single 5-micron FFPE sample. The ability to use this high-plex panel in routine clinical samples (e.g. diagnostic biopsies) promises to enable interrogation of the proteomic landscape of both normal and pathological samples at a level of spatial resolution and analytical depth that has not previously been possible.

Figure 4: A) Samples were interrogated with the CosMx Human Universal Cell Characterization RNA Panel **B)** CosMx images of tumor and stroma niches with segmentation markers: CD45 (magenta), PanCK (green), Cell Membrane (cyan), CD68 (yellow), nuclei (grey) **C)** HER2 protein in the CK+ niche (IPA) correlates with ERBB2 mRNA in the tumor niche (CosMx). **D)** Differential expression as a function of response (pCR) in the CK+ and CK- niches.