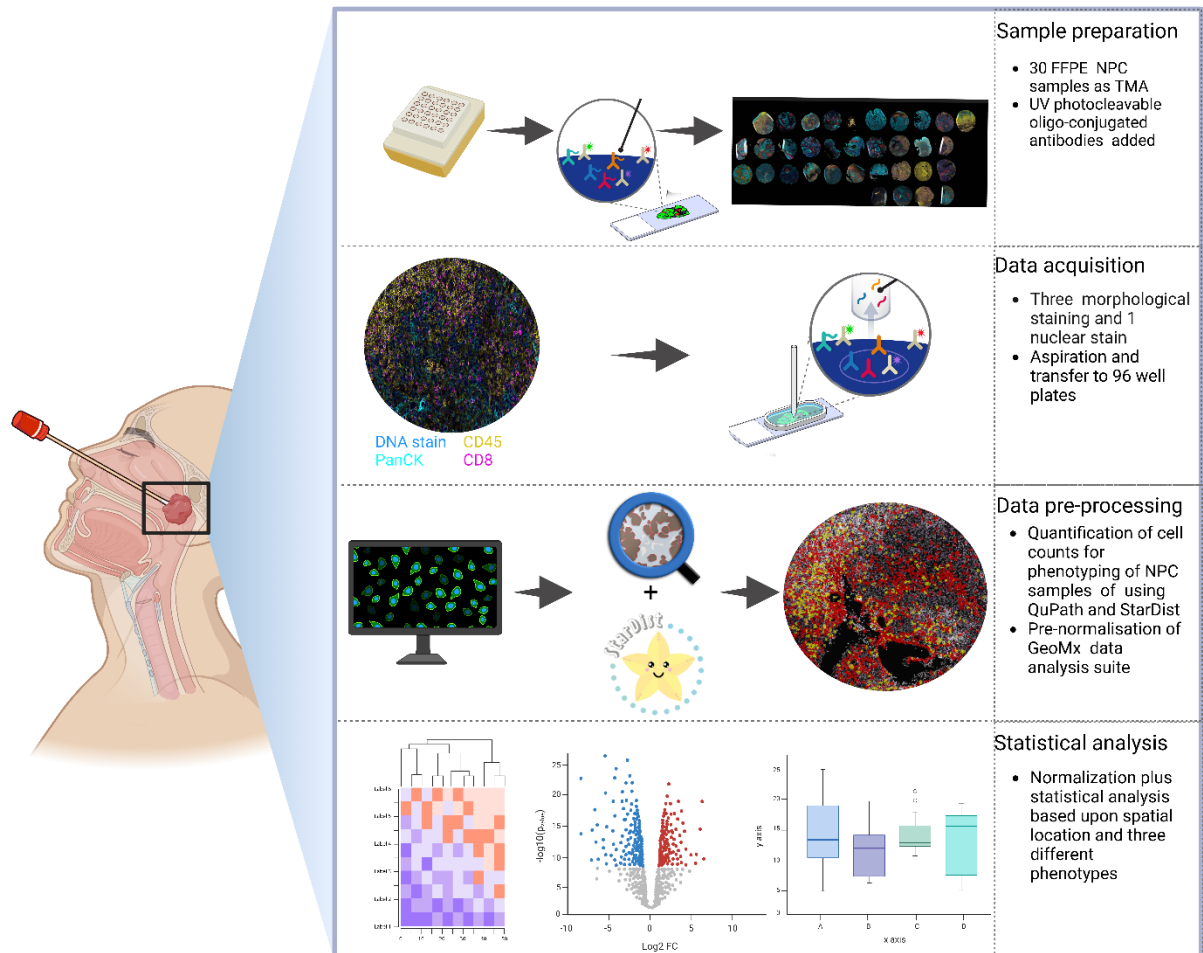# Supplementary Figures and Tables

Figure S1. Experimental setup. Tissue morphology for the 30 FFPE NPC samples was investigated using PanCK, CD45, CD8, and nuclear staining. ROIs were selected and further profiled with a 43-plex oligo-conjugated antibody cocktail using the GeoMx® DSP system. Protein expression profiles were further analysed based on their spatial distribution in the three tumor immune phenotypes ('inflamed', 'immune-excluded', and 'desert').
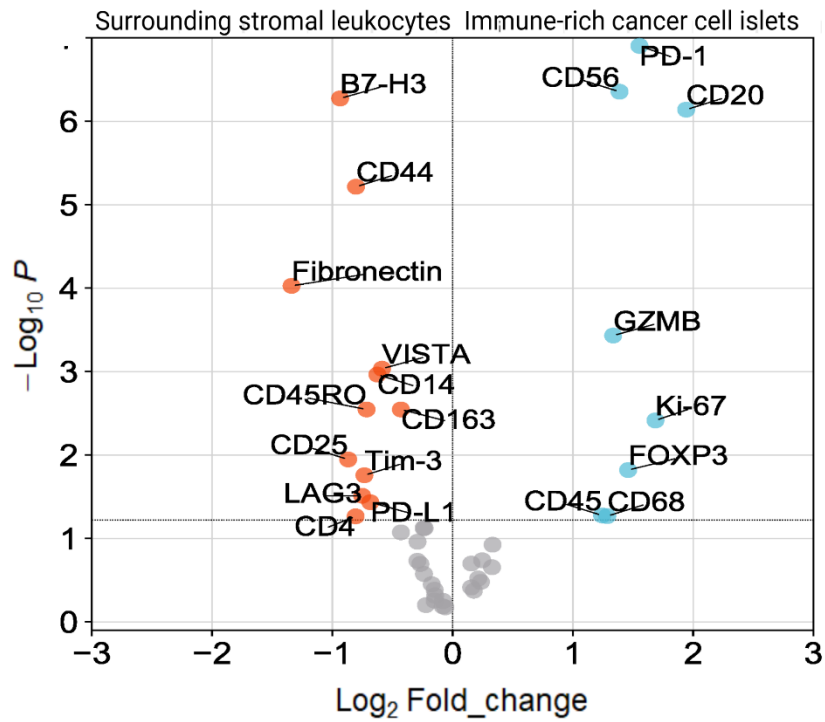
Figure S2. Two-group comparison in areas of interest. Volcano plot showing the differentially expressed proteins between the regions 'immune-rich cancer cell islets' and 'surrounding stromal leukocytes'. The values are based upon adjusted p-values, (Mann-Whitney test, Benjamini, Krieger, and Yekutieli correction, adjusted p-value<0.05).
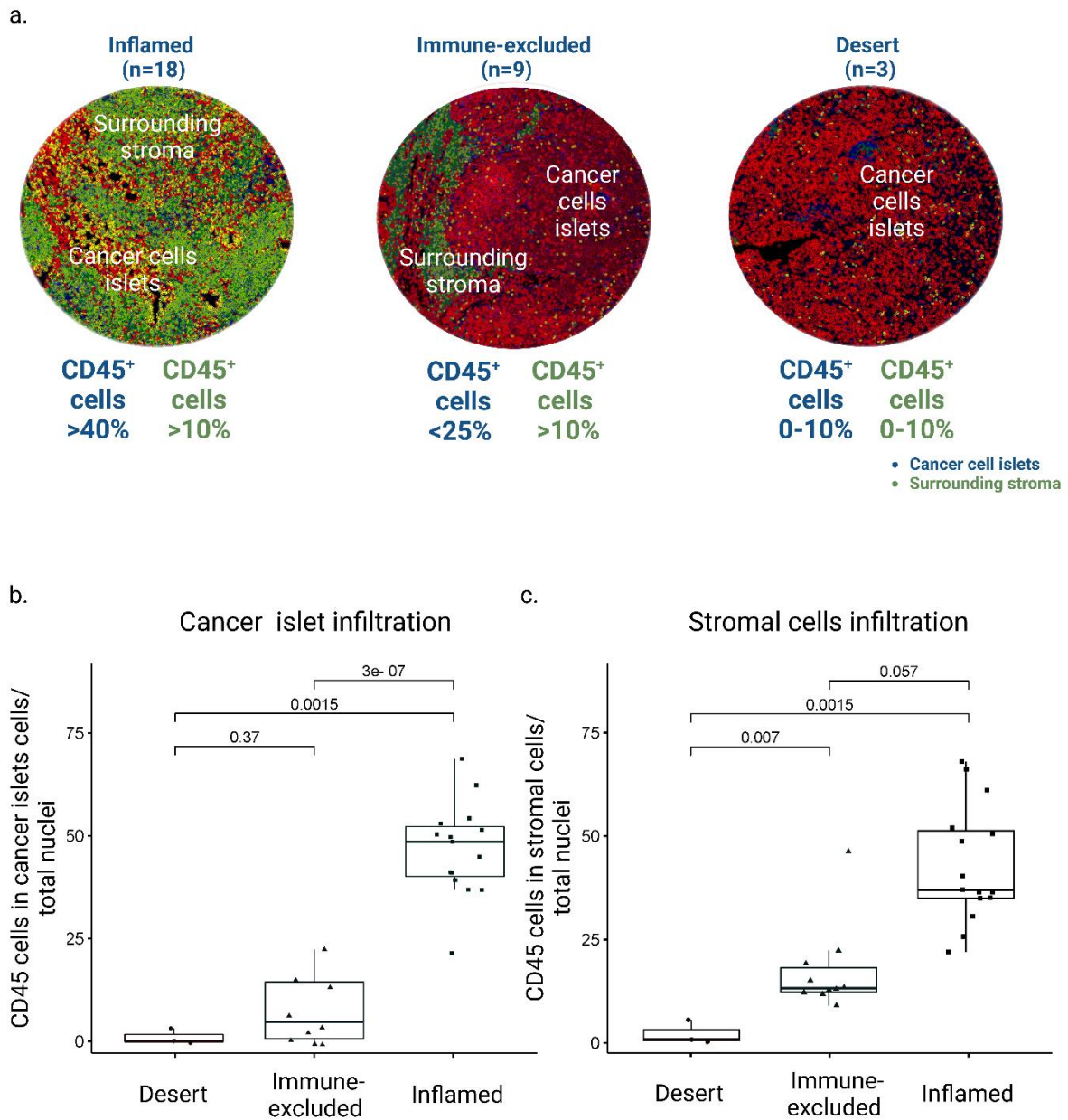
Figure S3. Quantification of CD45$^+$ cells in NPC biopsies. (**a**). Quantification of the CD45$^+$ and CD8$^+$ cells correlating with the phenotypes. The text below the figures depicts the percentages on which the classification was based. Box plots showing the percentage of CD45$^+$ cells in (**b**) the 'immune-rich cancer cell islets' and (**c**) 'surrounding stromal leukocytes' regions, respectively, within the three phenotypes ('inflamed', 'immune-excluded', and 'desert') (Kruskal-Wallis test, p-value<0.05).
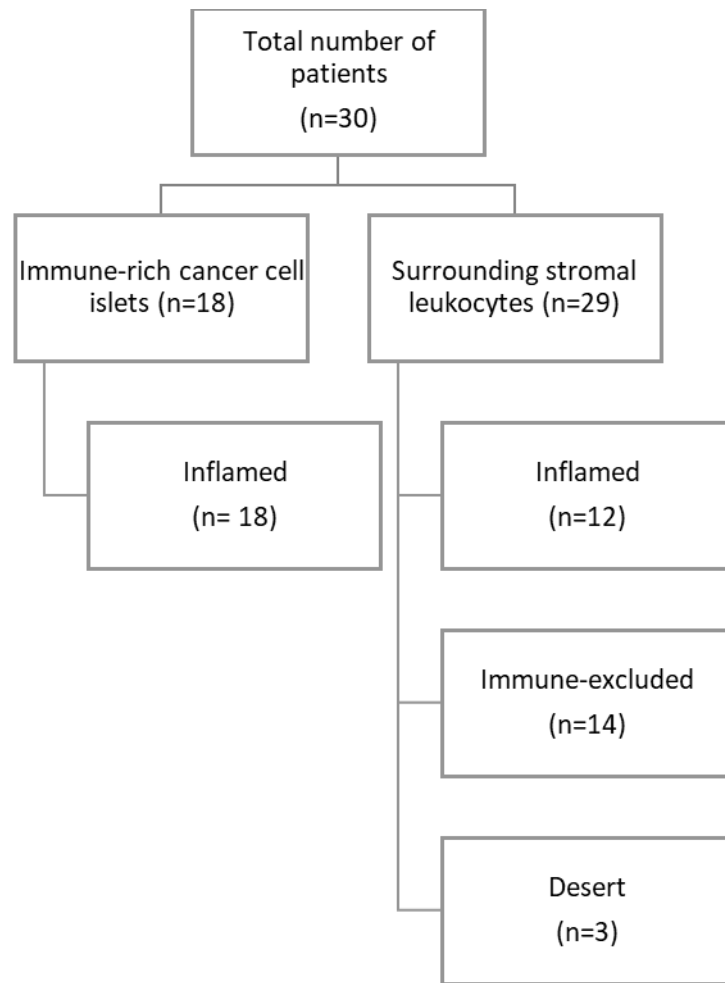
Figure S4. **Distribution of obtained areas of interest**. Different regions ('immune-rich cancer cell islets' and surrounding stromal segments') and their further division as seen in the identified phenotypes ('inflamed', 'immune-excluded' and 'desert').
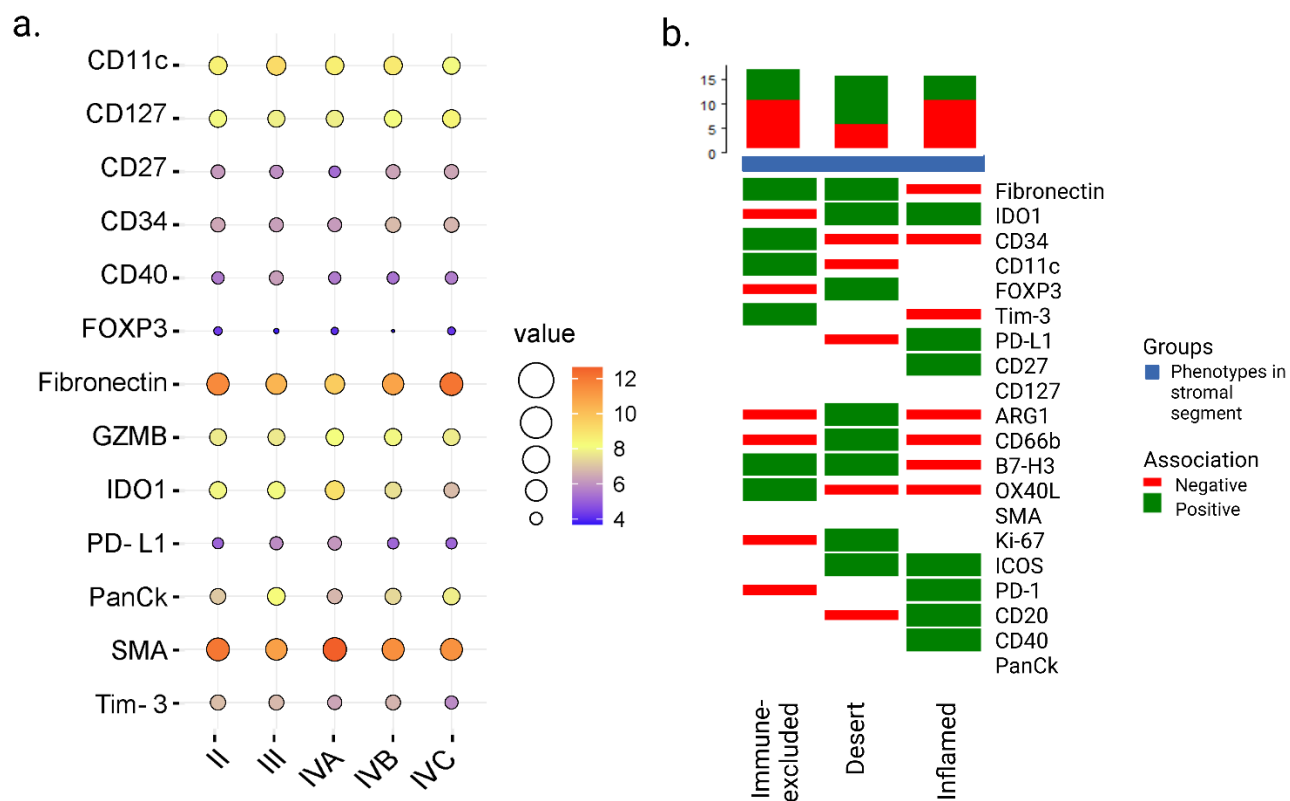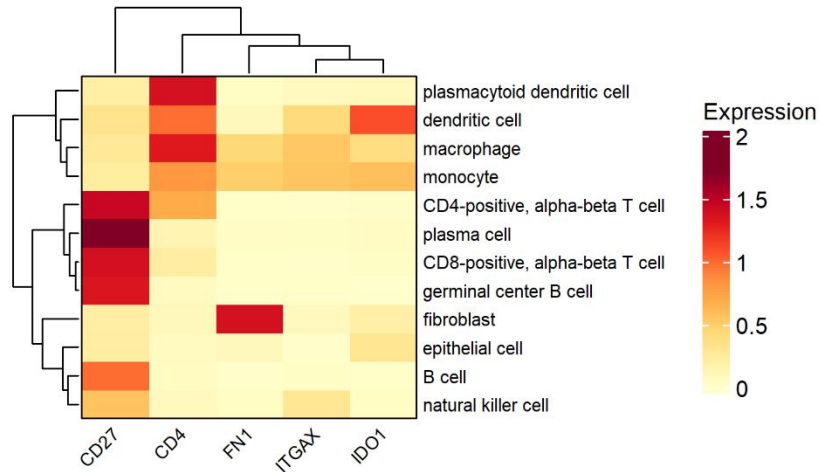
Figure S5. Differential expression in TNM stages. (**a**). Balloon plots showing average expression of significant protein markers in different TNM stages. The balloon's size and color correlate with each protein's expression within different clinical stages. (**b**). On an Oncoprint map, the most significant stromal regions' proteins associated with TNM staging among the three phenotypes: 'inflamed,' 'immune-excluded,' and 'desert', are represented.
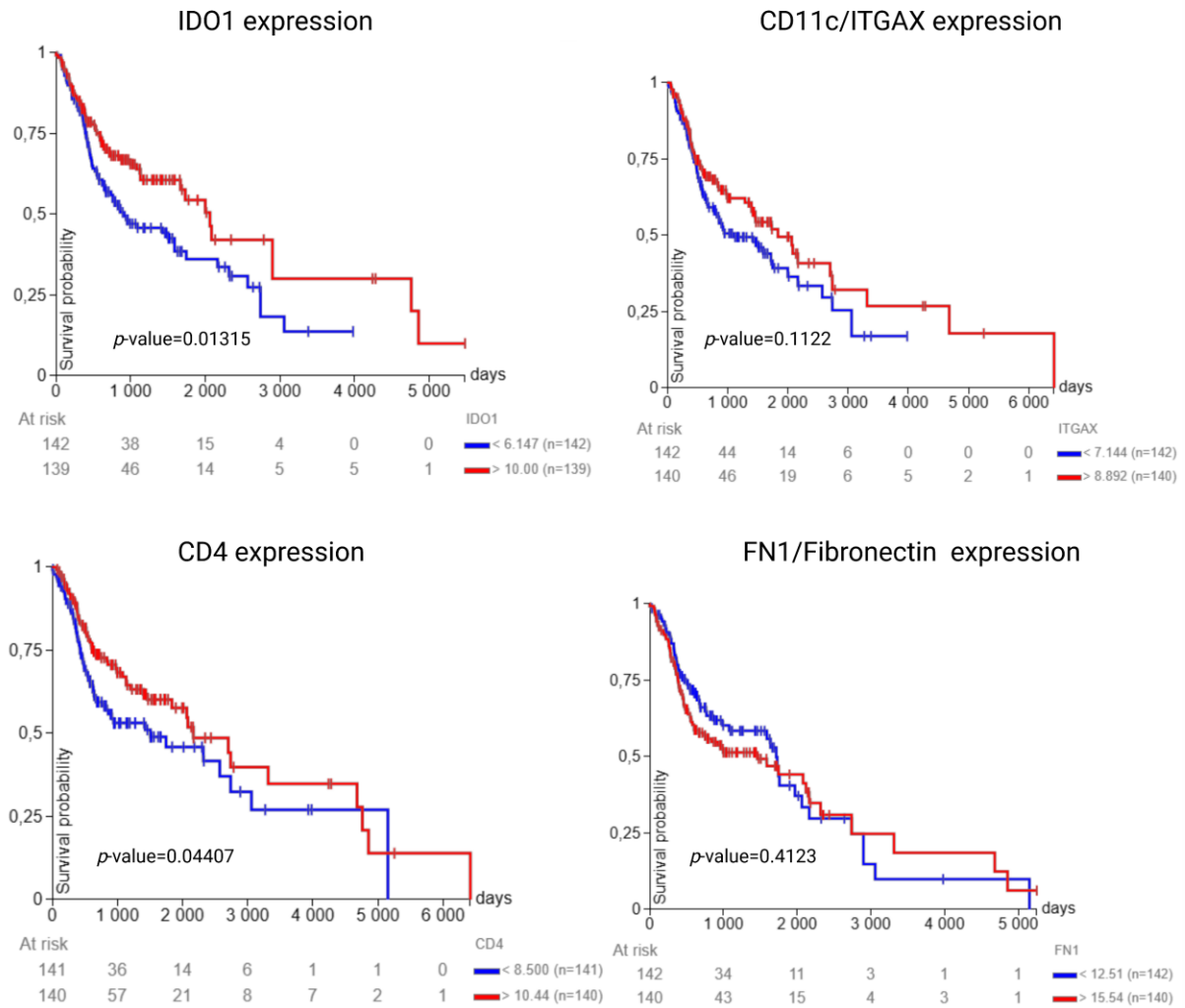
Figure S6. **Survival analysis using the TCGA HNSC dataset**. Kaplan Meier plots demonstrating the survival analysis based on gene expression of IDO1, CD11c/ITGAX, CD4, and Fibronectin/FN1 from the TCGA HNSC cohort. The groups were divided based on quartile differences, where the group in red represents the 4th quartile expression and the group in blue represents the 1st quartile.

a.

b.
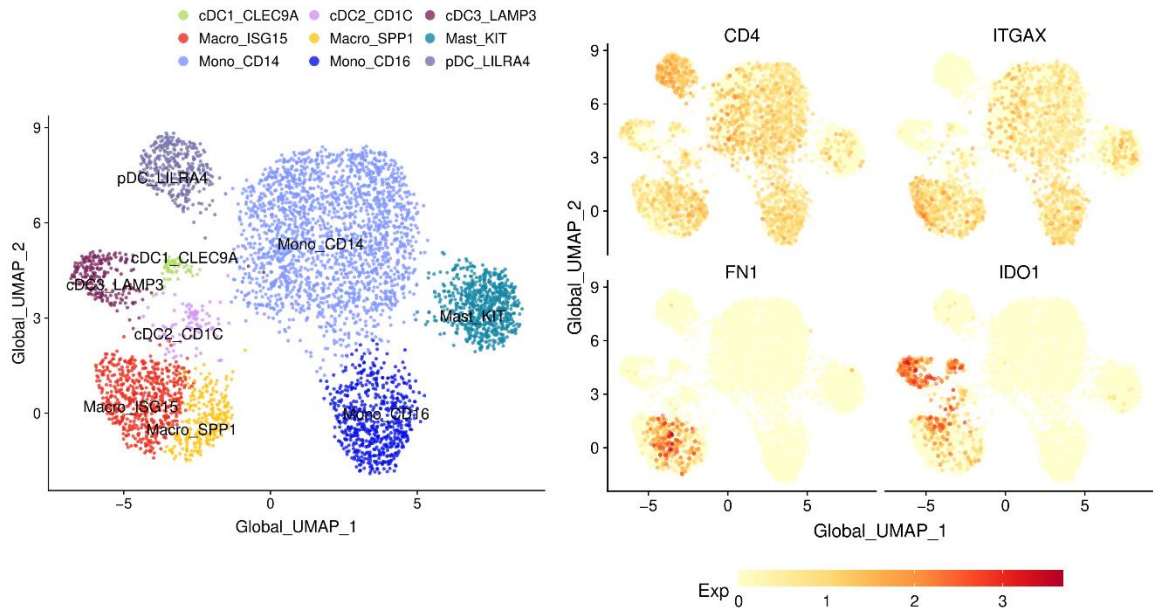
Figure S7. Differential expression in TNM stages. (**a**). Balloon plots showing average expression of significant protein markers in different TNM stages. The balloon's size and color correlate with each protein's expression within different clinical stages. (**b**). On an Oncoprint map, the most significant stromal regions' proteins associated with TNM staging among the three phenotypes: 'inflamed,' 'immune-excluded,' and 'desert', are represented.

Table S1: Clinicopathological characteristics of the cohort used for DSP analysis.

	Patient characteristics	No. of patients (n=30)
Sex	Female/Male	10/20
Age Median	57 years	
EBV status	Negative/Positive	4/26
Tumor stage (TNM-7)	I/II/III/IVA/IVB/IVC	2/3/5/7/7/5
Disease-specific mortality	Alive/Deceased	26/4

Table S2: Protein profiling panels included in the DSP analysis.

Panels	Proteins
Immune cell profiling core	PD-1, CD68, HLA-DR, Ki-67, Beta-2-microglobulin, CD11c, CD20, CD3, CD4, CD45, CD56, CD8, CTLA4, GZMB, PD-L1, PanCK, SMA, Fibronectin
Immune-oncology drug target module	4-1BB, LAG3, OX40L, Tim-3, VISTA, ARG1, B7-H3, IDO1, STING, GITR
Immune activation status module	CD127, CD25, CD80, ICOS, PD-L2, CD40, CD44, CD27
Immune cell typing module	CD45RO, FOXP3, CD34, CD66b, FAP-alpha, CD14, CD163

Table S3: Average expression values of DE markers by TNM stages associated with Figure S4a.

Protein markers	Stage II	Stage III	Stage IVA	Stage IVB	Stage IVC
CD11c	8.567133	9.2302	8.64545714	8.77455714	8.06038
CD127	8.042133	7.8629	7.85697143	8.10445714	8.4376
CD27	6.1626	5.9374	5.25081429	6.36157143	6.40662
CD34	6.427667	6.276	6.18667143	6.83295714	6.69566
CD40	5.543833	6.27012	5.54591429	5.38925714	5.59814
Fibronectin	11.562	10.35106	9.67524286	10.8241	12.1148
FOXP3	4.3069	3.78368	4.07895714	3.68227143	4.18768
GZMB	7.7489	7.73156	8.11188571	8.01465714	7.78162
IDO1	7.996933	8.06104	9.08818571	7.56065714	6.84828
PanCK	7.082233	8.27256	6.74314286	7.39724286	7.82662
PD-L1	5.078367	5.87858	6.10664286	5.149	5.12592
SMA	12.06367	10.955	12.6378571	11.417	11.3492
Tim-3	6.890967	6.788	6.3753	6.68394286	5.8624

TNM staging according to UICC's TNM classification system, 7th version

Table S4: Adjusted p-values of significant proteins obtained after cox regression analysis

Protein	Overall-survival		Overall-survival (7-year)		Disease-free survival		Disease-free survival (7-year)	
	Univariate analysis	Multivariate analysis	Univariate analysis	Multivariate analysis	Univariate analysis	Multivariate analysis	Univariate analysis	Multivariate analysis
CD11c	0.014	0.002	0.029	0.028	0.0365	0.04	0.026	0.026
Fibronectin	0.029	0.03	0.003	0.003	0.0001	0.002	0.0021	0.002
IDO1	0.003	0.023	0.0042	0.0039	0.0035	0.04	0.004	0.0039
CD4	0.022	0.001	0.014	0.0138	0.023	0.03	0.011	0.01
CD27	0.004	0.03	0.022	0.0218	0.038	0.04	0.023	0.022

The values represent p-values associated with hazard ratio of significant proteins for each survival parameter.

Table S5: Kaplan-Meier analysis of the significant proteins obtained via cox regression analysis by survival parameters.

Protein	Overall-survival	Overall-survival (7-year)	Disease-free survival	Disease-free survival (7-year)
CD11c	0.028	0.0024	0.0001	0.0044
Fibronectin	0.035	0.0026	0.0023	0.007
IDO1	0.045	0.0079	0.0051	0.019
CD4	0.037	0.043	0.036	0.03
CD27	0.34	0.38	0.3	0.38

The values represent p-values associated with each survival parameter.