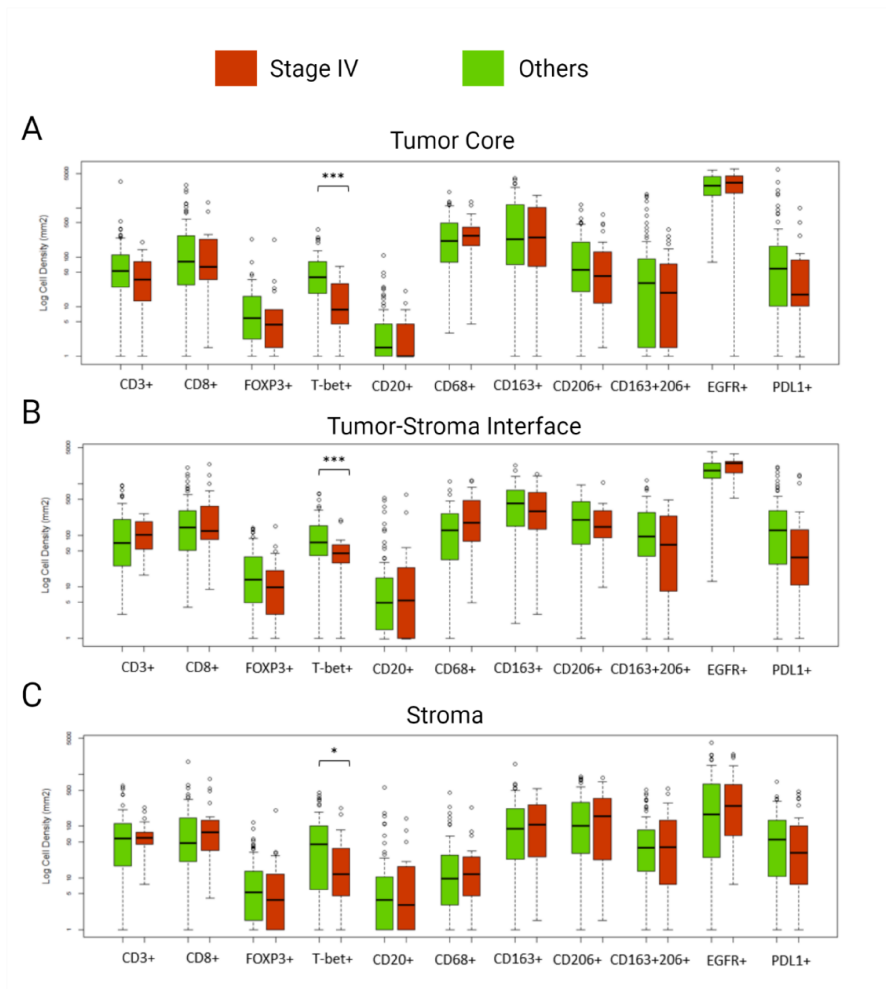
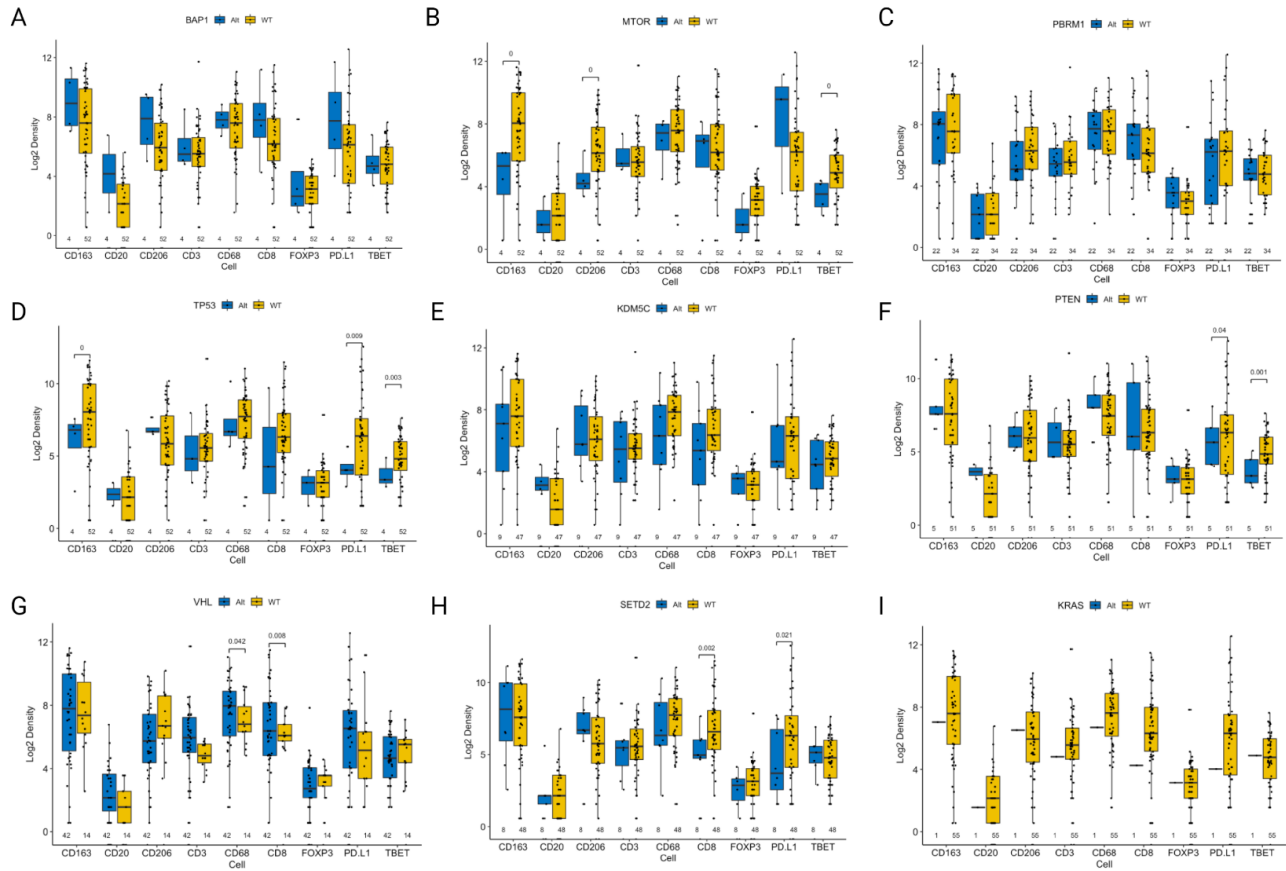


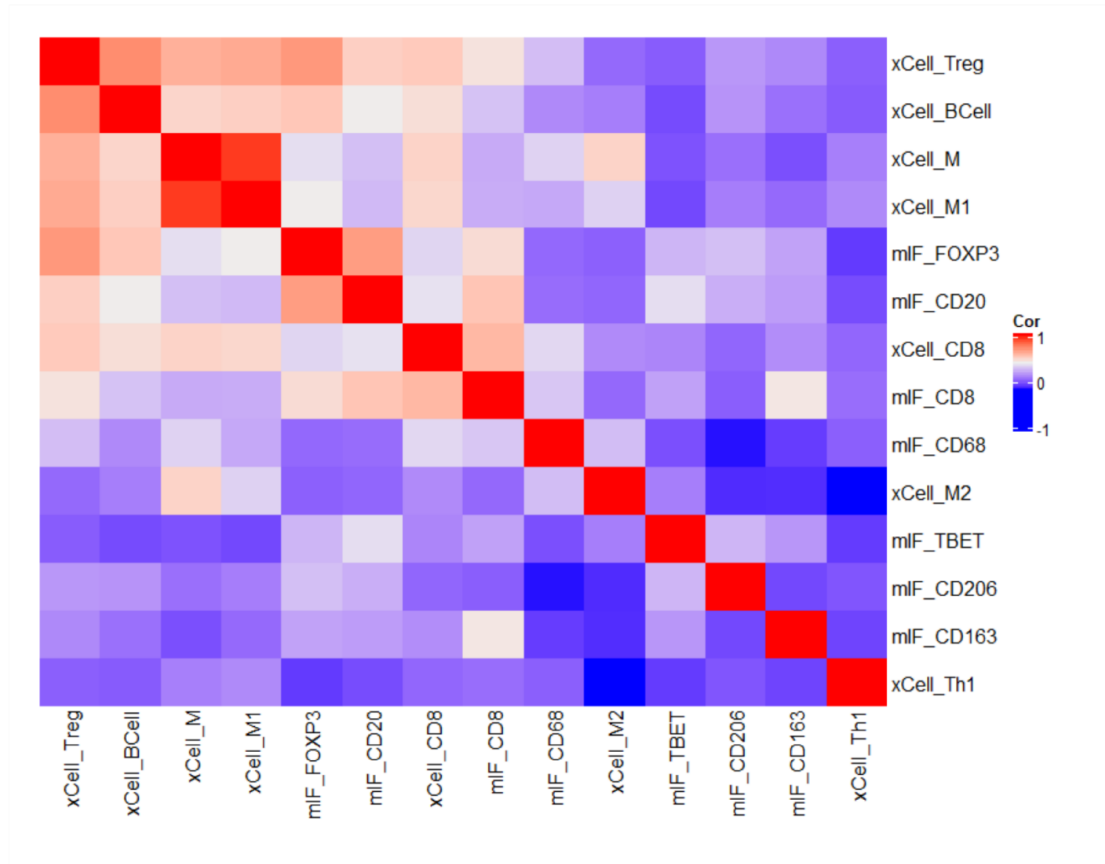
Supplemental Information



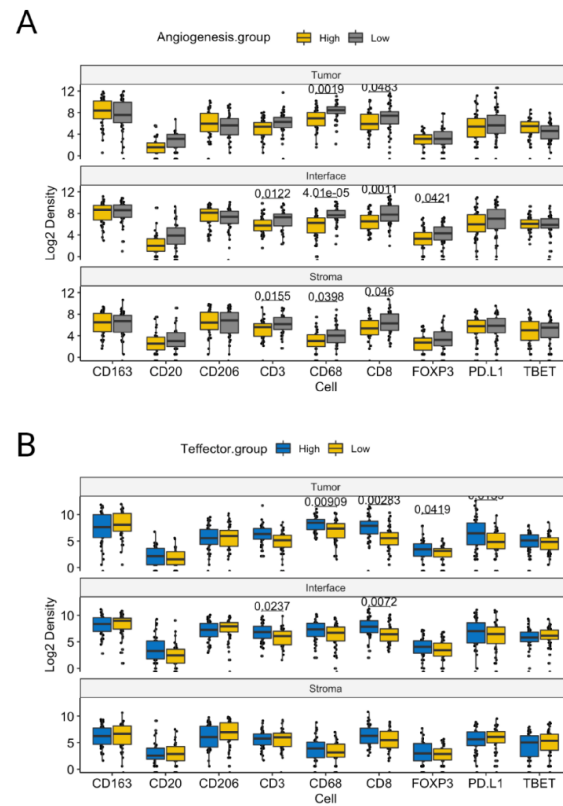
Supplemental Figure 1: Cell density (cells/mm²) of marked cell-types, stratified by clinical stage (Stage IV versus Stages I-III). Single asterisk denotes Wilcoxon $p < 0.05$, double-asterisk denotes $p < 0.01$, triple-asterisk denotes $p < 0.001$.



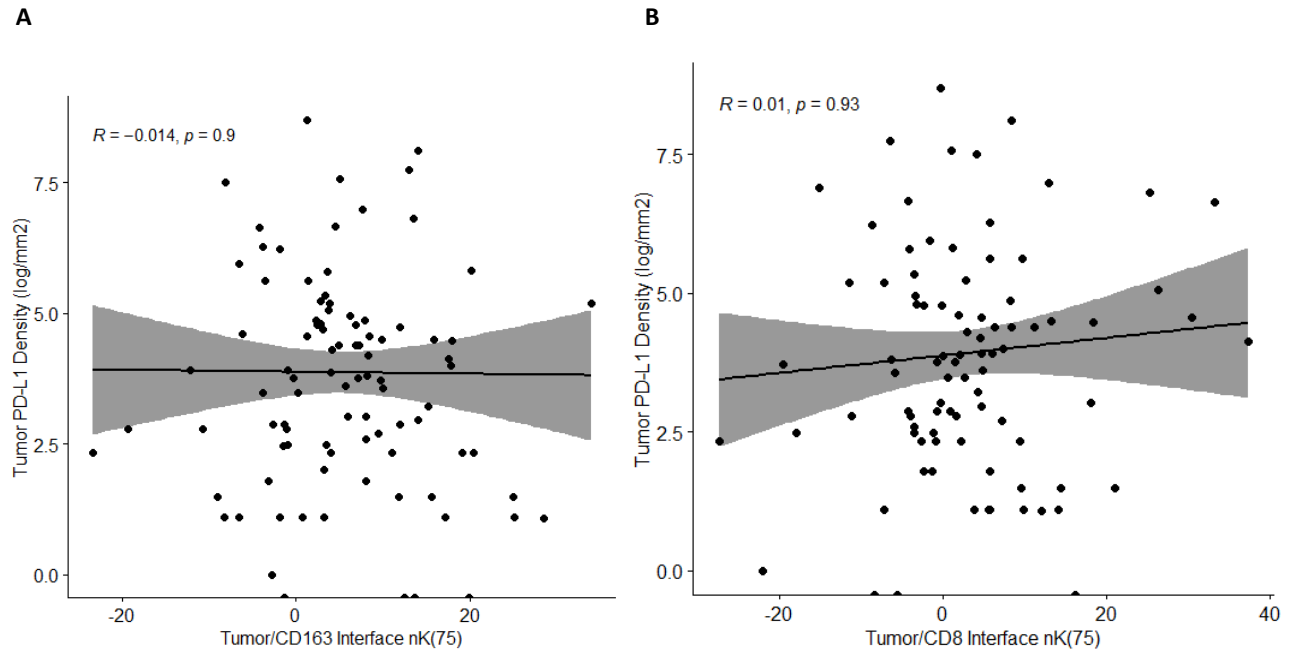
Supplemental Figure 2: Cell density (cells/mm²) of marked cell-types, stratified by somatic mutation status, Wilcoxon p-values listed within the figure. Somatic mutation stratification listed within each corresponding figure legend. A: BAP1. B: MTOR. C: PBRM1. D: TP53. E: KDM5C. F: PTEN. G: VHL. H: SETD2. I: KRAS.



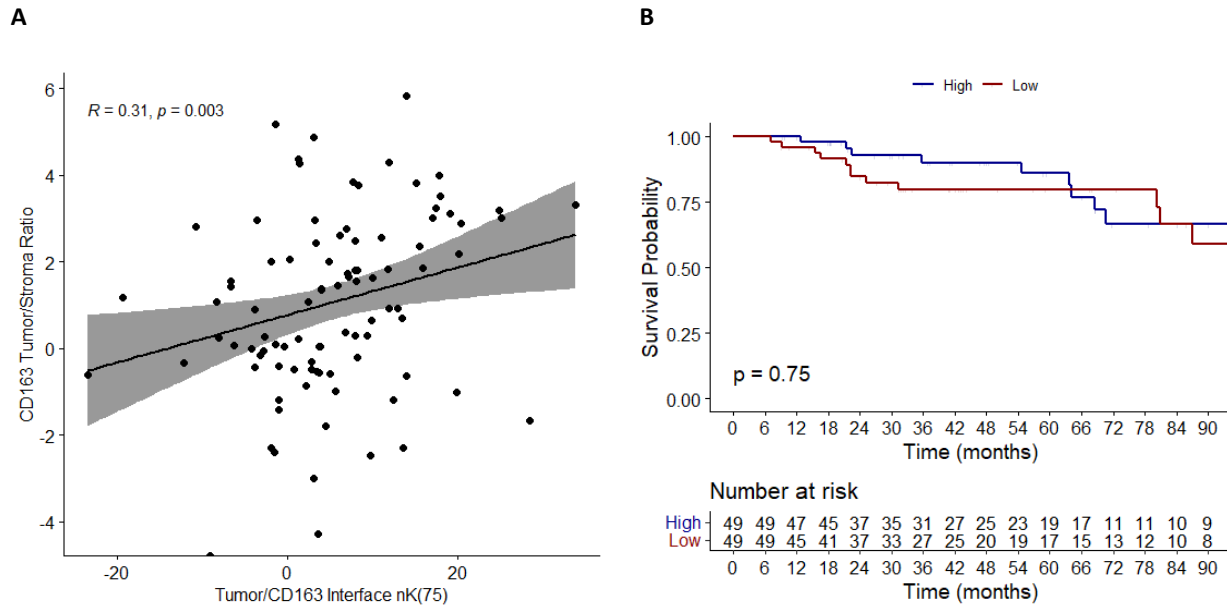
Supplemental Figure 3: Correlation matrix of xCell and mIF scores. Color scale indicates Spearman's correlation.



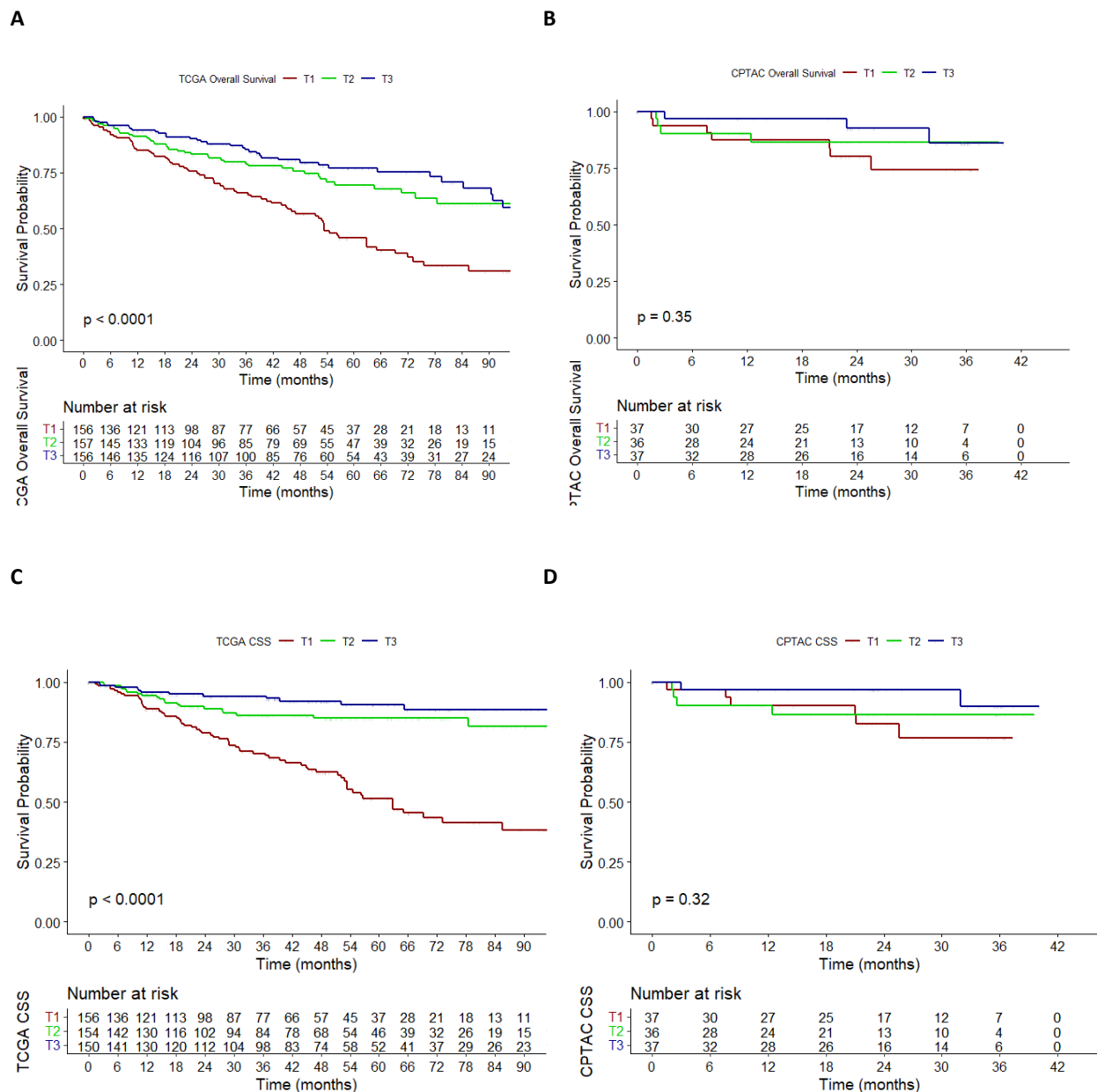
Supplemental Figure 4: Cell density (cells/mm²) of marked cell-types, stratified by Angiogenesis and T-effector gene signatures from the IMmotion150 clinical trial. Wilcoxon p-values reported within the figure.



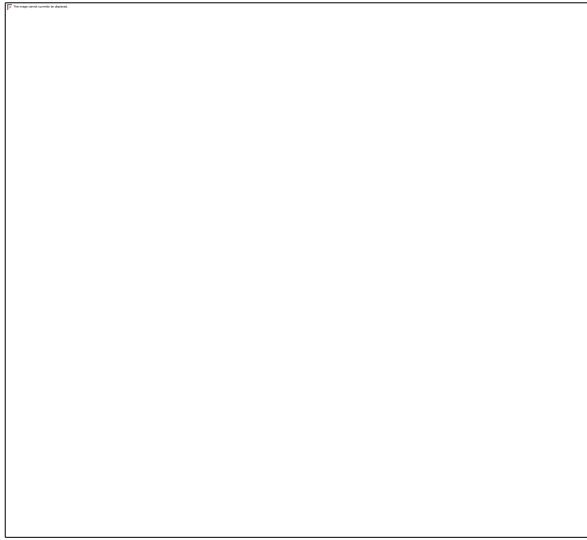
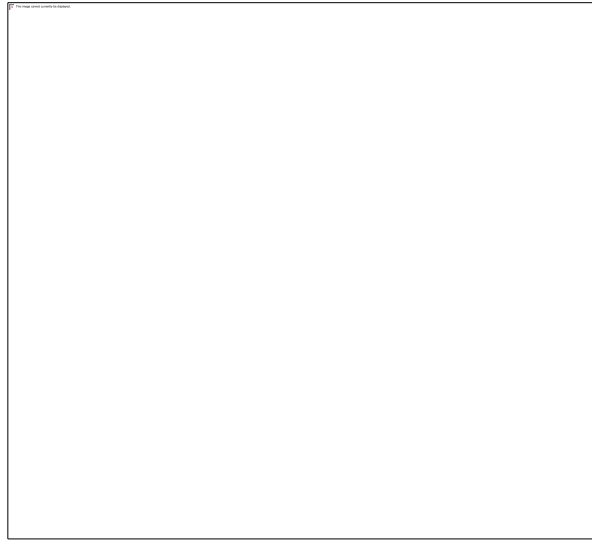
Supplemental Figure 5: A: Spearman's correlation between tumor-core PD-L1 density and Tumor/CD163 interface nK(75). B: Spearman's correlation between tumor-core PD-L1 density and Tumor/CD8 interface nK(75).



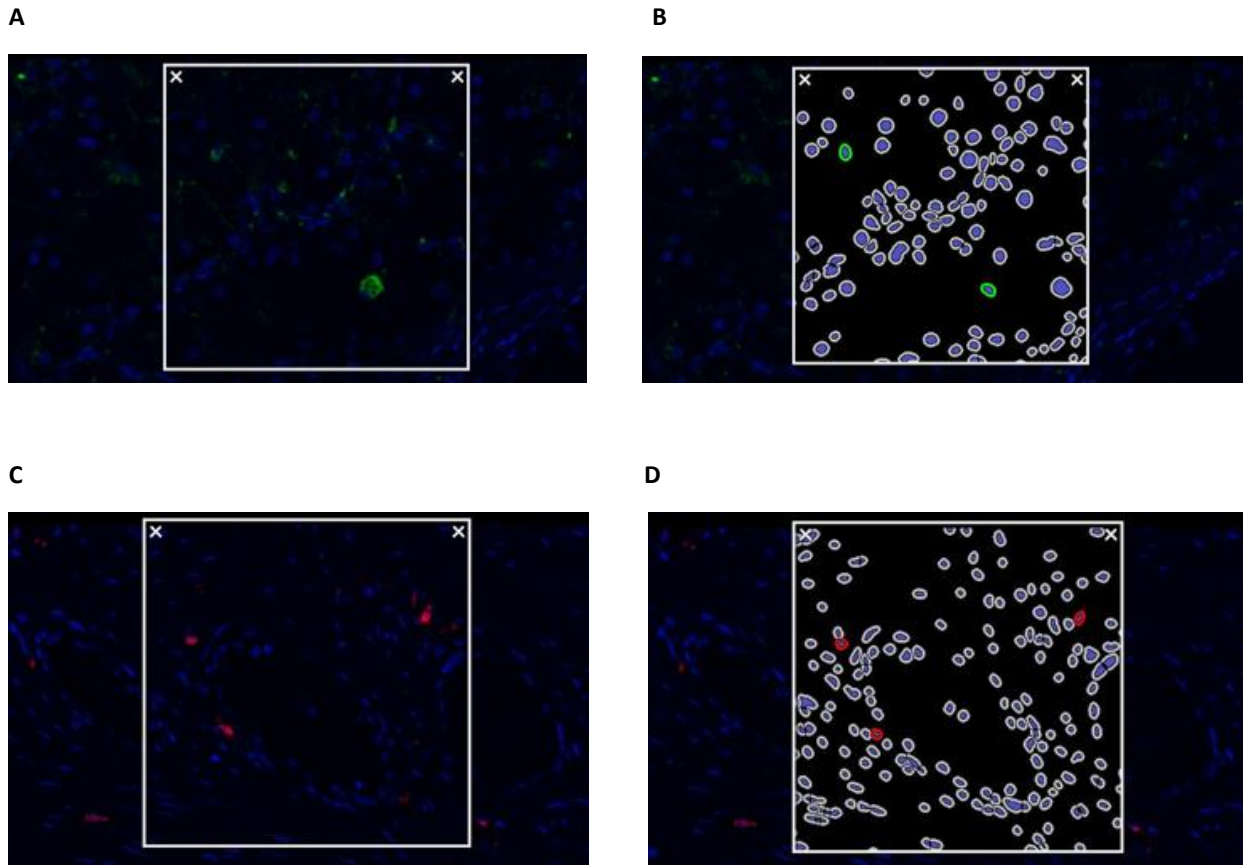
Supplemental Figure 6: A: Spearman's correlation between a ratio of tumor-core/stromal CD163 density, and tumor/CD163 interface nK(75). B: KM analysis for OS stratified by tumor-core/stromal CD163 density ratio median cut-points. Log-rank p-value shown within the plot.



Supplemental Figure 7: A: TCGA cohort - KM analysis for OS, stratified by tertiles of the CD163 Clustering Gene Signature. B: CPTAC cohort - KM analysis for OS, stratified by tertiles of the CD163 Clustering Gene Signature. C: TCGA cohort - KM analysis for CSS, stratified by tertiles of the CD163 Clustering Gene Signature. D: CPTAC cohort - KM analysis for CSS, stratified by tertiles of the CD163 Clustering Gene Signature.

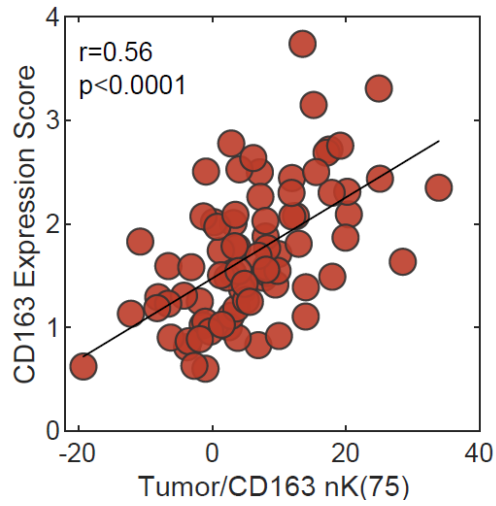
A**B**

Supplemental Figure 8: Ridgeline density distribution plots demonstrating cell counts per ROI for each marked cell type. Square root normalized cell counts on the x-axis. Solid vertical line indicates a cutoff of 10 cells ($\text{sqrt}=3.16$), which was utilized in this analysis as criteria for inclusion in geospatial analysis.

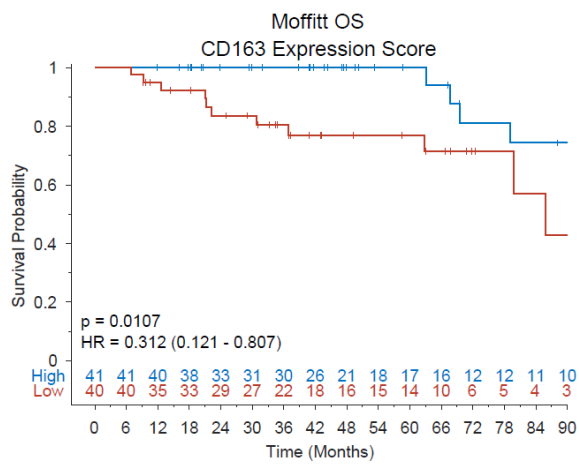


Supplemental Figure 9: Examples of HALO images pre- and post-markup utilizing the classification algorithm after setting nucleus and cytoplasm thresholds. Representative images were selected from regions where staining artifact occurs beyond the cellular boundary to demonstrate classification thresholds. A and B: Corresponding pre- and post-markup imaging stained for DAPI (blue) and CD68+ (green). C and D: Corresponding pre- and post-markup imaging stained for DAPI (blue) and CD163+ (red).

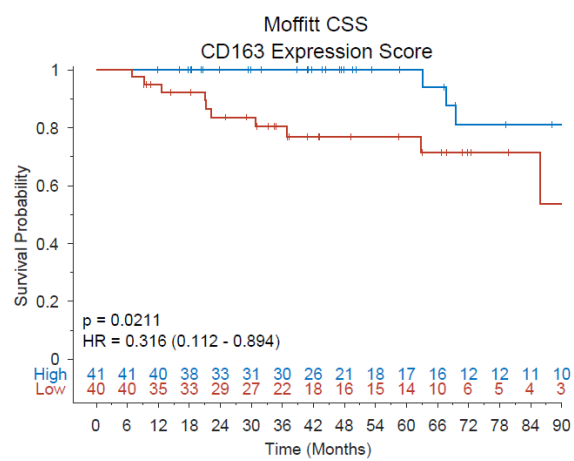
A



B



C



Supplemental Figure 10. A: scatter plot and Spearman's correlation between Tumor/CD163 nK(75) and CD163 gene expression score metrics within the Moffitt cohort. B: KM estimates of OS using the CD163 expression score within the Moffitt cohort. C: KM estimates of CSS using the CD163 expression score within the Moffitt cohort.

Characteristic	N = 97 ¹
Age	66 (59, 72)
Gender	
Male	65 (67.0%)
Female	32 (33.0%)
Race	
White	85 (87.6%)
Black	4 (4.1%)
Other	8 (8.3%)
Histology	
ccRCC	97 (100%)
Tumor Size (cm)	6.2 (4.5, 9.5)
SSIGN	7.0 (3.0, 7.8)
Clinical Stage	
Stage I/II	24 (24.7%)
Stage III	49 (50.5%)
Stage IV	24 (24.7%)
¹ Statistics presented: median (IQR); n (%)	

Supplemental Table 1. Baseline patient and tumor demographics

Overall Survival

Metric	Median	N Low	N High	Surv. Hi (mos.)	Surv. Lo (mos.)	Log-Rank P	FDR-adjusted Cox P
InterfaceROI.Tumor.CD163.K.75	4.656111	46	46	148.9956	85.84016	0	0
InterfaceROI.Tumor.CD8.K.75	2.02284	43	44	148.9956	67.59378	0.002	0
InterfaceROI.CD68.Density	143.1823	48	49	138.5409	148.99563	0.013	0.1
InterfaceROI.Tumor.CD163.206.K.75	4.009733	38	39	148.9956	138.54095	0.014	0.292
InterfaceROI.Stroma.CD163.K.Area	-381.158	46	46	114.2124	148.99563	0.03	0.356

ccRCC Specific Survival

Metric	Median	N Low	N High	Surv. Hi (mos.)	Surv. Lo (mos.)	Log-Rank P	FDR-adjusted Cox P
InterfaceROI.Tumor.CD8.K.75	2.02284	43	44	173.7811	69.08818	0	0
InterfaceROI.Tumor.CD163.K.75	4.656111	46	46	173.7811	85.20984	0	0
InterfaceROI.Tumor.CD163.206.K.75	4.009733	38	39	173.7811	137.52366	0.004	0.084
InterfaceROI.Tumor.CD68.K.75	8.134679	42	42	173.7811	137.52366	0.007	0.116
InterfaceROI.CD68.K.75	19.71405	42	42	173.7811	113.3738	0.011	0.23
InterfaceROI.CD68.Density	143.1823	48	49	137.5237	173.78108	0.016	0.079
InterfaceROI.Stroma.CD163.K.75	-3.34679	46	46	113.3738	173.78108	0.025	0.22
InterfaceROI.Tumor.CD3.K.75	-2.97018	42	42	173.7811	113.3738	0.046	0.076

Recurrence Free Survival

Metric	Median	N Low	N High	Surv. Hi (mos.)	Surv. Lo (mos.)	Log-Rank P	FDR-adjusted Cox P
InterfaceROI.Tumor.CD20.K.75	-8.29682	8	8	79.65633	15.73717	0.001	0.269
StromaROI.CD3.Density	57.01469	36	36	49.95206	79.65633	0.001	0.409
TumorROI.Stroma.CD8.K.75	-1.09861	25	26	60.02464	147.65754	0.018	0.269
StromaROI.Stroma.CD3.K.75	-0.10601	25	26	49.83984	147.65754	0.019	0.409
InterfaceROI.FOXP3.K.75	12.57078	13	13	57.62628	NA	0.025	0.41

Supplemental Table 2. Overall survival, ccRCC specific survival, and recurrence-free survival tables for the metrics that demonstrated the highest associations within each outcome measurement.

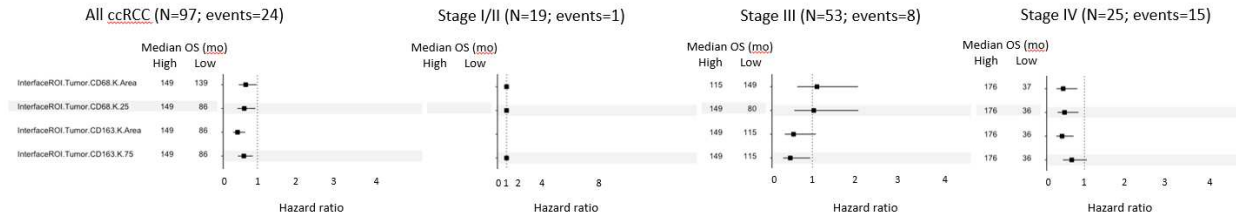
mIF Stain	Cell Type	Panel 1	Panel 2	Panel 3
DAPI	Nucleus	+	+	+
PCK	Tumor cell	+	+	+
CD3	T-cell	+	-	-
CD8	Cytotoxic T-cell	+	-	+
FOXP3	FOXP3+ Treg cell	+	-	-
T-bet	T-bet+ Th cell	+	-	-
CD20	B-cell	-	+	-
PD-L1	PD-L1+ cell	-	+	+
CD68	Macrophage	-	+	-
CD163	CD163+ M2 Macrophage	-	+	+
CD206	CD206+ M2 Macrophage	-	+	-
TIM-3	T-cell Terminal Exhaustion (w/ CD8+)	-	-	+

Supplemental Table 3. Summary table of the mIF stains utilized in this analysis, with their generalized corresponding cell-types, as well as their presence in each of the 3 mIF panels utilized in this analysis.

Gene Symbol	GeneID	Map_location	Description	R	Cancer-Specific Context
GABRB1	2560	4p12	gamma-aminobutyric acid type A receptor subunit beta1	0.354779	Weakly associated with colon adenocarcinoma as compared with normal tissue (PMID: 32565954)
NEBL	10529	10p12.31	nebullette	0.33575	Inhibited pancreatic cancer cell progression through TGF-B suppression (PMID: 30945341)
C17orf107	100130311	17p13.2	chromosome 17 open reading frame 107	0.33374	Minimal prior characterization
TMEM125	128218	1p34.2	transmembrane protein 125	0.331452	Associated with improved prognosis in papillary RCC (PMID: 33154194)
CCDC85C	317762	14q32.2	coiled-coil domain containing 85C	0.32089	A mutation of CCDC85C has been utilized as a potent tumor-rejection neo-epitope in in-vitro studies (PMID: 31219806)
PTPN13	5783	4q21.3	protein tyrosine phosphatase non-receptor type 13	0.319264	Well described tyrosine phosphatase associated with suppression of tumor growth in multiple primary sites, breast cancer in particular (PMID: 21235435)
TUFT1	7286	1q21.3	tuftelin 1	0.318564	Overexpression has been associated with cell proliferation and EMT progression in RCC (PMID: 34257606)
HOXC9	3225	12q13.13	homeobox C9	0.317886	Well described homeobox transcription factor that has been consistently shown to regulate tumor angiogenesis (PMID: 32456563)
LTF	4057	3p21.31	lactotransferrin	-0.317886	Suppresses AKT signalling, decreases cellular migration in ccRCC (PMID 32244557)
CORO2A	7464	9q22.33	coronin 2A	-0.318293	Promotes migration and proliferation of breast cancer cells (PMID 32695665)
SCN3A	6328	2q24.3	sodium voltage-gated channel alpha subunit 3	-0.322846	Minimal prior characterization
VTN	7448	17q11.2	vitronectin	-0.325326	Cell-adhesion basement membrane glycoprotein, found to be an oncogenic mediator in gastric cancer, regulated by VEGFR2 (PMID 30819137)
CNTN5	53942	11q22.1	contactin 5	-0.327104	Minimal prior characterization
IFNGR1	3459	6q23.3	interferon gamma receptor 1	-0.335388	Receptor for IFN-G, which is a major cytokine regulating macrophage polarization and angiogenesis. Both pro- and anti-tumor effects have been identified. (PMID 27667683).
CHI3L2	1117	1p13.2	chitinase 3 like	-0.343428	In breast cancer, overexpression has

			2		been associated with infiltrating M1 and M2 macrophages, TGF-B, T-cell exhaustion (PMID 33937022).
BMP7	655	20q13.31	bone morphogenetic protein 7	-0.345505	Well described molecule in TGF-B superfamily, found to be associated with immunotherapy resistance in RCC via BMP7 secretion by tumor cells and acting on macrophages in the microenvironment (PMID 32973129).
TTPAL	79183	20q13.12	alpha tocopherol transfer protein like	-0.362669	Overexpressed in colon adenocarcinoma, promotes tumorigenesis by activating WNT/B-catenin signalling (PMID 31018940). Overexpressed in gastric cancer, found to activate P13K/AKT signaling (PMID 34642500).
KRT32	3882	17q21.2	keratin 32	-0.362933	Minimal prior characterization
ADPRHL1	113622	13q34	ADP-ribosylhydrolase like 1	-0.36409	Minimal prior characterization
SP5	389058	2q31.1	Sp5 transcription factor	-0.394021	Diminishes expression of genes previously activated by the WNT pathway, particularly in stem cells (PMID: 29044119)

Supplemental Table 4. Summary table of the 20 genes included in the CD163 Clustering Gene Signature that was applied to the TCGA and CPTAC cohorts.

Median OS

Supplemental Table 5. Overall survival outcomes, Cox proportional hazards regression, for the primary outcomes of interest (CD68 and CD163 bivariate tumor clustering at the interface), stratified by clinical stage.

