Supplementary Table 1. 3D Histech fluorescence microscope scanner optical specifications (A) Lumencor Spectra-6 LED Light engine specifications. (B & C) Semrock epifluorescence cube excitation (B) and emission (C) specifications. (D) Semrock epifluorescence cube part numbers.

A. Lumencor Spectra-6 LED Light Engine

Fluor	Channel Name	Bandpass Filter	Power (mW)
DAPI	Violet	386/23	186
FITC	Cyan	475/28	174
AF555	Green	550/88	909
AF594	Green	550/88	909
Cy5	Red	650/13	96

B. Semrock Epifluorescence Filter Cube – Excitation Optics

Semrock Cube	Fluor	Excitation Filter Transmission Range	Dichroic Beamsplitter Reflection Ravg > 95%
LED-DA/FI/TR/Cy5-A-000	DAPI	378.3 - 406.8 nm	380 - 404 nm
	FITC	459.0 - 489.7 nm	461 - 487.5 nm
	Cy5	623.1 - 646.9 nm	626 - 644 nm
SpGold-B-000	AF555/Cy3	521.2 - 546.8 nm	350 - 544 nm
SpRed-B-000	AF594	573.1 - 599.0 nm	350 - 596 nm

C. Semrock Epifluorescence Filter Cube – Emission Optics

Semrock Cube	Fluor	Emission Filter Transmission Range	Dichroic Beamsplitter Reflection Tavg > 93%
LED-DA/FI/TR/Cy5-A-000	DAPI	412.2 - 451.9 nm	414 - 450 nm
	FITC	497.1 - 532.6 nm	499.5 - 530 nm
	Cy5	656.9 - 804.1 nm	659.5 - 800 nm
SpGold-B-000	AF555/Cy3	555.0 - 589.1 nm	558 - 950 nm
SpRed-B-000	AF594	608.9 - 647.1 nm	612 - 950 nm

D. Semrock Epifluorescence Filter Cube Details

Semrock Cube	Excitation Filter	Dichroic	Emission Filter
LED-DA/FI/TR/Cy5-A-000	FF01-392/474/554/635	FF409/493/573/652-Di01	FF01-432/515/595/730
SpGold-B-000	FF01-534/20	FF552-Di02	FF01-572/28
SpRed-B-000	FF01-586/20	FF605-Di02	FF01-628/32

Supplementary Table 2. Image capture and adjustment settings. The following camera capture speeds and display levels were used to acquire and analyse fluorescence scans for this study.

Camera Exposure and Display Levels

Channel	Marker	Shutter Speed (ms)	Black Level	White Level
DAPI	DAPI	10	0	24,050
FITC	CD8 TSA-FITC	15	0	18,939
SpGold	Cytokeratin AF555	25	0	21,503
SpRed	CD11c AF594	30	0	9,920
Cy5	PD-L1 TSA- Cy5	12	0	8,000

Supplementary Table 3 . Median and interquartile range (IQR) of each variable by tissue core and region analysed.

Marker	Core	Region	N	Median (IQR)
CD8 (cells/mm^2)	Tumour	Tissue	221	205 (76 - 464)
		Epithelium	221	60 (22 - 227)
		Stroma	221	315 (121 - 616)
	Leading Edge	Tissue	143	298 (126 - 744)
		Epithelium	143	72 (11 - 309)
		Stroma	143	372 (165 - 849)
	Normal	Tissue	134	464 (228 - 722)
		Epithelium	134	261 (131 - 585)
		Stroma	134	564 (312 - 930)
CD11c (cells/mm^2)	Tumour	Tissue	221	85 (31 - 186)
		Epithelium	221	23 (9 - 59)
		Stroma	221	127 (45 - 337)
	Leading Edge	Tissue	143	76 (33 - 172)
		Epithelium	143	27 (7 - 69)
		Stroma	143	101 (36 - 222)
	Normal	Tissue	134	56 (21 - 100)
		Epithelium	134	9 (3 - 23)
		Stroma	134	116 (38 - 185)
CD11c+PDL1+ (cells/mm^2)	Tumour	Tissue	221	12 (2 - 55)
		Epithelium	221	0 (0 - 5)
		Stroma	221	23 (2 - 101)
	Leading Edge	Tissue	143	12 (1 - 89)
		Epithelium	143	0 (0 - 14)
		Stroma	143	15 (1 - 103)
	Normal	Tissue	134	2 (0 - 13)
		Epithelium	134	0 (0 - 0)
		Stroma	134	4 (0 - 27)
CD11c+PDL1- (cells/mm^2)	Tumour	Tissue	221	50 (20 - 111)
		Epithelium	221	16 (7 - 39)
		Stroma	221	78 (29 - 176)
	Leading Edge	Tissue	143	44 (20 - 82)
		Epithelium	143	17 (3 - 38)
		Stroma	143	49 (18 - 112)
	Normal	Tissue	134	49 (19 - 88)
		Epithelium	134	9 (3 - 21)
		Stroma	134	88 (34 - 167)
PDL1+/CD11c+ (%)*	Tumour	Tissue	218	18 (3 - 42)
		Epithelium	210	0 (0 - 20)
		Stroma	218	20 (3 - 48)
	Leading Edge	Tissue	143	25 (2 - 61)
		Epithelium	121	6 (0 - 50)
		Stroma	140	25 (2 - 67)
	Normal	Tissue	131	4 (0 - 18)
		Epithelium	110	0 (0 - 0)
		Stroma	130	4 (0 - 22)

*Variable classified as zero if no double positives were found. If no CD11c+ cells were found then variable was excluded.

Supplementary Table 4 . Univariate Cox regression for immune-related variables in leading edge cores for T stage 3 and 4 cases only

Marker	Region	N	HR	L 95%CI	U 95%CI	Wald P	Log-Rank P
$CD8^+$ cell density - Leading Edge (high vs low)	Tumour	125	0.53	0.32	0.87	**0.012**	**0.011**
	Stroma	125	0.66	0.41	1.07	0.093	0.091
$CD11c^+$ cell density - Leading Edge (high vs low)	Tumour	125	0.80	0.49	1.29	0.361	0.361
	Stroma	125	0.47	0.28	0.77	**0.003**	**0.002**
$CD11c^+PD\text{-}L1^+$ cell density - Leading Edge (high vs low)	Tumour	125	0.58	0.35	0.96	**0.033**	**0.031**
	Stroma	125	0.46	0.28	0.75	**0.002**	**0.002**

Supplementary Table 5. Univariate Cox regression for immune-related variables in central tumour and matched normal tissue cores. Significant P-values highlighted in bold.

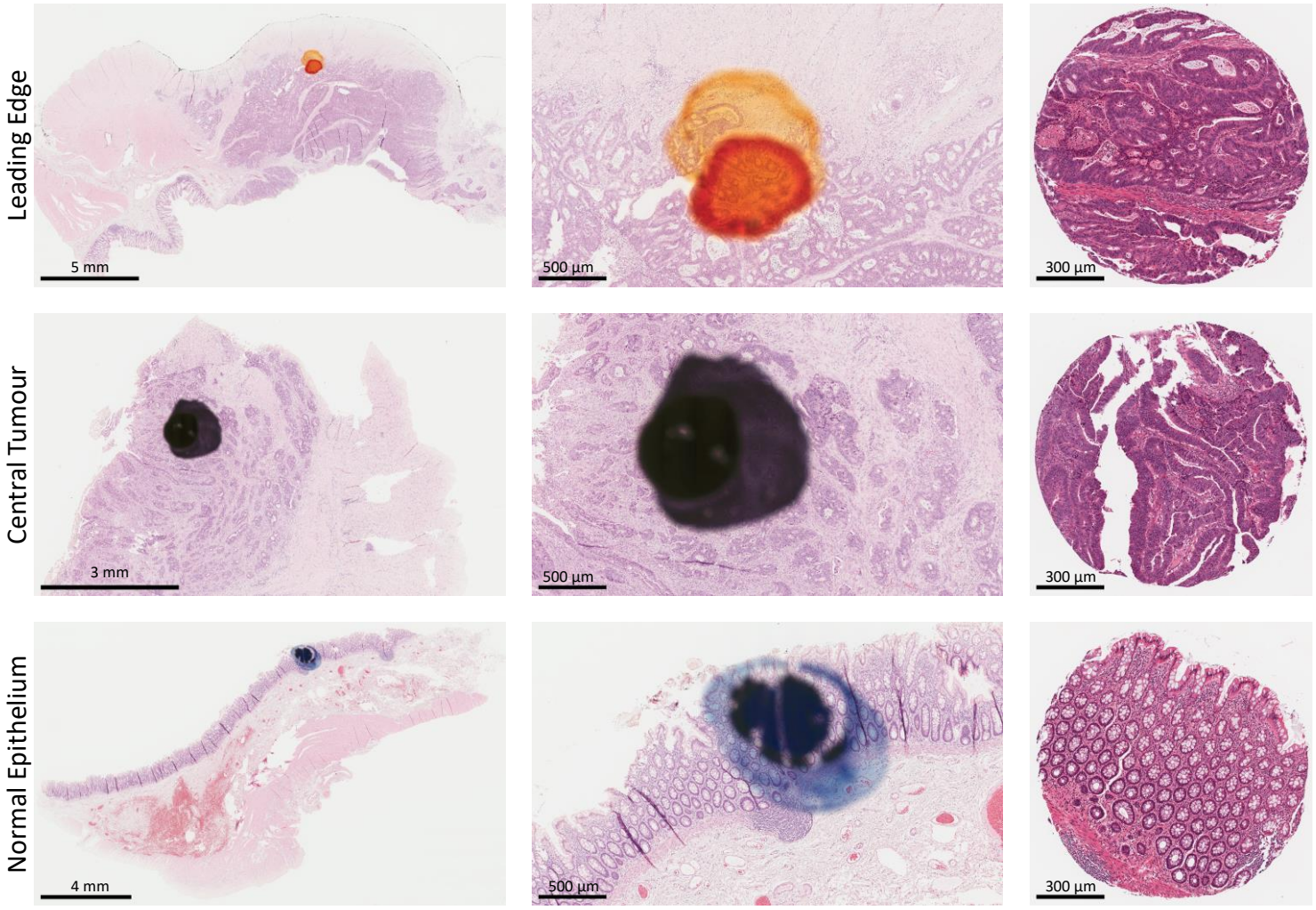
Marker	Region	N	HR	L95% CI	U95%CI	Wald P
$CD8^+$ cell density - Central Tumour	Tumour	221	0.68	0.47	0.97	**0.035**
	Stromal	221	0.79	0.55	1.14	0.204
$CD11c^+$ cell density - Central Tumour	Tumour	221	1.01	0.70	1.46	0.941
	Stromal	221	0.77	0.54	1.11	0.164
$CD11c^+PDL1^+$ cell density - Central Tumour	Tumour	221	0.84	0.58	1.21	0.348
	Stromal	221	0.73	0.51	1.05	0.090
$CD11c^+PDL1^-$ cell density - Central Tumour	Tumour	221	1.07	0.73	1.55	0.742
	Stromal	221	0.89	0.62	1.28	0.523
% $PDL1^+/CD11c^+$ cells - Central Tumour	Tumour	210	0.88	0.60	1.28	0.502
	Stromal	218	0.72	0.50	1.04	0.078
$CD8^+$ cell density - Normal	Epithelium	134	1.64	1.03	2.61	**0.037**
	Lamina propria	134	1.37	0.87	2.18	0.177
$CD11c^+$ cell density - Normal	Epithelium	134	1.22	0.77	1.93	0.404
	Lamina propria	134	0.94	0.59	1.49	0.789
$CD11c^+PDL1^+$ cell density - Normal	Epithelium	134	1.25	0.60	2.60	0.555
	Lamina propria	134	1.35	0.85	2.14	0.201
$CD11c^+PDL1^-$ cell density - Normal	Tumour	134	1.21	0.77	1.92	0.409
	Stromal	134	0.89	0.56	1.41	0.627
% $PDL1^+/CD11c^+$ cells - Normal	Tumour	110	1.28	0.61	2.69	0.520
	Stromal	130	1.29	0.81	2.06	0.285

All variables are reported as high vs low, split at the median value

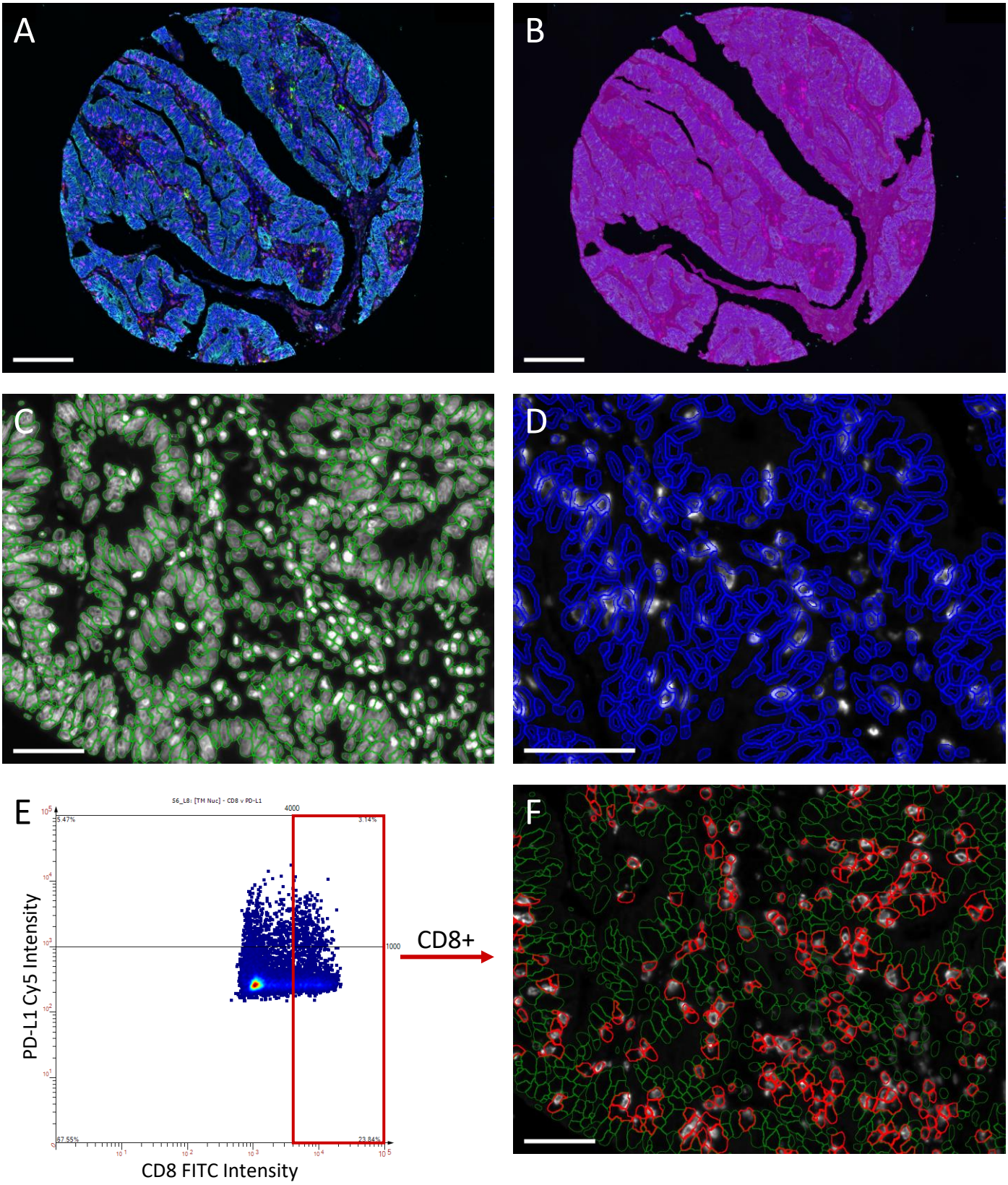
Supplementary Table 6. Univariate Cox regression for the density and proportion of $CD8^+$ cells within distance zones from the closest $CD11c^+$ or $CD11c^+PD\text{-}L1^+$ cells on leading edge cores.

Marker	Mask	Distance	Continuous					Categorical			
			N	*Adj. HR	L95% CI	U95%CI	*Adj. P	*Adj. HR	L95% CI	U95%CI	*Adj. P
CD8 Density	$CD11c^+$	0-10um	143	1.00	1.00	1.00	0.677	1.04	0.64	1.68	0.881
		0-100um	143	1.00	1.00	1.00	0.964	0.88	0.55	1.41	0.587
		>100um	143	1.00	1.00	1.00	0.385	0.75	0.47	1.20	0.234
	$CD11c^+PD\text{-}L1^+$	0-10um	44	1.00	1.00	1.00	0.262	2.52	0.87	7.30	0.088
		0-100um	44	1.00	1.00	1.00	0.454	0.58	0.21	1.61	0.297
		>100um	44	1.00	1.00	1.00	0.630	1.09	0.41	2.92	0.869
CD8 Proportion	$CD11c^+$	0-10um	143	1.01	0.99	1.03	0.341	1.16	0.66	2.05	0.606
		0-100um	143	1.01	0.99	1.02	0.287	1.29	0.78	2.13	0.320
		>100um	143	0.99	0.98	1.01	0.287	0.81	0.49	1.34	0.416
	$CD11c^+PD\text{-}L1^+$	0-10um	44	0.98	0.91	1.06	0.624	1.82	0.60	5.55	0.289
		0-100um	44	1.02	0.99	1.05	0.168	2.33	0.79	6.92	0.127
		>100um	44	0.98	0.96	1.01	0.168	0.43	0.15	1.27	0.127

*Adjusted for total $CD11c^+$ or $CD11c^+PD\text{-}L1^+$ cell density

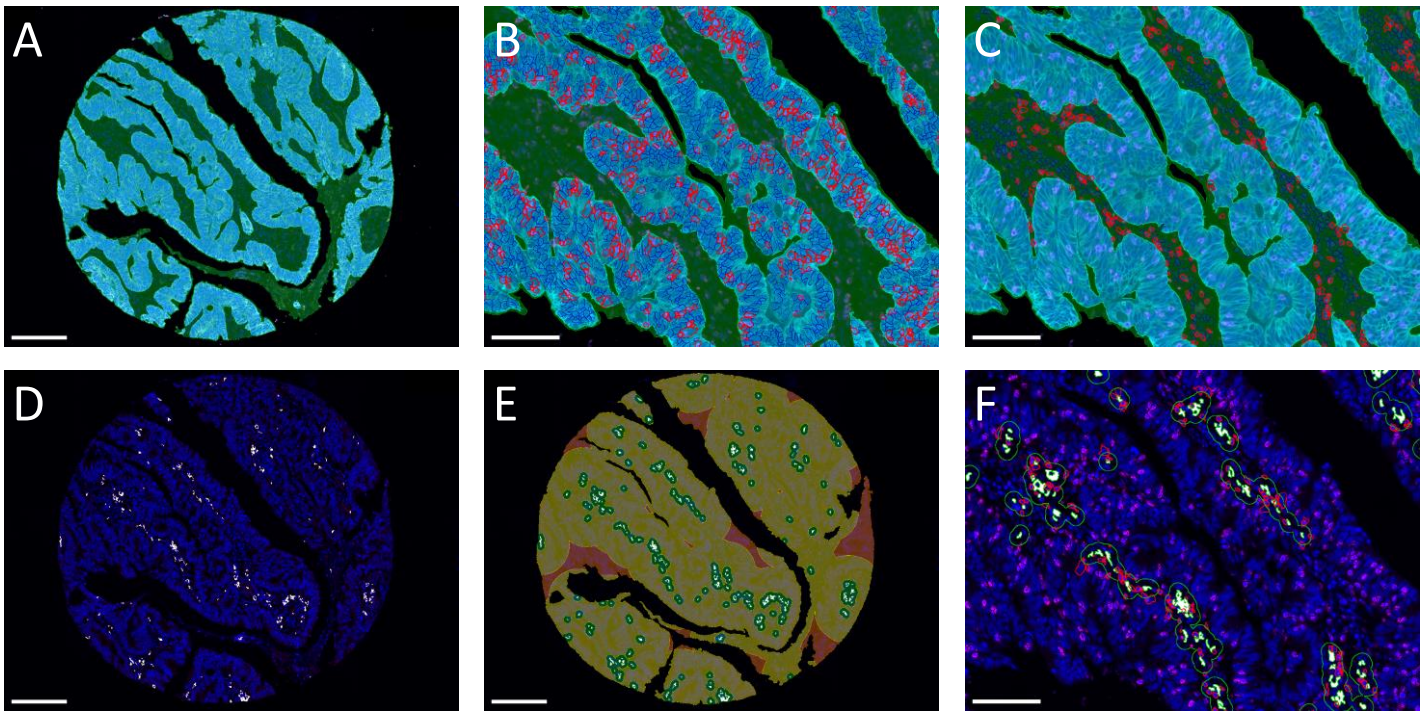


Supplementary Figure 1. Selection of regions of interest.
Areas of leading edge tumour (top row), central tumour (middle row) and normal colonic epithelium (bottom row) were identified by a pathologist using H&E-stained sections (left and centre columns). TMAs were constructed using marked slides as a guide. The resultant cores from the marked areas are shown in the far right column. Scale bars as annotated.



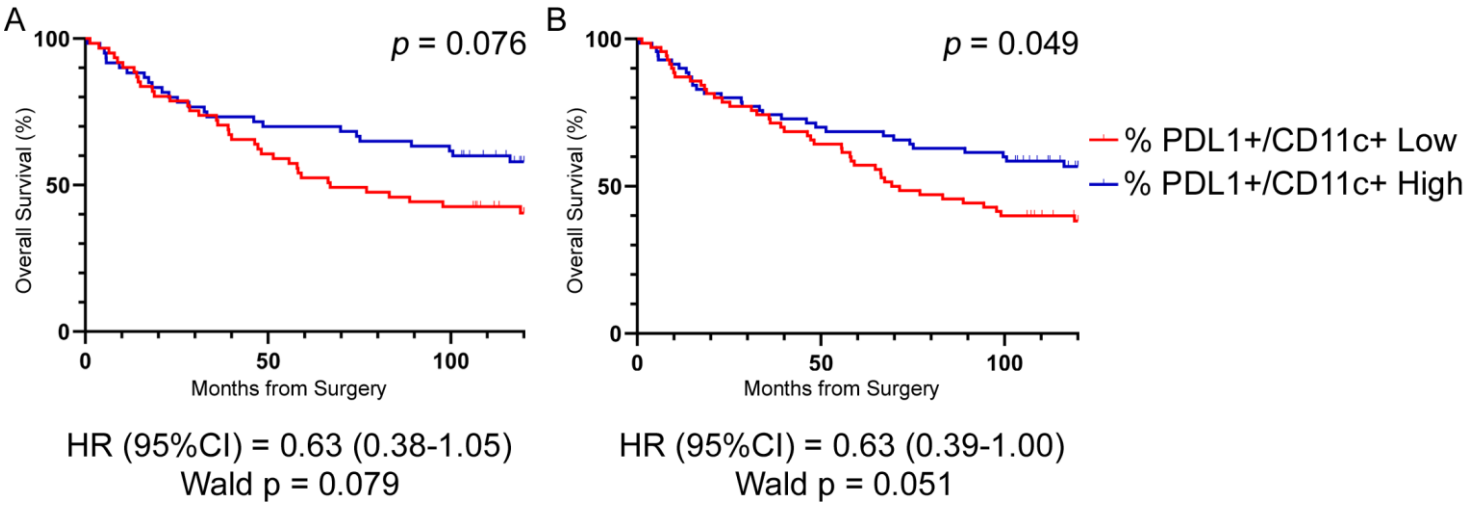
Supplementary Figure 2. Cytometric Quantitation of Scanned Images.

A. Colon cancer tissue core displaying five fluorescent channels. **B.** Automated tissue mask detection (purple overlay) allows measurement of the tissue area, and limits analysis to cells on the tissue mask. **C.** Nuclear segmentation (green outline) using DAPI staining identifies individual cells. **D.** A cellular membrane mask (blue ring) allows quantitation of membranous markers e.g. CD8 (displayed) for each cell. **E.** Scattergrams are used to display cell characteristics for each marker, and allow identification of single, double and triple-positive cells. Here the staining intensity of CD8 (x-axis) and PD-L1 (y-axis) within each cell mask are displayed. **F.** CD8+ cells (red nuclei) can be visualised by 'backgating' cells with a FITC intensity above a user-defined threshold (red box) using scattergrams. Scale bars A & B 200µm, C, D & F 50µm.



Supplementary Figure 3. Spatial Distribution

Spatial distribution of cell populations was assessed by creating masks and limiting cell counts to these compartments. **A.** Epithelial (cyan) and stromal (green) masks based on the presence or absence of cytokeratin staining. **B.** Intratumoral $CD8^+$ cells (red nuclei) located on the epithelial mask (cyan). **C.** Stromal $CD8^+$ cells (red nuclei). **D.** A CD11c mask (white) created by thresholding on the CD11c channel. **E.** The distance transform algorithm allows creation of distance bands 0-10µm (green), 10-100µm (yellow) and >100µm (red) from the CD11c mask (white), allowing quantitation of $CD8^+$ densities within each zone. **F.** DAPI (blue) and CD8 (magenta) stains overlaid with the CD11c mask (white) and 0-10µm distance zone (green contour). $CD8^+$ cells within 10µm of the CD11c mask are highlighted red. Scale bars A, D & E 200µm, B, C & F 100µm.



Supplementary Figure 4. Overall survival by percentage of CD11c$^+$ cells expressing PD-L1
Survival curves for the percentage of PD-L1-expressing CD11c$^+$ cells in the tumor (**A**) and stromal (**B**) regions of leading edge cores. Log-Rank p-values and corresponding univariate Cox regression analysis results are presented.