

Supplemental Materials

Table S1. Characteristics of the different pancreatic cancer precursor lesions and pancreatic adenocarcinoma.

	MCN	IPMN	PanIN	PDAC
Cystic	yes	yes	no	no
Location	body/tail>head	head>body/tail	head=body=tail	head>body/tail
Pancreatic duct communication	no	yes	yes	yes
Association with invasive cancer	rarely	yes	yes	-
Cytologic atypia	low grade – high grade	low grade – high grade	low grade – high grade	-
Molecular mutations	KRAS, SMAD4, TP53	GNAS, KRAS, RNF43, TP53	KRAS, TP53, SMAD4/DPC4, BRCA2	KRAS, TP53, CDKN2A, SMAD4
Age, decade	4 th -5 th	6 th -7 th	6 th -7 th	7 th -8 th
Sex predilection	F>>>M	M>F	F>M	M>F

Figure S1. Composite images of multiplex fluorescent immunohistochemistry. A - F. Composite multispectral images of cystic lesions MCN (A) and IPMN (B), IPMN-associated PDAC (IAPA) (C), normal pancreatic tissue (NNPT) (D), PanIN (E), and PDAC (F) (original magnification, x20). White, ECs; green, CD4+ (helper) T cells; yellow, CD8+ (cytotoxic) T cells; red, Tregs; magenta, APCs.

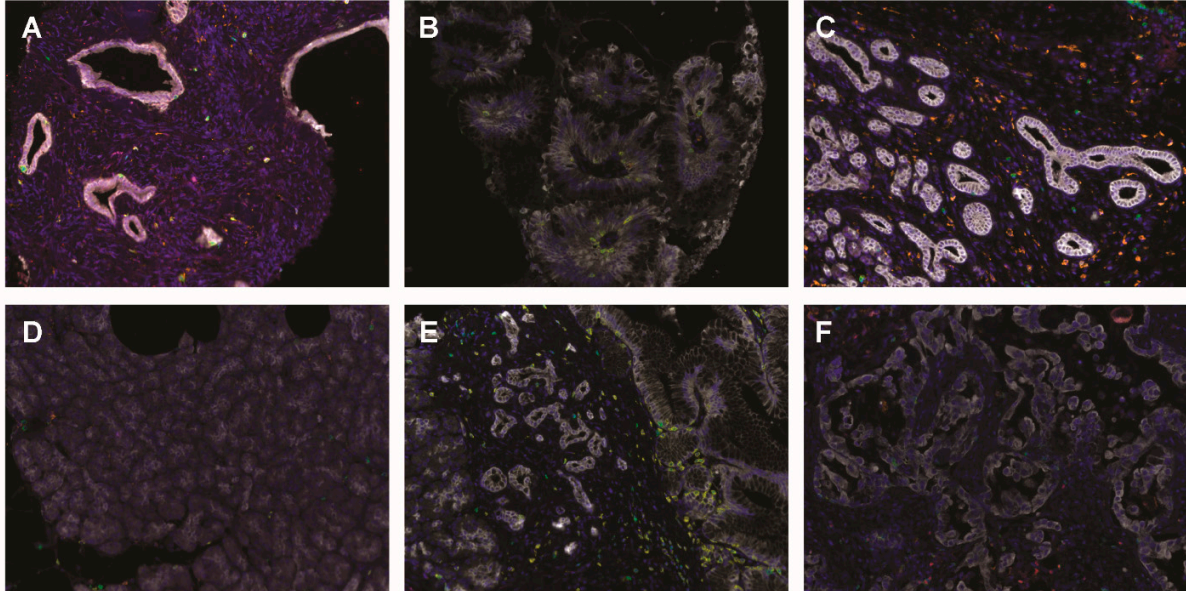


Figure S2. Composite image of PD-L1 staining (PD-L1+ cells) in PDAC.

White, ECs; green, CD4+ (helper) T cells; yellow, CD8+ (cytotoxic) T cells; red, Tregs; magenta, APCs; blue, PD-L1 positive cells (original magnification, x20).

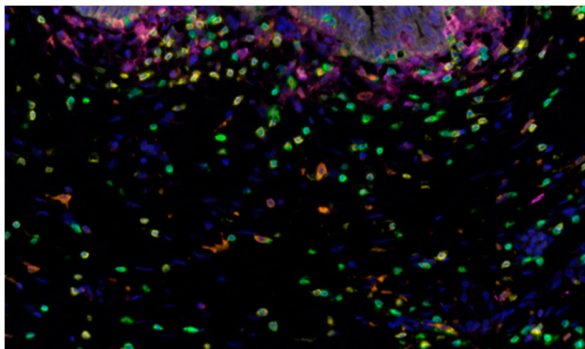


Figure S3. Schema depicting cells that are engaged or non-engaged. Cells are considered engaged if they are within a 15 μm radius from the center of a nucleus of a T cell or within a 40 μm radius from the center of a nucleus of another cell. If cells are outside that radius, they are considered non-engaged.

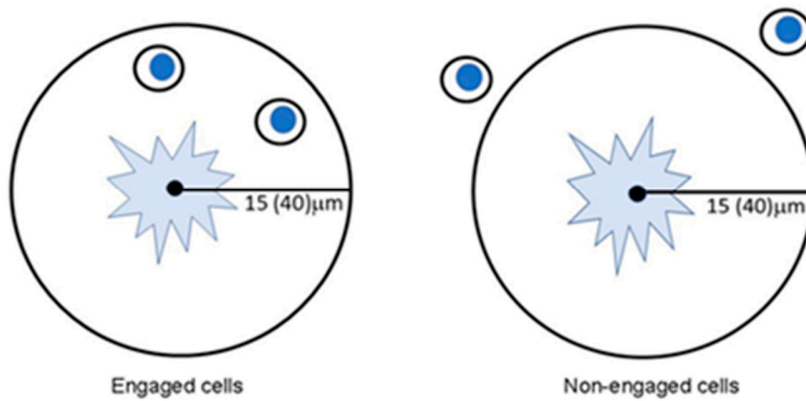


Figure S4. Engagement of CD8+ T cells with PD-L1 positive APCs in *cystic* lesions (A) and *non-cystic* lesions (C). Engagement of CD8+ T cells with PD-L1 positive ECs in *cystic* lesions (B) and *non-cystic* lesions (D). Normal pancreatic tissue used as a reference. * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$.

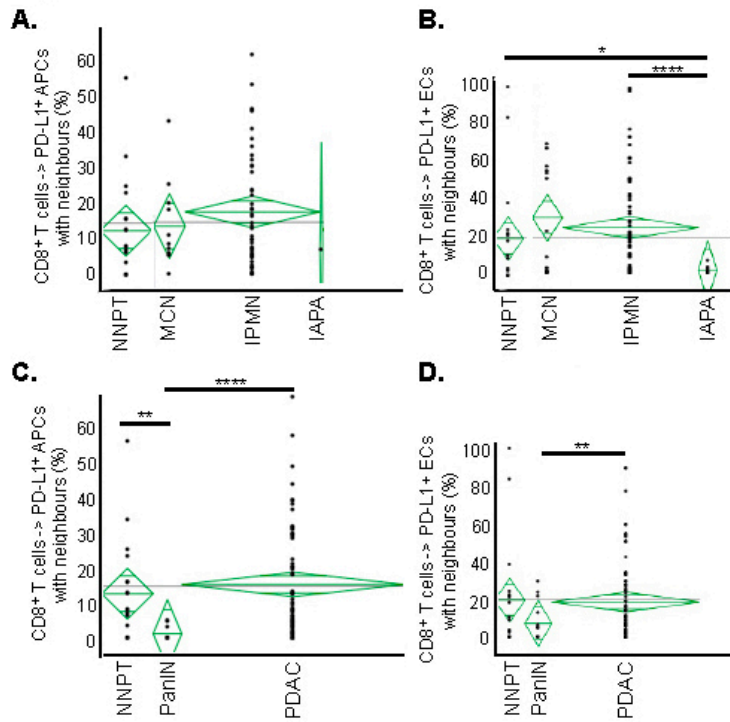


Table S2. Numbers of tissue samples of each tissue type used for the individual experiments.
Individual experiments are referred as to figures/images.

Figure	Image/Graph	NNPT	MCN	IPMN	IAPA	PanIN	PDAC
1	G, J	36	17	71	8	29	104
	H, K	36	17	71	8	29	104
	I, L	36	17	71	8	29	104
2	A, C	21	16	49	8	20	100
	B, D	21	16	49	8	20	100
3	A, C	36	17	71	8	29	106
	B, D	19	12	47	2	10	80
4	A, G	36	17	70	8	26	96
	B, H	36	15	66	8	27	98
	C, I	15	9	37	1	5	43
	D, J	11	8	35	8	13	75
	E, K	34	14	67	8	28	97
	F, L	36	15	71	8	29	93
5	A, C	36	14	72	8	28	104
	B, D	16	8	45	6	15	64
S4	A, C	14	11	44	1	4	69
	B, D	12	11	50	5	9	58

Figure S5. Neighbourhood analysis. The steps below represent the different steps we used to determine nearest neighbors. One representative example is shown.

First-order statistics

All cells

Number of cells: 2035

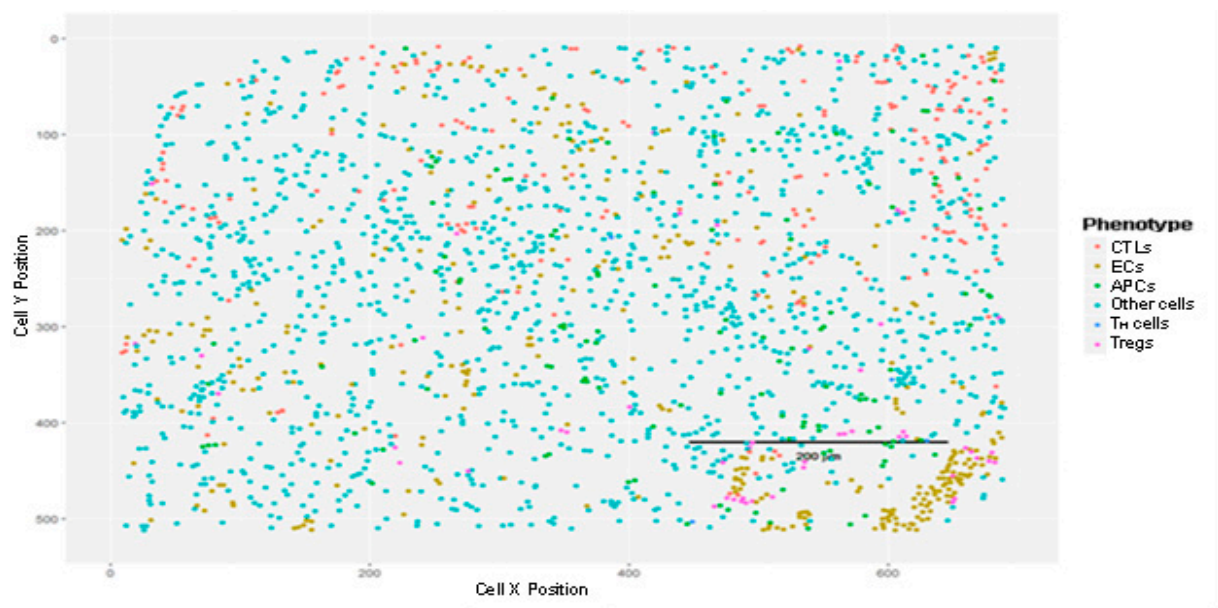
Average intensity: 0.006 cells per square micron

Frequency and intensity per phenotype:

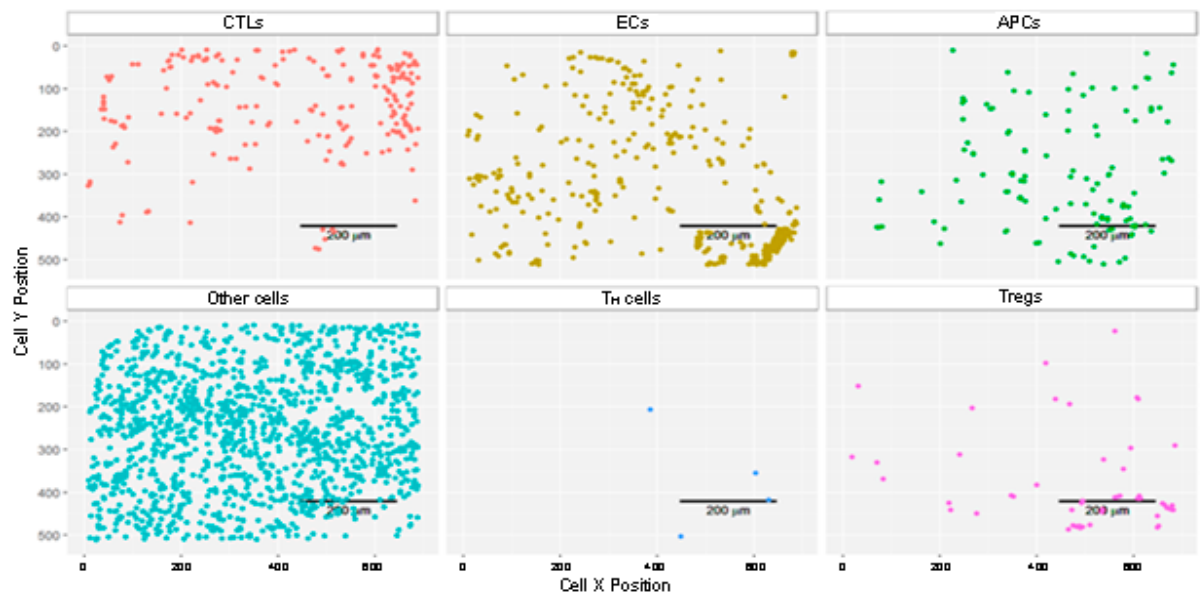
	frequency	proportion	intensity
Cytotoxic T cells	217	0.107	0.001
Epithelial cells	346	0.170	0.001
APC	119	0.058	0.000
Other cell types	1299	0.638	0.004
T helper cells	4	0.002	0.000
T regs	50	0.025	0.000

Cell and phenotype locations:

1) Locations of all cells



2) Individual cells



3) Nearest neighbours, selected phenotype

