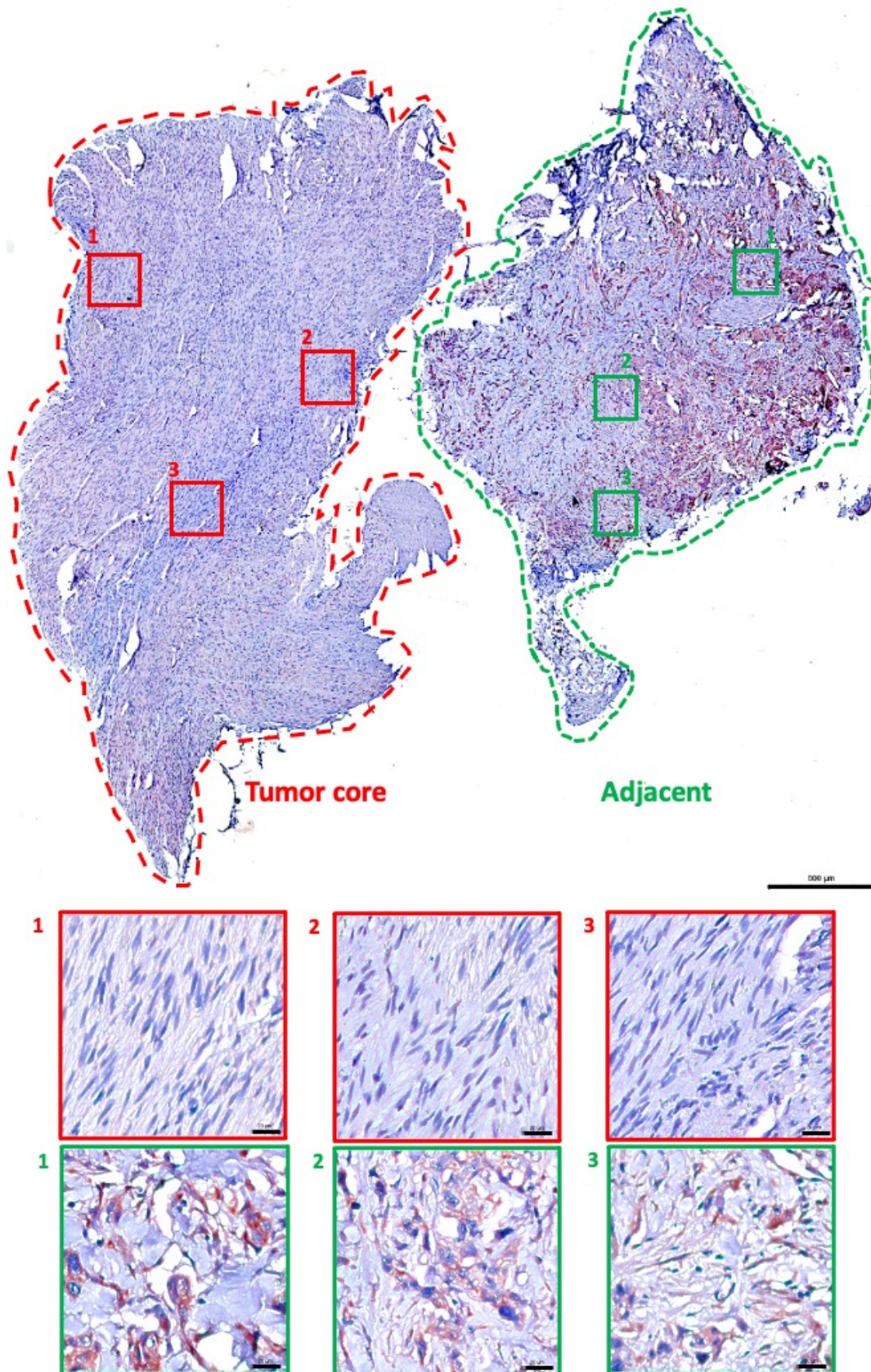


Appendix for
"CD106⁺ pericyte stem cells induce Ly6G⁺ cell accumulation and resistance to
immunotherapy in pancreatic cancer"

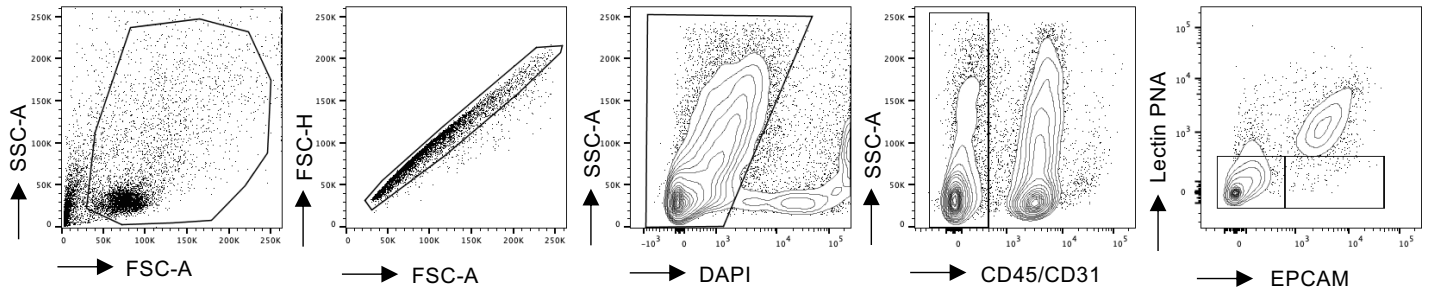
Appendix Figure S1.....	2
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Appendix Figure S1



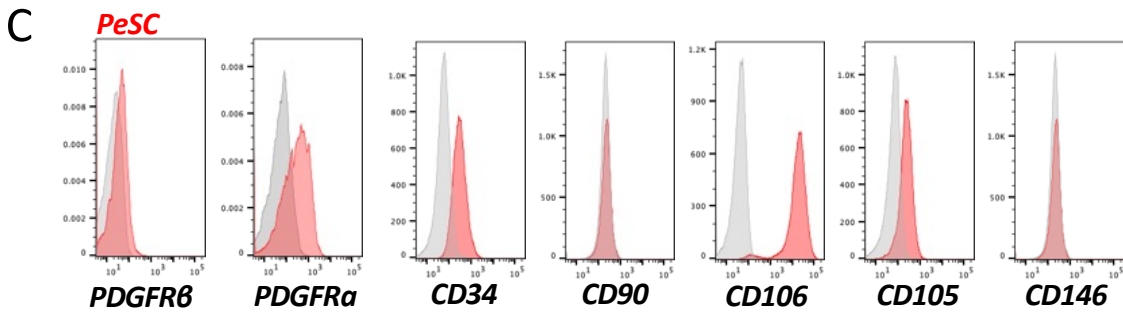
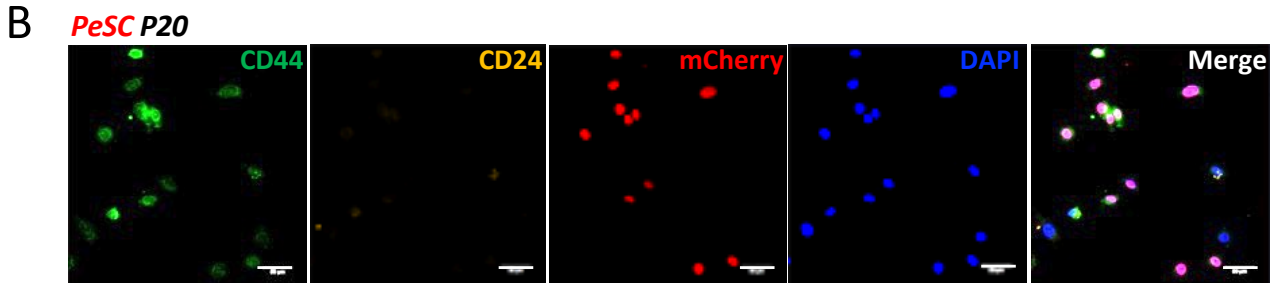
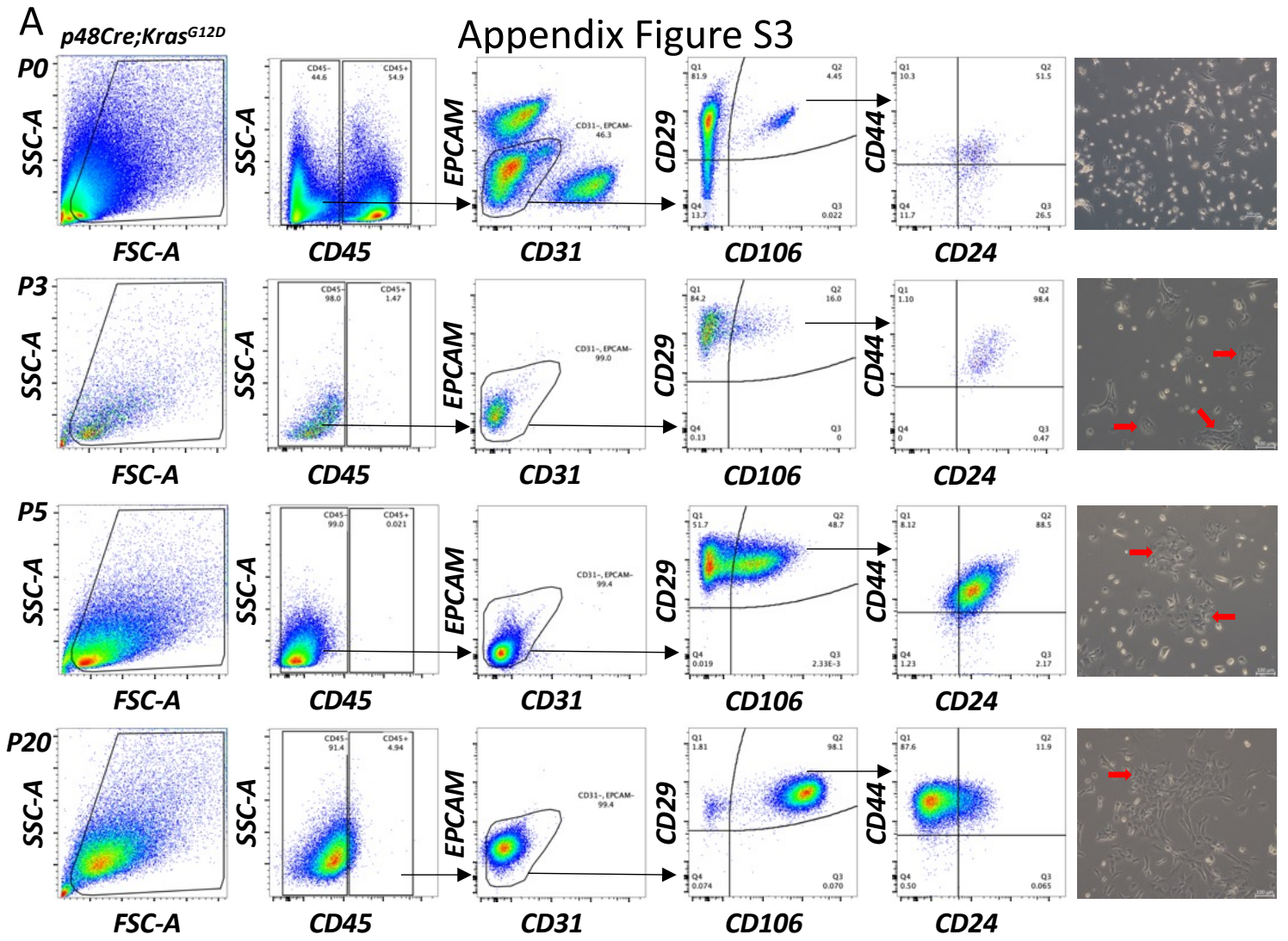
Appendix Figure S1: The CD106⁺ population is uniquely localized in pancreatic injury and precancerous lesions. Representative IHC staining of tumor core and adjacent region in PDAC patient (stage III) for CD106⁺. The corresponding magnification insets of tumor core (red dotted line) or adjacent region (green dotted line) are displayed below the gross figure. Scale bar, 500 μm in gross, 20 μm in magnification.

Appendix Figure S2

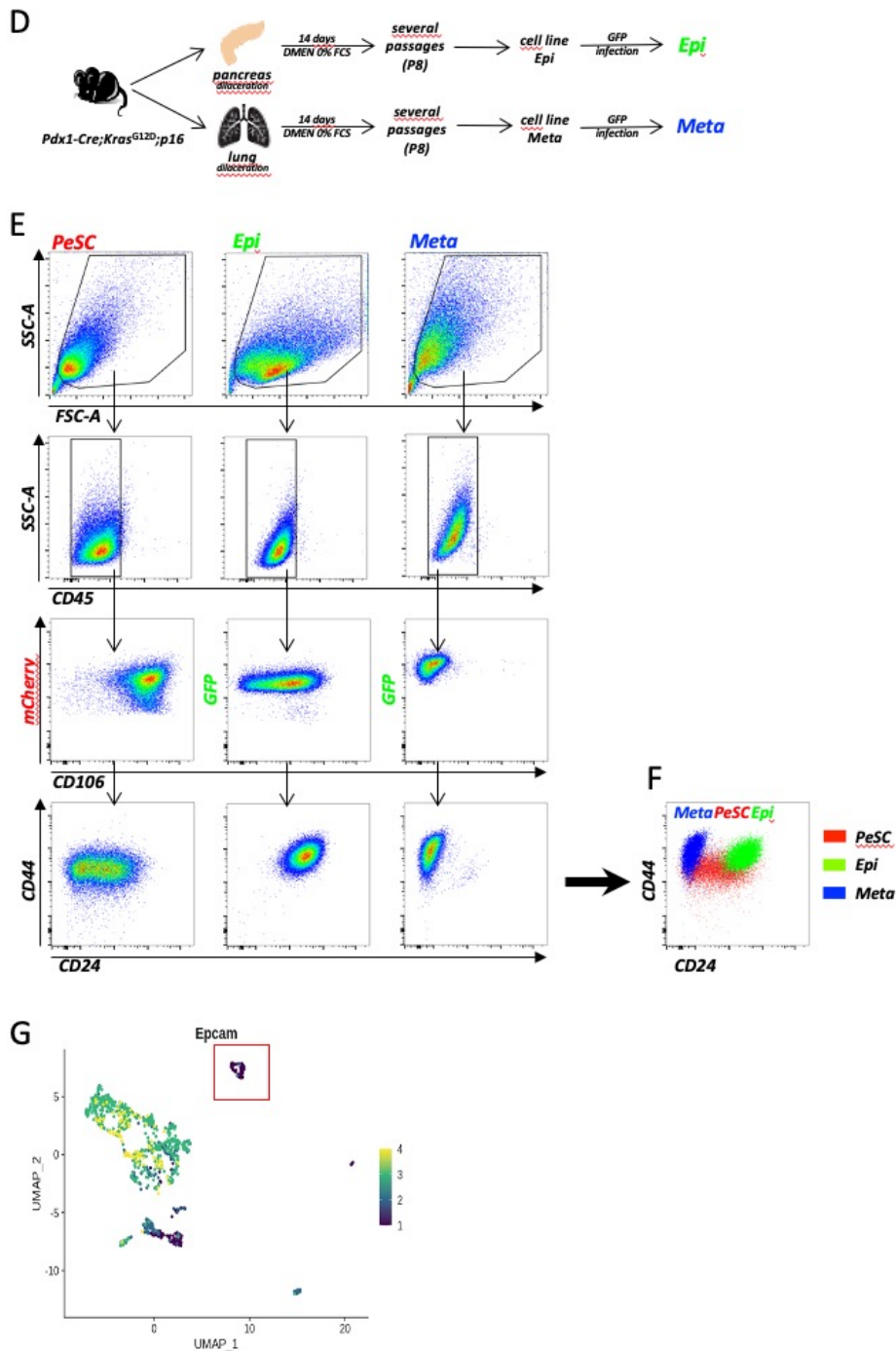


Appendix Figure S2: Gating strategy for FACS sorting and single cell RNAseq.

Appendix Figure S3

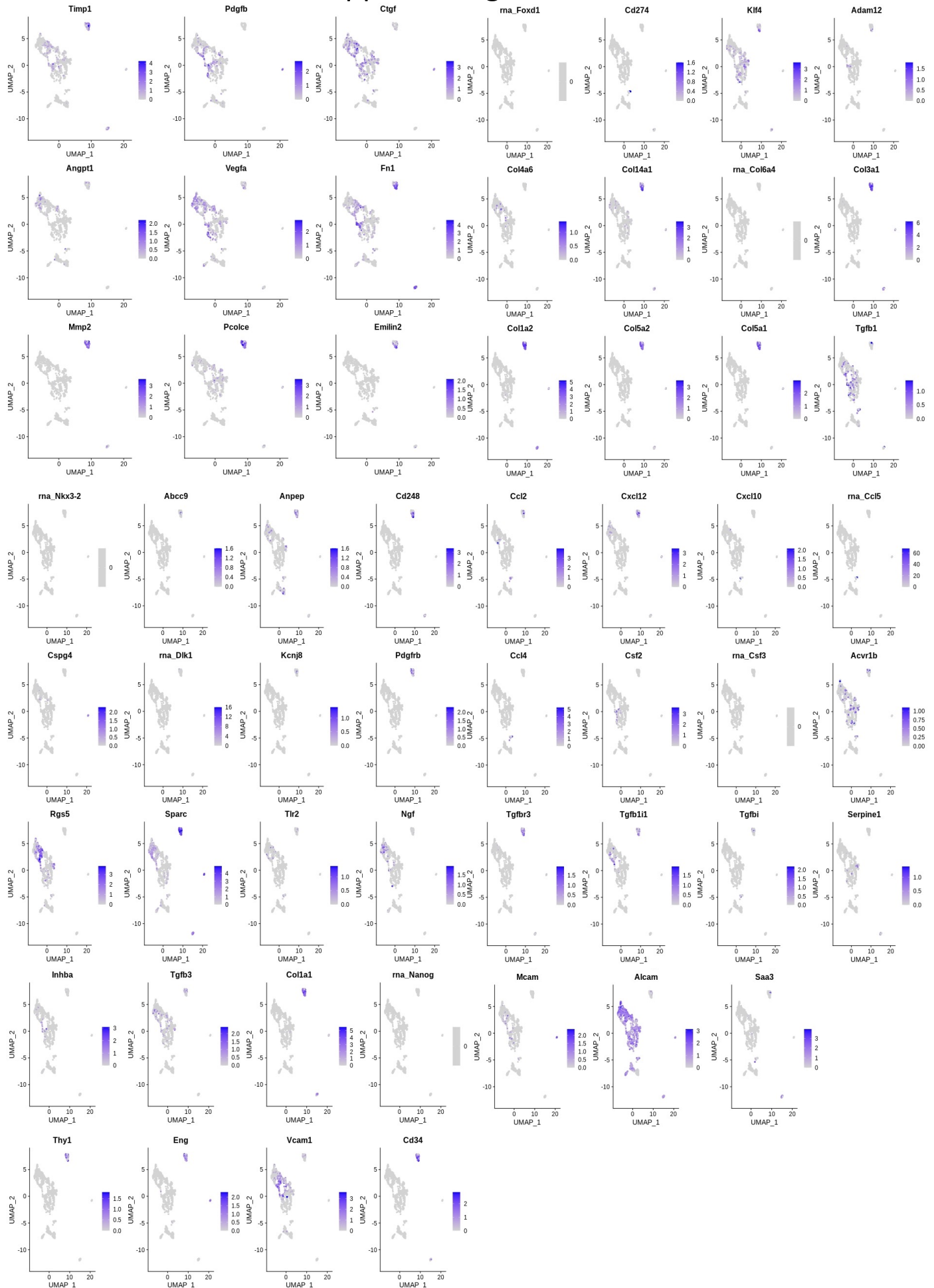


Appendix Figure S3



Appendix Figure S3: Generation of the PeSC line and its phenotype. (A) Gating strategy for the identification and kinetic phenotype-progression of the $CD45^+EPCAM^+CD31^+CD106^+CD24^{low}CD44^{high}$ population after cell line generation at P0, P3, P5 and P20. The red arrows indicate morphologically similar PeSC population clusters. Scale bar, 100 μ m. (B) Representative IF staining of the PeSC cell line on coverslip at P20 for CD44 (green), CD24 (yellow), mCherry (red), DAPI (blue). Scale bar, 50 μ m. (C) Representative histogram analysis of PDGFR β , PDGFR α , CD34, CD90, CD106, CD105, and CD146 by FACS staining in PeSCs. Generation of the Epi and Meta cell lines and their phenotypes. (D) Schematic representation of the experimental setting for Epi and Meta cell line generation. (E) Gating strategy for PeSCs, Epi cells and Meta cells based on CD45, mCherry/GFP, CD106, CD24 and CD44 staining. (F) Representative merged dot plots of PeSCs, Epi cells and Meta cells for CD24 and CD44 staining. (G) Single cell analysis UMAP plots shows that cluster 7 (PeSC fraction) had no expression of EPCAM.

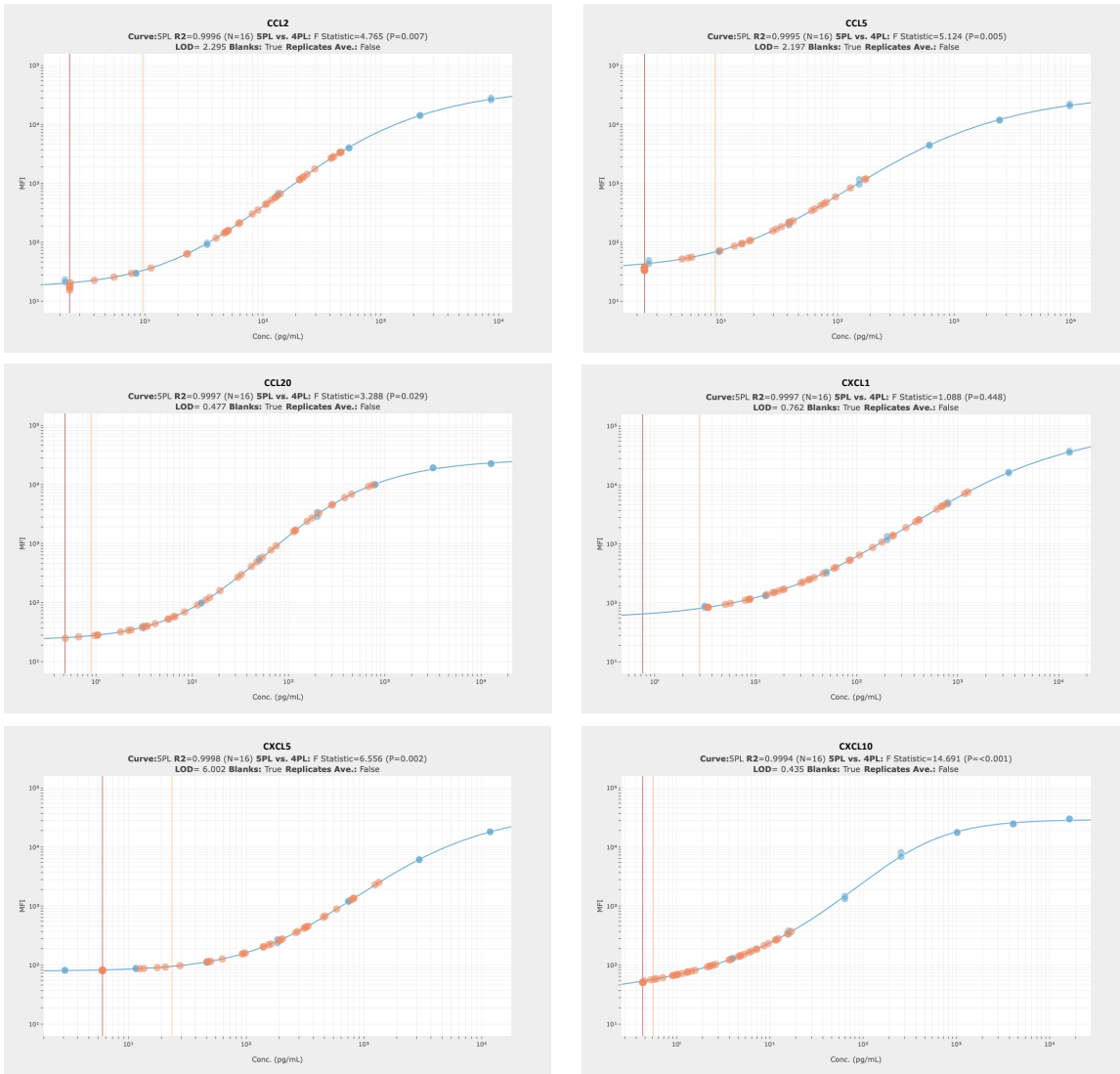
Appendix Figure S4



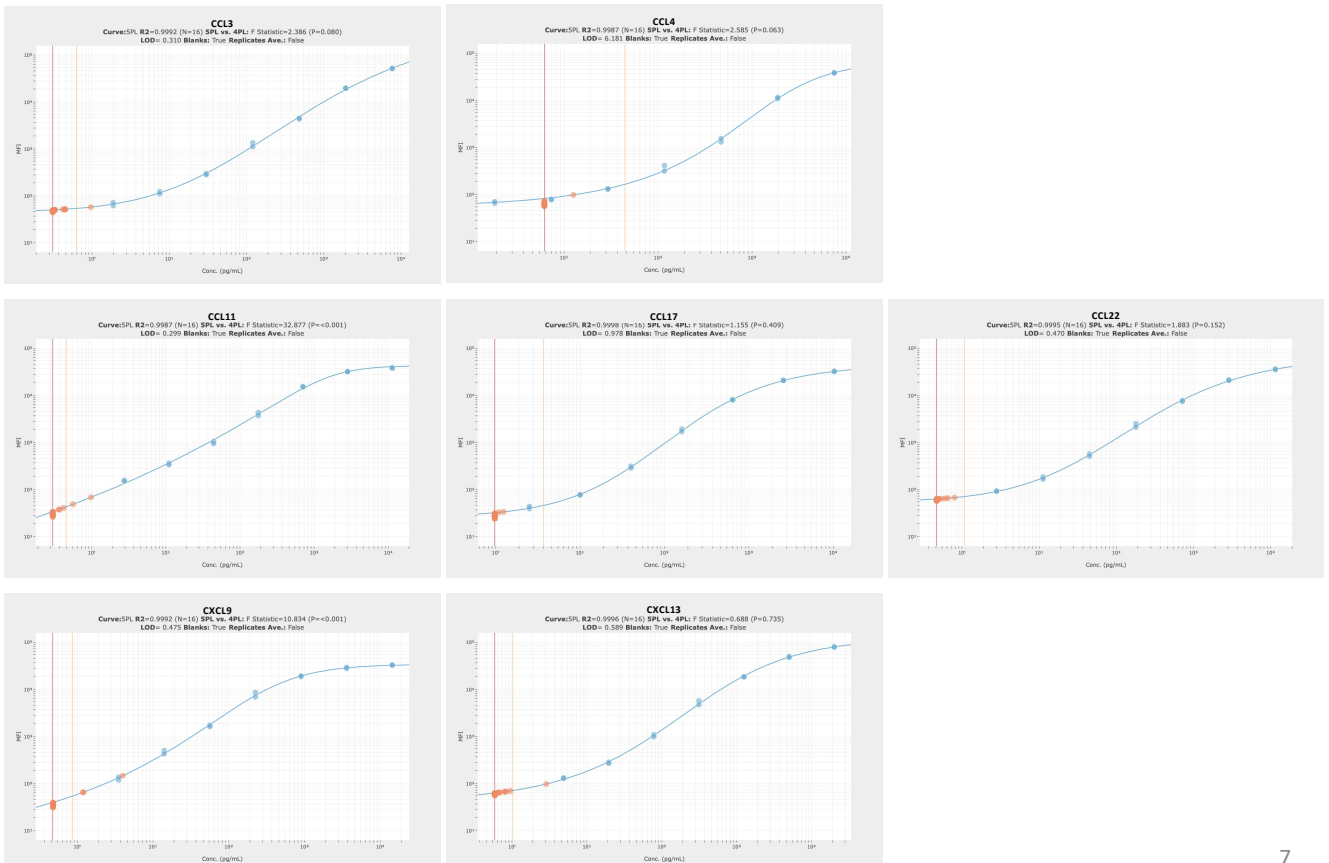
Appendix Figure S4: Single cell analysis reveals a distinct cluster 7 with PeSC signature. Single cell analysis UMAP plots of the PeSC signature genes extracted from the bulk RNAseq analysis and applied to the single cell analysis UMAP dots.

Appendix Figure S5

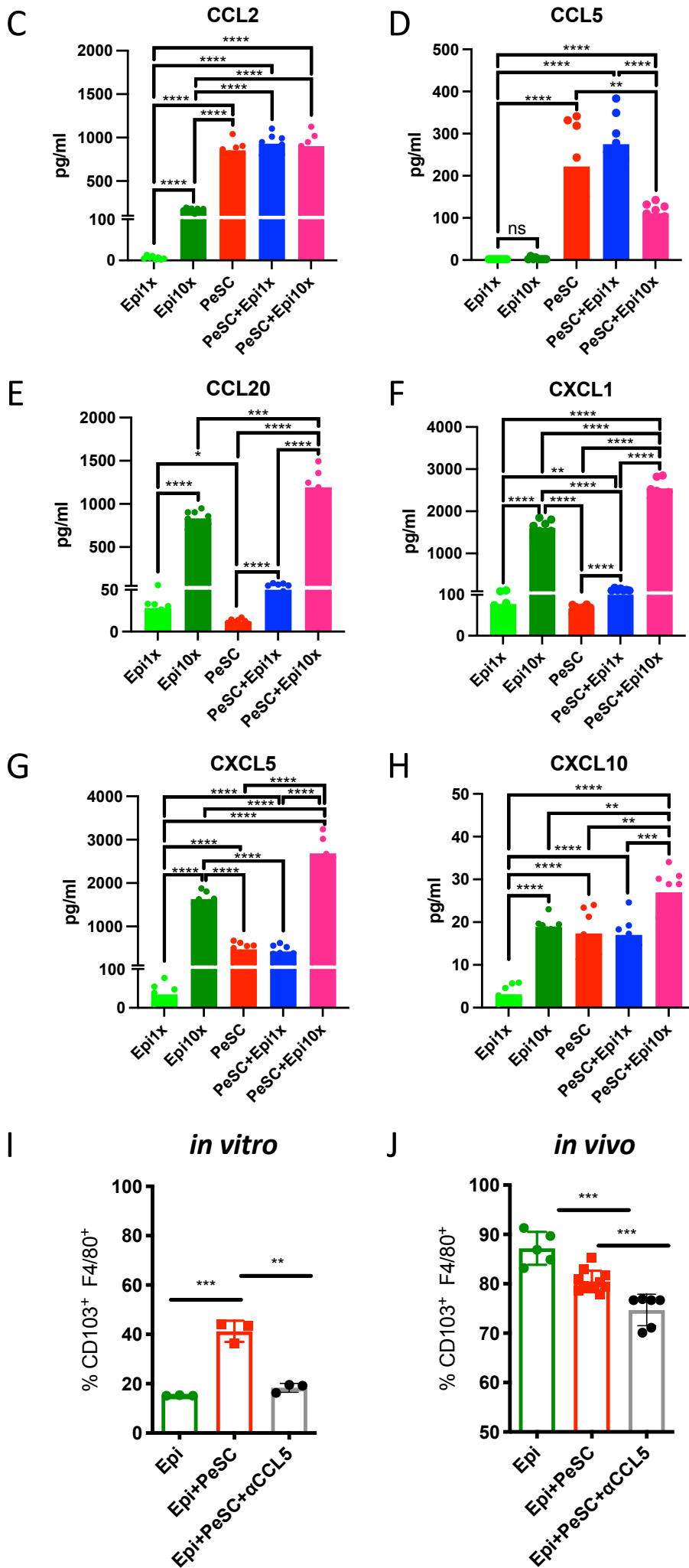
A



B



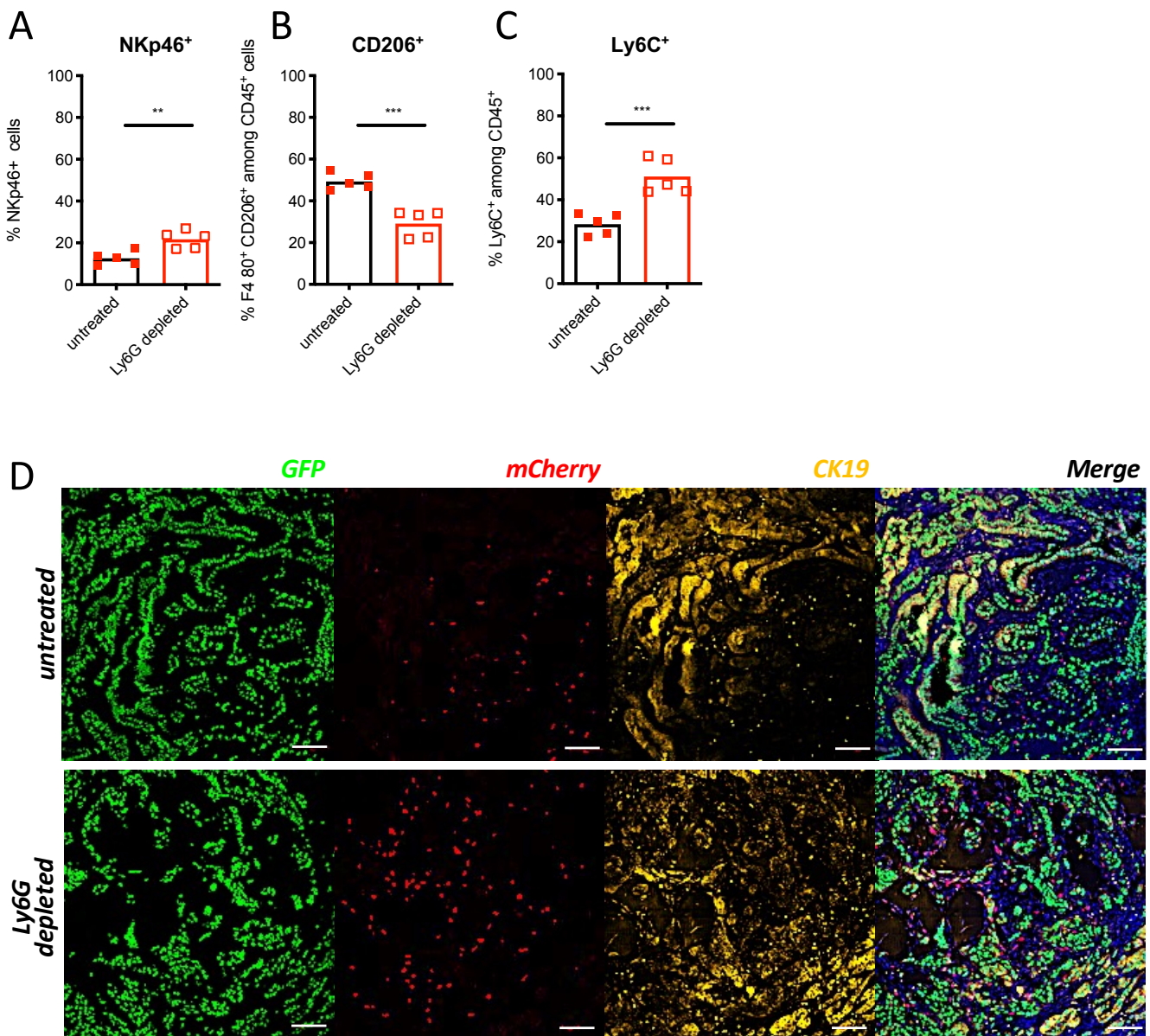
Appendix Figure S5



Appendix Figure S5

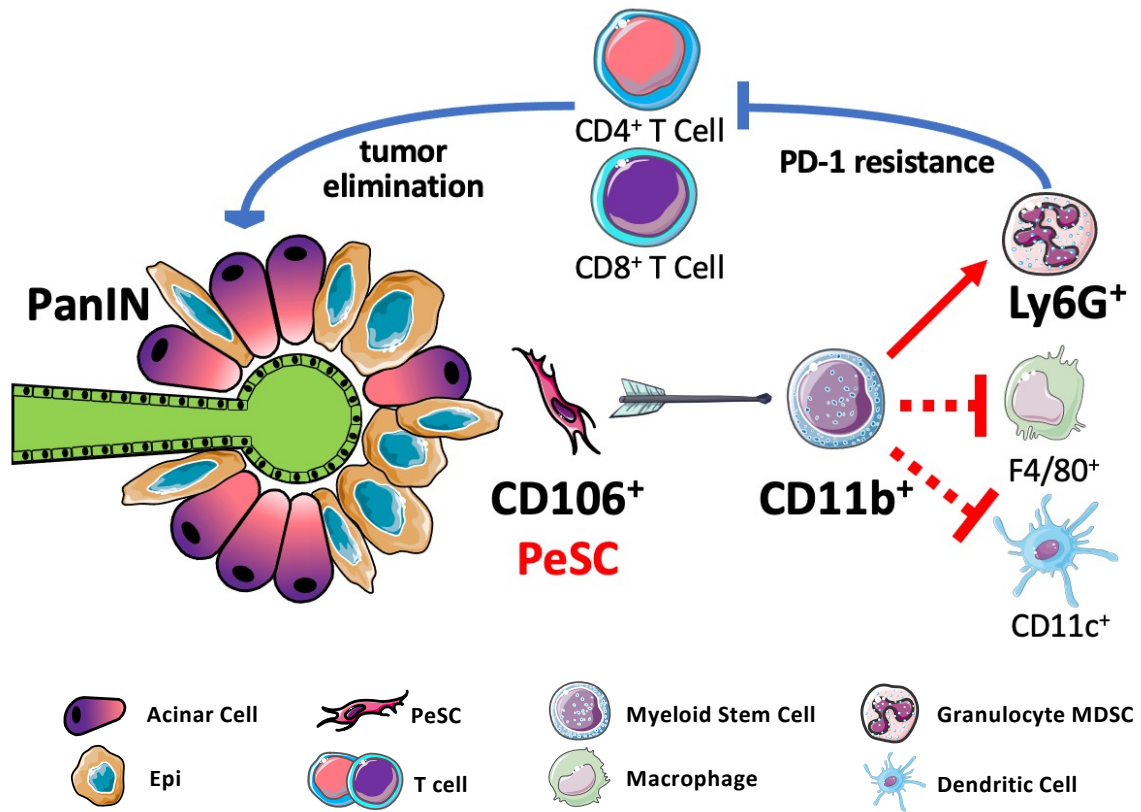
Appendix Figure S5: Tumor-PeSC crosstalk affects the immune system. (A and B) The standard curve of each chemokine is derived from the LEGENDplex™ data analysis software (blue dots and curves). All non-zero standard curve points are displayed as blue dots on the graph. All logistic regression line derived by the curve fitting algorithm is performed by 5-parameter logistic regression (5PL), displaying on the graph as a solid blue line. The theoretical limit of detection (LOD) and limit of quantification (LOQ) are depicted as red and orange vertical lines, respectively. Chemokine CCL2, CCL5, CCL20, CXCL1, CXCL5, and CXCL10 can be detected from the cell culture supernatant in condition of Epi1x, Epi10x, PeSC, PeSC + Epi1x and PeSC + Epi10x, as showed by the orange dots in line with the curve which were mostly above the LOD and LOQ (A). Chemokine CCL3, CCL4, CCL11, CCL17, CCL22, CXCL19 and CXCL13 are not detected from the cell culture supernatant in condition of Epi1x, Epi10x, PeSC, PeSC + Epi1x and PeSC + Epi10x, as all the sample dots (orange) were below the LOD and LOQ (B). Quantification of chemokine CCL2 (C), CCL5 (D), CCL20 (E), CXCL1 (F), CXCL5 (G), and CXCL10 (H) detected from the cell culture supernatant in conditions of Epi1x, Epi10x, PeSC, PeSC + Epi1x and PeSC + Epi10x, respectively. The LEGENDplex assays have been performed twice. The displayed results are extracted from the second experiment. Biological duplicates were applied in each culture condition and the supernatant of each condition was then distributed into 4 different dilutions for FACS analysis quantified as technical replicates. (I) *In vitro* FACS analysis of the percentage of CD103⁺F4/80⁺ cells among CD45⁺ cells under the indicated culture conditions (biological triplicates in each condition). (J) *In vivo* FACS analysis of the percentage of CD103⁺F4/80⁺ cells among CD45⁺ cells under the indicated graft and treatment conditions. Each dot represents 1 grafted mouse, 5-6 mice in each group. **P < 0.01, ***P < 0.001, ****P < 0.0001 The P-values were calculated using Student's t-test with GraphPad Prism.

Appendix Figure S6



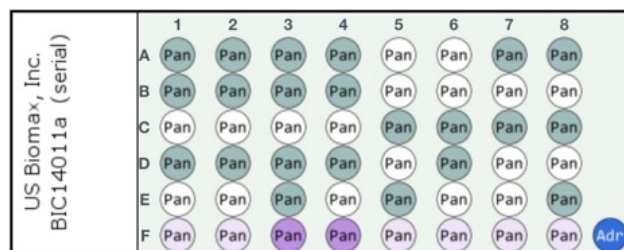
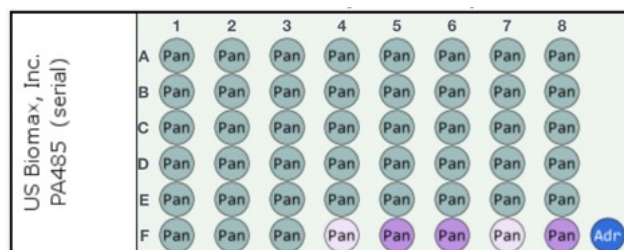
Appendix Figure S6: *In vivo* depletion of Ly6G⁺ MDSCs diminishes tumor growth. FACS analysis of the percentage of NKp46⁺ cells (A), CD206⁺F4/80⁺ cells (B), and Ly6C⁺ cells (C). **P < 0.01 and *** P < 0.001. The P-values were calculated using Student's t-test with GraphPad Prism. (D) Representative IF staining of the implanted tumor graft in Rag2KO mouse for GFP (green), mCherry (red), CK19 (yellow) and DAPI (blue). Scale bar, 50μm. 8 mice in each group, 5 for FACS analysis and 3 for histological analysis. Each dot represents 1 mouse.

Appendix Figure S7



Appendix Figure S7: Interaction of CD106⁺ PeSCs with CD11b⁺ myeloid stem cells in the neoplastic microenvironment of PDAC

Appendix Table S1



No.	Age	Sex	Organ/ Anatomic Site	Pathological diagnosis	TNM	Grade	Stage	Type
A1	68	M	Pancreas	AP	-	-	-	Inf
A2	47	M	Pancreas	AP	-	-	-	Inf
A3	60	M	Pancreas	AP	-	-	-	Inf
A4	44	M	Pancreas	CP	-	-	-	Inf
A5	49	F	Pancreas	CP	-	-	-	Inf
A6	62	M	Pancreas	CP	-	-	-	Inf
A7	40	M	Pancreas	CP	-	-	-	Inf
A8	51	F	Pancreas	CP	-	-	-	Inf
B1	58	M	Pancreas	CP	-	-	-	Inf
B2	47	M	Pancreas	CP	-	-	-	Inf
B3	51	M	Pancreas	RCP	-	-	-	Inf
B4	39	M	Pancreas	RCP	-	-	-	Inf
B5	59	M	Pancreas	RCP	-	-	-	Inf
B6	50	F	Pancreas	RCP	-	-	-	Inf
B7	51	M	Pancreas	RCP	-	-	-	Inf
B8	61	M	Pancreas	RCP	-	-	-	Inf
C1	51	F	Pancreas	RCP	-	-	-	Inf
C2	50	F	Pancreas	RCP	-	-	-	Inf
C3	67	M	Pancreas	RCP	-	-	-	Inf
C4	60	M	Pancreas	RCP	-	-	-	Inf
C5	67	M	Pancreas	RCP	-	-	-	Inf
C6	53	F	Pancreas	RCP	-	-	-	Inf
C7	33	F	Pancreas	RCP	-	-	-	Inf
C8	47	F	Pancreas	RCP	-	-	-	Inf
D1	57	M	Pancreas	RCP	-	-	-	Inf
D2	33	F	Pancreas	RCP	-	-	-	Inf
D3	49	M	Pancreas	RCP	-	-	-	Inf
D4	46	F	Pancreas	RCP	-	-	-	Inf
D5	59	M	Pancreas	RCP	-	-	-	Inf
D6	66	F	Pancreas	RCP	-	-	-	Inf
D7	66	F	Pancreas	RCP	-	-	-	Inf
D8	65	F	Pancreas	RCP	-	-	-	Inf
E1	55	M	Pancreas	RCP	-	-	-	Inf
E2	76	M	Pancreas	RCP	-	-	-	Inf
E3	35	F	Pancreas	RCP	-	-	-	Inf
E4	60	M	Pancreas	RCP	-	-	-	Inf
E5	49	M	Pancreas	RCP	-	-	-	Inf
E6	47	F	Pancreas	RCP	-	-	-	Inf
E7	61	M	Pancreas	RCP	-	-	-	Inf
E8	76	F	Pancreas	RCP	-	-	-	Inf
F1	53	M	Pancreas	RCP	-	-	-	Inf
F2	55	F	Pancreas	RCP	-	-	-	Inf
F3	68	M	Pancreas	RCP	-	-	-	Inf
F4	60	M	Pancreas	PDAC	T2N0M0	2	IB	Mal
F5	49	M	Pancreas	PDAC	T3N0M0	3	II	Mal
F6	47	F	Pancreas	PDAC	T2N0M0	2	IIB	Mal
F7	61	M	Pancreas	PDAC	T2N0M0	2	IB	Mal
F8	76	F	Pancreas	PDAC	T3N0M0	1	I	Mal
-	42	M	Adrenal gland	PCC	-	-	-	Mal

AP = Acute Pancreatitis
CP = Chronic Pancreatitis
RCP = Reactive Chronic Pancreatitis
PCC = Pheochromocytoma
Inf = Inflammation
Mal = Malignant

<https://www.biomax.us/tissue-arrays/pancreas/PA485>

No.	Age	Sex	Organ/ Anatomic Site	Pathologica l diagnosis	TMN	Grade	Stage	Type
A1	42	M	Pancreas	CP	-	-	-	Inf
A2	42	M	Pancreas	CP	-	-	-	Inf
A3	50	F	Pancreas	CP	-	-	-	Inf
A4	50	F	Pancreas	CP	-	-	-	Inf
A5	66	F	Pancreas	PanIN2	-	-	-	PanIN
A6	66	F	Pancreas	PanIN2	-	-	-	PanIN
A7	70	F	Pancreas	CP	-	-	-	Inf
A8	70	F	Pancreas	CP	-	-	-	Inf
B1	51	M	Pancreas	CP	-	-	-	Inf
B2	51	M	Pancreas	CP	-	-	-	Inf
B3	57	M	Pancreas	CP	-	-	-	Inf
B4	57	M	Pancreas	CP	-	-	-	Inf
B5	33	F	Pancreas	PanIN1	-	-	-	PanIN
B6	33	F	Pancreas	PanIN1	-	-	-	PanIN
B7	44	M	Pancreas	PanIN1	-	-	-	PanIN
B8	44	M	Pancreas	PanIN1	-	-	-	PanIN
C1	53	M	Pancreas	PanIN1	-	-	-	PanIN
C2	53	M	Pancreas	PanIN1	-	-	-	PanIN
C3	51	F	Pancreas	PanIN1	-	-	-	PanIN
C4	51	F	Pancreas	PanIN1	-	-	-	PanIN
C5	51	M	Pancreas	CP	-	-	-	Inf
C6	51	M	Pancreas	CP	-	-	-	Inf
C7	55	F	Pancreas	CP	-	-	-	Inf
C8	55	F	Pancreas	CP	-	-	-	Inf
D1	53	M	Pancreas	CP	-	-	-	Inf
D2	53	M	Pancreas	CP	-	-	-	Inf
D3	67	M	Pancreas	CP	-	-	-	Inf
D4	67	M	Pancreas	CP	-	-	-	Inf
D5	73	F	Pancreas	PanIN1	-	-	-	PanIN
D6	73	F	Pancreas	CP	-	-	-	Inf
D7	65	M	Pancreas	PanIN2	-	-	-	PanIN
D8	65	M	Pancreas	PanIN2	-	-	-	PanIN
E1	64	M	Pancreas	PanIN2	-	-	-	PanIN
E2	64	M	Pancreas	PanIN2	-	-	-	PanIN
E3	76	M	Pancreas	CP	-	-	-	Inf
E4	76	M	Pancreas	PanIN1	-	-	-	PanIN
E5	15	F	Pancreas	CP	-	-	-	Inf
E6	15	F	Pancreas	PanIN2	-	-	-	PanIN
E7	55	F	Pancreas	PanIN2	-	-	-	PanIN
E8	55	F	Pancreas	CP	-	-	-	Inf
F1	54	F	Pancreas	PDAC	T2N0M0	1	I	Mal
F2	54	F	Pancreas	PDAC	T2N0M0	1	I	Mal
F3	51	M	Pancreas	PDAC	T3N1M0	1	IIB	Mal
F4	51	M	Pancreas	PDAC	T3N1M0	1	IIB	Mal
F5	68	F	Pancreas	PDAC	T2N0M0	2	IB	Mal
F6	68	F	Pancreas	PDAC	T2N0M0	2	IB	Mal
F7	55	F	Pancreas	PDAC	T2N0M0	2	IB	Mal
F8	55	F	Pancreas	PDAC	T2N0M0	-	IB	Mal
-	42	M	Adrenal gland	PCC	-	-	-	Mal

PanIN = Pancreatic Intraepithelial Neoplasia
PDAC = Pancreatic Duct Adenocarcinoma

<https://www.biomax.us/tissue-arrays/pancreas/BIC14011a>

Appendix Table S1: TMA characteristics.