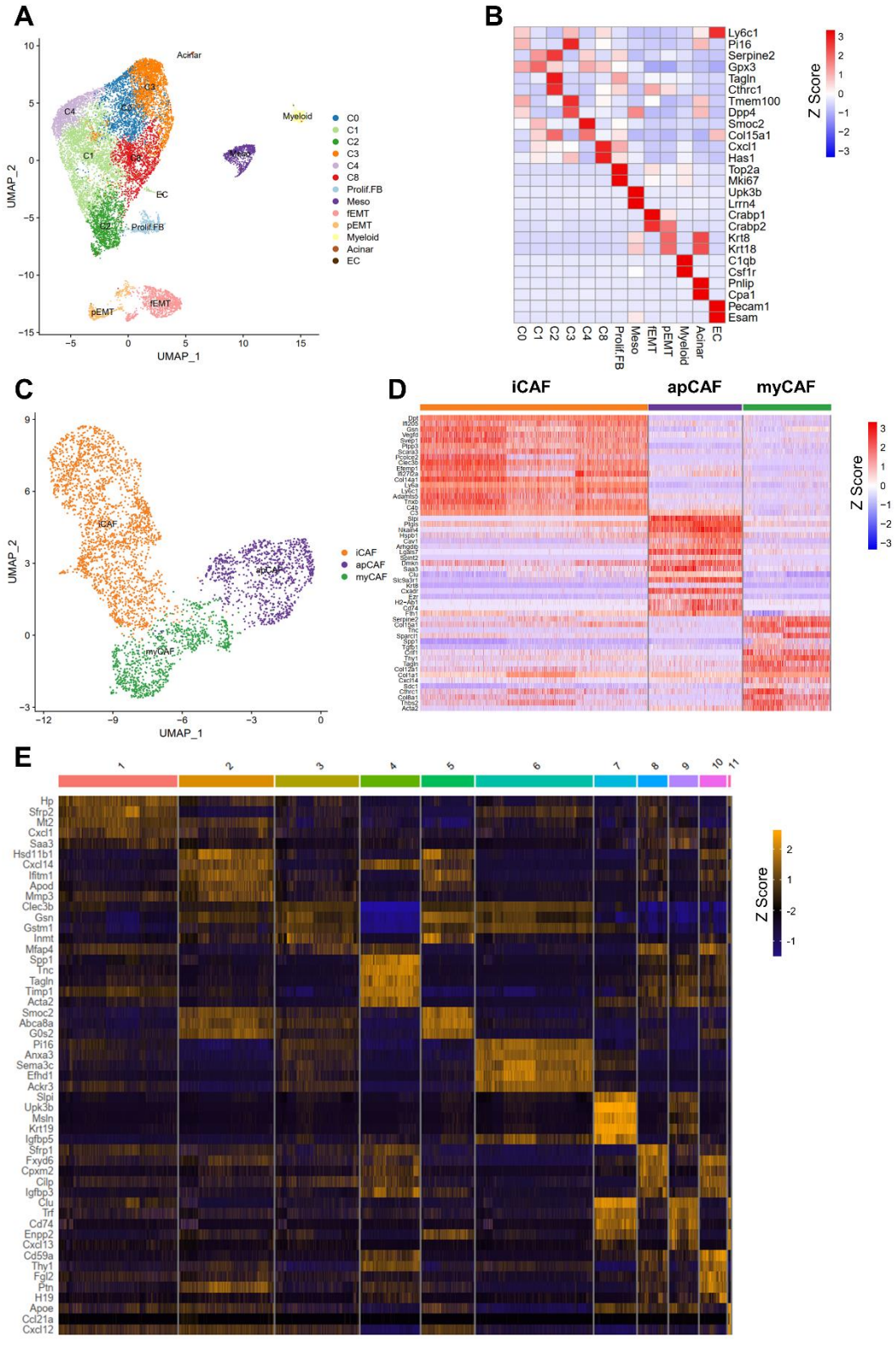

A
B
C
D
E
UMAP_1
UMAP_2
Acinar
Myeloid
Meso
EC
Prolif.FB
fEMT
pEMT
C0
C1
C2
C3
C4
C8
iCAF
apCAF
myCAF
Z Score
Ly6c1
Pi16
Serpine2
Gpx3
Tagln
Cthrc1
Tmem100
Dpp4
Smoc2
Col15a1
Cxcl1
Has1
Top2a
Mki67
Upk3b
Lrrn4
Crabp1
Crabp2
Krt8
Krt18
C1qb
Csf1r
Pnlip
Cpa1
Pecam1
Esam

Figure S1. Preprocessing of the scRNA-seq Datasets for the Integrated Analyses, Related to Figure 1.

(A) scRNA-seq data of the *KIC* PDA by Dominguez et al. were reprocessed. Identities of the cell clusters were matched to the previously reported cluster names based on the expression of genes mentioned in Dominguez et al. including clusters 0, 1, 2, 3, 4, 8, proliferating fibroblasts (Prolif.FB), normal mesothelial cells (Meso), fEMT tumor cells (fEMT), pEMT tumor cells (pEMT), myeloid cells, acinar cells and endothelial cells (EC).

(B) The heatmap displaying the top marker genes for each cell cluster of the reprocessed data from Dominguez et al.

(C) scRNA-seq data of the *KPC* PDA by Elyada et al. were reprocessed. Identities of the cell clusters were matched to the previously reported cluster names based on the expression of genes mentioned in Elyada et al. including iCAFs, apCAFs and myCAFs.

(D) The heatmap displaying the top marker genes for each cell cluster of the reprocessed data from Elyada et al.

(E) Three scRNA-seq datasets of fibroblasts in PDA (Hosein et al., Dominguez et al. and Elyada et al.) were integrated. Graph-based clustering of cells with UMAP was performed with the integrated data and 11 clusters of fibroblasts were identified (Figure 1G). The top marker genes for each cell cluster of the integrated data were shown as the heatmap.

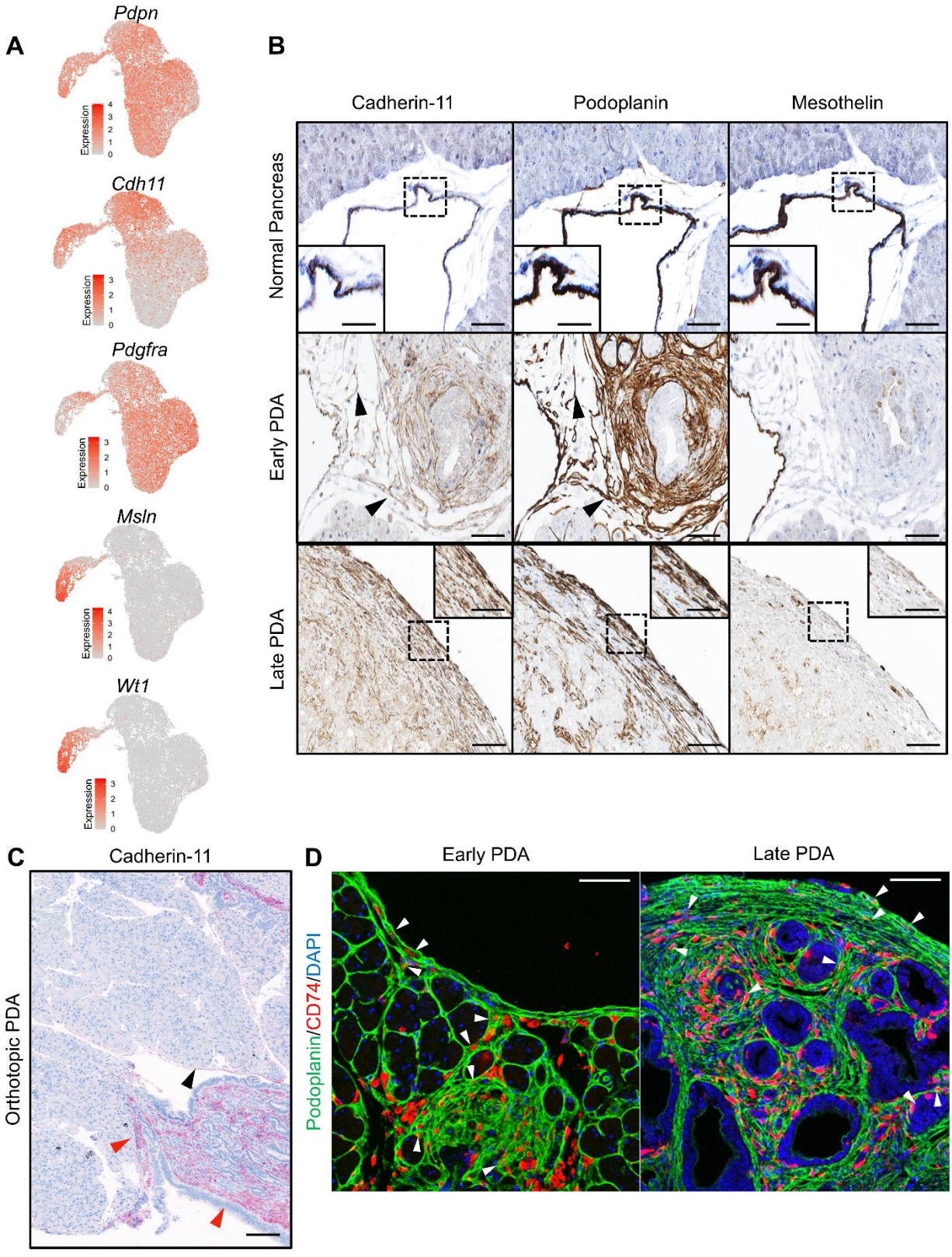

A
Pdpn
Cdh11
Pdgfra
Msln
Wt1
Expression
B
Cadherin-11
Podoplanin
Mesothelin
Normal Pancreas
Early PDA
Late PDA
C
Cadherin-11
Orthotopic PDA
D
Early PDA
Late PDA
Podoplanin/CD74/DAPI

Figure S2. Mesothelial Cells Form a Lining of Normal Pancreas and Contribute to the Peripheral CAF Formation in PDA, Related to Figure 2.

(A) UMAP plots of *Pdpn*, *Cdh11*, *Pdgfra*, *Msln* and *Wt1* from the integrated fibroblast data. Color keys indicate log normalized expression.

(B) IHC staining of mesothelium in normal pancreata, early *KPfC* tumors (40-day-old) and late *KPfC* tumors (60-day-old) by cadherin-11, podoplanin and mesothelin. Arrows, expanding mesothelial cells. Original scale bar 50 μm. Inset scale bar 12.5 μm.

(C) The primary mouse PDA cell line BMFA3 derived from *KPfC* tumor was orthotopically injected into the pancreata of C57BL/6 mice. Mouse pancreata were harvested one week after the implantation and subjected to IHC staining for cadherin-11. The cadherin-11^+ mesothelium lining the uninvolved normal pancreas tissues (black arrow). The cadherin-11^+ mesothelium adjacent to the tumor lesions (red arrows). Scale bar 200 μm.

(D) Multiple IHC staining of podoplanin (green), CD74 (red) and DAPI (blue) in early *KPfC* tumors (40-day-old) and late *KPfC* tumors (60-day-old). Arrows, podoplanin$^+$CD74$^+$ mesothelial cells or apCAFs. Scale bar 50 μm.

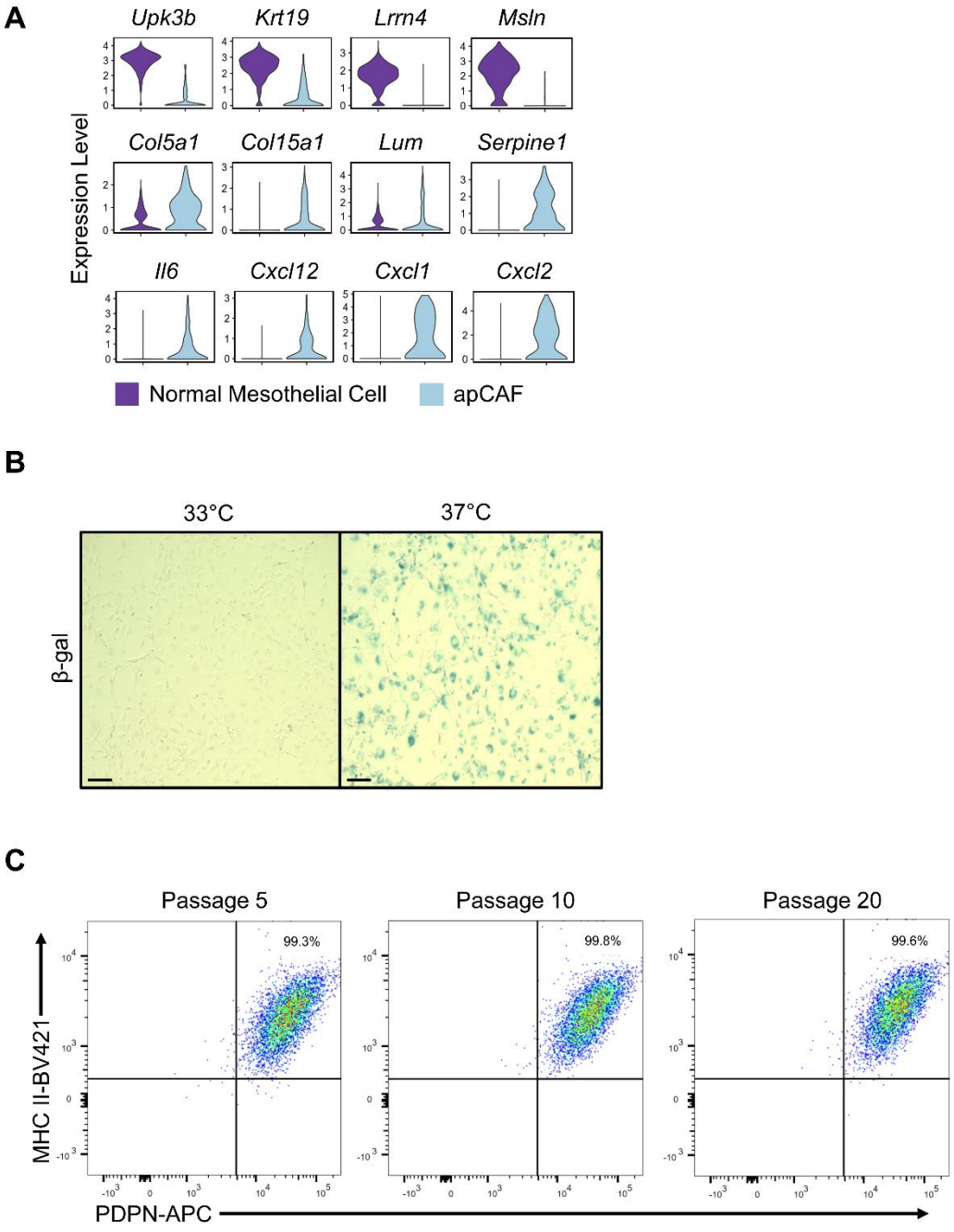
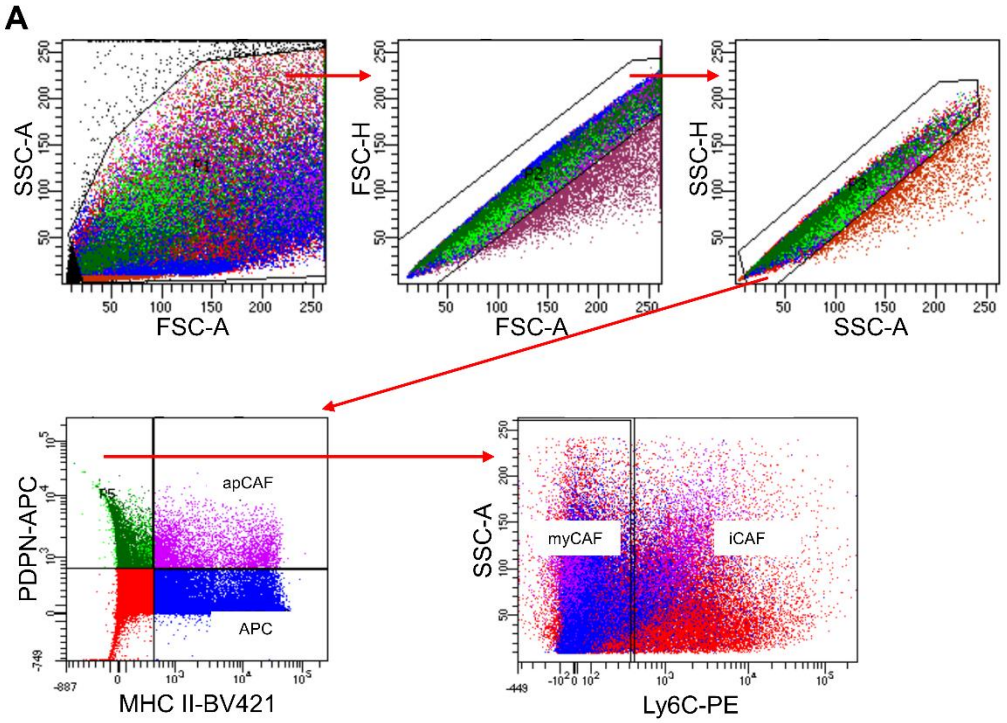

A
Upk3b
Krt19
Lrrn4
Msln
Col5a1
Col15a1
Lum
Serpine1
Il6
Cxcl12
Cxcl1
Cxcl2
Expression Level
Normal Mesothelial Cell
apCAF
B
33°C
37°C
β-gal
C
Passage 5
Passage 10
Passage 20
99.3%
99.8%
99.6%
MHC II-BV421
PDPN-APC

Figure S3. Gene Signature of Normal Mesothelial Cells and apCAFs and Characterization of PanMeso Cells, Related to Figures 2 and 4.

(A) Violin plots showing the mesothelial genes (*Upk3b*, *Krt19*, *Lrrn4*, *Msln*) and fibroblastic genes (*Col5a1*, *Col15a1*, *Lum*, *Serpine1*, *Il6*, *Cxcl12*, *Cxcl1*, *Cxcl2*) in normal mesothelial cells and apCAFs from the integrated data (Figure 1G). The width of the violin plots represents frequency of cells in each region. Values of Y axis indicate log normalized expression of genes.

(B) PanMeso cells were cultured at 33 °C or 37 °C for seven days and subjected to staining for β-Galactosidase to examine cell senescence. Scale bar 50 μm.

(C) PanMeso cells were cultured at 33 °C for 5, 10 and 20 passages. Cells from different passages were subjected to flow cytometry analysis for podoplanin and MHC II.


A
SSC-A
FSC-A
FSC-H
FSC-A
SSC-H
SSC-A
PDPN-APC
MHC II-BV421
apCAF
APC
SSC-A
Ly6C-PE
myCAF
iCAF

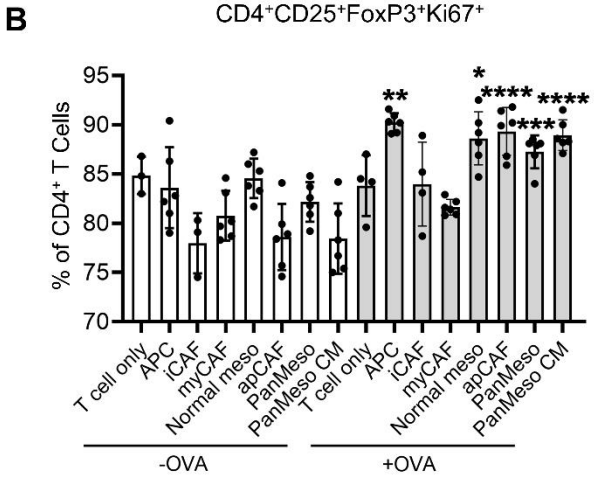

B
CD4⁺CD25⁺FoxP3⁺Ki67⁺
% of CD4⁺ T Cells
95
90
85
80
75
70
T cell only
APC
iCAF
myCAF
Normal meso
apCAF
PanMeso
PanMeso CM
-OVA
+OVA
**
*
****
****
****

Figure S4. apCAFs Induce Treg Expansion, Related to Figure 5.

(A) Late stage *KPfC* tumors were digested into cell suspension. APCs (podoplanin^{-}MHC II^{+}), apCAFs (podoplanin^{+}MHC II^{+}), iCAFs (podoplanin^{+}MHC II^{-}Ly6C^{+}), myCAFs (podoplanin^{+}MHC II^{-}Ly6C^{-}) from *KPfC* tumors were sorted by FACS.

(B) CD4^{+} T cells after being co-cultured with APCs/iCAFs/myCAFs/normal mesothelial cells/apCAFs were subjected to flow cytometry for the analysis of CD25, FoxP3 and Ki67. CD4^{+}CD25^{+}FoxP3^{+}Ki67^{+} T cells were quantified, n=3-6, data shown as mean ± SD, statistical analysis, t-test, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 vs without OVA control.

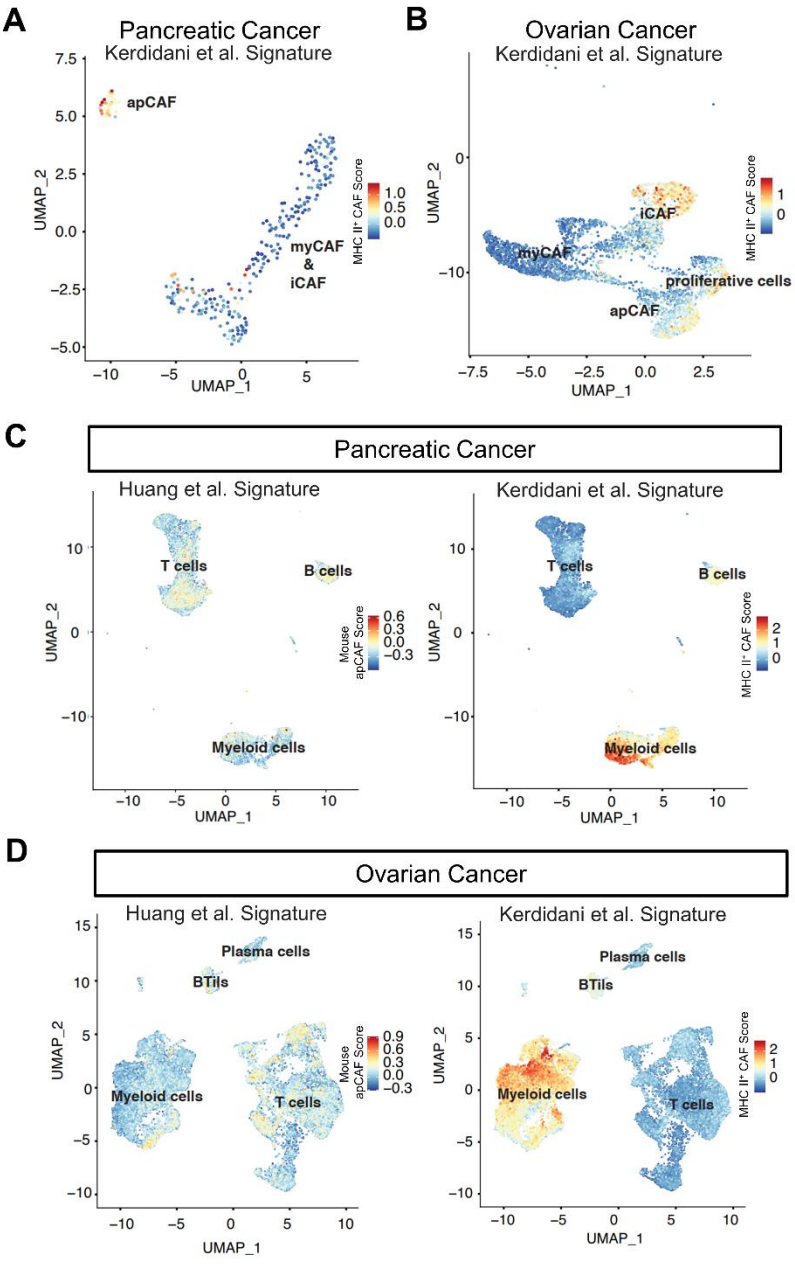
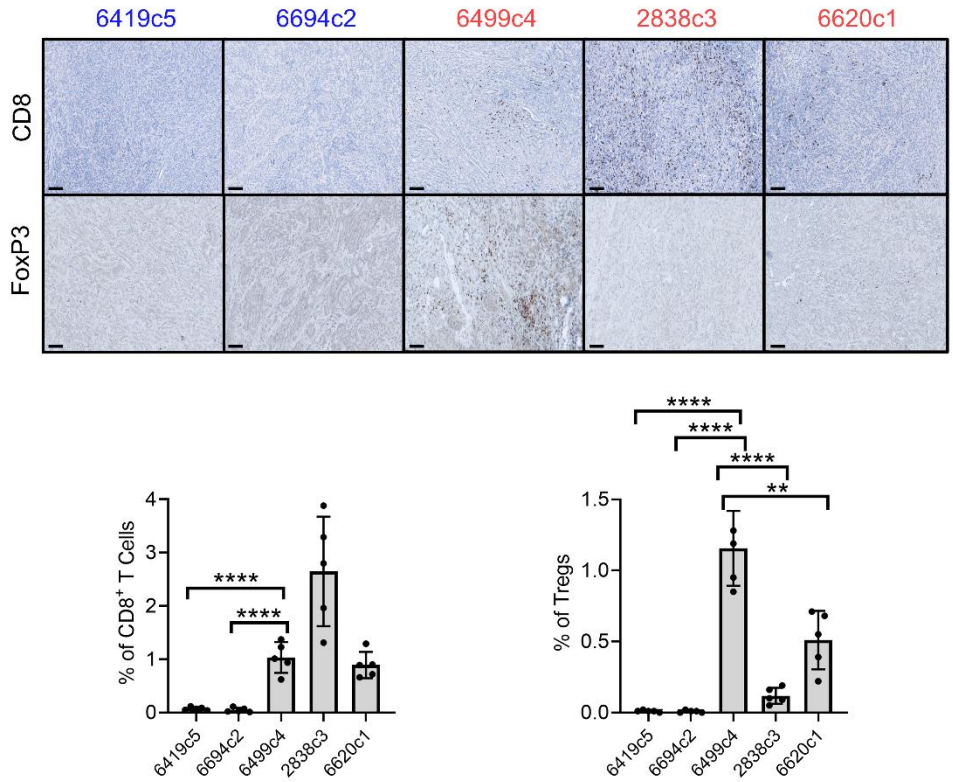

A
Pancreatic Cancer
Kerdidani et al. Signature
apCAF
myCAF
&
iCAF
MHC II+ CAF Score
UMAP_1
UMAP_2
B
Ovarian Cancer
Kerdidani et al. Signature
iCAF
myCAF
proliferative cells
apCAF
MHC II+ CAF Score
C
Pancreatic Cancer
Huang et al. Signature
Kerdidani et al. Signature
T cells
B cells
Myeloid cells
Mouse apCAF Score
MHC II+ CAF Score
D
Ovarian Cancer
Huang et al. Signature
Kerdidani et al. Signature
Plasma cells
BTils
Myeloid cells
T cells
Mouse apCAF Score
MHC II+ CAF Score

Figure S5. Scoring of apCAF Signatures in Immune Compartments of Human Cancer, Related to Figure 6.

(A-B) The MHC II^{+} CAF gene signature generated from Kerdidani et al. was scored in the fibroblast compartments of two human cancer scRNA-seq datasets including PDA (A) and ovarian cancer (B).

(C-D) The immune cell subpopulations from the human PDA and ovarian cancer scRNA-seq datasets were first identified based on the cluster annotations mentioned in the original publications. We then scored our mouse apCAF signature and Kerdidani et al MHC II^{+} CAF signature in the immune cell compartments of the human PDA (C) and ovarian cancer (D) datasets.


6419c5
6694c2
6499c4
2838c3
6620c1
CD8
FoxP3

% of CD8+ T Cells
****
****
0
1
2
3
4
6419c5
6694c2
6499c4
2838c3
6620c1

% of Tregs
****
****
****
**
0.0
0.5
1.0
1.5
6419c5
6694c2
6499c4
2838c3
6620c1

Figure S6. T Cell Characterization of Tumors Derived from the *KPC* Clones, Related to Figure 8.

T cell-inflamed (red) and non-T-cell-inflamed (blue) *KPC* cells were injected orthotopically. Tumors were harvested three weeks after injection and subjected to IHC staining for CD8 and FoxP3. Scale bar 50 μm. $CD8^+$ T cells and $FoxP3^+$ Tregs were quantified, n=5, data shown as mean ± SD, statistical analysis, t-test, **P<0.01, ****P<0.0001.

Table S1. qRT-PCR primer sequences, Related to STAR Methods

Mouse Gene Name	Primer Sequence
Msln	Forward Primer: CTTGGGTGGATACCACGTCTG Reverse Primer: CTTCTGTCTTACAGCCATAGCC
Upk3b	Forward Primer: AGACCTGATTGCCTACGTGC Reverse Primer: GGTGTCCTTAGTTGAGACATGCT
Ezr	Forward Primer: CAATCAACGTCCGGGTGAC Reverse Primer: GCCAATCGTCTTTACCACCTGA
Nkain4	Forward Primer: CTCTGGAACGGCAAGTCTTTG Reverse Primer: GTGGCCGGTATTGAATGGTG
Pdpn	Forward Primer: ACCGTGCCAGTGTTGTTCTG Reverse Primer: AGCACCTGTGGTTGTTATTTTGT
Cdh11	Forward Primer: CTGGGTCTGGAACCAATTCTTT Reverse Primer: GCCTGAGCCATCAGTGTGTA
Cd74	Forward Primer: AGTGCGACGAGAACGGTAAC Reverse Primer: CGTTGGGGAACACACACCA
H2-Ab1	Forward Primer: AGCCCCATCACTGTGGAGT Reverse Primer: GATGCCGCTCAACATCTTGC
Cdh2	Forward Primer: AGCGCAGTCTTACCGAAGG Reverse Primer: TCGCTGCTTTCATACTGAACTTT
Vim	Forward Primer: CGTCCACACGCACCTACAG Reverse Primer: GGGGGATGAGGAATAGAGGCT
Snai1	Forward Primer: CACACGCTGCCTTGTGTCT Reverse Primer: GGTCAGCAAAAGCACGGTT
Snai2	Forward Primer: TGGTCAAGAAACATTTCAACGCC Reverse Primer: GGTGAGGATCTCTGGTTTTGGTA
Zeb1	Forward Primer: GCTGGCAAGACAACGTGAAAG Reverse Primer: GCCTCAGGATAAATGACGGC
Il6	Forward Primer: TAGTCCTTCCTACCCCAATTTCC Reverse Primer: TTGGTCCTTAGCCACTCCTTC
Cxcl1	Forward Primer: CTGGGATTCACCTCAAGAACATC Reverse Primer: CAGGGTCAAGGCAAGCCTC
Pdgfrb	Forward Primer: TTCCAGGAGTGATACCAGCTT Reverse Primer: AGGGGGCGTGATGACTAGG
Tagln2	Forward Primer: CCTGGCCGTGAGAACTTCC Reverse Primer: GTCCGTGGTGTTAATGCCATAG
Col1a1	Forward Primer: GCTCCTCTTAGGGGCCACT Reverse Primer: CCACGTCTCACCATTGGGG
Col12a1	Forward Primer: AAGTTGACCCACCTTCCGAC Reverse Primer: GGTCCACTGTTATTCTGTAACCC
Tgfb1	Forward Primer: CTCCCGTGGCTTCTAGTGC Reverse Primer: GCCTTAGTTTGGACAGGATCTG
Il1a	Forward Primer: CGAAGACTACAGTTCTGCCATT Reverse Primer: GACGTTTCAGAGGTTCTCAGAG
Cxcl12	Forward Primer: TGCATCAGTGACGGTAAACCA Reverse Primer: TTCTTCAGCCGTGCAACAATC
Col1a2	Forward Primer: GTAACTTCGTGCCTAGCAACA Reverse Primer: CCTTTGTCAGAATACTGAGCAGC