Expanded View Figures

Figure EV1. The CD106$^+$ population is uniquely localized to pancreatic injury and precancerous lesions.

A Representative IHC staining for CD106$^+$ in normal pancreas. Scale bar, 50 μm.

B Representative IHC staining for CD106$^+$ in IPMN+PanIN I-III and IPMT human samples. The adjacent region (above) and tumor core (below) were paired from the same patient respectively, corresponding to the diagnosis. The red arrow indicated the CD106$^+$ spindle-like cells. Scale bar, 50 μm.

C Representative IHC staining of pancreatic lesions of human TMA for CD106$^+$. The magnification region indicates representative CD106$^+$ cells. Scale bar, 200, 50 μm. Quantification of CD106 expression in pancreatitis, PanIN and PDAC lesions according to patients' pathological references of TMA (Appendix Table S1). *$P < 0.05$ and **$P < 0.01$. ****$P < 0.0001$. The P-values were calculated using Student's t-test.

D Representative IF staining of the PeSC cell line on a coverslip for CD106 (green), mCherry (red), and DAPI (blue). Scale bar, 50 μm.

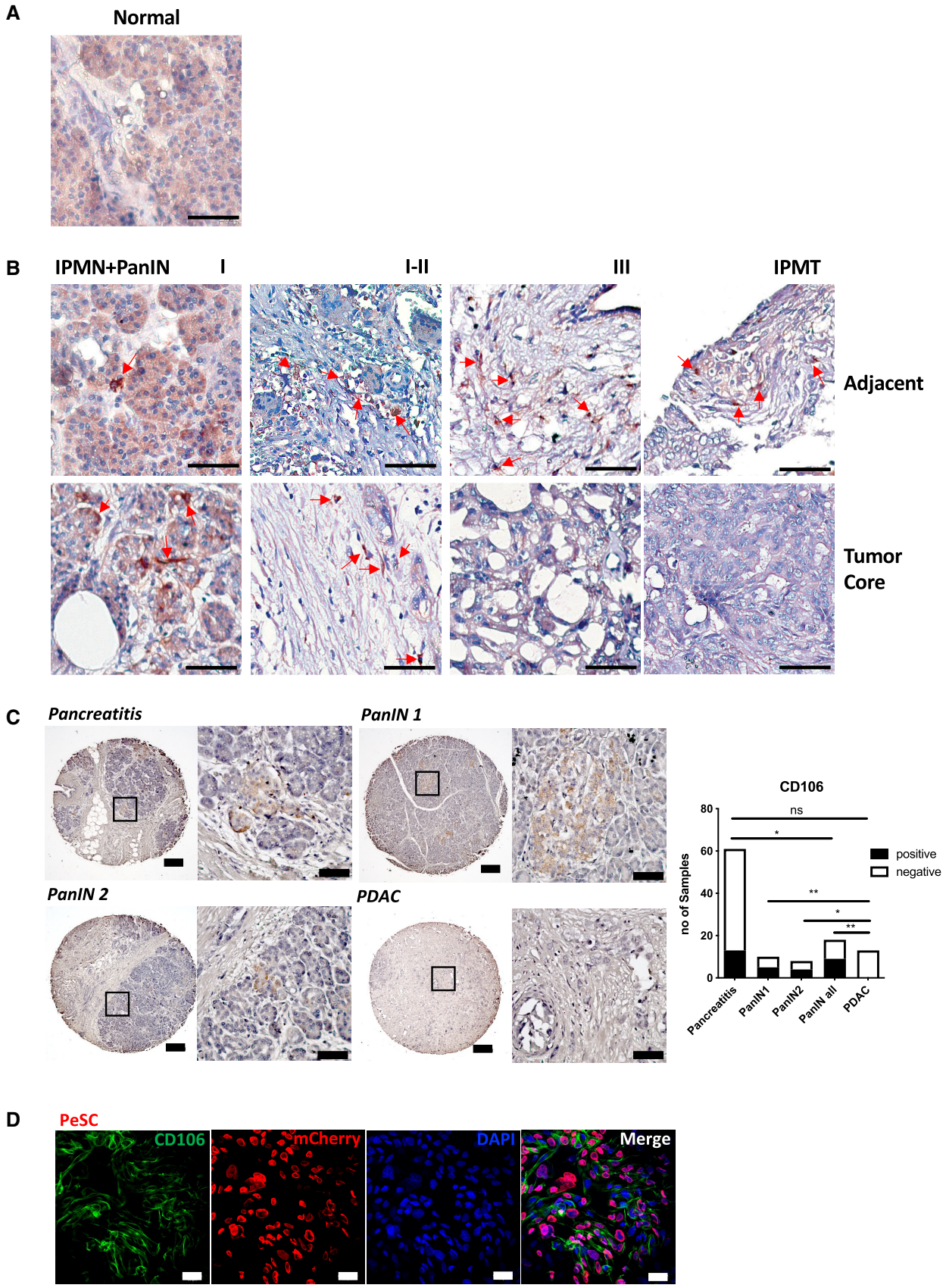
A
Normal
B
IPMN+PanIN
I
I-II
III
IPMT
Adjacent
Tumor Core
C
Pancreatitis
PanIN 1
PanIN 2
PDAC
CD106
ns
*
**
*
**
positive
negative
no of Samples
80
60
40
20
0
Pancreatitis
PanIN1
PanIN2
PanIN all
PDAC
D
PeSC
CD106
mCherry
DAPI
Merge

Figure EV1.

Figure EV2. PeSC signature in human cancer single-cell data.

A, B Application of ovarian cancer pericyte (A) and brain cancer pericyte score (B) to our mouse single-cell RNAseq analysis showing enrichment in the unique cluster of PeSC.

C Pooled single-cell RNAseq data to UMAP analysis from 35 samples (11 normal pancreas and 24 PDAC patients), including a total number of over 80,000 cells. The UMAP shows the distribution of each cell cluster.

D Cell types of each cluster were identified based on Muraro's single-cell dataset as a reference.

E, F Unsupervised clustering UMAP analysis distinguishes 23 subclusters in the integration of whole 35 samples (E), pooled from 11 normal pancreas samples and 24 PDAC samples (F).

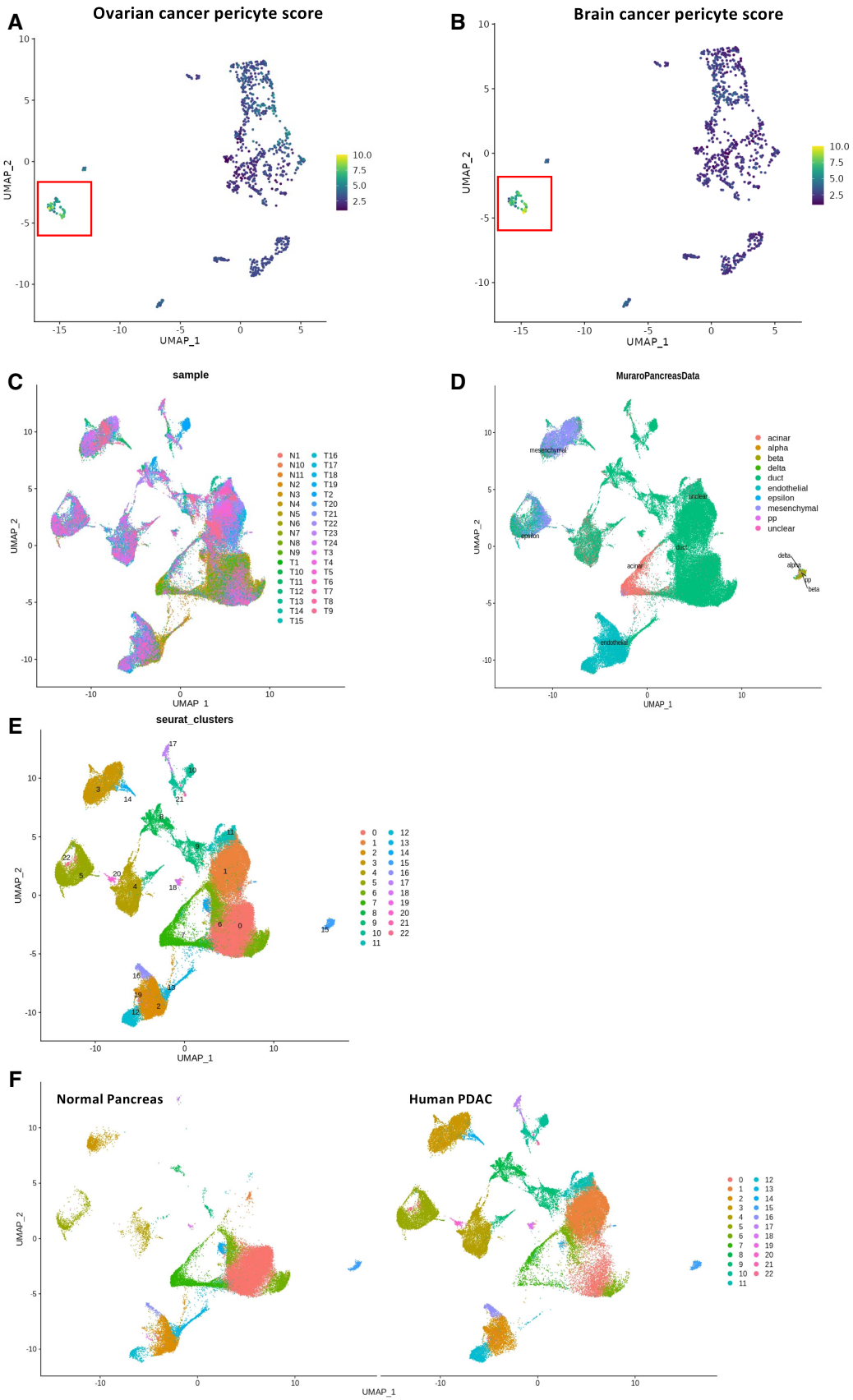
A
Ovarian cancer pericyte score
B
Brain cancer pericyte score
UMAP_1
UMAP_2
10.0
7.5
5.0
2.5
C
sample
D
MuraroPancreasData
acinar
alpha
beta
delta
duct
endothelial
epsilon
mesenchymal
pp
unclear
E
seurat_clusters
F
Normal Pancreas
Human PDAC

Figure EV2.

Figure EV3. Chimera approach identifies the origin of PeSC.

A Experimental protocol for bone marrow chimeras. One month old KC mice were sublethally irradiated and reconstituted with bone marrow cells from a Tomato expressing mouse. After reconstitution, the chimeras were sacrificed on day 60.

B Representative dot plots of reconstituted bone marrow cells (Tomato$^+$) in KC chimeras compared to the nonreconstituted KC mice as control by FACS analysis. Quantification of reconstituted cells (Tomato$^+$) among CD45$^+$ immune cells in either pancreas or periphery of KC chimeras compared to the control. Three mice in each condition.

C Representative dot plots and gating strategy of distinguishing the reconstituted CD106$^+$ cells (Tomato$^+$) and nonreconstituted CD106$^+$ cells (Tomato$^-$) in KC chimeras. Three mice in the group, each dot represents one mouse.

D Representative IF staining for CD106 (green) and Tomato (red) in the pancreas of chimera mice. Inset shows colocalization of CD106- and Tomato-stained cells. Scale bar, 50 μm (upper), 10 μm (lower).

Data information: ns not significant, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$. The P-values were calculated using Student's t-test.

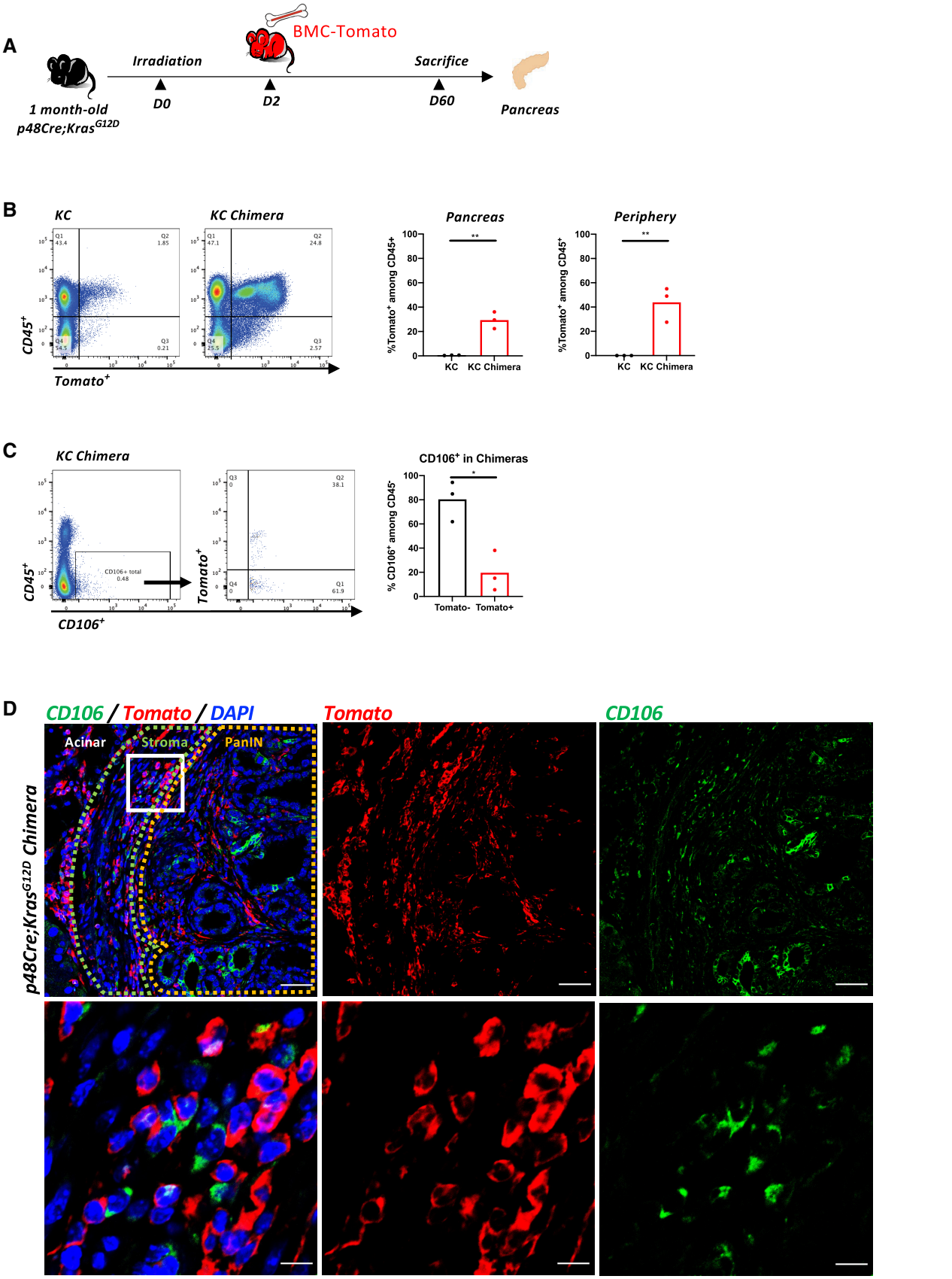
A
BMC-Tomato
Irradiation
Sacrifice
1 month-old
p48Cre;Kras^G12D
D0
D2
D60
Pancreas
B
KC
KC Chimera
CD45+
Tomato+
Pancreas
Periphery
%Tomato+ among CD45+
KC
KC Chimera
C
KC Chimera
CD106+ total
0.48
CD45+
Tomato+
CD106+
CD106+ in Chimeras
% CD106+ among CD45-
Tomato-
Tomato+
D
CD106 / Tomato / DAPI
Tomato
CD106
Acinar
Stroma
PanIN
p48Cre;Kras^G12D Chimera

Figure EV3.

Figure EV4. Stem properties of PeSCs and their impact on tumor cells.

A Proliferation curves of Epi cells in the indicated culture conditions based on the analysis of total green (GFP) object area in each well observed by IncuCyte. The data of each curve are summarized from six biological replicates of each condition. The bars are representing the mean ± SD.

B FACS analysis of the percentage of $Ki67^{+}$ Epi cells in each culture condition at 48 h. FACS analysis was performed by biological triplicates corresponding to the indicated culture condition.

C, D The bars are representing the mean ± SD (C, D) Proliferation curves of PeSCs in the indicated culture conditions based on the analysis of the total red (mCherry) object area in each well observed by IncuCyte. The data of each curve are summarized from six biological replicates of each condition. The bars are representing the mean ± SD.

E qPCR analysis of EMT-related genes expressed by PeSCs after the treatment of PDGF-BB and TGF-β compared to the untreated ones (UT). Treatment was performed in biological triplicates and qPCR analysis was performed by technical duplicates. The bars are representing the mean ± SD.

F Quantification of Epi (GFP^{+}) or PeSC ($mCherry^{+}$) cells in the s.c. tumor grafts in the indicated conditions based on FACS analysis. Five mice in each group.

Data information: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$. The *P*-values were calculated using Student's *t*-test.

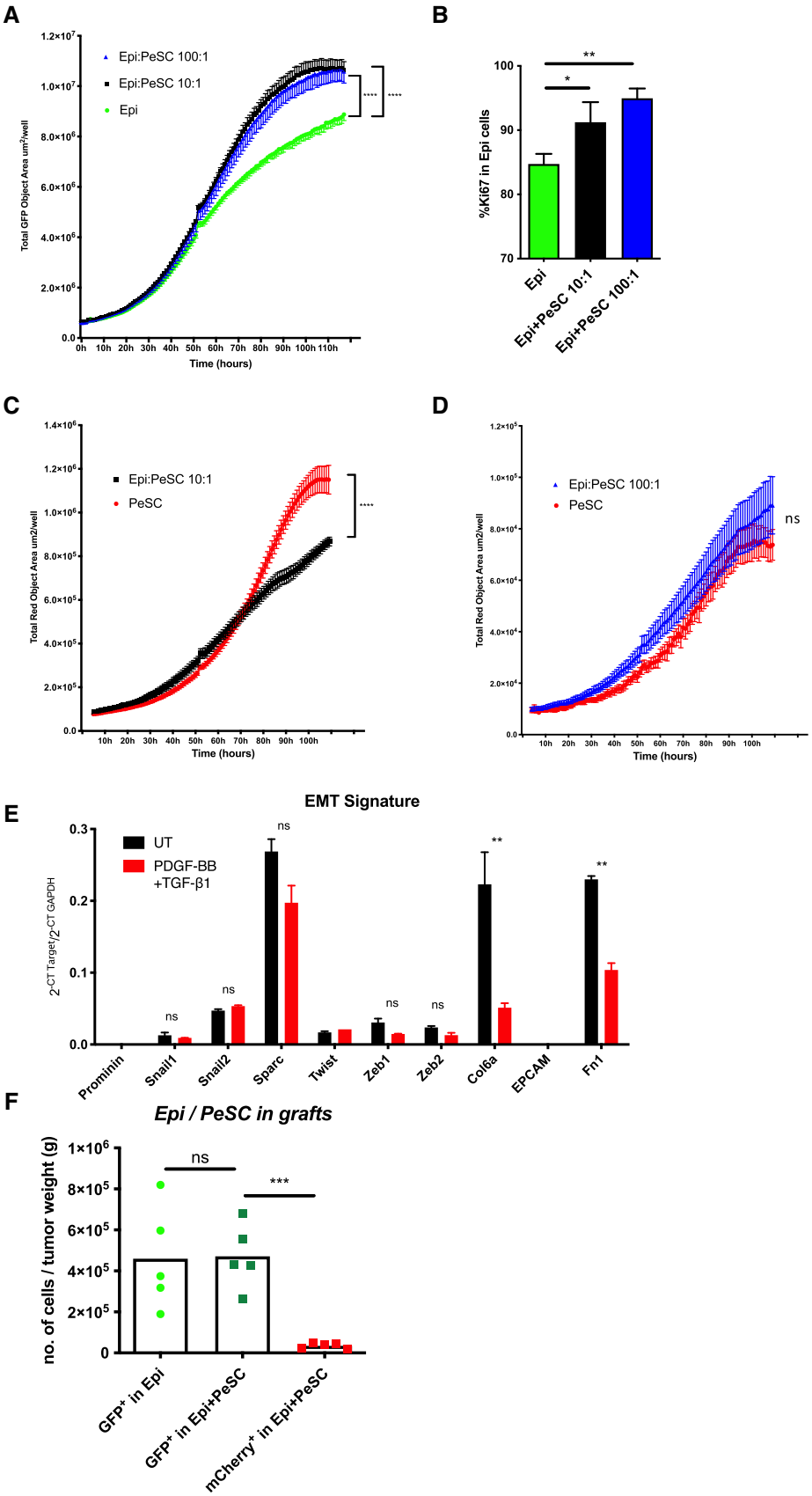
A
Epi:PeSC 100:1
Epi:PeSC 10:1
Epi
Total GFP Object Area um2/well
Time (hours)
B
%Ki67 in Epi cells
Epi
Epi+PeSC 10:1
Epi+PeSC 100:1
C
Epi:PeSC 10:1
PeSC
Total Red Object Area um2/well
Time (hours)
D
Epi:PeSC 100:1
PeSC
ns
Total Red Object Area um2/well
Time (hours)
E
EMT Signature
UT
PDGF-BB
+TGF-β1
2-CT Target/2-CT GAPDH
Prominin
Snail1
Snail2
Sparc
Twist
Zeb1
Zeb2
Col6a
EPCAM
Fn1
F
Epi / PeSC in grafts
no. of cells / tumor weight (g)
GFP+ in Epi
GFP+ in Epi+PeSC
mCherry+ in Epi+PeSC

Figure EV4.

Figure EV5. Tumor-PeSC crosstalk affects the immune system.

A, B *In vivo* neutralization of CD106: (A) Experiment setting. (B) Tumor weight, FACS analysis of % $PeSC^+$ among $CD45^-$ cells, % Epi^+ among $CD45^-$ cells, % $CD11b^+$ among $CD45^+$ cells, % $Ly6G^+CD11b^+$ among $CD45^+$ cells, % $CD11b^+F4/80^+$ among $CD45^+$ cells, % $CD11b^+F4/80^+CD103^+$ among $CD45^+$ cells (eight mice in each group, five for FACS analysis, and three for histological analysis. Quantification of PeSCs and Epi cells were cumulative from two FACS panels as technical duplicates).

C–L *In vivo* depletion of CCL5 impact the macrophages differentiation: (C) Experimental setting. (D) Tumor weight of the grafts formed by Epi + PeSC in CCL5-depleted conditions compared with undepleted conditions. FACS analysis of the percentage of $CD11b^+$ cells (E), $Ly6G^+CD11b^+$ (F), $F4/80^+CD11b^+$ (G), $F4/80^+CSFR1^+$ (H), $F4/80^+CD163^+$ (I), $F4/80^+CD11b^+CD103^+$ (J), $F4/80^+CD11b^+PD\text{-}L1^+$ (K) and $CD8^+$ cells (L) among $CD45^+$ cells derived from the tumor grafts. Ten mice in each group, six for FACS analysis and four for histological analysis.

Data information: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, and $****P < 0.0001$. The *P*-values were calculated using Student's *t*-test.

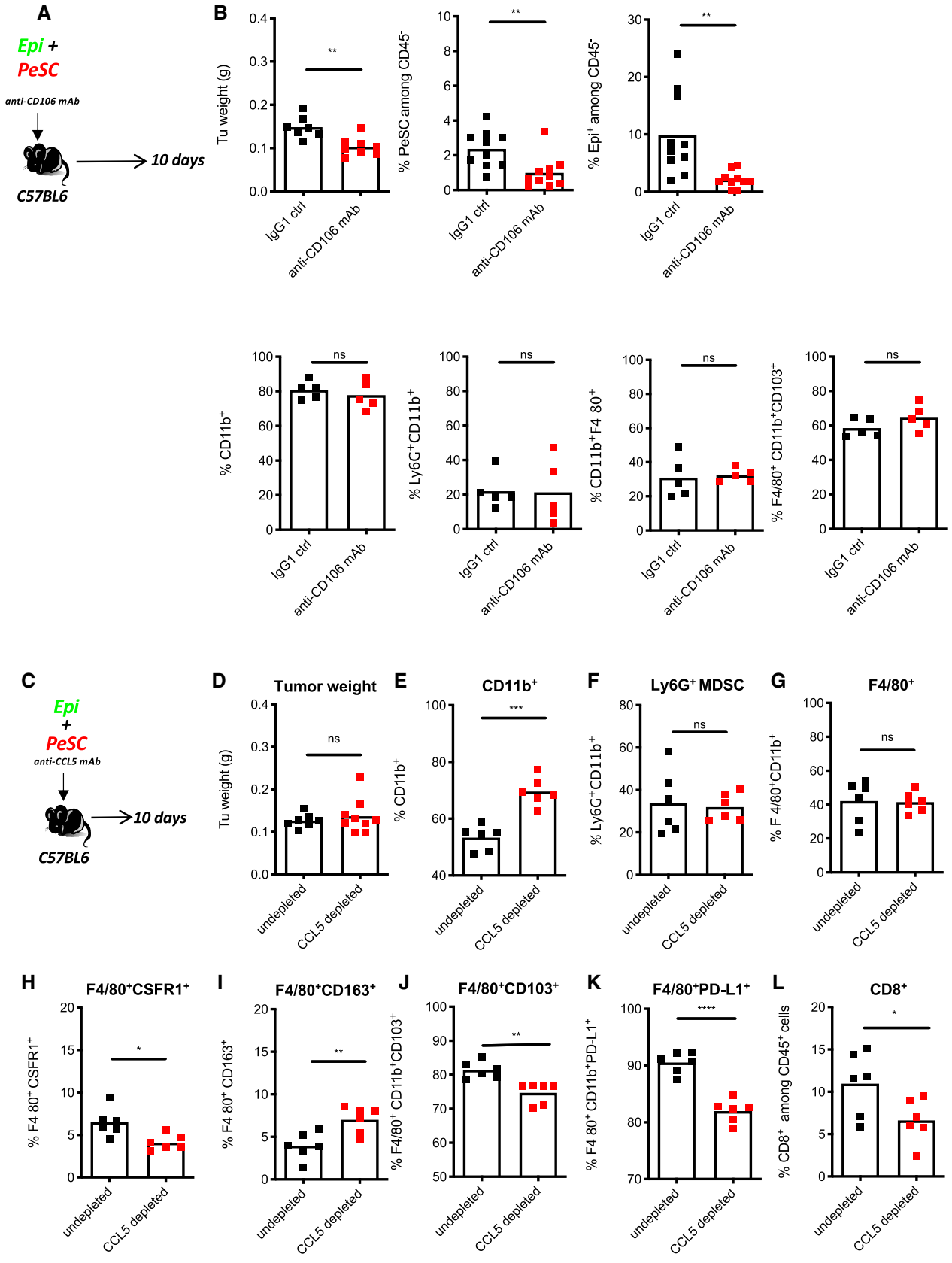
A
Epi +
PeSC
anti-CD106 mAb
10 days
C57BL6
B
Tu weight (g)
% PeSC among CD45-
% Epi+ among CD45-
% CD11b+
% Ly6G+CD11b+
% CD11b+F4 80+
% F4/80+ CD11b+CD103+
IgG1 ctrl
anti-CD106 mAb
ns
C
Epi
+
PeSC
anti-CCL5 mAb
10 days
C57BL6
D
Tumor weight
E
CD11b+
F
Ly6G+ MDSC
G
F4/80+
undepleted
CCL5 depleted
H
F4/80+CSFR1+
I
F4/80+CD163+
J
F4/80+CD103+
K
F4/80+PD-L1+
L
CD8+
% F4 80+ CSFR1+
% F4 80+ CD163+
% F4/80+ CD11b+CD103+
% F4 80+ CD11b+PD-L1+
% CD8+ among CD45+ cells

Figure EV5.