

Supporting Information

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Transcriptome Landscape of Cancer-Associated Fibroblasts in Human PDAC

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Supplementary Information

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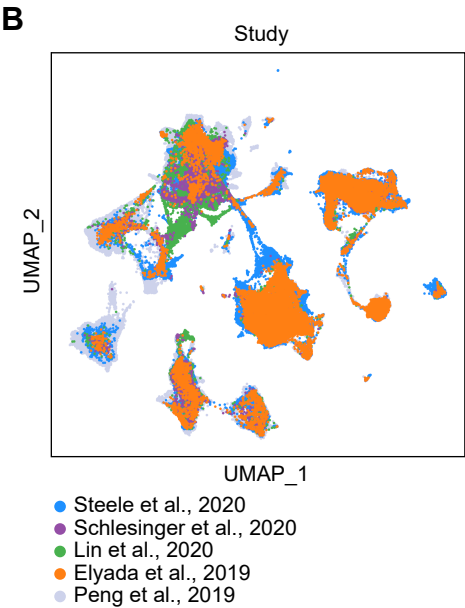
Table S3. Primer and siRNA sequence.

Figure S1

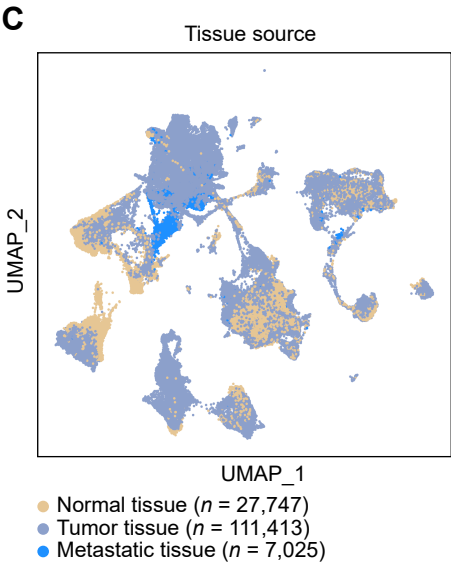
A

Data label	Platform	Data accession	Sample	Cell number	Reference
Steele et al., 2020	10×	GSE155698	PDAC tissue (<i>n</i> = 16) Normal tissue (<i>n</i> = 3)	48,279	Multimodal mapping of the tumor and peripheral blood immune landscape in human pancreatic cancer (Nature Cancer, 2020)
Schlesinger et al., 2020	10×	GSE141017	PDAC tissue (mixed)	5,018	Single-cell transcriptomes of pancreatic preinvasive lesions and cancer reveal acinar metaplastic cells' heterogeneity (Nature Communications, 2020)
Lin et al., 2020	10×	GSE154778	PDAC tissue (<i>n</i> = 10) Metastatic tissue (<i>n</i> = 6)	15,076	Single-cell transcriptome analysis of tumor and stromal compartments of pancreatic ductal adenocarcinoma primary tumors and metastatic lesions (Genome Medicine, 2020)
Elyada et al., 2019	10×	Request for authors	PDAC tissue (<i>n</i> = 6) Normal tissue (<i>n</i> = 2)	20,369	Cross-species single-cell analysis of pancreatic ductal adenocarcinoma reveals antigen-presenting cancer-associated fibroblasts (Cancer Discovery, 2019)
Peng et al., 2019	10×	Request for authors	PDAC tissue (<i>n</i> = 24) Normal tissue (<i>n</i> = 11)	57,443	Single-cell RNA-seq highlights intra-tumoral heterogeneity and malignant progression in pancreatic ductal adenocarcinoma (Cell Research, 2019)

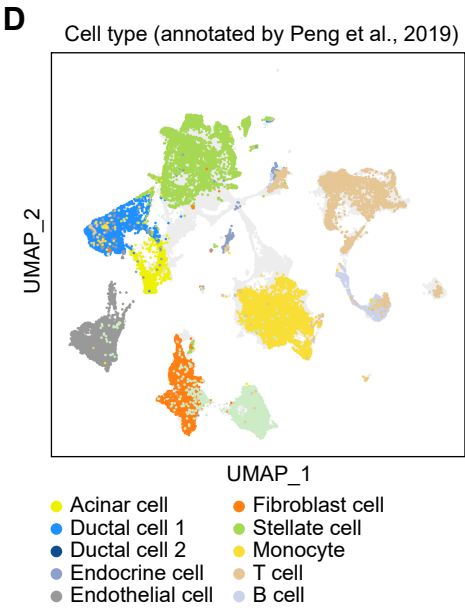
B



C



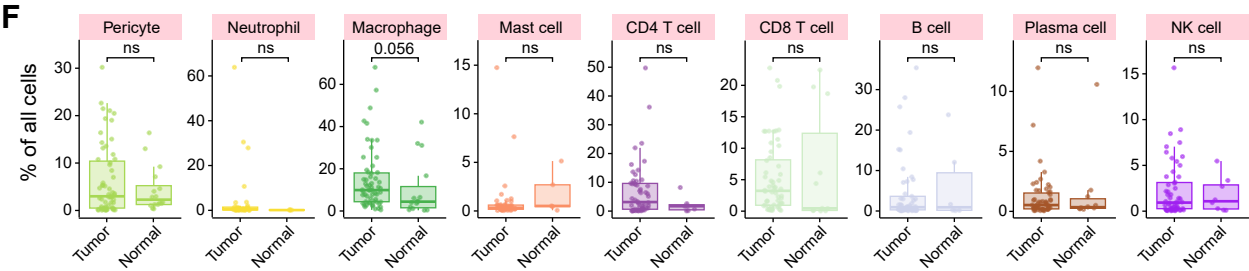
D



E



F



G

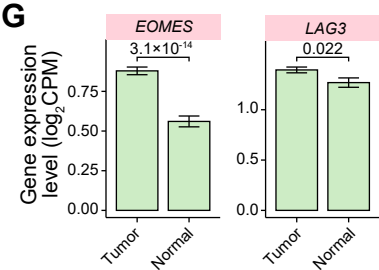


Figure S1. Quality control information of collected single-cell RNA-seq data.

(A) Table showing the detail information of collected single-cell RNA-seq data.

(B and C) UMAP plots showing the clustering of all cells. The dot color indicates data sources (B) or tissue sources (C). The number of cells in each data source or tissue source is indicated in brackets, respectively.

(D) UMAP plot showing the clustering of all cells. The dot color indicates cell types which are directly obtained from the previous study (Peng et al., 2019).

(E) Stacked bar plots showing the composition of distinct cell types in tumor (left) and normal (right) tissues.

(F) Boxplots showing the percentage of representative cell types among all cells from tumor and normal tissues. The two-tailed Wilcoxon-ranked *P* value is indicated.

(G) Bar plots showing the expression level of exhausted markers in CD8 T cells. The two-tailed Student's *t*-test *P* value is indicated. Data are shown with the mean value $\pm$ SEM.

Figure S2

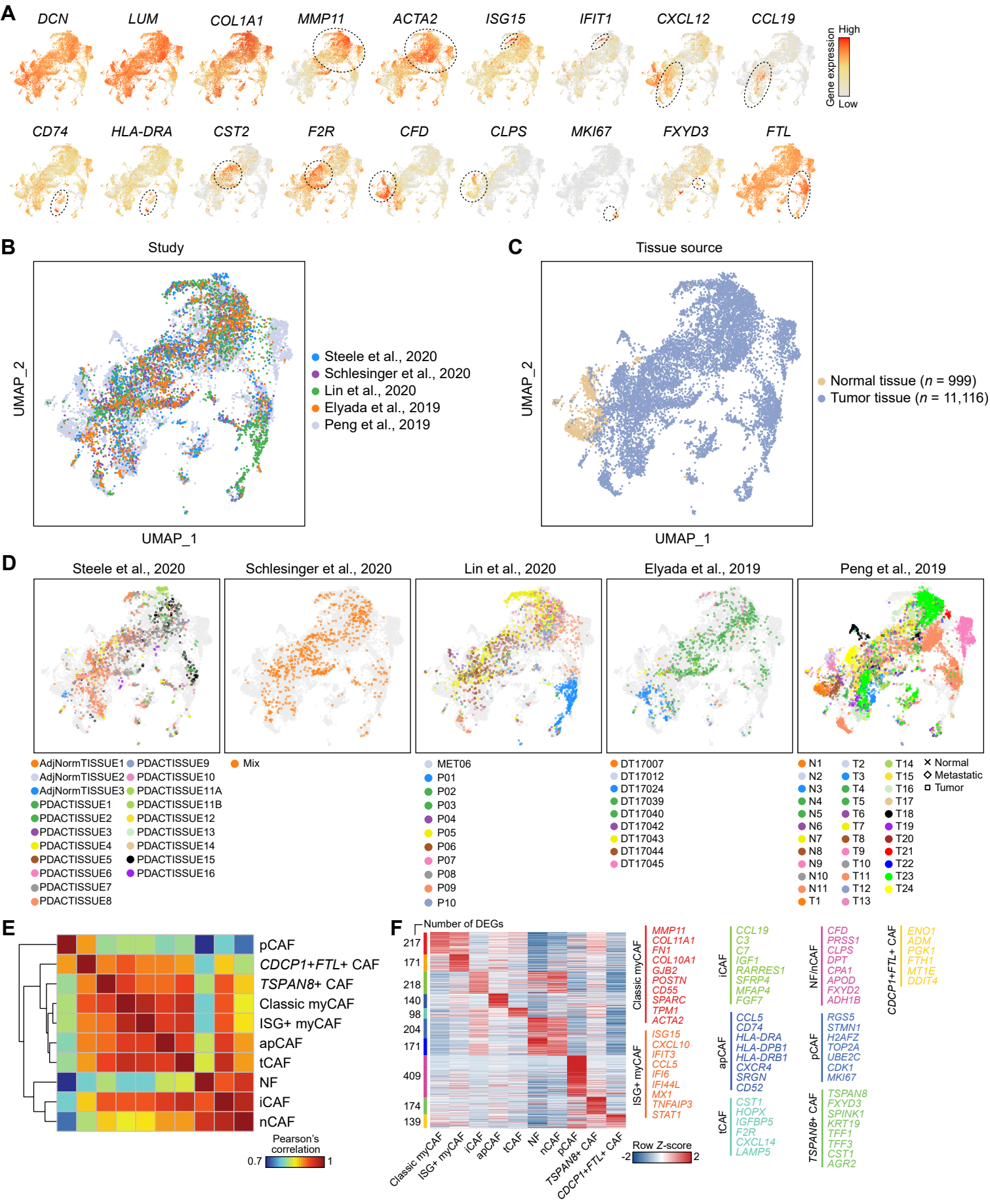


Figure S2. Identification CAF subtypes.

(A) UMAP plots showing the expression level of pan-CAF marker genes and CAF subtype signature genes.

(B and C) UMAP plots showing the clustering of fibroblasts collected from normal or tumor tissues. The dot color indicates data sources (B) or tissue sources (C). The number of cells in each tissue source is indicated in brackets.

(D) UMAP plot showing the clustering of fibroblasts collected from normal or tumor tissues. The dot color indicates samples in each study.

(E) Heatmap showing the Pearson's correlation coefficient of distinct CAF subtypes in pairwise manner.

(F) Heatmap showing the expression level of DEGs in each CAF subtype. Values are scaled in each gene with Z-score. The number of DEGs in each subtype is indicated in the left, and represented DEGs are indicated in the right.

Figure S3

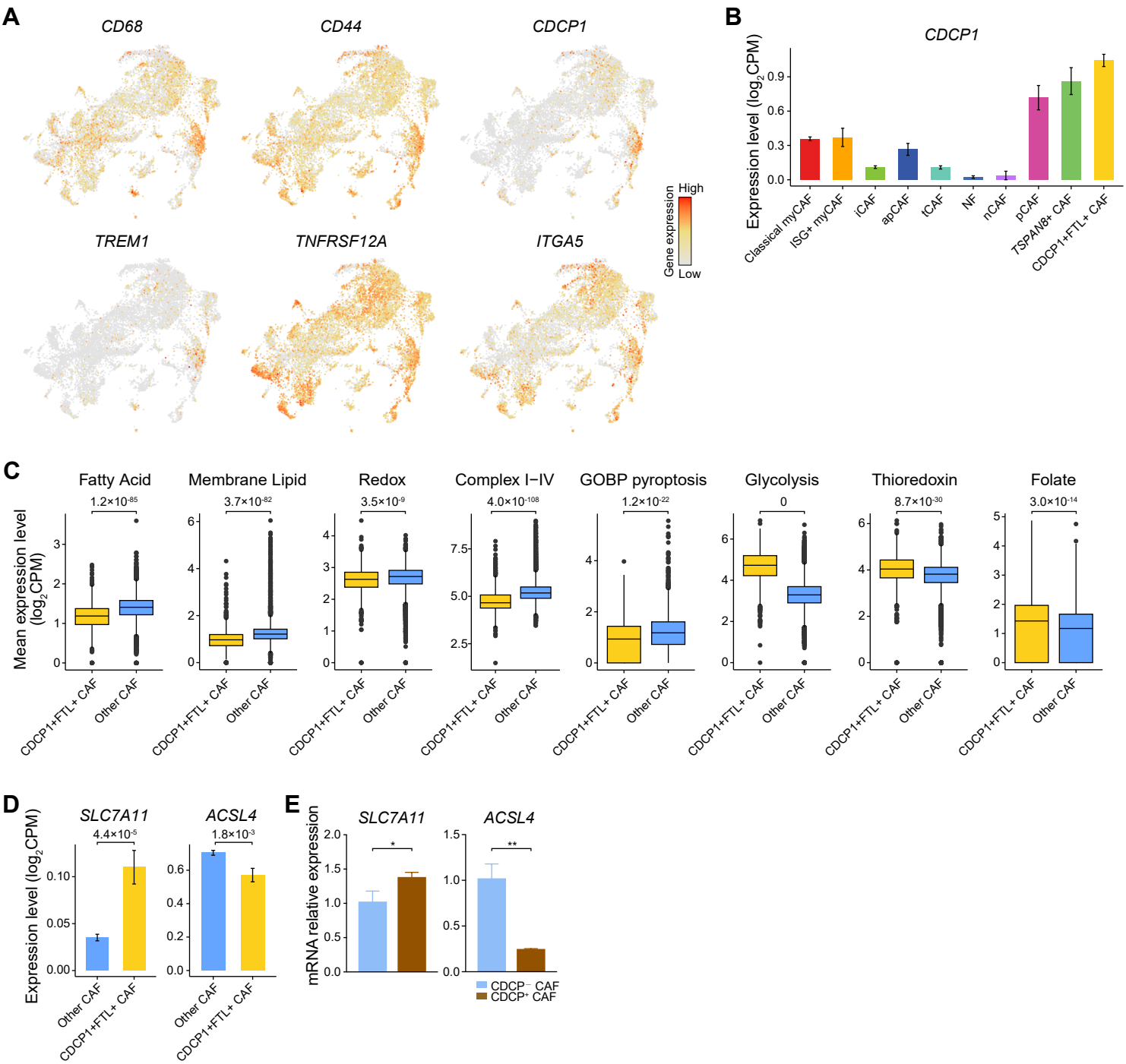


Figure S3. Identification and functional role of CDCP1⁺FTL⁺ CAF in PDAC.

(A) UMAP plots showing the expression level of marker genes.

(B) Bar plots showing the expression level of *CDCP1* in CAF subtypes. Data are shown with the mean value $\pm$ SD.

(C) Boxplots showing the expression level of ferroptosis-related metabolic pathways. Two-tailed Student's *t*-test *P* values are indicated.

(D) Bar plot showing the expression level of ferroptosis-related genes *SLC7A11* and *ACSL4*. Two-tailed Student's *t*-test *P* value is indicated.

(E) mRNA expression of ferroptosis related genes *SLC7A11* and *ACSL4* of sorted CDCP1⁺/⁻ CAFs. Two-tailed Student's *t*-test *P* value is calculated, **P* < 0.05, ***P* < 0.01.

Figure S4

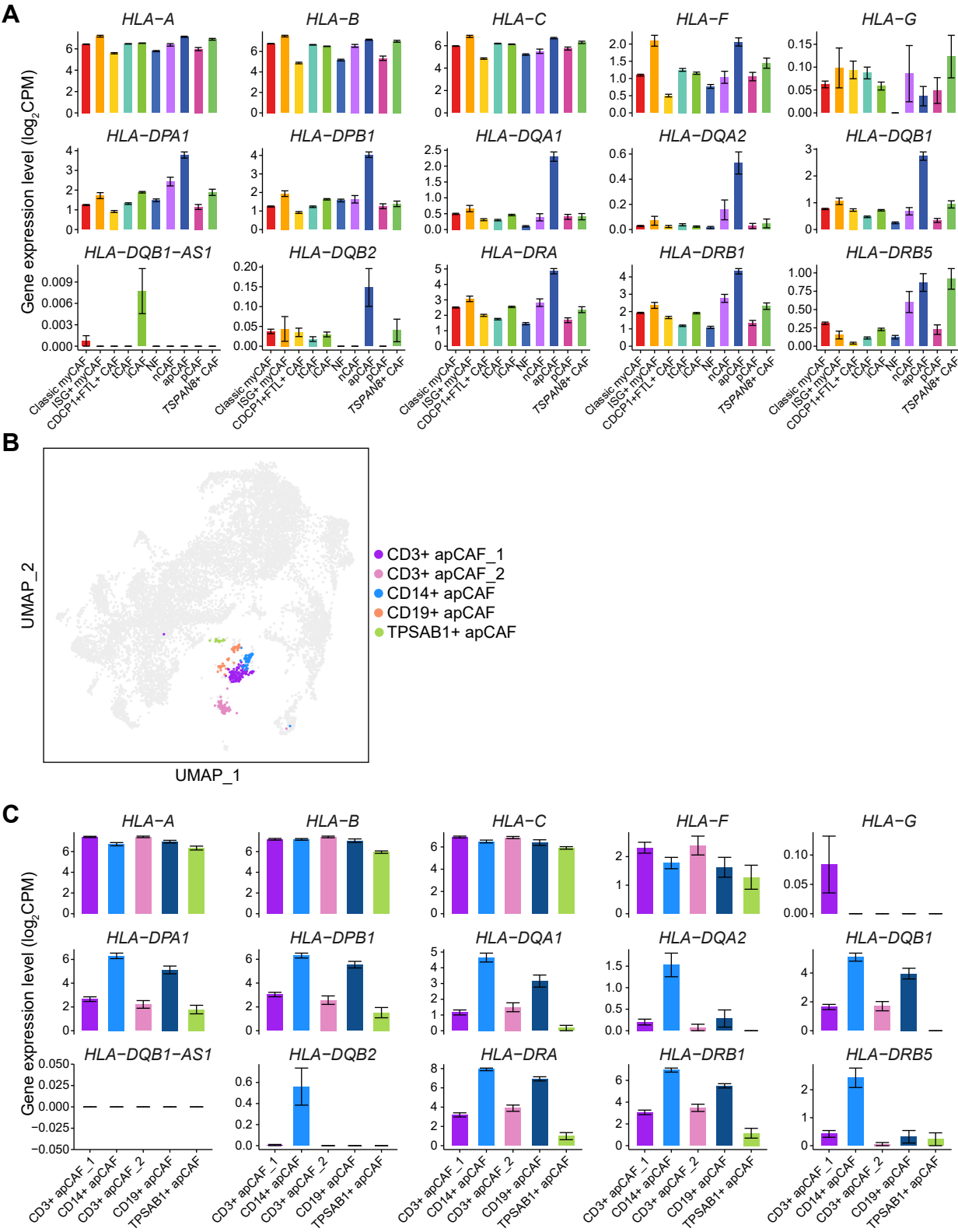


Figure S4. Identification of apCAF subtypes and immune cells co-expressed CAF marker genes.

(A and C) Barplots showing the expression level of MHC-I and MHC-II genes in all fibroblasts (A) or apCAF subtypes (C). Data are shown with the mean value $\pm$ SEM.

(B) UMAP plot showing the distribution of apCAFs in all fibroblasts from normal and tumor tissues. The dot color indicates apCAF subtypes.

Figure S5

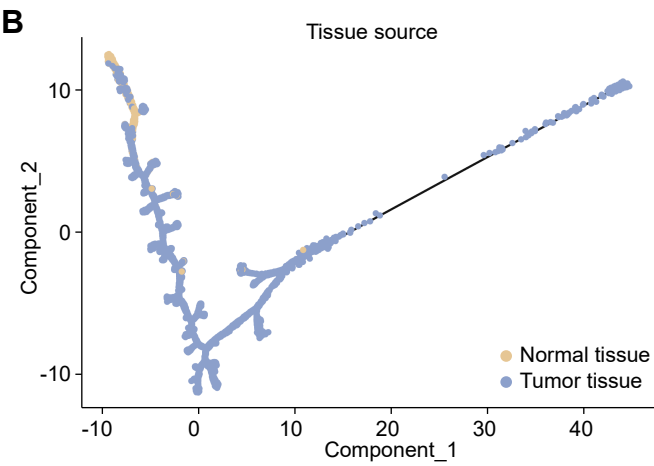
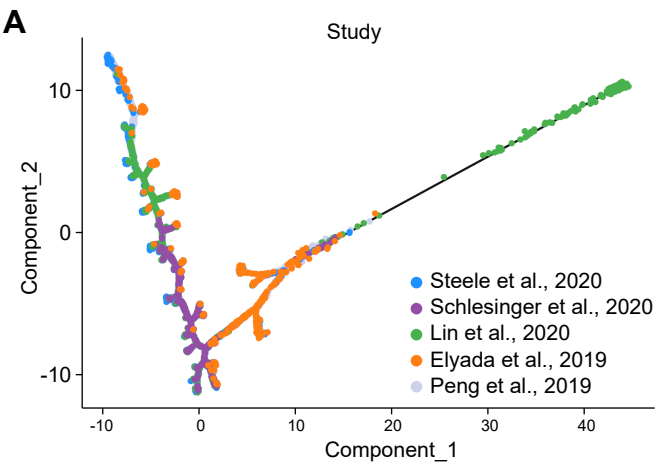


Figure S5. Pesudotime analysis of distinct CAF subtypes.

Pesudotime analysis showing the differentiated trajectory of distinct CAF subtypes. The dot color indicates data sources (A) or tissue sources (B).

Figure S6

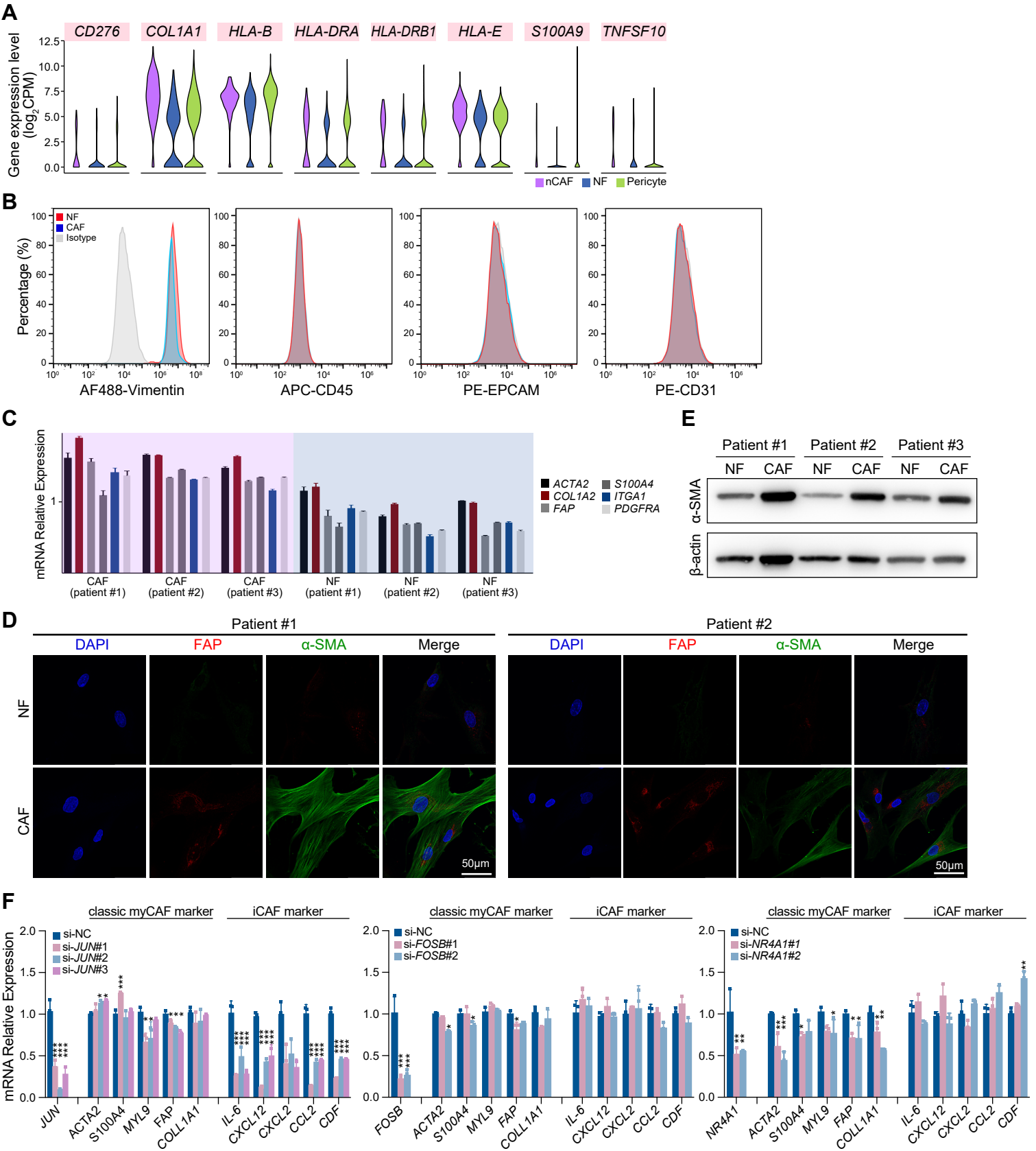


Figure S6. AP-1 members triggering the malignant phenotype conversion of NFs.

(A) Violin plots showing the expression level of representative genes in nCAFs, NFs, and pericytes.

(B) Flow cytometry indicating the expression of vimentin in human primary CAF and the absence of CD45, EPCAM and CD31.

(C) Bar plot showing the mRNA expression of representative genes in paired human primary PDAC CAFs and NFs. Data are shown with the mean value $\pm$ SEM.

(D) Representative of confocal microscopy images of FAP and α -SMA staining in paired human primary PDAC CAFs and NFs. Scale bar, 50 μ m.

(E) Western blots showing α -SMA levels in paired human primary PDAC CAFs and NFs.

(F) Bar plots showing the mRNA expression of representative genes of classic myCAF and iCAF in human primary CAFs after knocking-down *JUN*, *FOSB*, and *NR4A1* by si-RNA. Two or three different siRNAs were used for avoiding off-target effect. For each group, $n = 3$ biological replicates. Data are shown with the mean value $\pm$ SD. P value by one-way ANOVA, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$.

Figure S7

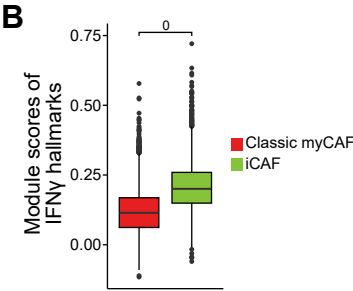
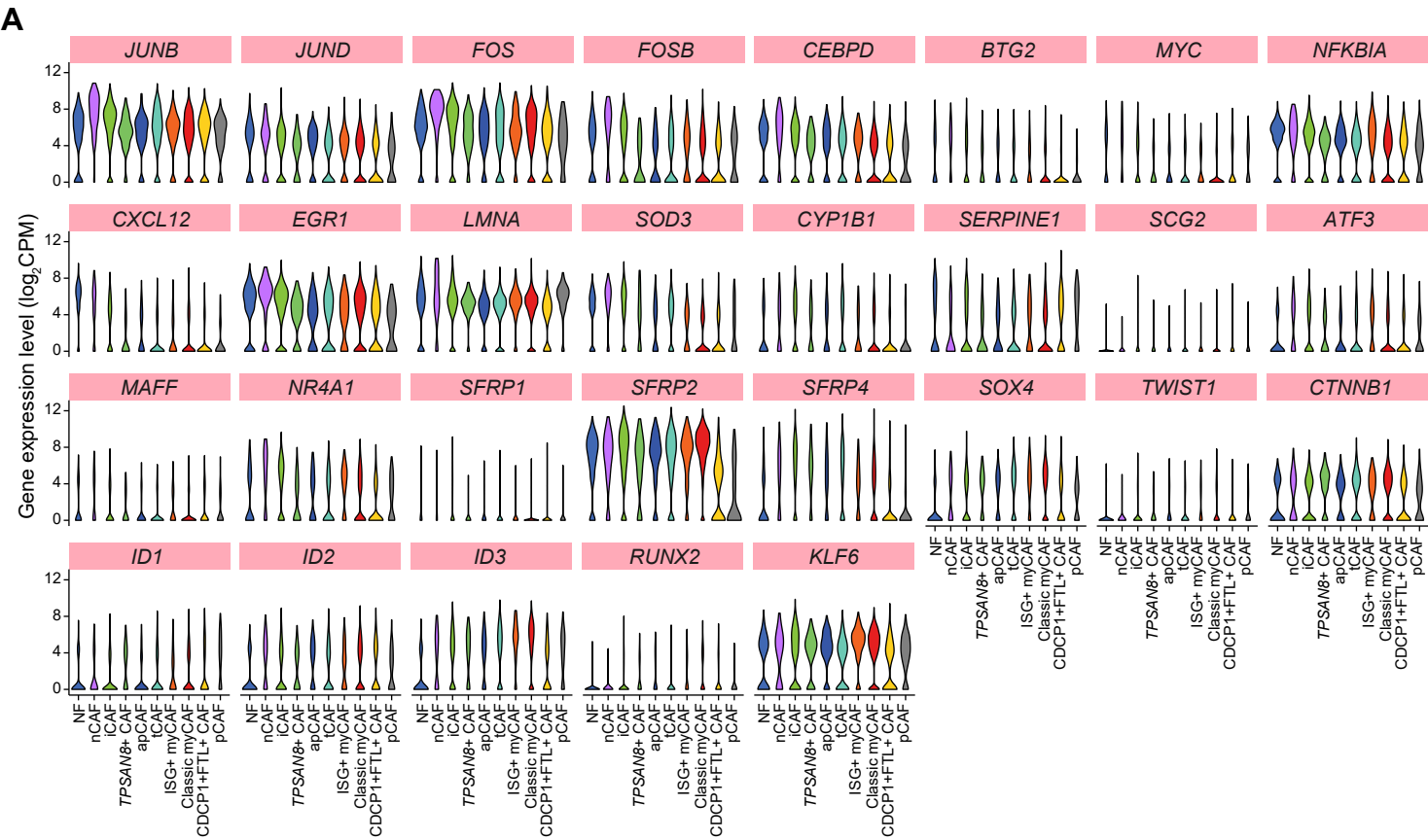


Figure S7. IFN γ contributing to the fate transformation from classic myCAFs to iCAFs

(A) Violin plots showing the expression level of representative genes across CAF subtypes.

(B) Boxplot showing the module score of IFN γ hallmarks in classic myCAFs and iCAFs. The two-tailed Student's t -test P value is indicated.

Figure S8

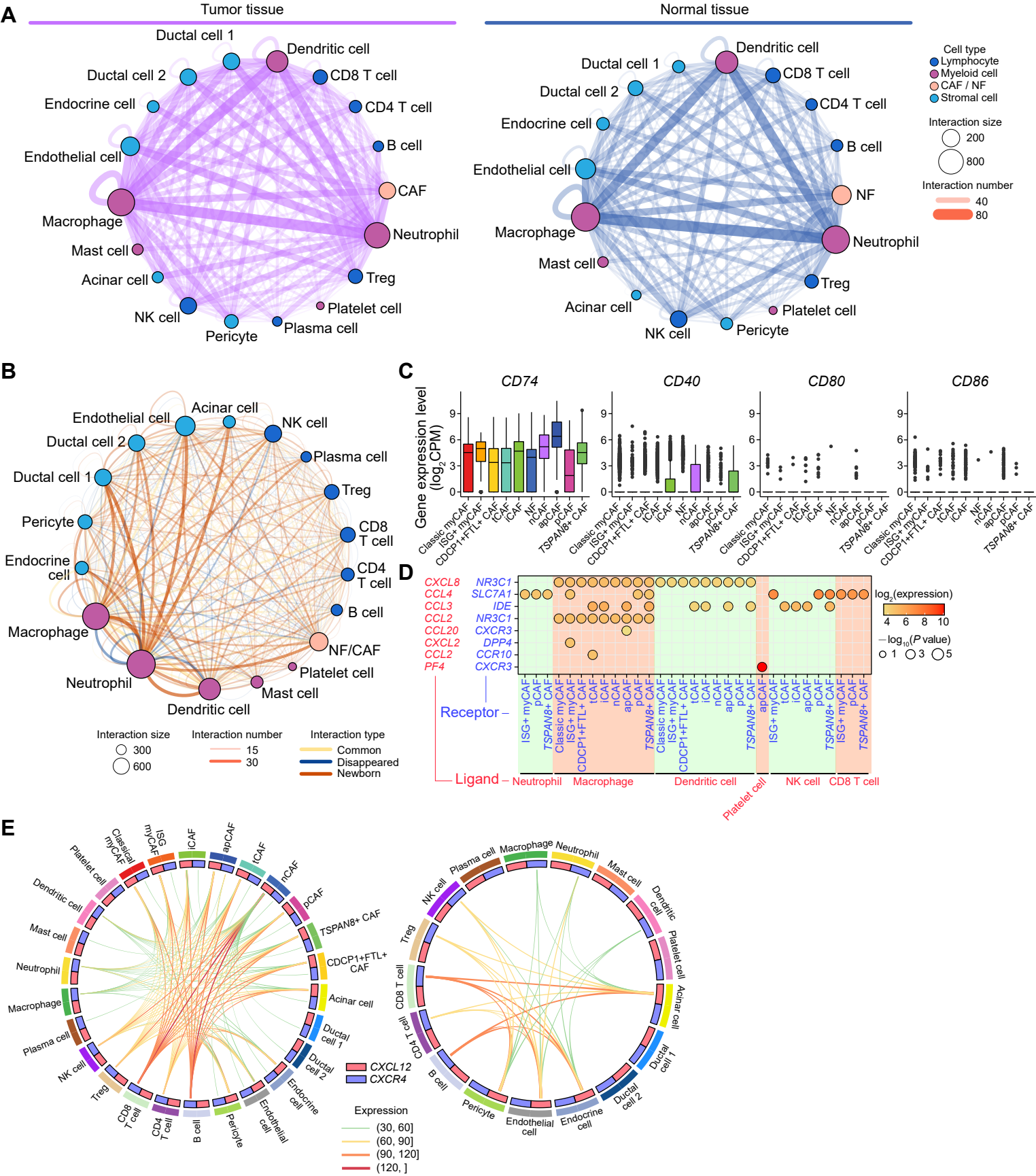


Figure S8. Cell-cell interactions between CAF subtypes and other cells.

(A) Cell-cell interaction network showing interactions between CAF subtypes and other cells in tumor (left) and normal (right) tissues. The dot color indicates the cell type, and the dot size indicates the interaction number of a given cell type. The line thickness indicates the interaction number of a given cell type pair.

(B) Cell-cell interaction network showing distinct types of interactions between CAFs (or NFs) and other cells. The line color indicates the interaction type, newborn interactions (tumor specific interactions) in red, disappeared interactions (normal tissue specific interactions) in blue, and common interactions (coexisted in tumor and normal tissues) in yellow. The dot color indicates the cell type, the dot size indicates the interaction number of a given cell type, and the line thickness indicates the interaction number of a given cell type pair.

(C) Boxplots showing the expression of costimulatory genes in NFs and distinct CAFs.

(D) Dot plot showing the expression level of ligand-receptor gene pair related with chemotaxis and cytokine within lymphocyte-CAF interactions. The dot color and size indicate the expression levels and statistical significance, respectively. Red words indicate the ligands (row) expressed in the corresponding cell type (column), and blue words indicate the receptors (row) expressed in the corresponding cell type (column).

(E) Circle plots showing *CXCL12-CXCR4* gene pair in pairwise cell types within tumors (left), or in pairwise cell types (exclude CAFs) within tumors (right). For a give line in this plot, the endpoint indicates the cell type expressing ligand *CXCL12*, and the other endpoint indicates the cell type expressing receptor *CXCR4*.