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Figure S1. Quality control and overall/subcluster single-cell transcriptome profile. a. Quality check pipeline of the pan-cancer single-cell profile. b/c. Distribution of TME cells represented by tissue types and malignant status. d. Distribution of epithelial cells represented by tissue types. e. The UMAP clustering result of myeloid cells. The alteration in the proportion of *IL1B*⁺, *SPP1*⁺ and *APOE*⁺ macrophages along adjacent normal lung (Lung N: n = 11), lung tumor (Lung T: n = 11), advanced stage of tumor (tLB: n = 4), and brain metastasized (mBrain: n = 10) tissues. The box is bounded by the first and third quartile with a horizontal line at the median and whiskers extend to the maximum and minimum value. **f.** Top pathways enriched in SPP1⁺ TAMs (upper) and $C1QC^+$ TAMs (lower) as determined by KEGG. g. Bubble plot of markers in each dendrite cell (DC) subcluster and the violin plot of CD274, LAMP3, CCL22 and CD3D, Kruskal-Wallis two-sided test is used to test the significance of gene expression level among different DC cells clusters. ***: *p* < 0.001. **h.** Subclusters of plasmacytoid DCs (pDCs) and feature plot and violin plot of GZMB, CD3D, and CD3E. i/j. Confocal images of multiplexed immunofluorescence staining of CD3, CD11c and CD86 in anaplastic thyroid (n = 3), gastric (n = 3) and colorectal cancer (n = 3). The quantification results in each of them. The blue arrow indicates CD3⁺ T-cell, and the red arrow indicates CD3⁺ DCs (CD3⁺CD11c⁺CD86⁺). Scale bar: 20 µm. Multiplexed immunofluorescence assays are performed twice on tumor samples following assay optimization. k. Subclustering tumor-infiltrated B-cell and violin plot with specific gene expression in subclusters. Source data are provided as a Source Data file.





Figure S2. Profile of epithelial clusters across different cancer types. a. The clustering of epithelial cells in pan-cancer profile. **b.** Histography of the composition proportion of different tissue types in each epithelial cluster. **c.** Bubble plot of specific expressed marker in each epithelial cluster. **d.** The feature plot of selected genes in specific epithelial clusters (E3, E5, E8, E9, E10 and E13).



Figure S3. General characteristics of CAF. a. Hierarchical clustering of TME components in all cancer types. **b.** Tissue type-specific interaction quantification of the main TME components represented in tumor (Fibroblast: n = 10, Lymphocyte: n = 10, Myeloid: n = 10, Endothelium: n = 10, Plasma: n = 10), adjacent (Fibroblast: n = 8, Lymphocyte: n = 8, Myeloid: n = 8, Endothelium: n = 8, Plasma: n = 8), and normal tissues (Fibroblast: n = 7, Lymphocyte: n = 7, Myeloid: n = 7, Endothelium: n = 7, Plasma: n = 7). The box is bounded by the first and third quartile with a horizontal line at the median and whiskers extend to the maximum and minimum value, Anova two-sided test is used to test the significance of counts of interaction among different cell type categories. Tumor *p*-value is 3.93×10^{-14} . Adjacent *p*-value is 2.38×10^{-4} , Normal tissue *p*-value is 8.19×10^{-5} . **c.** Differentially expressed genes between cancerassociated fibroblasts (CAFs) and normal fibroblasts (NFs). **d.** Feature plots of *ACTA2*, *ACTG2*, *FAP*, and *TGFB1* in the fibroblast component of the TME. **e.** Gene ontology enrichment of all components of CAFs. Source data are provided as a Source Data file.



Figure S4. Characteristics of three CAF states. a. Hierarchical clustering of fibroblasts in all cancer types. b. The constitution ratio of each cancer type in each state. c. Left: CREB3L1 expression was enriched along the evolutionary trajectory of CAFs. Right: comparison of CREB3L1 expression among the three CAF states, state1: n = 2133, state2: n = 2476, state3: n = 2891, The box is bounded by the first and third quartile with a horizontal line at the median and whiskers extend to the maximum and minimum value. Mann-Whitney two-sided test is used to test the significance of expression level of *CREB3L1* between CAF states. ****: *p* < 0.0001. **d.** Comparison of the EMT score and CREB3L1 expression among the three states of CAFs in each tissue type, Bladder: n = 1448, Breast: n = 454, Colorectal: n = 706, Gastric: n = 821, Intrahepatic duct: n = 59, Lung: n = 668, Ovary: n = 1262, Pancreas: n = 1659, Prostate: n = 168, Thyroid: n = 248. e. Left: CREB3L1 downstream target set enriched along the evolutionary trajectory of CAFs. Right: comparison of the downstream target set of CREB3L1 among the three CAF states, state1: n = 2133, state2: n = 2476, state3: n = 2891. The box is bounded by the first and third quartile with a horizontal line at the median and whiskers extend to the maximum and minimum value. Mann-Whitney twosided test is used to test the significance of regulon activity of CREB3L1 between CAF states. ****: *p* < 0.0001. Source data are provided as a Source Data file.



Figure S5. Interactions of CAFs with other TME components. **a.** Left: Clusters of NK/T cells in pan-cancer analysis. Right: bubble plot showing the specific marker genes in each cluster and the histography of the composition proportion of each type of NK/T cell. **b.** Predicted and detailed interactions between the three CAF states and each B-cell subcluster. **c.** Predicted and detailed interaction between the three CAF states with DCs and pDCs. **d.** the heatmap of interaction between each cluster of epithelial cells and CAF state1-3. **e/f.** Predicted and detailed interactions between the three three three CAF states and each epithelial cluster.







CAF_{state2} CAF_{state2} high enriched group p = 0.65Time (week)

Urothelial carcinoma

CAFstate3	high (N=70)	mission					
enrichment	(N=270)	0.59 (0.344 - 1.00)				-	0.049*
Gender	1 (N=75)	reference					
	2 (N=272)	1.13 (0.704 - 1.01)			-		0.614
Mutation burden	(N=340)	0.95 (0.921 - 0.98)					0.002 **
	desert (N=75)	reference				•	
Immune phenotype	excluded (N=124)	0.61 (0.382 - 0.97)				-	0.037 *
	infamed (N=76)	0.50		-	-	-	0.025 *
BiopsyTime	POST (N=125)	reference					
	PRE (N=101)	1.54 (0.735 - 1.70)					0.551
Lund	MS1a (N=22)	reference				•	
	M51b (N=22)	0.68 (0.294 - 1.56)					0.36
	MS2x1 (N=45)	0.40			-	+	0.077
	MS2x2 (N=25)	0.24 (0.073 - 0.00)					0.02*
	MS-B1	0.43		-	-	÷ .	0.069
	MS262.1 (N=10)	0.05 (0.170 - 2.36)					0.51
	MS262.2 (N=00)	0.85					0.76
Tobacco History	CURRENT	reference				i	
	NEVER (Natifa)	1.00					0.997
	PREVIOUS (Nor127)	0.95				-	0.875
Stage	1 (N=150)	reference				i	
	N-80	(1074-3.92)					- 0.03 *
	(N=09)	1.02				÷	0.957
	N (N=95)	1.09			·		0.802
ECOG Score	(N=340)	2.78 (1.030 - 4.20)					
# Events: 117; Global p-value (Log-Rat AIC: 1063.49; Concordance Index: 0.69	k): 5.97e-05		0.1	0.2	0.5	1 2	5

Uterine Sarcomas

Melanoma

CAFstate3 enrichment	high (N=34)	reference								
	low (N=16)	0.096 (0.020 - 0.37)		-						<0.00
mitotic.index.group	High (N=23)	reference					1			
	Low (N=27)	0.227 (0.076 - 0.67)		-		-	-			0.008
hormone.receptor. expression	Negative (N=28)	reference								
	Positive (N=11)	0.224 (0.041 - 1.21)	-		-			-		0.083
rna.group	Developmental (N=21)	reference								
	ECM (N=8)	0.999 (0.275 - 3.63)						-	_	• 0.998
	LMS-like (N=10)	0.453 (0.114 - 1.80)			-	-	-			0.26
	Proliferative (N=11)	0.634 (0.162 - 2.48)			-		-		-	0.512
cnv.group	High (N=25)	reference								
	Low (N=15)	1.228 (0.401 - 3.77)				÷		-		0.719
# Events: 25; Global p-value AIC: 125.95; Concordance In	(Log-Rank): 0.000048 dex: 0.81	945								

CAFstate3	high (N=23)	reference
enrichment	low (N=78)	0.19 (0.084 - 0.42)
Mstage	MD (N=2)	reference
	M1a (N=21)	0.16 (0.027 - 0.89)
	M1b (N=17)	0.12 (0.016 - 0.96)
	M1c (N=44)	1.19 (0.214 - 6.64)
	UNKNOWN (N=10)	0.16 (0.017 - 1.47)
BiopsyTime	ON (N=50)	reference
	PRE (N=51)	0.89 (0.486 - 1.61)
Cohort	NIV3-NAIVE (N=46)	reference
	NIV3-PROG (N=55)	0.61 (0.291 - 1.29)
Mutational.Subtype	BRAF (N=28)	reference
	NF1 (N=5)	22.75 (4.384 - 118.10)
	RAS (N=18)	1.80 (0.608 - 5.33)
	TripleWt (N=41)	0.54 (0.233 - 1.25)
Mutation.Load	(N=101)	1.00 (0.999 - 1.00)
Cytolytic.Score	(N=101)	1.00 (1.000 - 1.00)
# Events: 48; Global p-value (L AIC: 357.54; Concordance Inde	og-Rank): 0.00001	5736
No. 531.54, CONCUMENTAL INC.		0.01

b

Figure S6. Association of CAF states with immunotherapy survival outcomes. a. Estimation of the prognostic value of the CAF_{state1/2} signature score in three immunotherapy cohorts (urothelial carcinoma, uterine sarcoma, and melanoma), Kaplan–Meier curves for overall survival in all patients according to the number of positive ligands. *p*-values for all survival analyses have been calculated using the logrank test. **b.** The forest plot of multivariate adjustment results in three cohorts, Error bars represent 95% confidence intervals.

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COAD

STAD

С



CD163 G-SMA PanCK DAPI 25tum CD163 CD163

а

Figure S7. Characteristics of CAF_{ap} and CAF_{PN}. **a.** The absolute proportion of CAF_{ap} to all fibroblasts in each cancer type. **b.** Genetic similarity heatmap of fibroblast-related clusters and mono-macrophage-related clusters in the TME. **c.** Confocal images of multiplexed immunofluorescence staining of PanCK, α -SMA, and CD163 in colorectal and gastric cancer tissues. Multiplexed immunofluorescence assays are performed twice on tumor samples following assay optimization. **d.** The absolute proportion of CAF_{PN} to all fibroblasts in each cancer type.

а



Proportion (%)



С

GeneRatio

0,08



0,00

 $\mathsf{CAF}_{\mathsf{EndMT}}$ Fibroblasts Endothelial Expression

2









10

15 20

25

30

1e-04

Figure S8. Characteristics of CAF_{EndMT}. **a.** Histography of the proportion of CAF_{EndMT} in each type of cancer and cell orgins. **b.** The heatmap of differentially expressed genes in CAF_{EndMT}, all other fibroblasts and endothelial cells. Violin plot of *ESM1* in fibroblast and endothelial clusters. Kruskal-Wallis two-sided test is used to test the significance of gene expression level among different fibroblast and endothelium clusters. ***: p < 0.001. **c.** The KEGG pathway enrichment plot of CAF_{EndMT}. FDR value is calculated by R package "clusterProfiler".



DAPI CD44 CD31 CD68 SPP1



SPP1+CD68+ density

Figure S9. Communications of CAF_{EndMT} with other TME components. a. Estimation of the prognostic value of the CAF_{EndMT} signature in colorectal, gastric and breast cancer in terms of overall survival, Kaplan–Meier curves for overall survival in all patients according to the number of positive ligands. *p*-values for all survival analyses have been calculated using the log-rank test. **b**. The detailed interaction heatmap among TME components in normal, adjacent, and tumor tissues. **c**. Dynamic alteration of *ITGA9* and *ITGB1* along the EndMT trajectory. **d**. Illustration of the phenotype map and density map. **e**. An example of a defective region where tissues were dropped or overturned on a slide is highlighted in a red square. Scale bar: 500 µm Multiplexed immunofluorescence assays are performed twice on tumor samples following assay optimization.



Figure S10. Correlation of CAF_{EndMT} with *SPP1*⁺ TAM across different cancer types. Correlation of the CAF_{EndMT} and *SPP1*⁺TAM signature enrichment scores in various cancer types in TCGA datasets, R represents Pearson's correlation and its coefficient of determination. Significantly positive correlations were detected in most cancer types (25 of 28).