

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 μ m. 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.

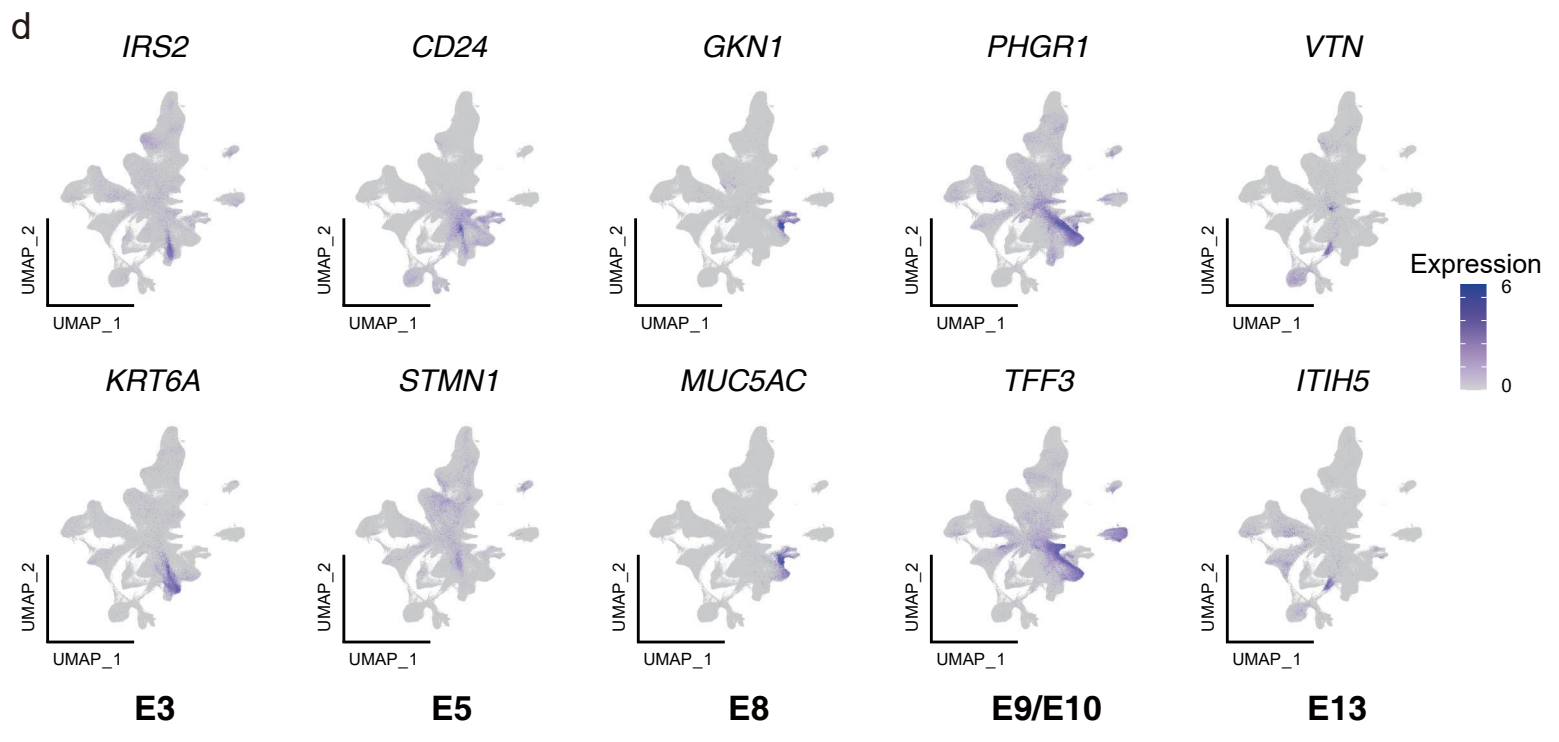
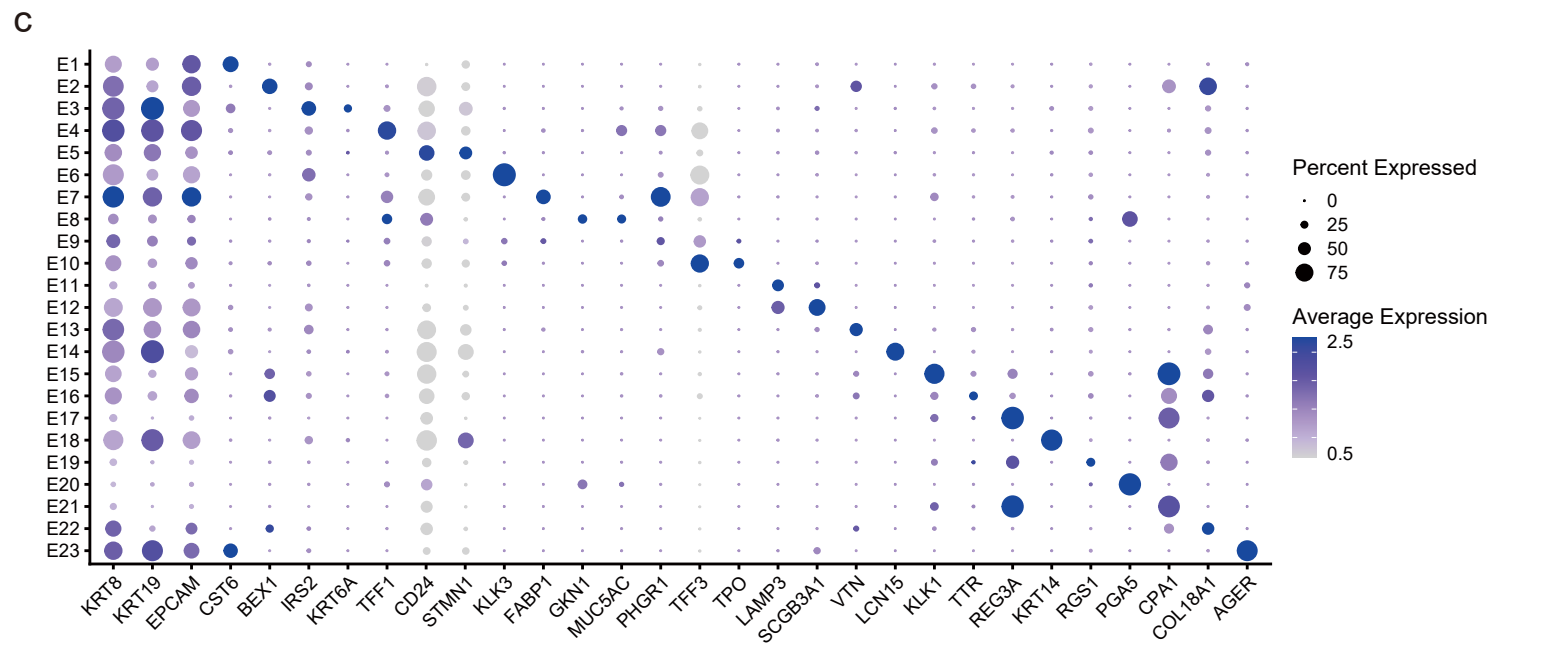
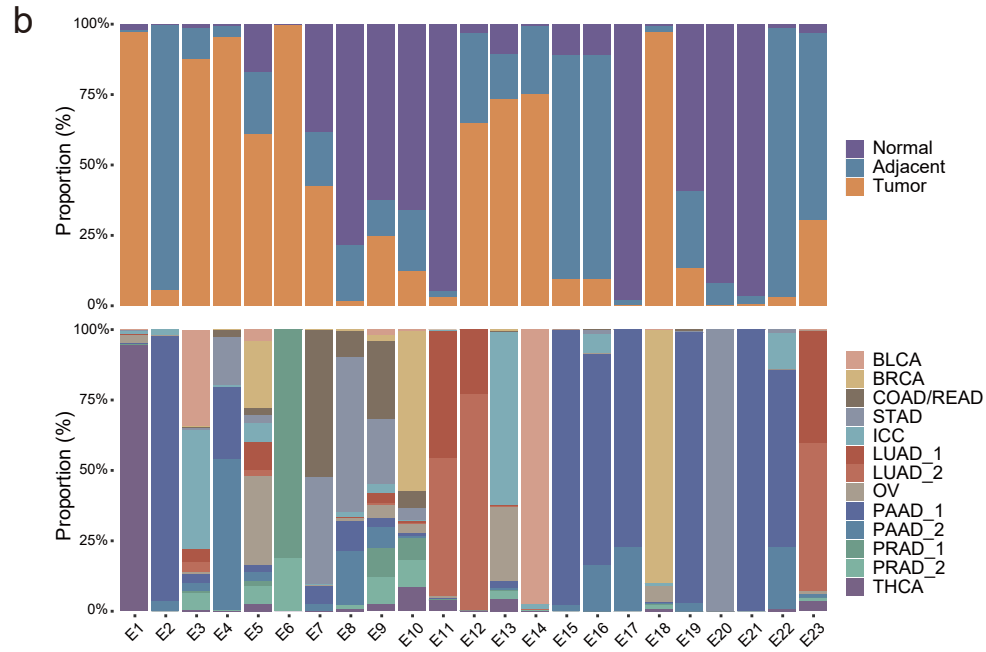
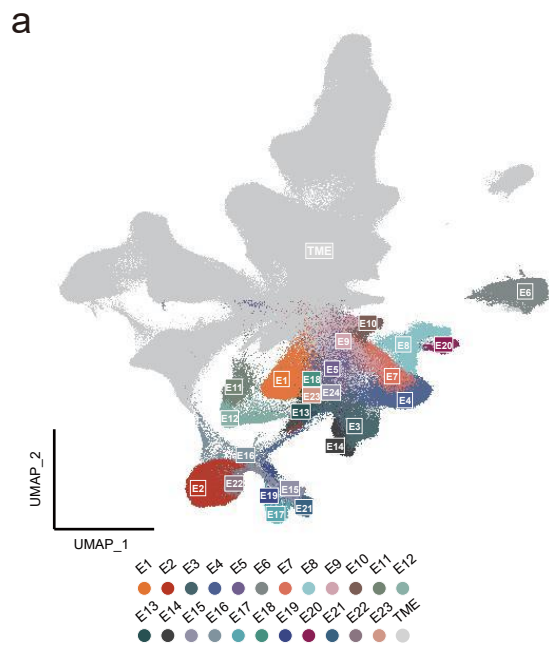


Figure S2. Profile of epithelial clusters across different cancer types. **a.** The clustering of epithelial cells in pan-cancer profile. **b.** Histogram of the composition proportion of different tissue types in each epithelial cluster. **c.** Bubble plot of specific expressed marker in each epithelial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

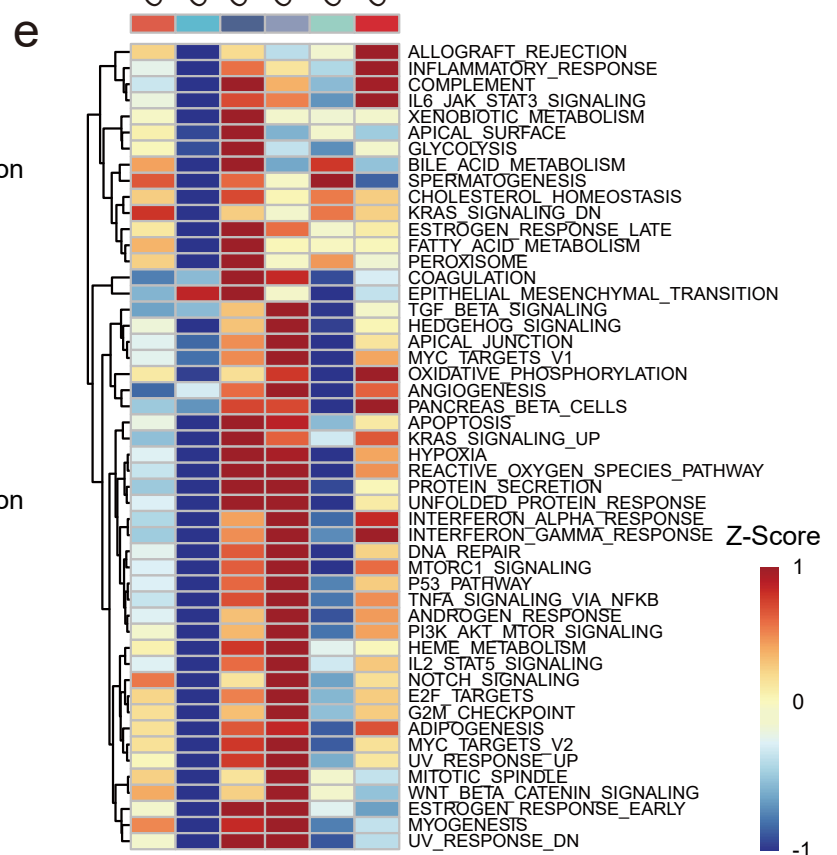
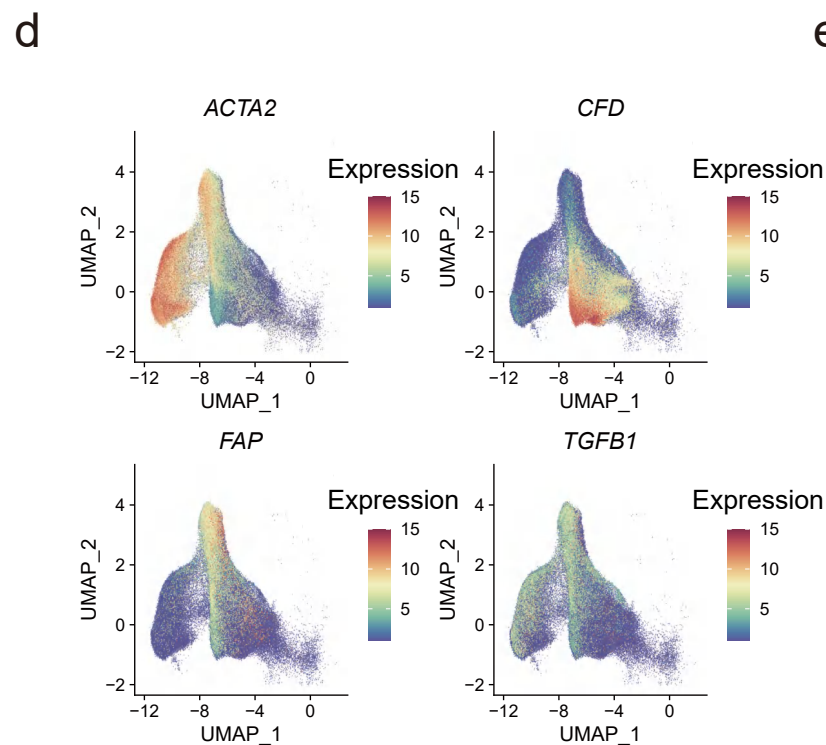
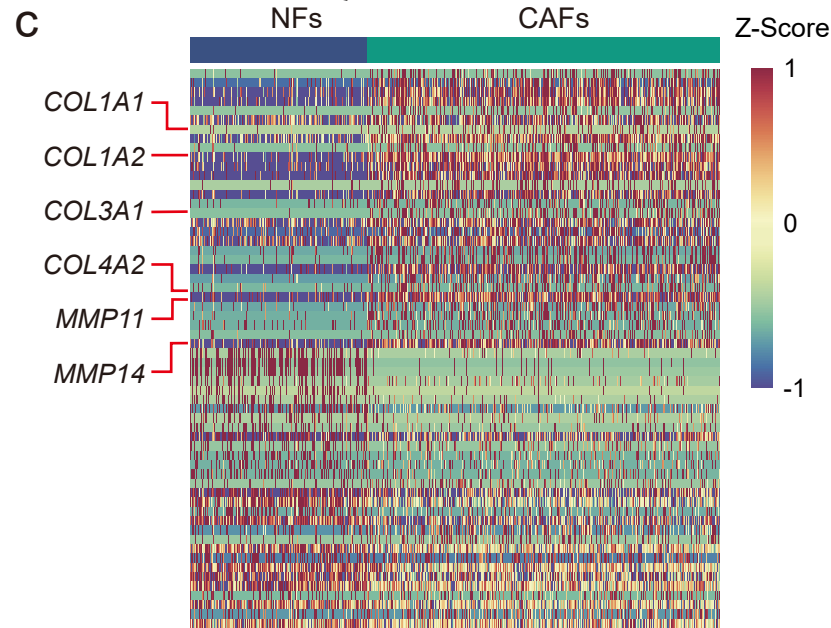
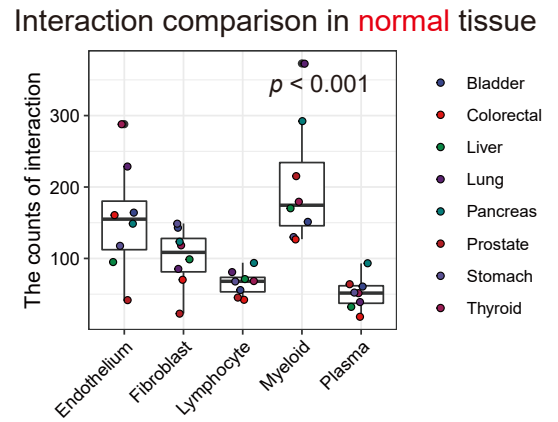
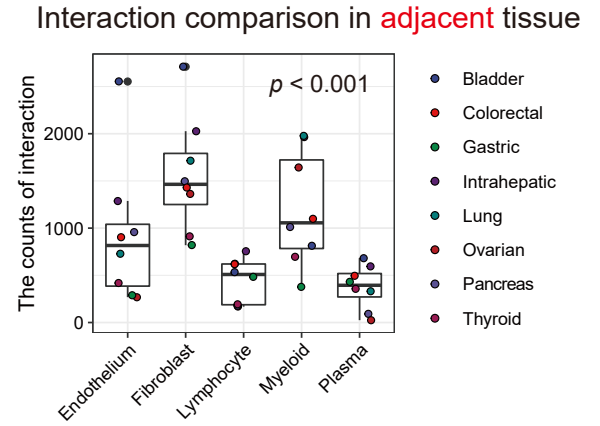
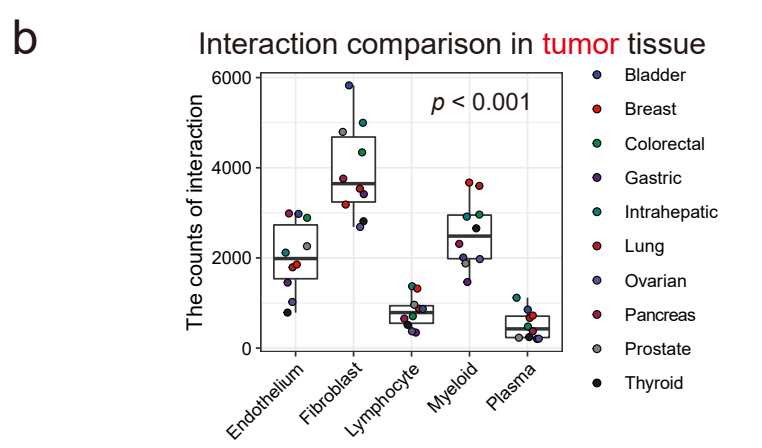
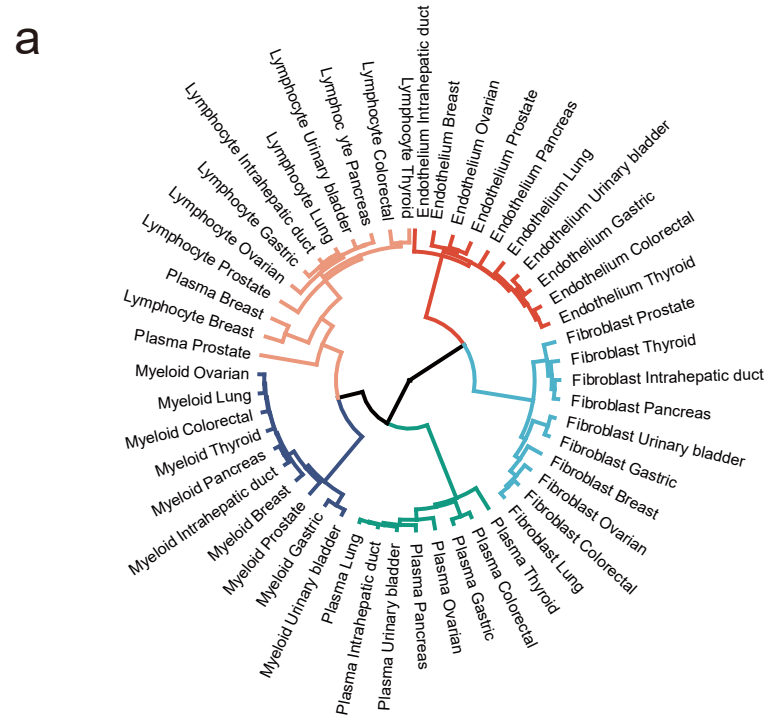
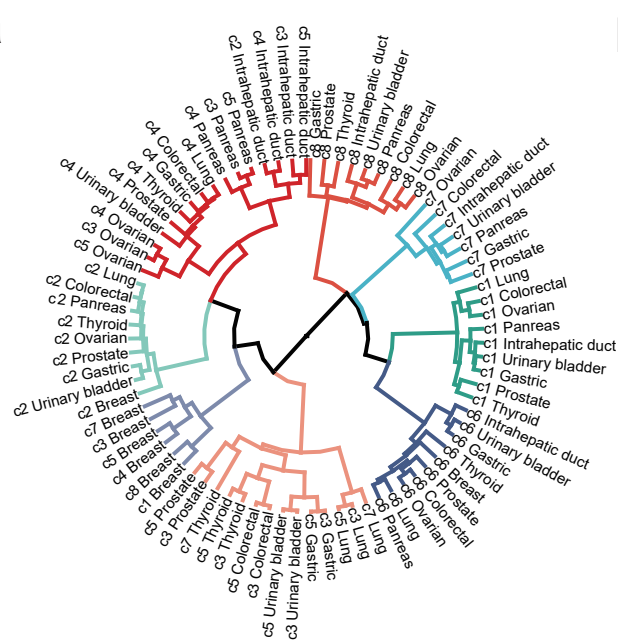
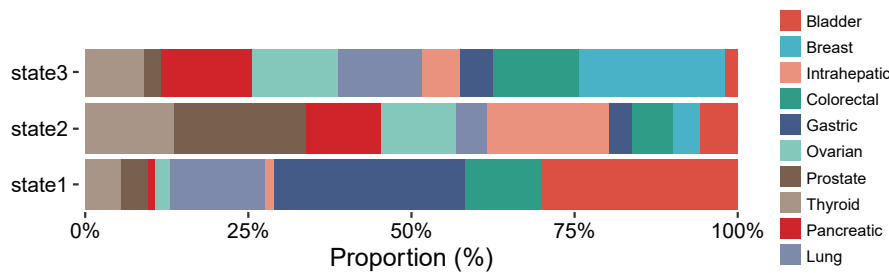


Figure S3. General characteristics of CAF. **a.** Hierarchical clustering of TME components in all cancer types. **b.** Tissue type-specific interaction quantification of the main TME components represented in tumor (Fibroblast: n = 10, Lymphocyte: n = 10, Myeloid: n = 10, Endothelium: n = 10, Plasma: 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 cancer-associated 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.

a

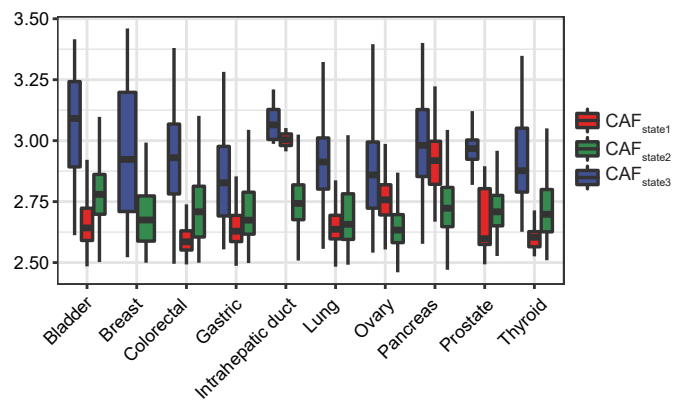
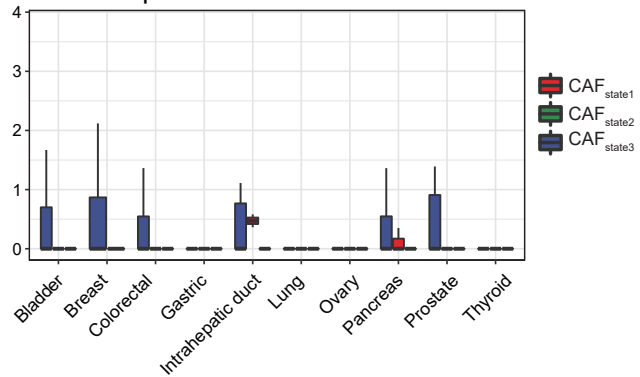


b

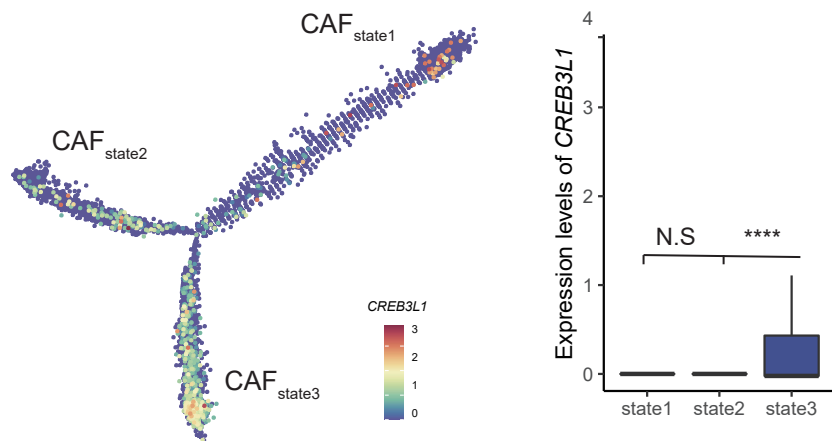


d

EMT scores

Expression levels of *CREB3L1*

c



e

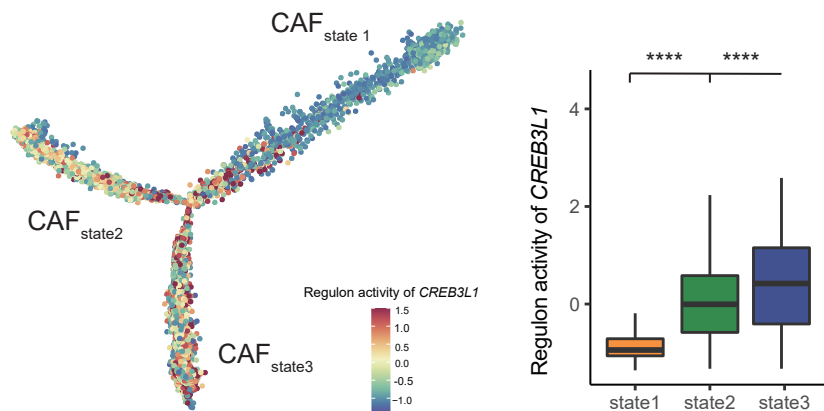


Figure S4. Characteristics of three CAF states. **a.** Hierarchical clustering of fibroblasts in all cancer types. **b.** The constitution ratio of each cancer type in each state. **c.** Left: *CREB3L1* expression was enriched along the evolutionary trajectory of CAFs. Right: comparison of *CREB3L1* 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 two-sided 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.

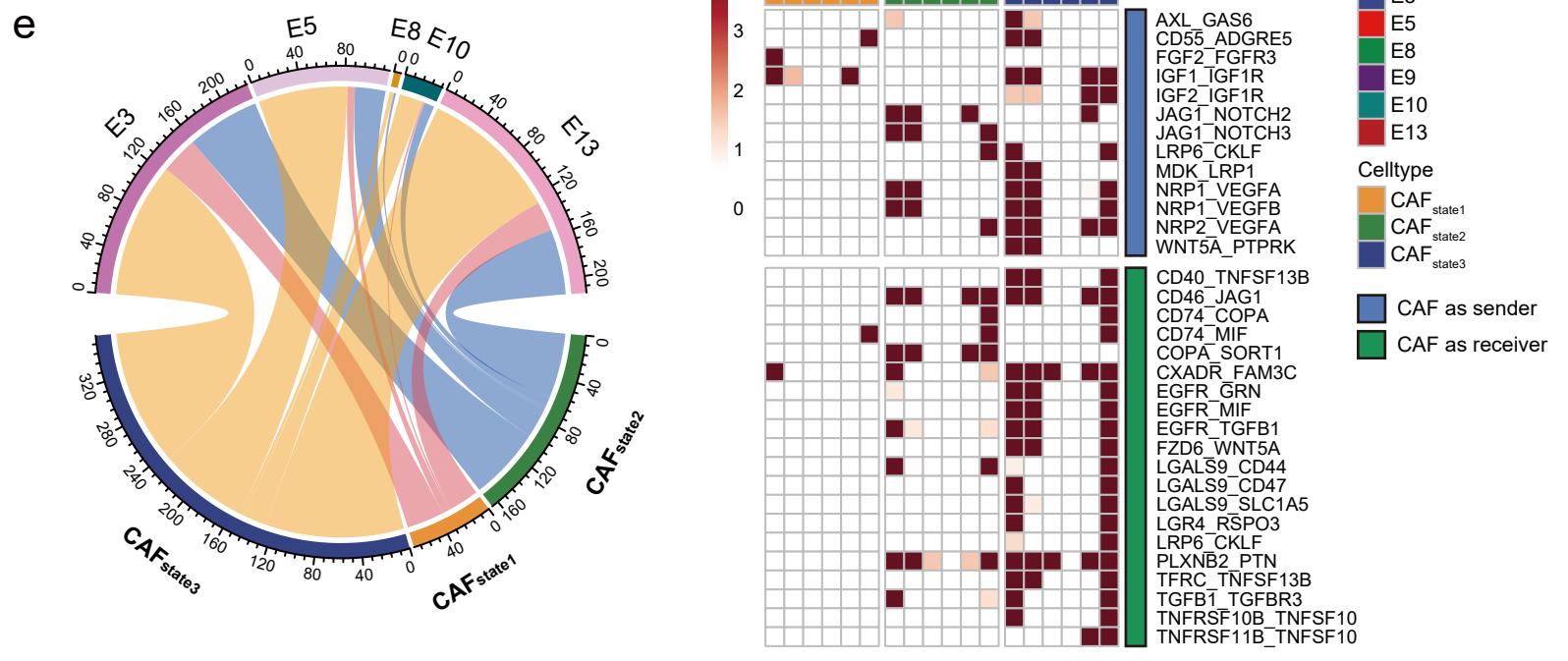
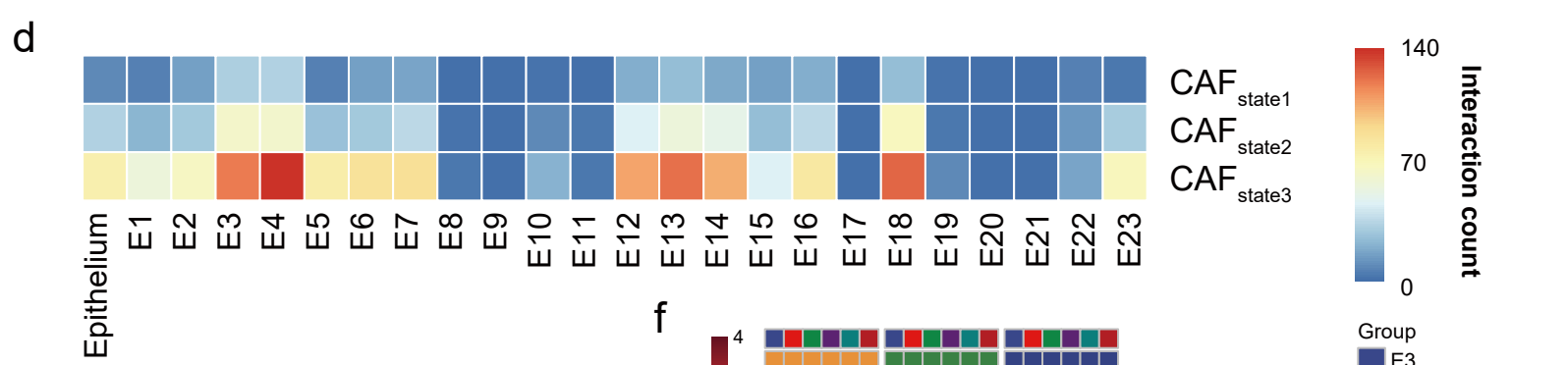
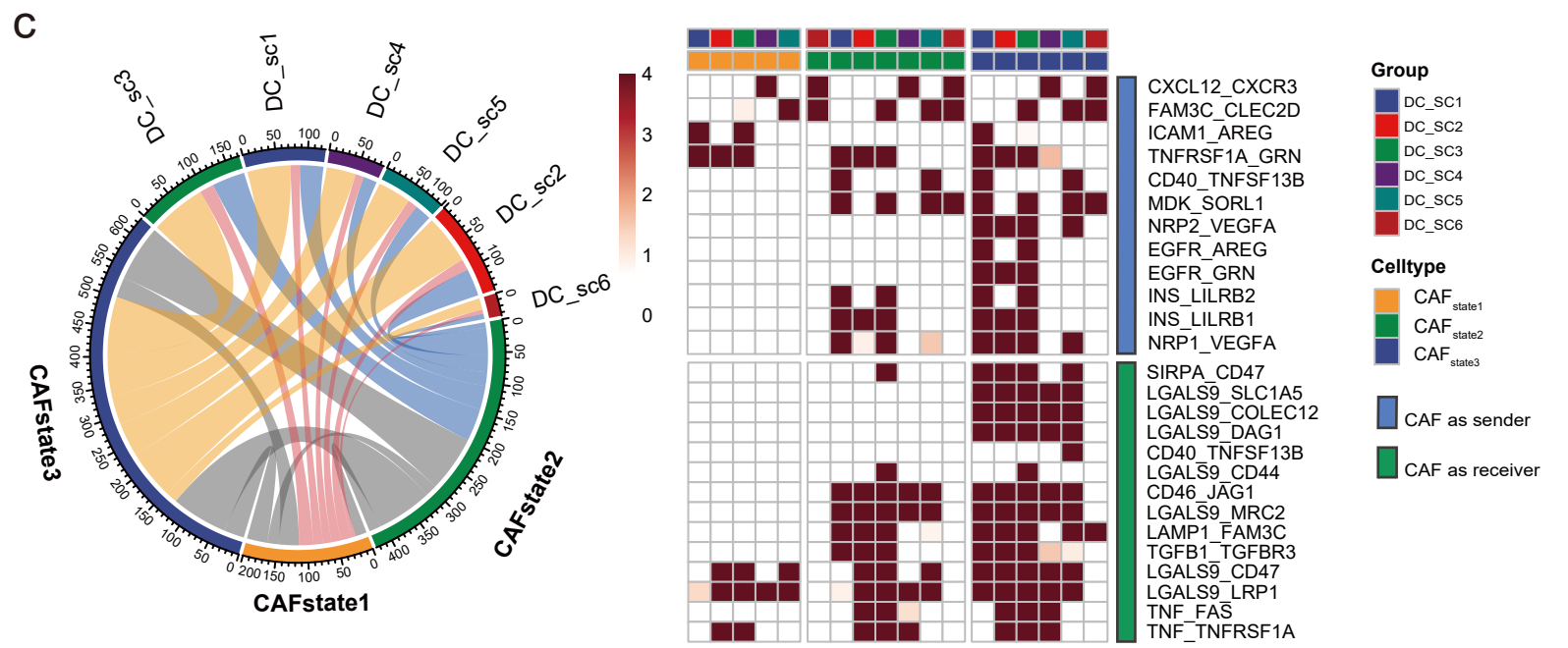
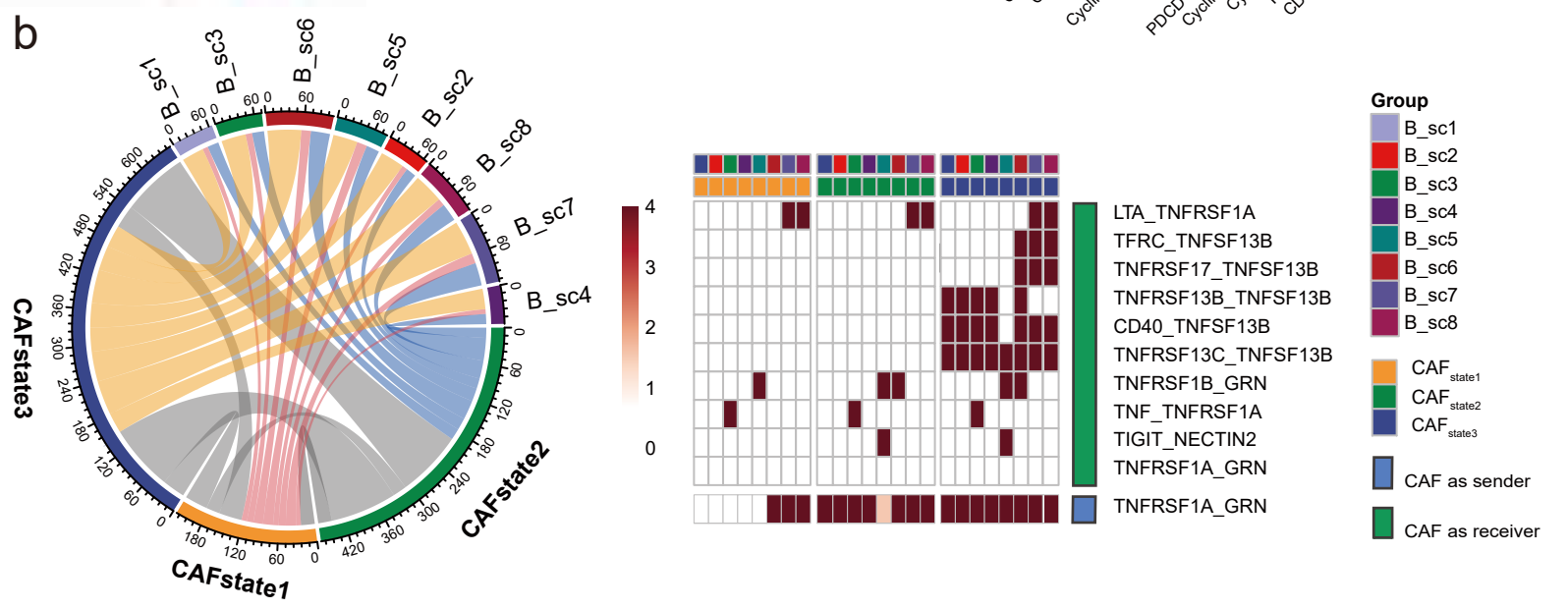
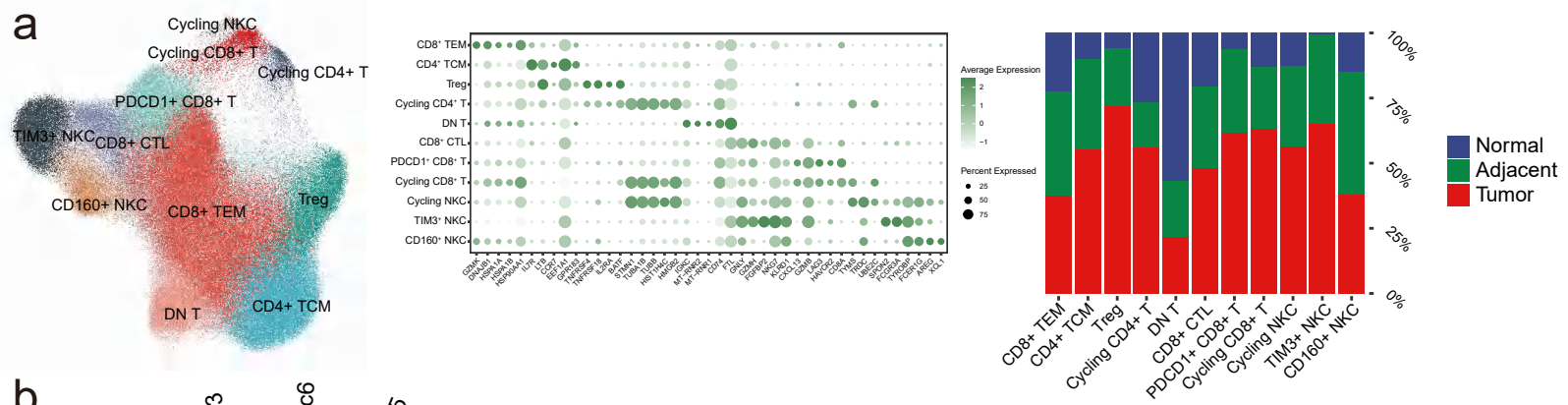
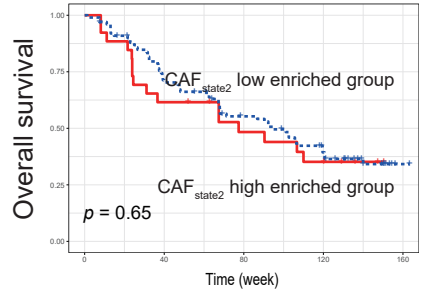
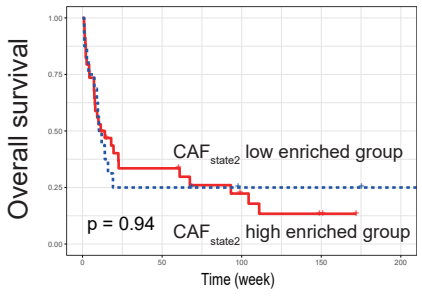
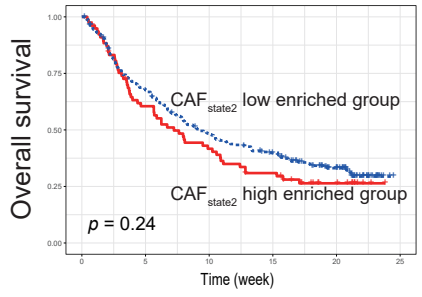
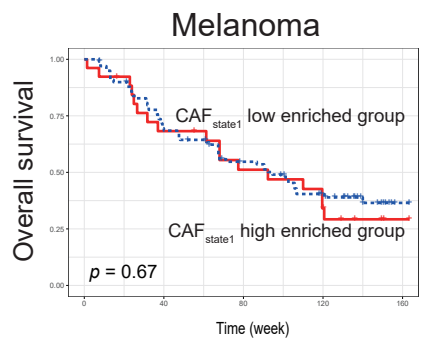
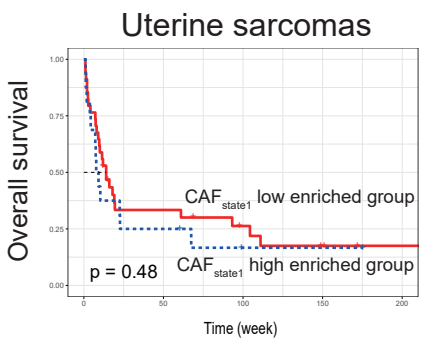
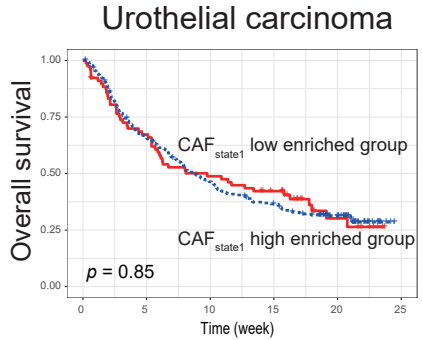


Figure S5. Interactions of CAFs with other TME components. **a.** Left: Clusters of NK/T cells in pan-cancer analysis. Right: bubble plot showing the specific marker genes in each cluster and the histogram of the composition proportion of each type of NK/T cell. **b.** Predicted and detailed interactions 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 CAF states and each epithelial cluster.

a



b

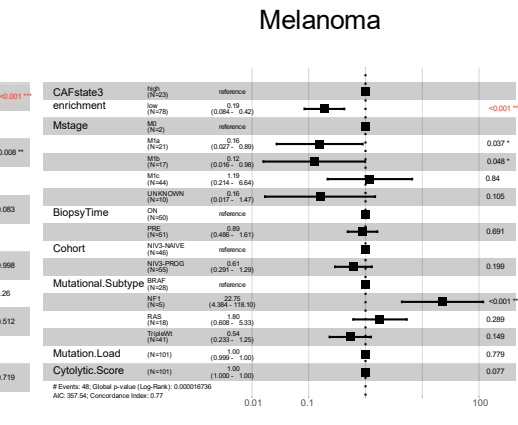
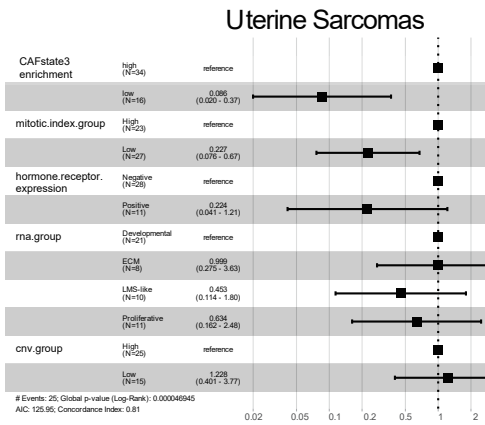
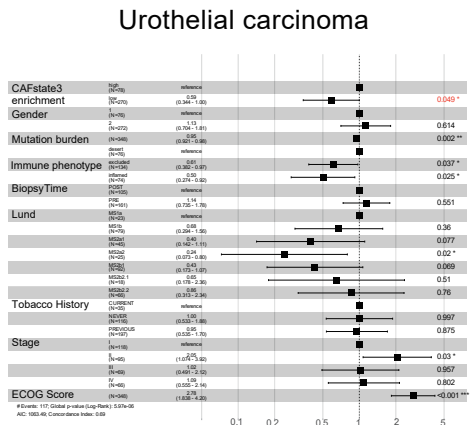


Figure S6. Association of CAF states with immunotherapy survival outcomes. a. Estimation of the prognostic value of the $CAF_{state1/2}$ signature score in three immunotherapy cohorts (urothelial carcinoma, uterine sarcoma, and melanoma), Kaplan–Meier curves for overall survival in all patients according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

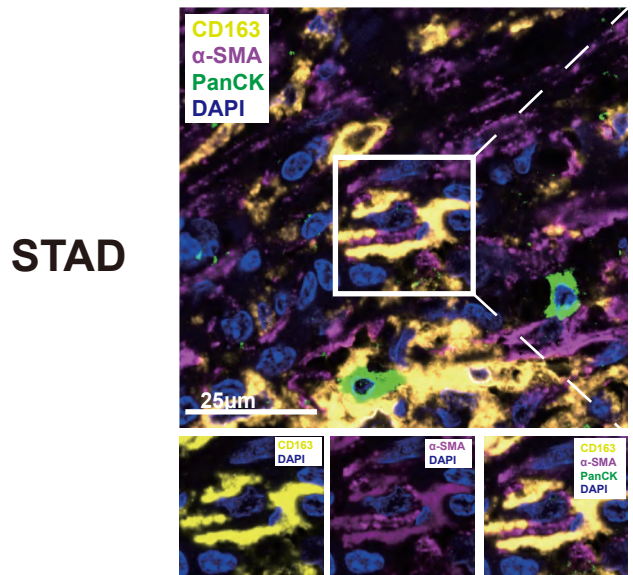
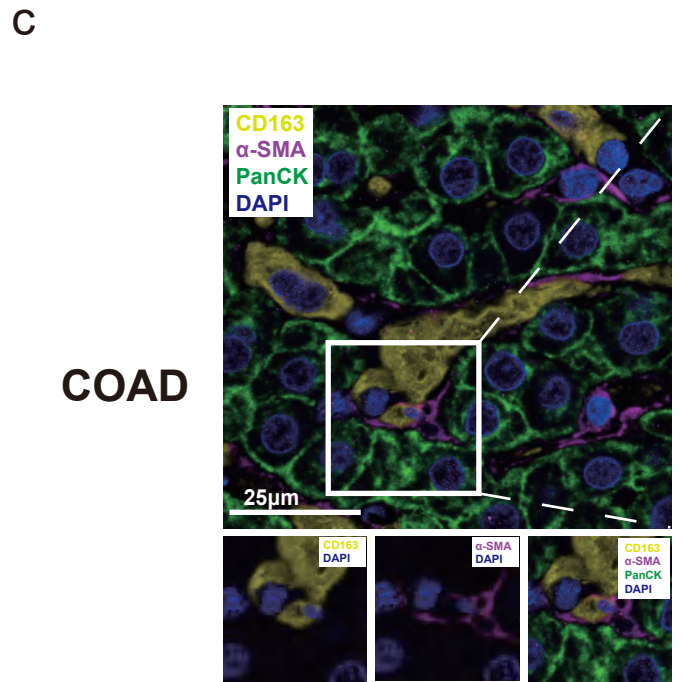
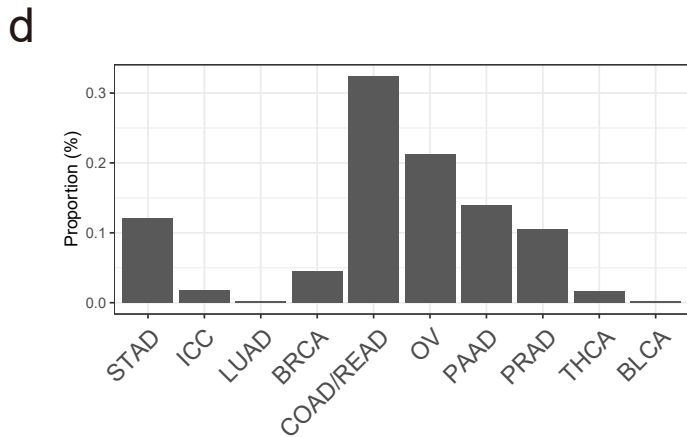
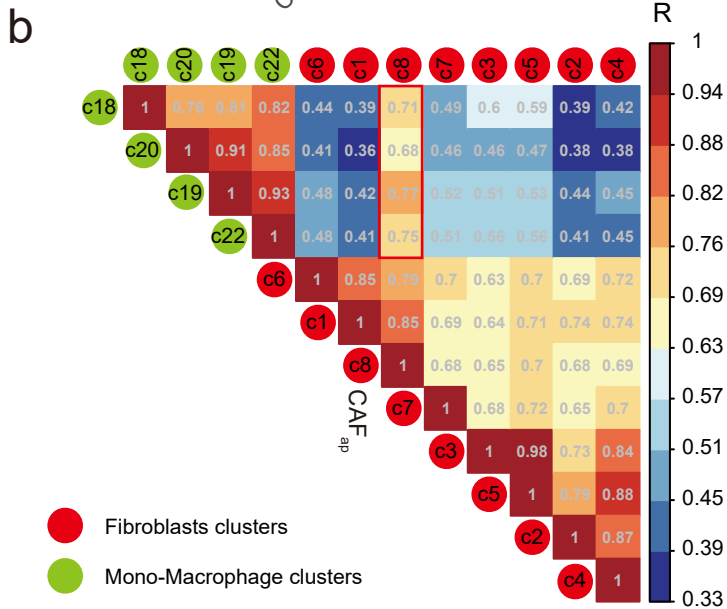
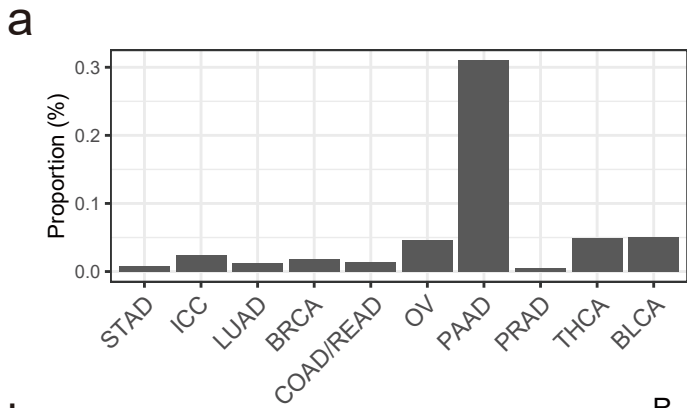


Figure S7. Characteristics of CAF_{ap} and CAF_{PN}. **a.** The absolute proportion of CAF_{ap} to all fibroblasts in each cancer type. **b.** Genetic similarity heatmap of fibroblast-related clusters and mono-macrophage-related clusters in the TME. **c.** Confocal images of multiplexed immunofluorescence 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.

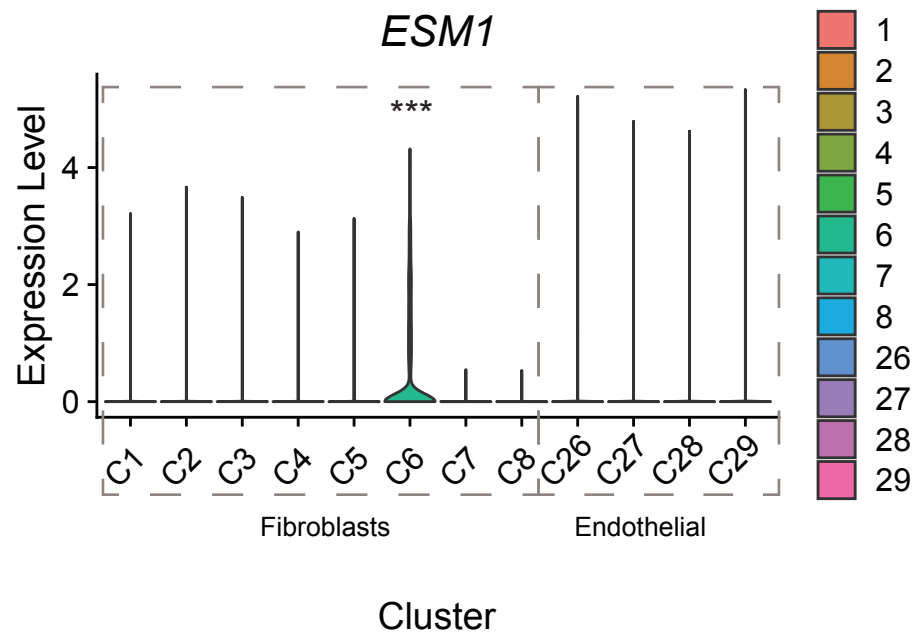
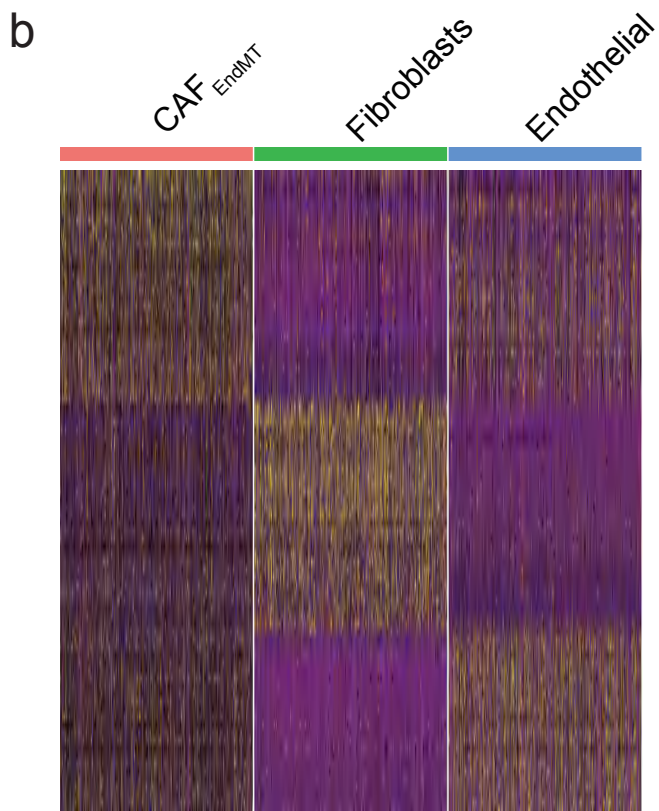
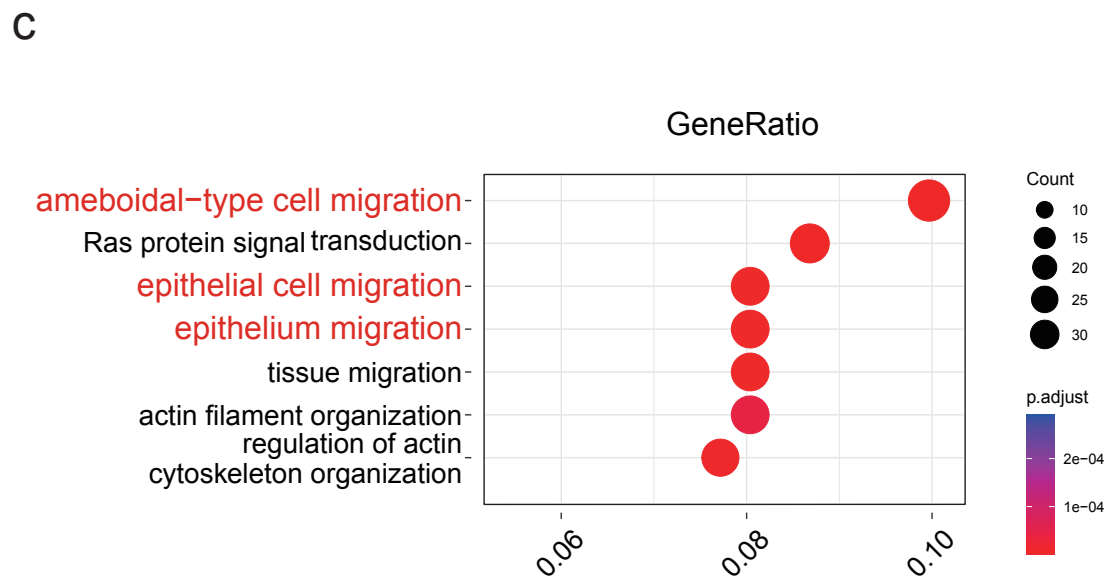
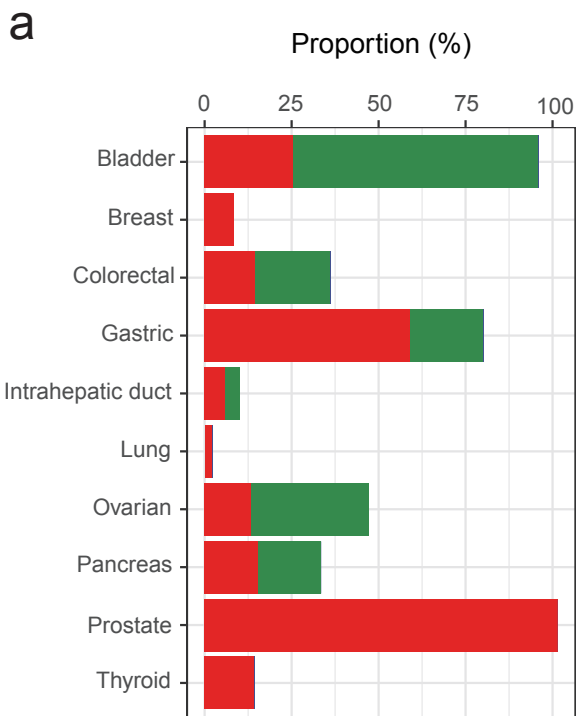


Figure S8. Characteristics of CAF_{EndMT}. **a.** Histogram of the proportion of CAF_{EndMT} in each type of cancer and cell origins. **b.** The heatmap of differentially expressed genes in CAF_{EndMT}, all other fibroblasts and endothelial cells. Violin plot of *ESM1* in fibroblast and endothelial clusters. 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”.

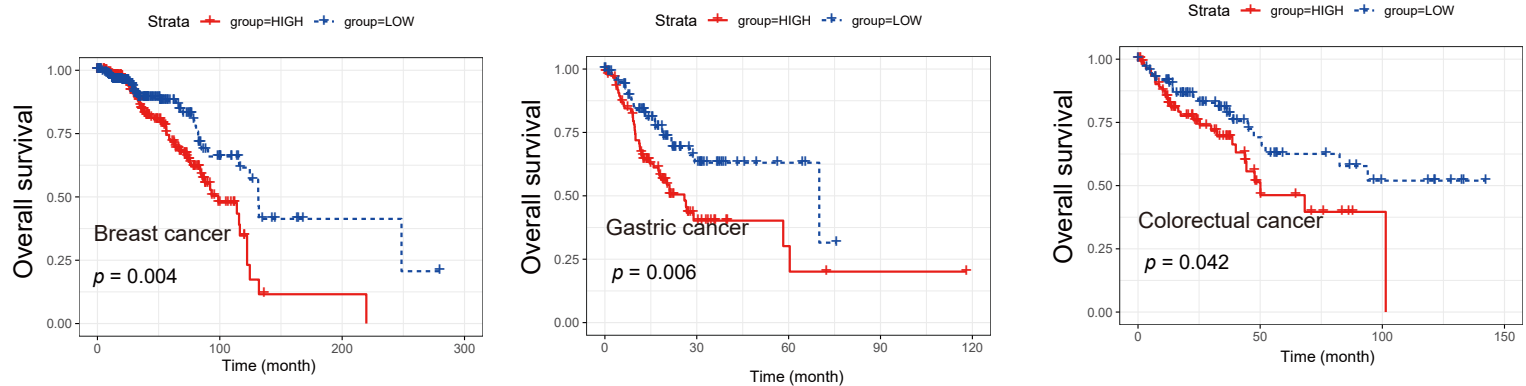
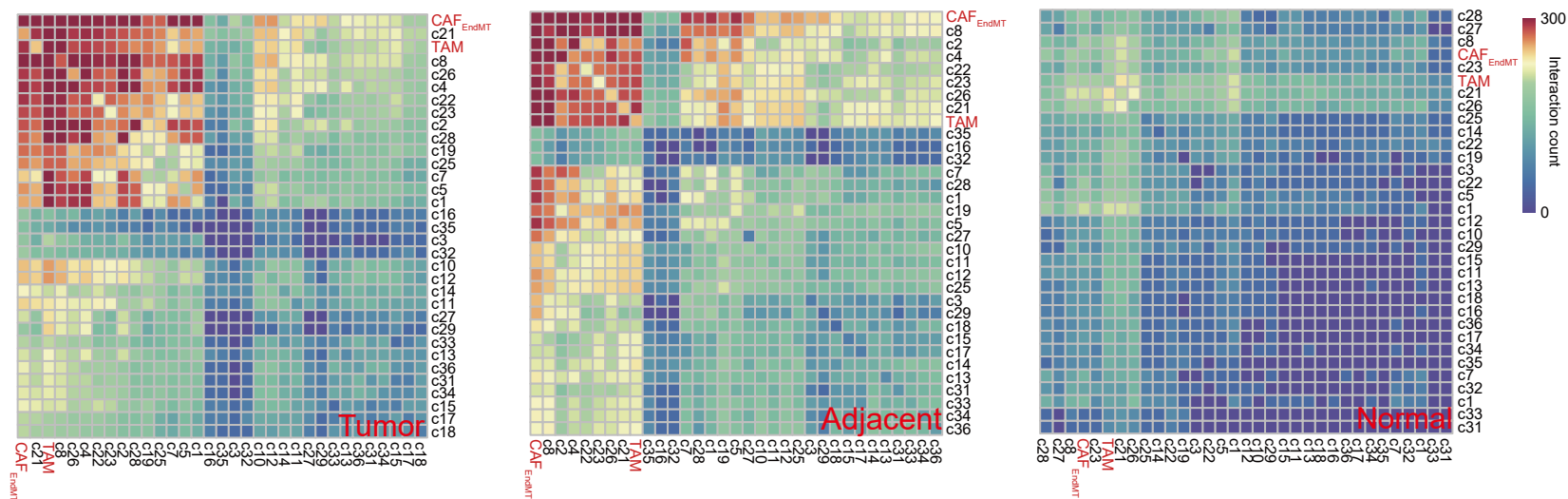
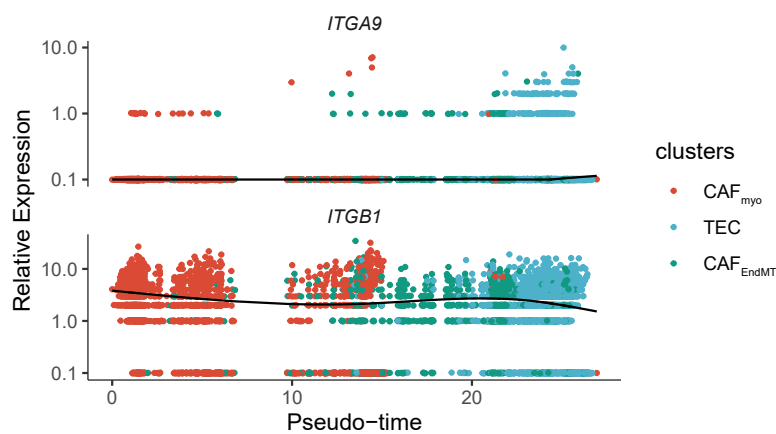
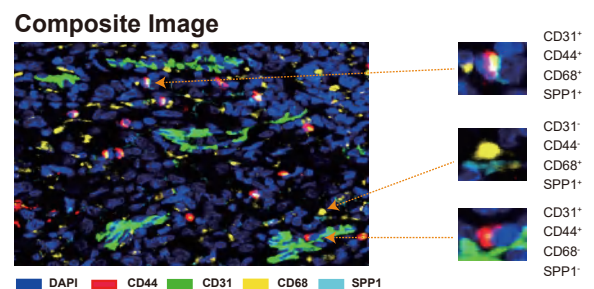
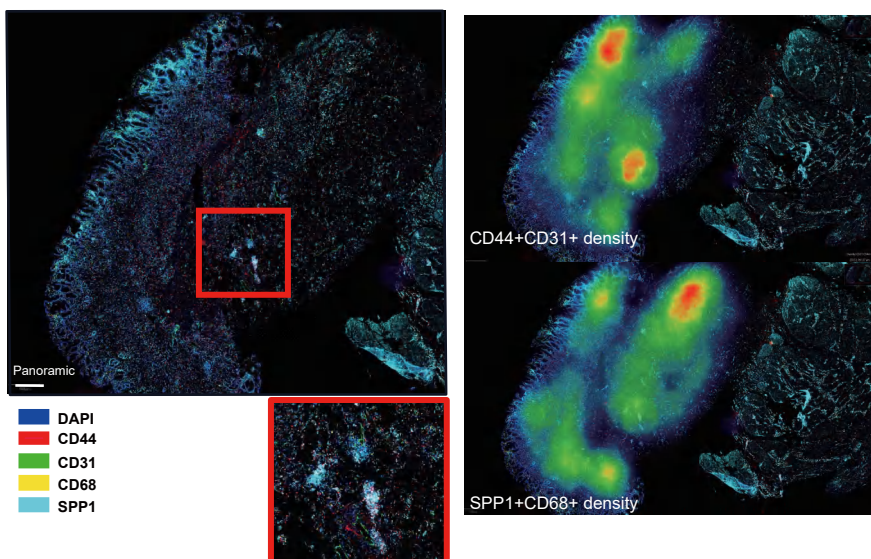
a**b****c****d****e**

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.

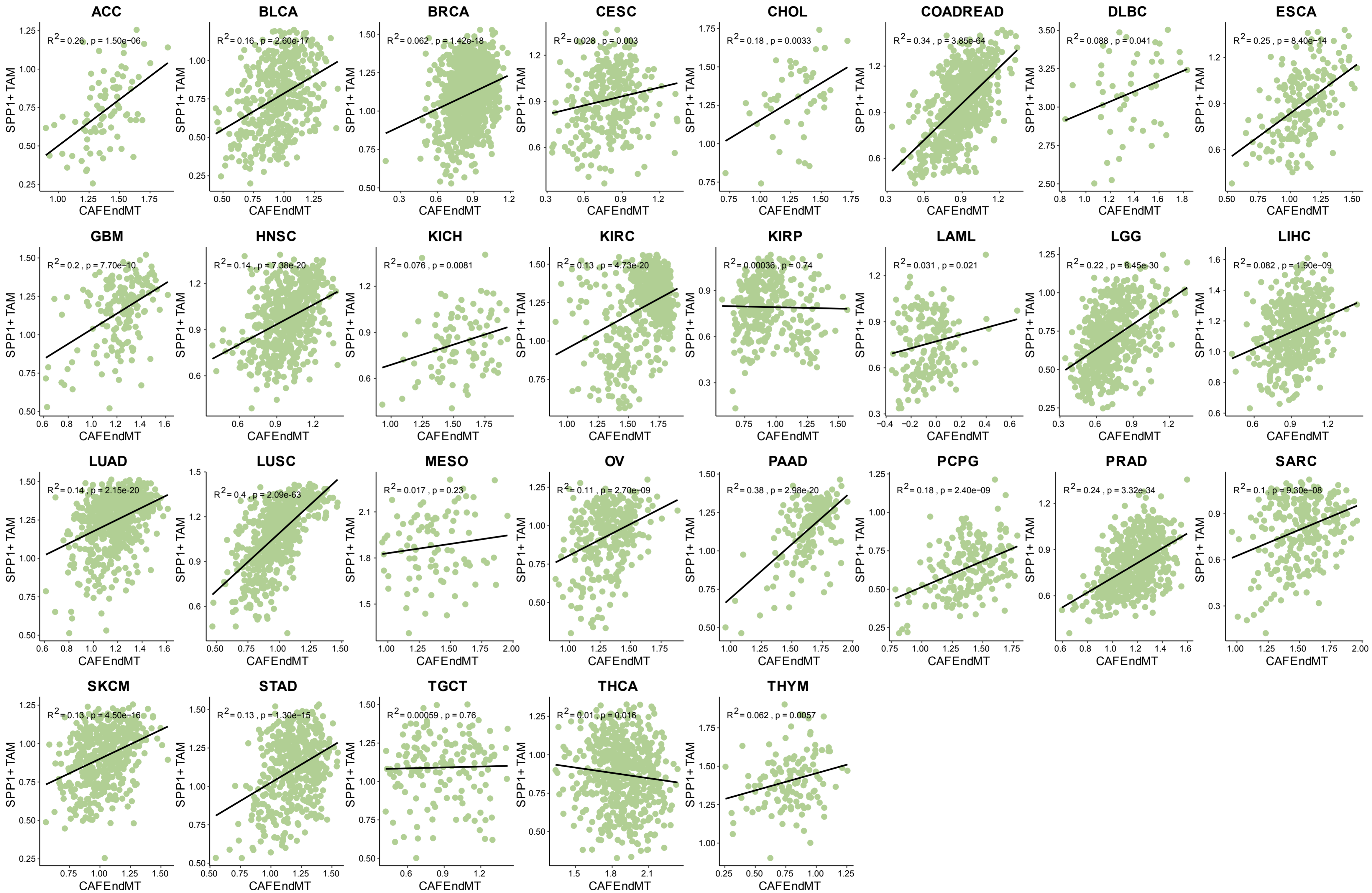


Figure S10. Correlation of CAF_{EndMT} with SPP1⁺ TAM across different cancer types. Correlation of the CAF_{EndMT} and SPP1⁺TAM signature enrichment scores in various cancer types in TCGA datasets, R represents Pearson's correlation and its coefficient of determination. Significantly positive correlations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