

SUPPLEMENTARY INFORMATION

FUSION-DRIVEN ONCOGENIC PROGRAMS SHAPE THE IMMUNE LANDSCAPE IN TRANSLOCATION RENAL CELL CARCINOMA

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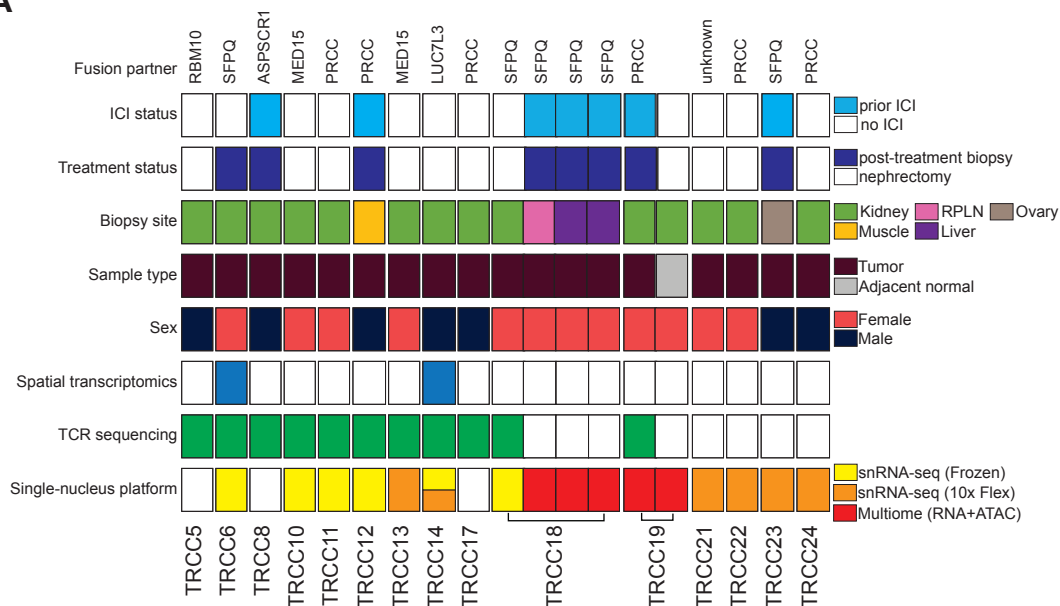
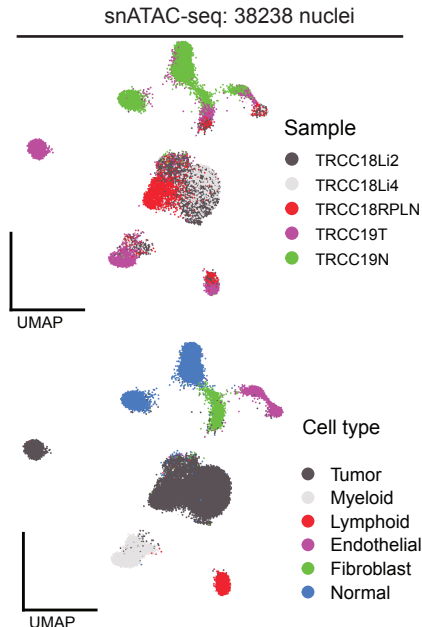
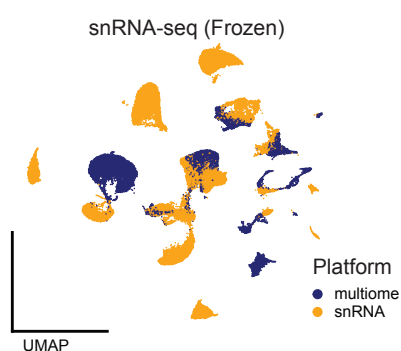
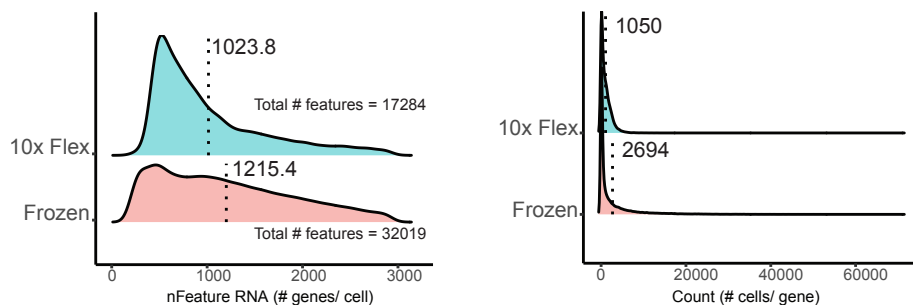
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TRCC14 frozen vs ffpe comparison

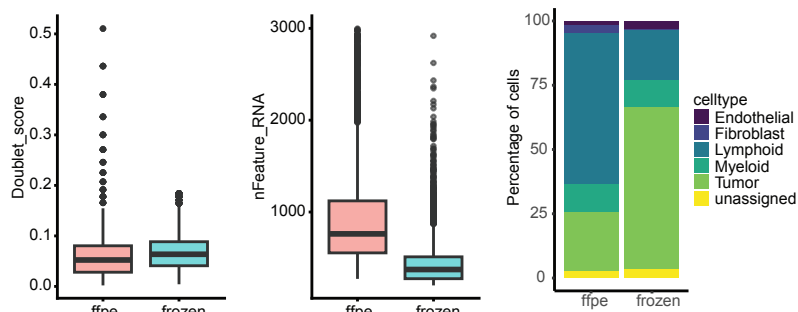
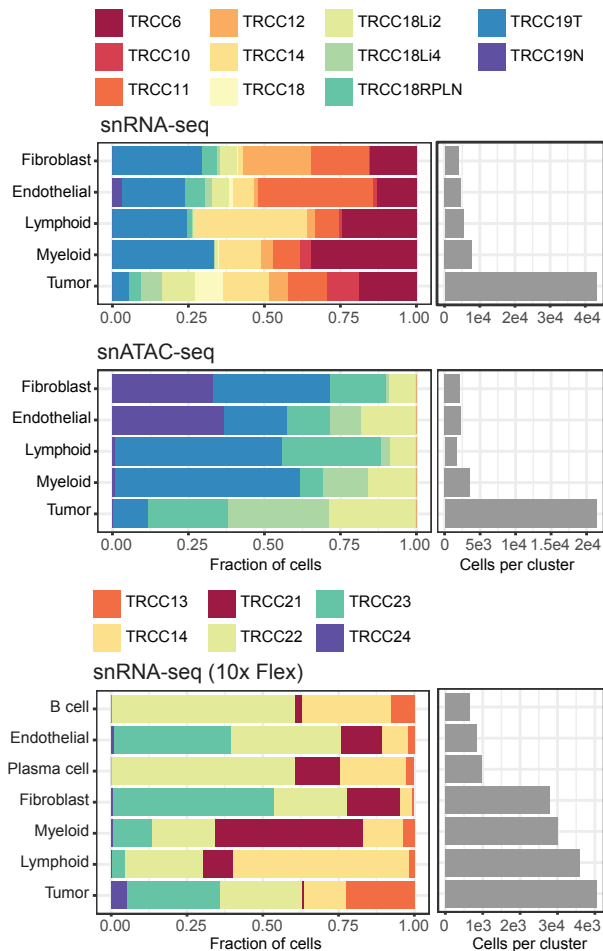
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Figure S1. Characteristics of tRCC snRNA-seq cohorts

- A. Summary of clinicopathological characteristics, treatment history, biopsy site, fusion partner, and molecular profiling approaches used for tRCC samples in this study.
- B. UMAPs of tRCC snATAC-seq data representing samples (top) and major cell lineages (bottom).
- C. UMAP representation of snRNA-seq data from the frozen cohort, highlighting samples processed via multiome (RNA+ATAC) or RNA-only platforms.
- D. Distribution of number of genes detected per nucleus in frozen and 10x flex fixed cohorts (left) and number of cells in which each gene was detected (right).
- E. Box plots comparing doublet score (left), nfeature_RNA (middle), and stacked barplot showing lineage composition (right) in TRCC14 frozen vs ffpe sample. Data is represented as median with 25th and 75th percentiles defining the box limits.
- F. Stacked bar plots showing lineage composition in three tRCC cohorts: snRNA-seq (Frozen), snATAC-seq, and snRNA-seq (10x Flex). Adjacent bar plots indicate total cell counts per lineage.

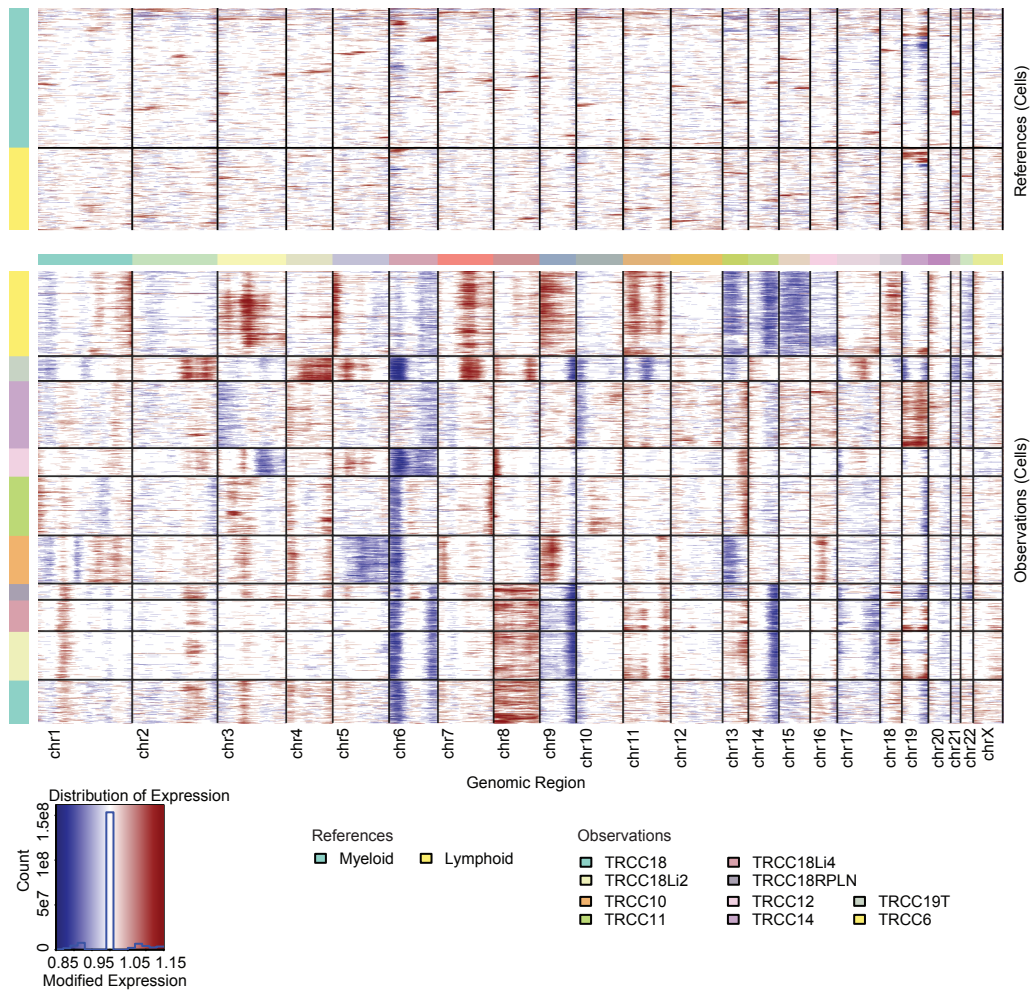
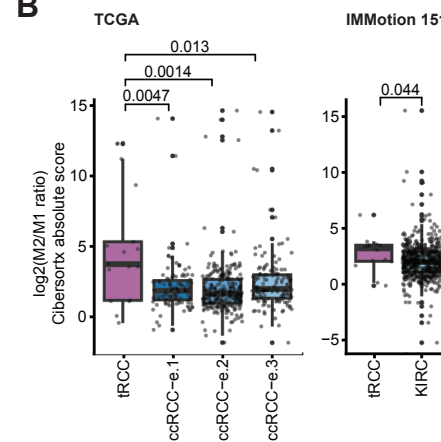
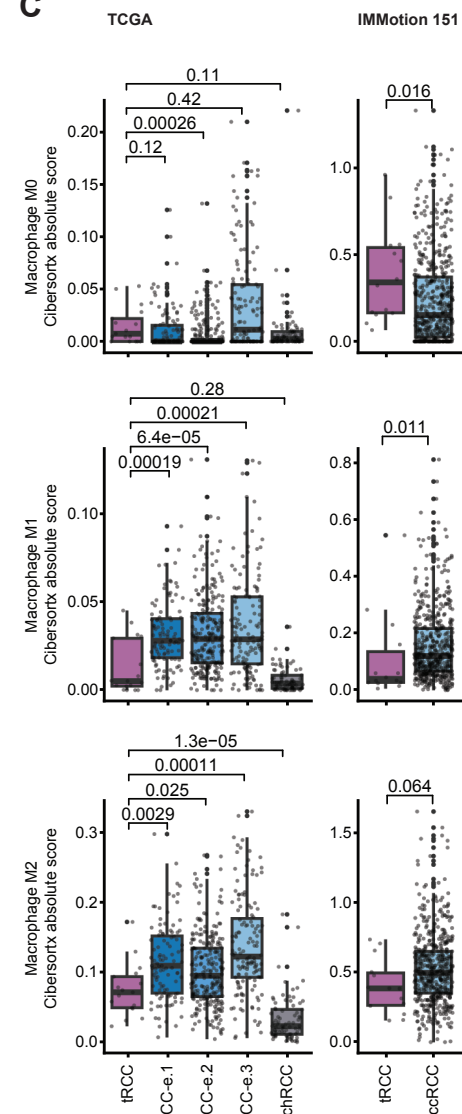
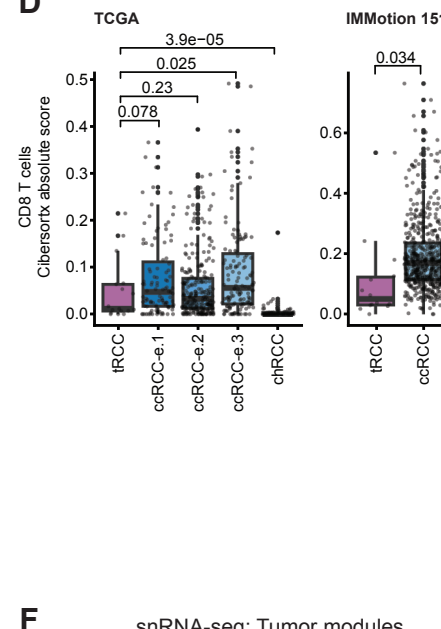
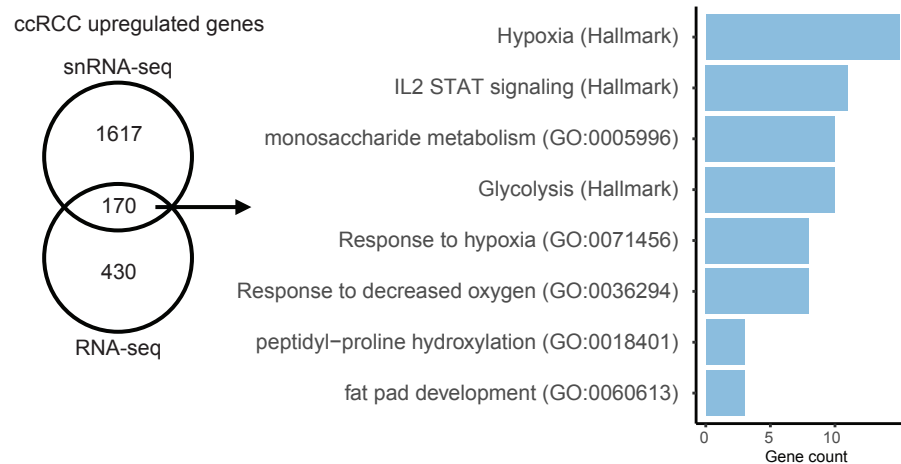
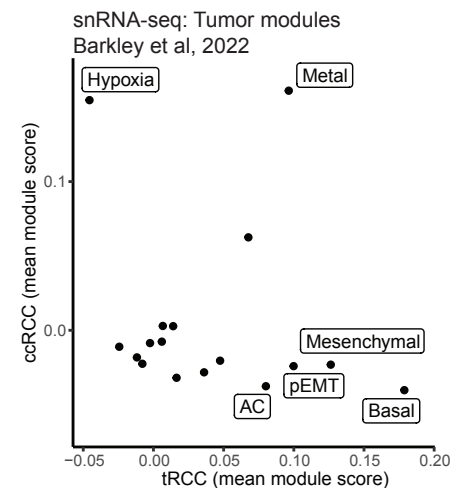
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Figure S2. Transcriptomic and immune landscape of tRCC and ccRCC

- A. Inferred copy number variants of malignant cells from the tRCC frozen cohort in this study. Reference myeloid and lymphoid cell populations in the upper panel; tRCC tumor cell observations in the lower panel, grouped by sample identity.
- B. Boxplot showing the $\log_2(M2/ M1)$ ratio from cibersortx absolute scores identified through bulk RNA-seq deconvolution in RCC subtypes. Data is represented as median with 25th and 75th percentiles defining the box limits. P values were calculated using two-sided Wilcoxon rank-sum tests.
- C. Boxplots showing the cibersortx absolute scores of macrophage cell types identified through bulk RNA-seq deconvolution in RCC subtypes. Data is represented as median with 25th and 75th percentiles defining the box limits. P values were calculated using two-sided Wilcoxon rank-sum tests.
- D. Boxplot showing the cibersortx absolute scores of CD8 T cells identified through bulk RNA-seq deconvolution in RCC subtypes. Data is represented as median with 25th and 75th percentiles defining the box limits. P values were calculated using two-sided Wilcoxon rank-sum tests.
- E. Venn diagram depicting genes upregulated in ccRCCs vs tRCC in snRNA-seq and bulk RNA-Seq datasets (left). Bar plot with top enriched pathways in ccRCCs based on gene-ontology (right).
- F. Scatterplot of module scores in tRCC vs ccRCC tumor cells from snRNA-seq data, based on a published study⁴⁶.

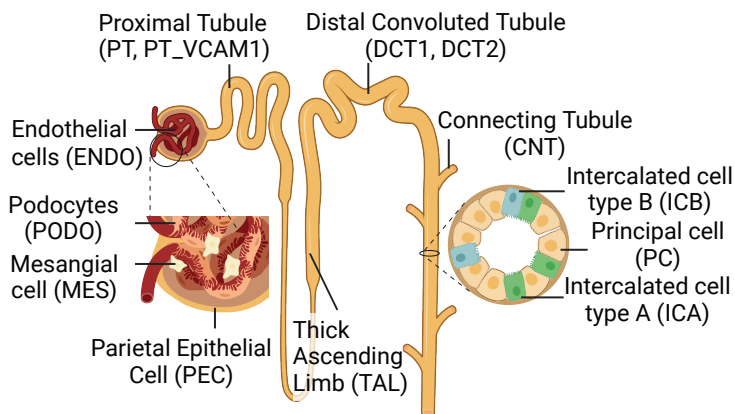
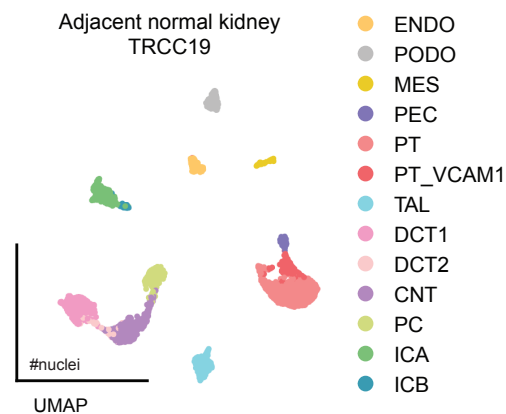
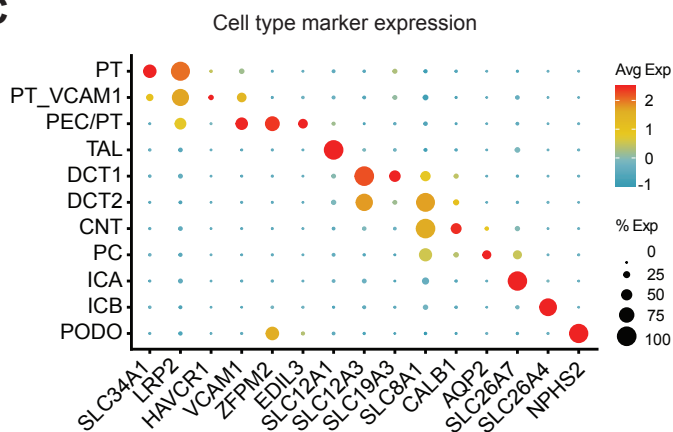
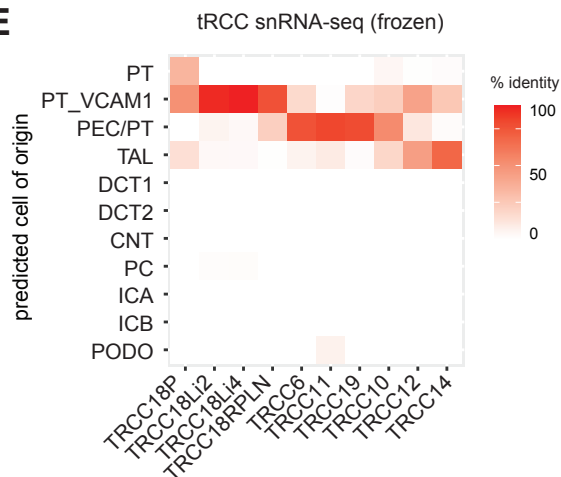
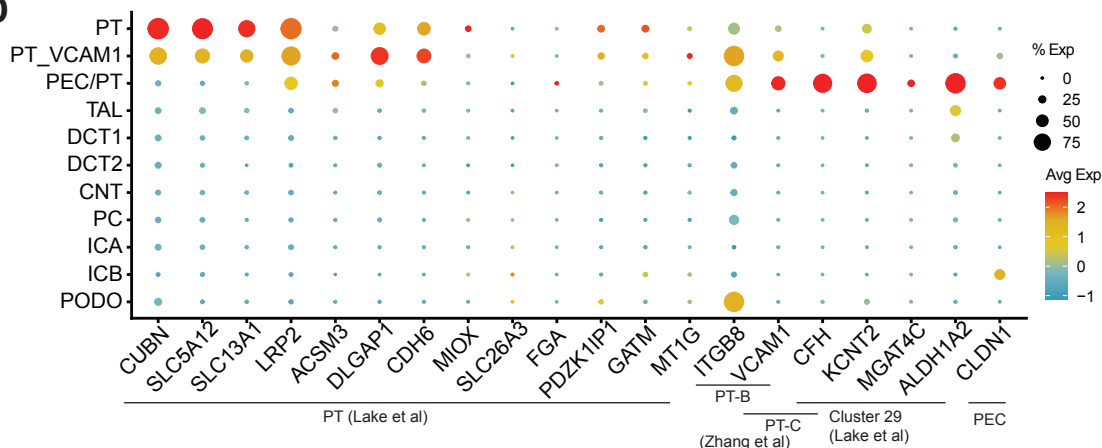
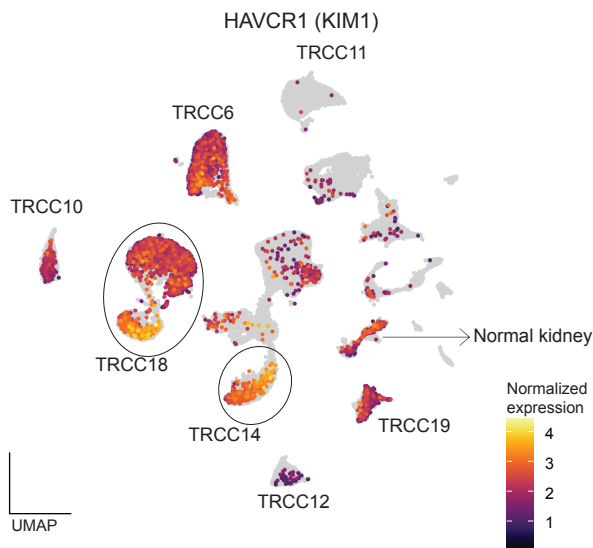
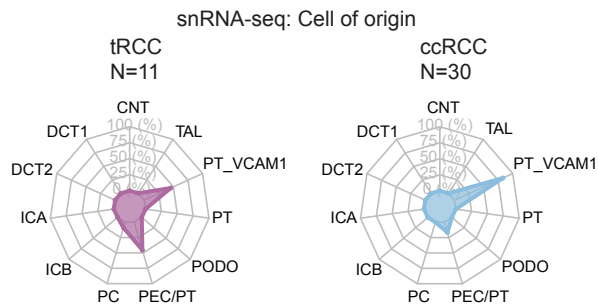
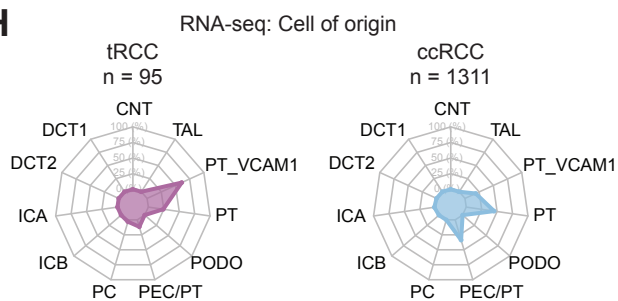
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Figure S3. tRCC and ccRCC tumors arise from a similar cell of origin

- A. Schematic of human nephron depicting different normal kidney cell types.
- B. UMAP from snRNA-seq of tumor-adjacent normal kidney sample, TRCC19N.
- C. Dot plot depicting the expression of marker genes in normal kidney cell types in TRCC19N.
- D. Dot plot depicting the expression of expanded marker gene panel in normal kidney cell types in TRCC19N, incorporating PT and cluster 29 markers from Lake et al, PT-B/PT-C markers from Zhang et al, and canonical PEC marker CLDN1.
- E. Heatmap depicting percent identity between tumor cells in individual tRCC samples and normal kidney cell types (tRCC snRNA-seq frozen cohort).
- F. UMAP showing expression of *HAVCR1* (KIM1) across tRCC tumor and non-tumor cells from the frozen cohort.
- G. Radar plots indicating the similarity of tRCC and ccRCC to different normal kidney cell types (snRNA-seq frozen cohorts).
- H. Radar plots indicating the similarity of samples from tRCC and ccRCC bulk RNA-seq cohorts to different normal kidney cell types.

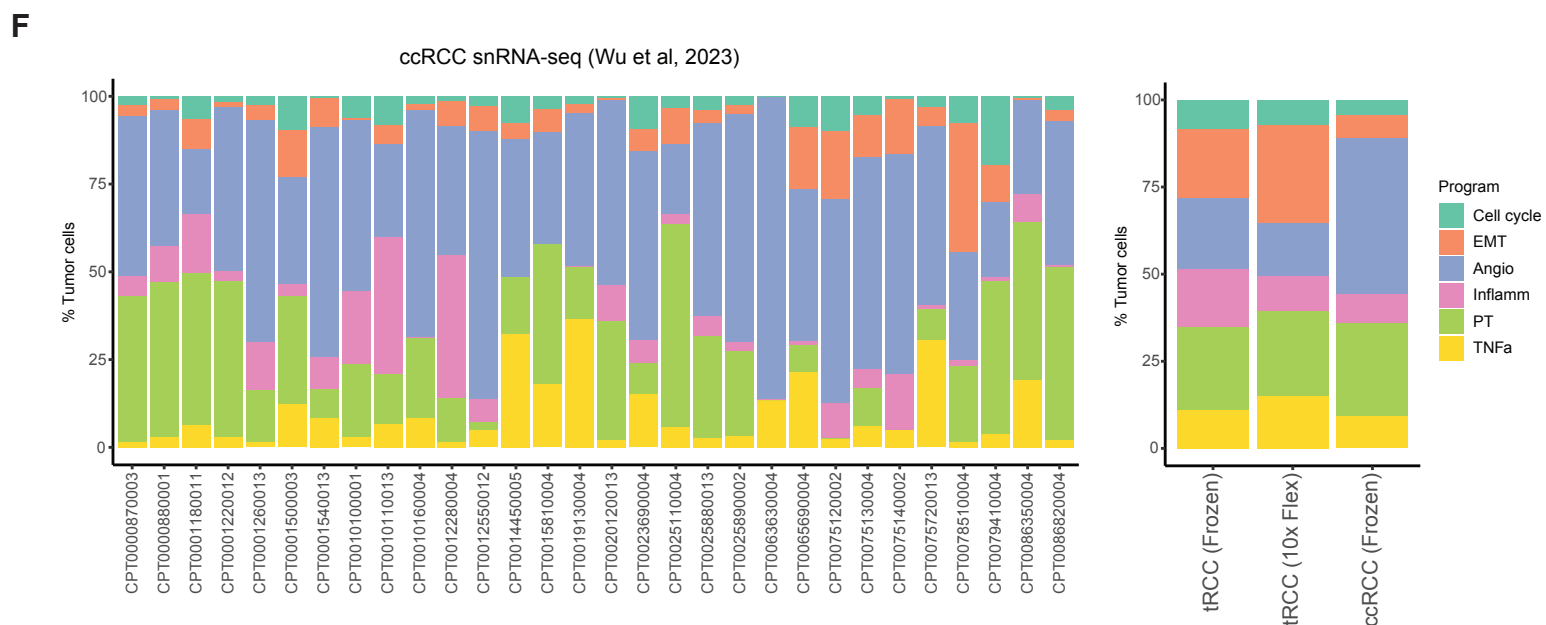
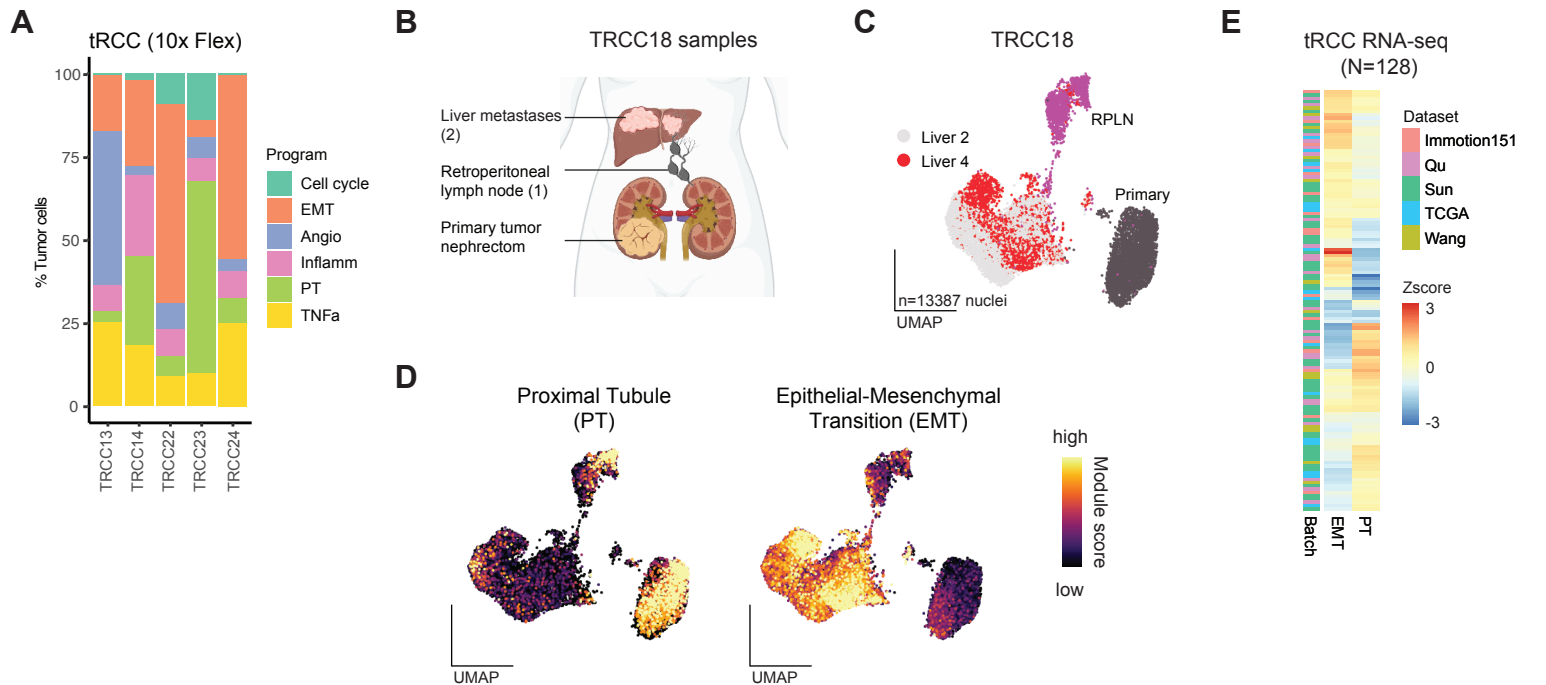


Figure S4. Characterization of tumor programs in tRCC and ccRCC

- A. Stacked bar plot representing the distribution of tRCC NMF programs across the tRCC 10x Flex cohort.
- B. Schematic of samples related to primary and metastatic sites from patient TRCC18.
- C. UMAP depicting distribution of cells from TRCC18 by biopsy site.
- D. UMAP depicting proximal tubule (PT) and epithelial-mesenchymal transition (EMT) program scores across primary and metastatic sites of TRCC18.
- E. Heatmap depicting distribution of EMT and PT program scores across tRCC samples from the bulk RNA-seq cohort, annotated by dataset.
- F. Stacked bar plot representing the distribution of identified NMF programs across ccRCC snRNA-seq cohort (left) and comparison of overall proportions between all tRCC and ccRCC cohorts (right).
- G.** Dot plot showing module scores of tumor NMF programs defined previously in a pan-cancer study by Gavish et al.⁴⁵ and association with tRCC NMF programs identified in this study.

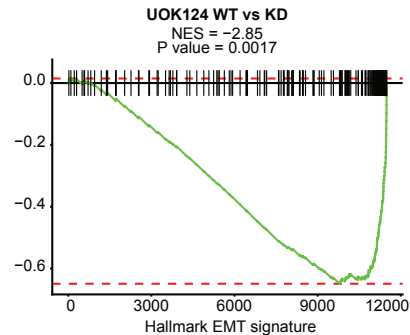
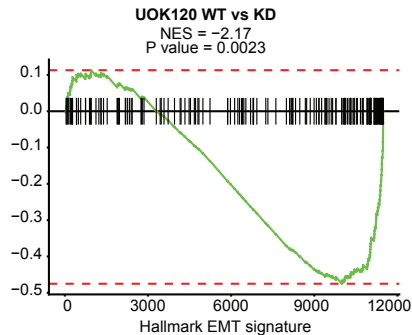
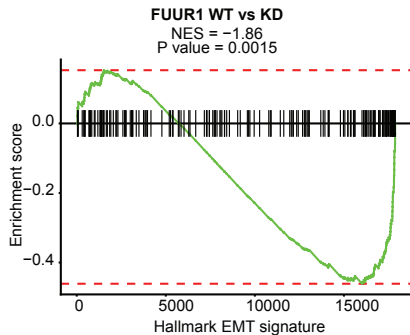
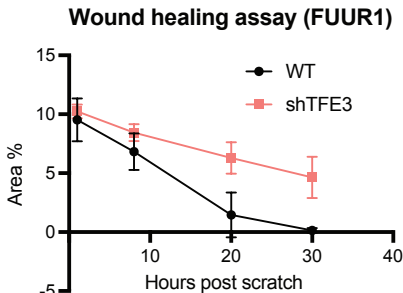
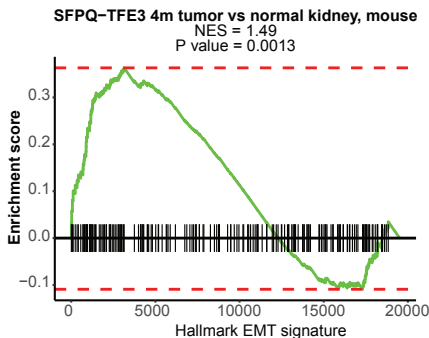
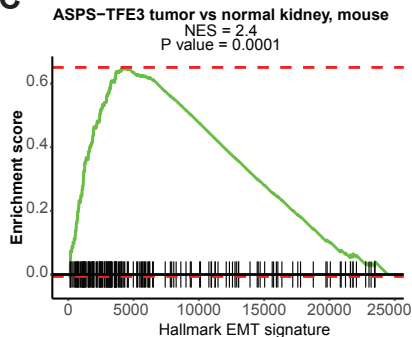
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Figure S5. Epithelial mesenchymal transition program in tRCC tumors

- A. Gene set enrichment analysis (GSEA) plots showing the Hallmark epithelial-mesenchymal transition (EMT) gene signature upon TFE3 fusion knockdown in tRCC cell lines harboring ASPSCR1-TFE3 (FUUR1; NES = -1.86, $P = 0.0015$), PRCC-TFE3 (UOK120; NES = -2.17, $P = 0.0023$), or PRCC-TFE3 (UOK124; NES = -2.85, $P = 0.0017$) fusions. WT, wild-type; KD, knockdown.
- B. Wound healing assay in FUUR1 cells expressing wild-type (WT) TFE3 fusion or TFE3 fusion knockdown (shTFE3), showing the percentage of open wound area remaining at 0, 6, 20, and 30 hours post-scratch. Data (N=6 replicates) represent mean $\pm$ SEM.
- C. GSEA plots showing enrichment of the Hallmark EMT gene signature in murine models of ASPSCR1-TFE3⁷⁶ (NES = 2.4, $P = 0.0001$) and SFPQ-TFE3⁷⁷ (NES = 1.49, $P = 0.0013$) tumors compared to normal kidney tissue.

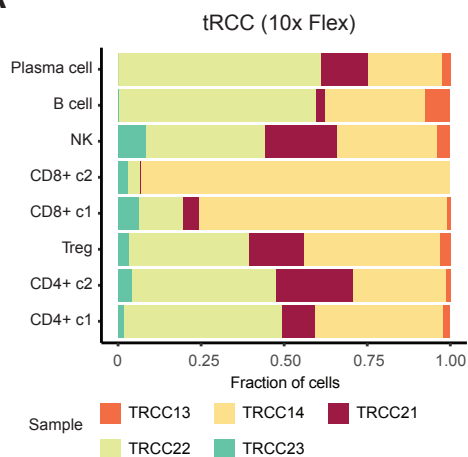
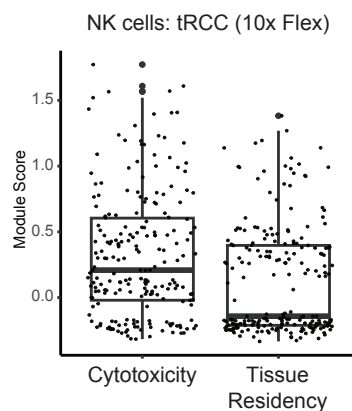
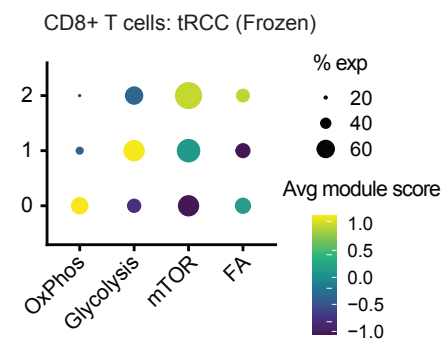
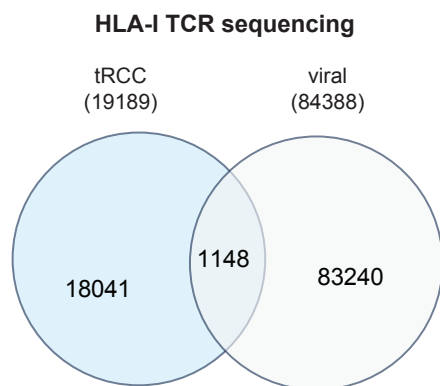
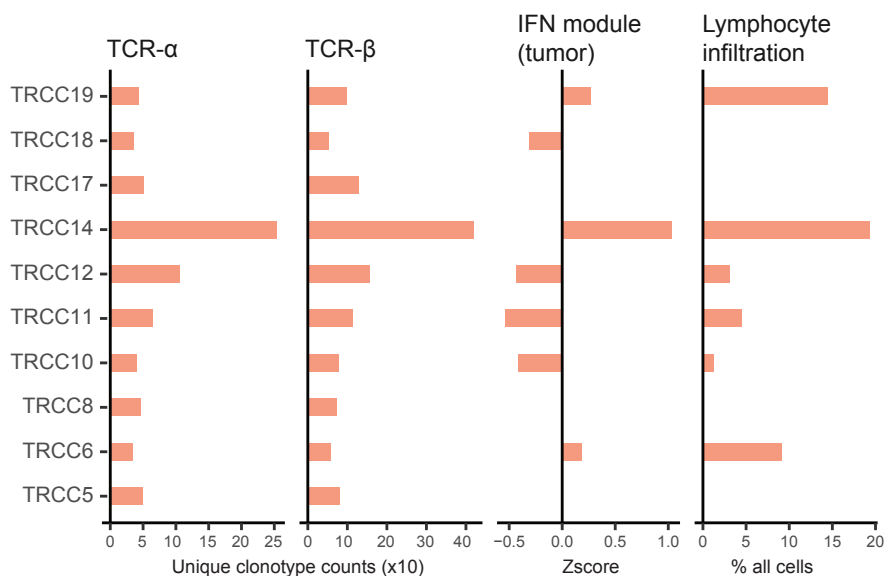
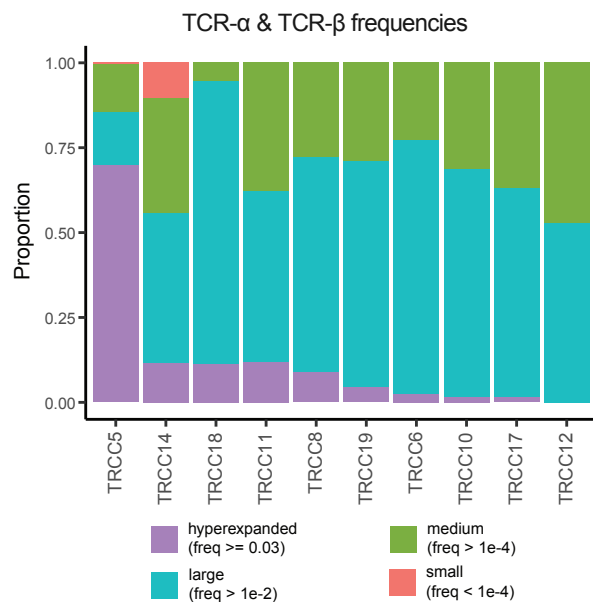
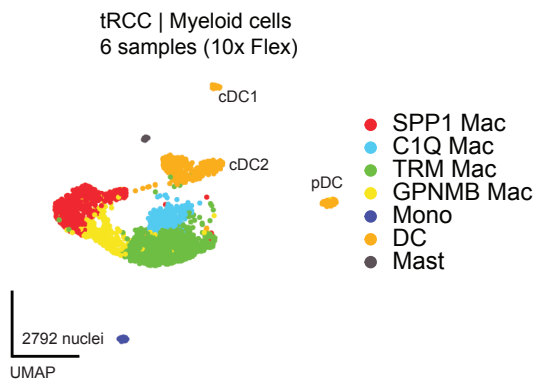
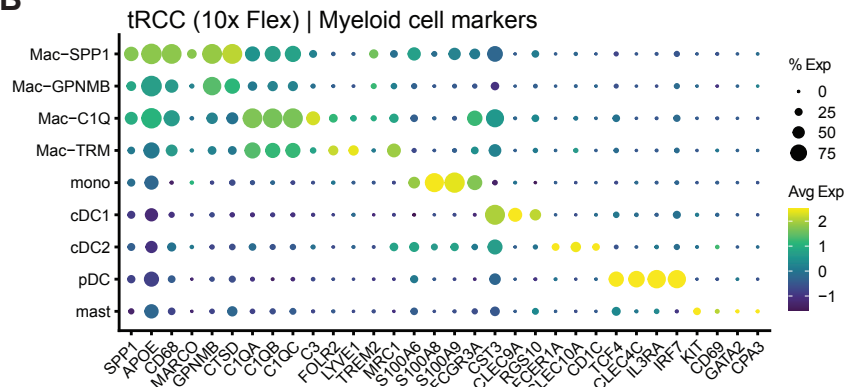
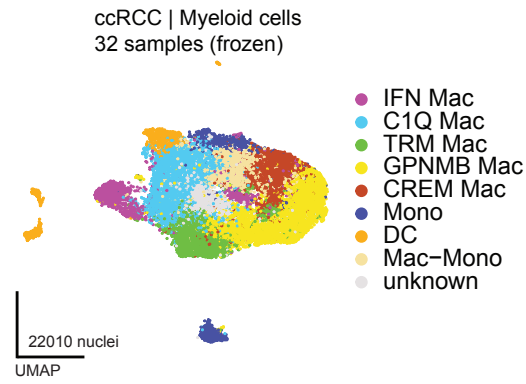
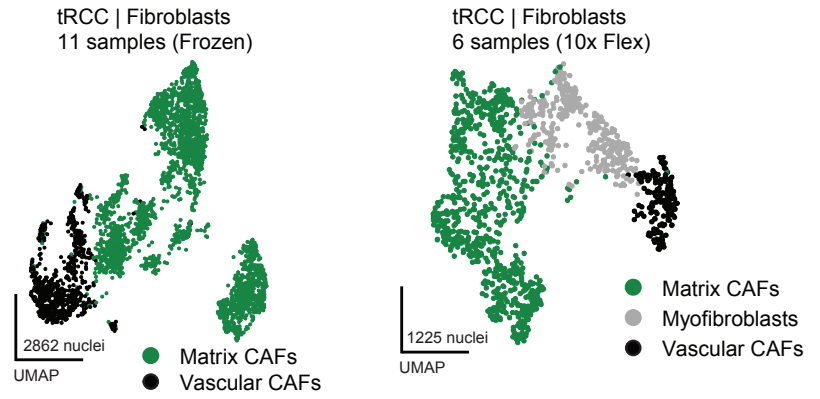
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Figure S6. Lymphocyte infiltration and T cell receptor (TCR) clonality in tRCC

- A. Stacked bar plot showing the composition of lymphocytes in tRCC 10x Flex cohort.
- B. Box plot of cytotoxicity and tissue residency module scores in NK cells from the tRCC 10x Flex cohort.
- C. Dot plot of metabolic pathway module scores in CD8 T cell subclusters from the tRCC frozen cohort.
- D. Venn plot showing intersection of TCR sequences from bulk-TCR α and TCR β sequencing of tRCC samples and publicly annotated viral TCR sequences.
- E. Bar plots of counts of unique TCR α and TCR β clonotypes identified in each tRCC sample, corresponding interferon (IFN) module scores in tumor cells, and percentage lymphocyte infiltration from snRNA-seq data.
- F. Proportions of TCR clonotypes in each tRCC sample based on their abundance: hyperexpanded, large, medium, and small. Proportions reflect values after removing overlapping viral sequences from D.

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		Significant interactions per direction	
		ccRCC	tRCC
Tumor →	Macrophages	45	83
	Fibroblast	112	181
	Lymphoid	30	76
Macrophages →	Tumor	61	75
	Fibroblast	60	74
	Lymphoid	16	30
Fibroblast →	Tumor	120	170
	Macrophages	31	75
	Lymphoid	17	65
Lymphoid →	Tumor	28	50
	Macrophages	17	31
	Fibroblast	25	56

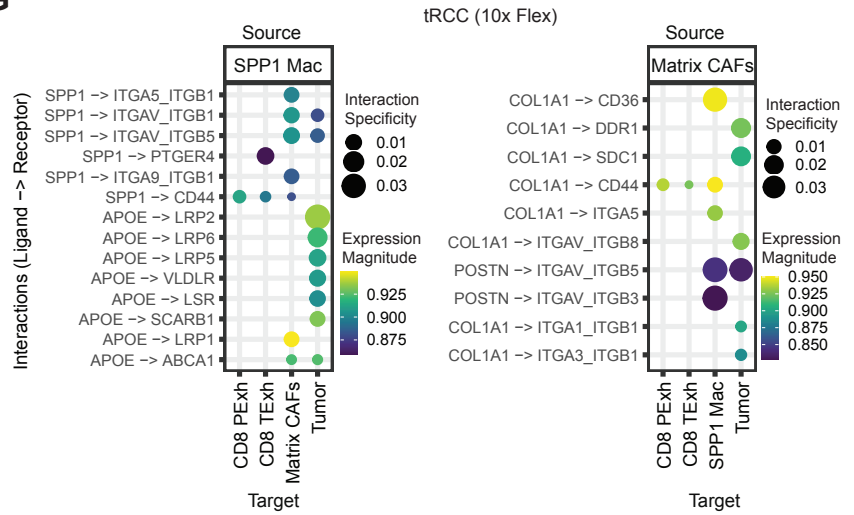
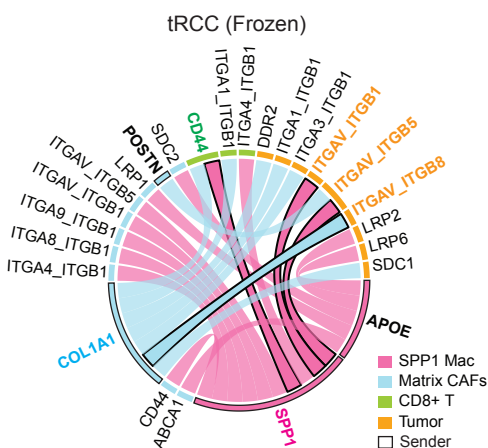
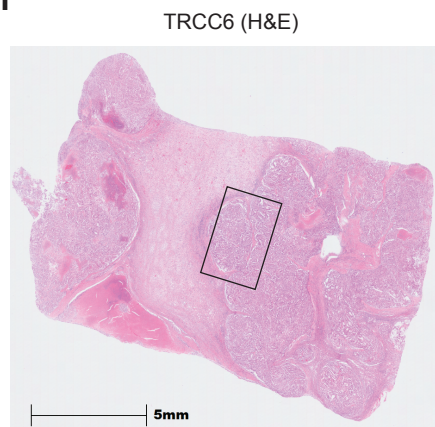
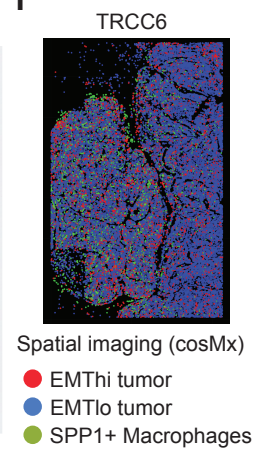
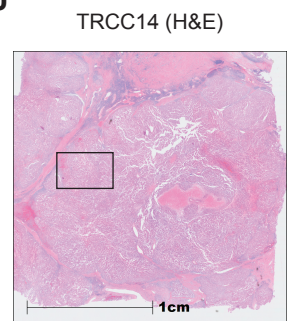
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Figure S7. Suppressive myeloid cells and fibroblasts in the tRCC TME

- A. UMAP of myeloid cell subtypes in the tRCC (10x Flex) cohort.
- B. Dot plot showing expression of marker genes in myeloid cell types in the tRCC 10x Flex cohort from A.
- C. UMAP depicting myeloid cell subtypes identified in the ccRCC (frozen) cohort.
- D. UMAPs depicting fibroblast subtypes identified in the tRCC frozen (left) & 10X flex (right) cohorts.
- E. Heatmap depicting significant interactions between different cell types in tRCC and ccRCC frozen cohorts.
- F. Chord diagram showing interactions of SPP1 macrophages and Matrix CAFs (senders) with tumor cells and CD8 T cells in the tRCC (frozen) cohort.
- G. Dot plots highlighting interaction specificity and expression magnitude of interactions of SPP1 macrophages and Matrix CAFs (senders) with tumor cells and CD8 progenitor exhausted and terminal exhausted T cells in the tRCC (10X flex) cohort.
- H. H&E staining of the full slide of sample TRCC6. The section highlighted indicates the field of view utilized for spatial imaging.
- I. Spatial image plot from sample TRCC6 highlighting distribution of EMT-high tumor cells, EMT-low tumor cells, and SPP1+ macrophages.
- J. H&E staining of full slide of sample TRCC14. The section highlighted indicates the field of view utilized for spatial imaging.