

Spatial single-cell landscape of tumor-associated macrophages and their crosstalk with the tumor microenvironment

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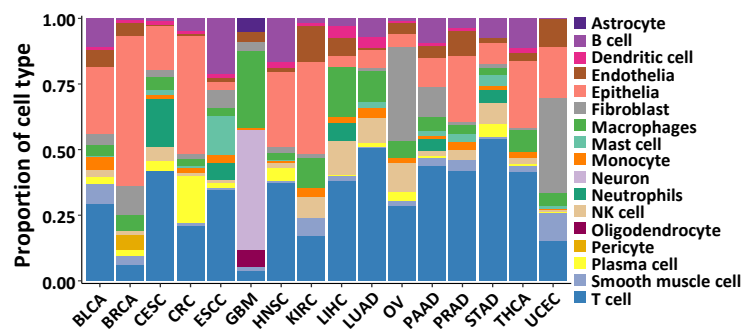
These authors contributed equally

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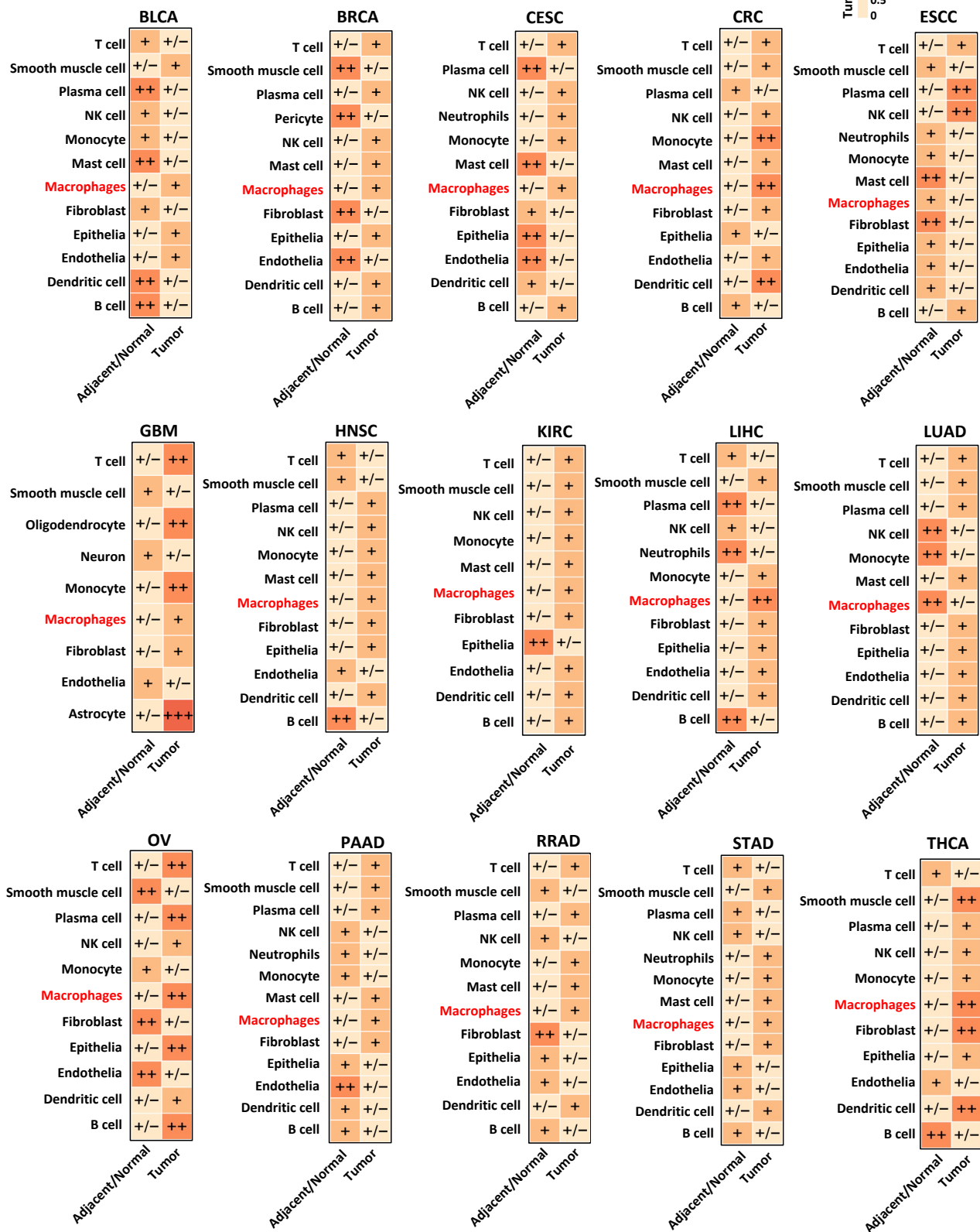
Supplementary Fig. S1. scRNA-seq preprocessing and single-cell subtype definition, related to Fig. 1.

a UMAP plot illustrating the integration of samples across different cancer types, with colors representing distinct samples. **b** Boxplot displaying the batch effect levels assessed using the LISI index, where a mean value greater than 1 indicates minimal batch effects ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$). **c** UMAP plot demonstrating the major cell types identified in the example dataset across different cancer types, with colors representing distinct cell types.

a

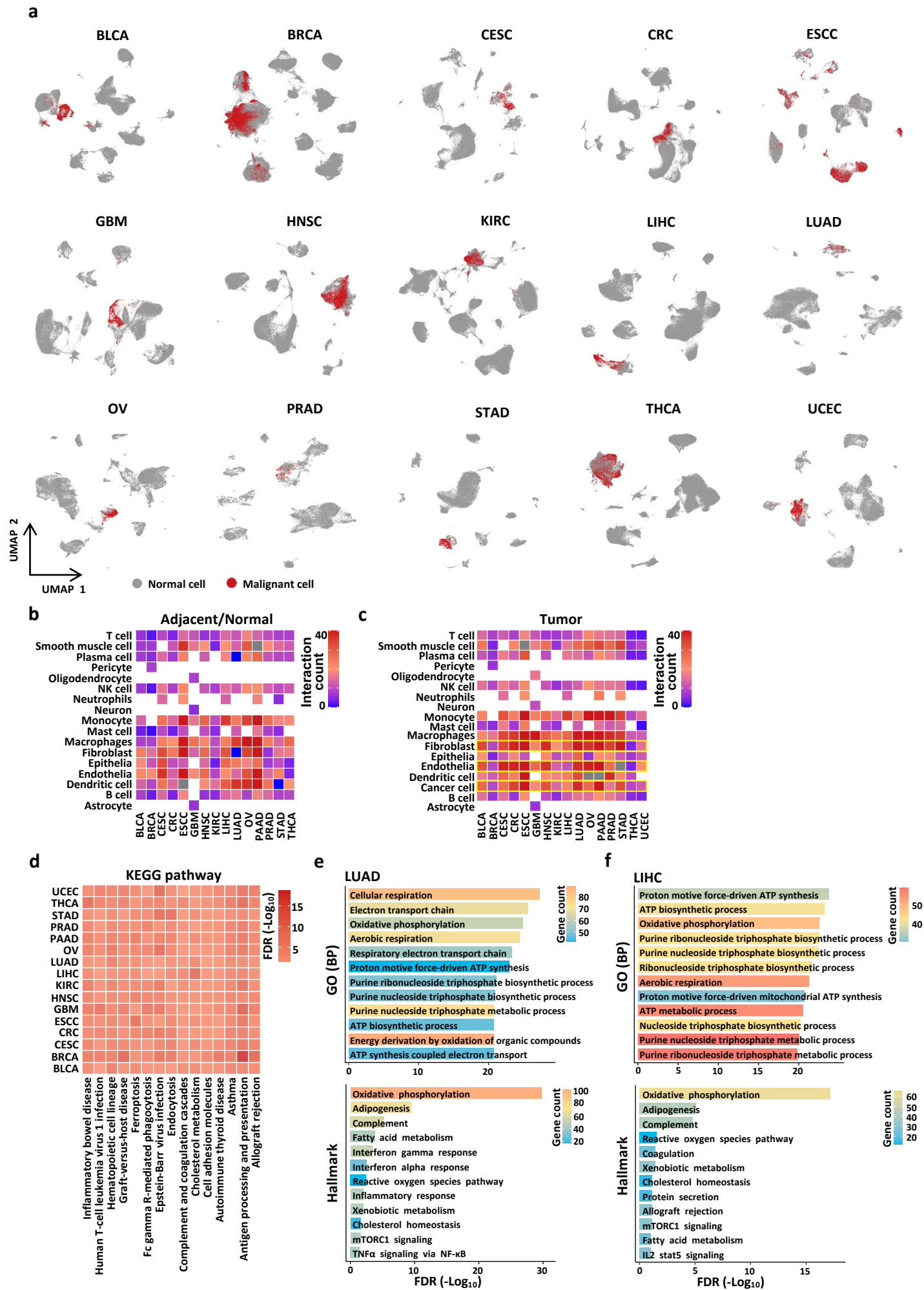


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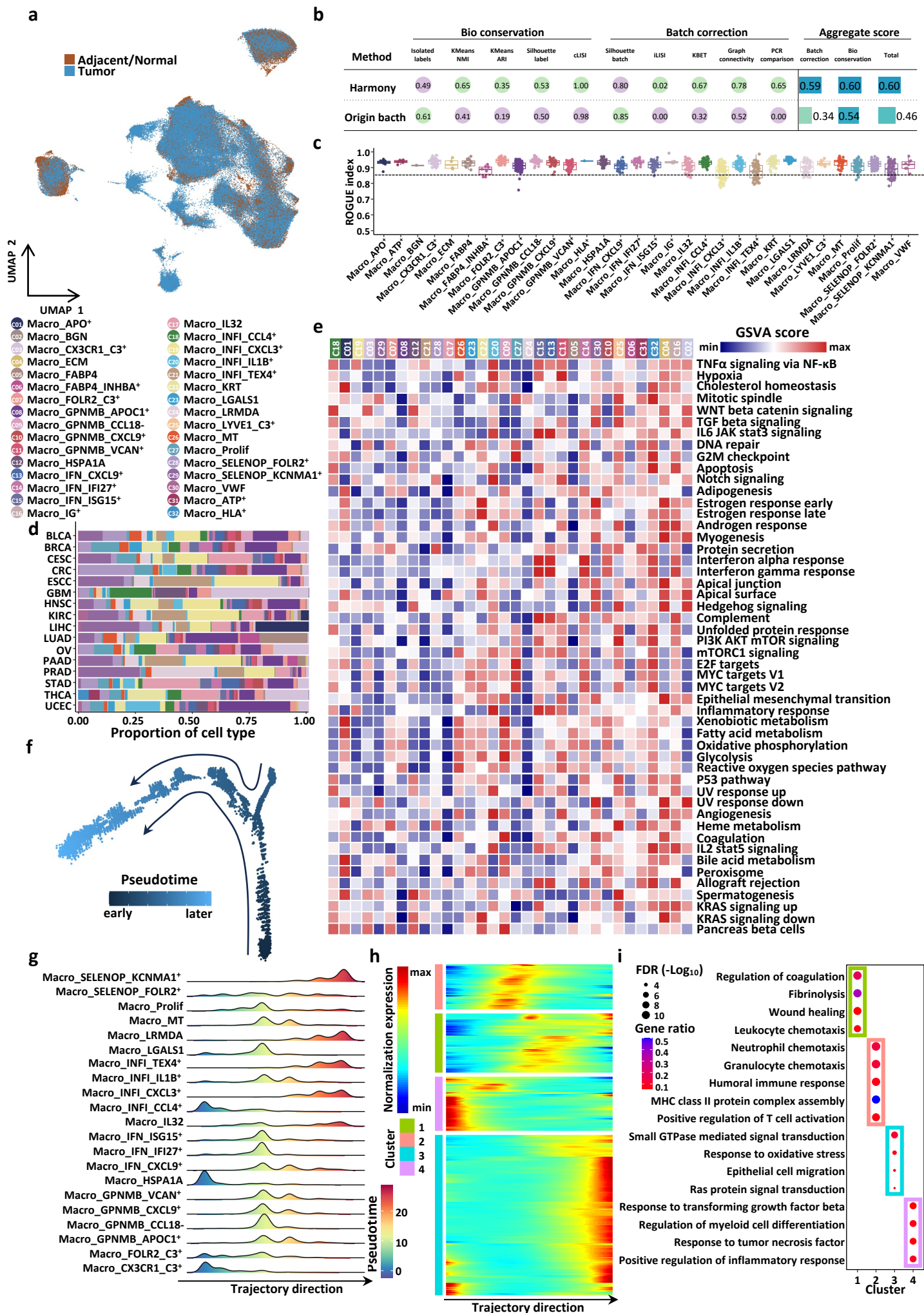
Supplementary Fig. S2. Pan-cancer cell type proportions and preference analysis, related to Fig. 1.

a Bar plot illustrating the proportion of different cell types across various cancer types. **b** Heatmap showing the tissue preference of different cell types across various cancer types, where values greater than 1 indicate significant tissue preference.



Supplementary Fig. S3. Malignant cell identification and macrophage signature analysis, related to Fig. 1.

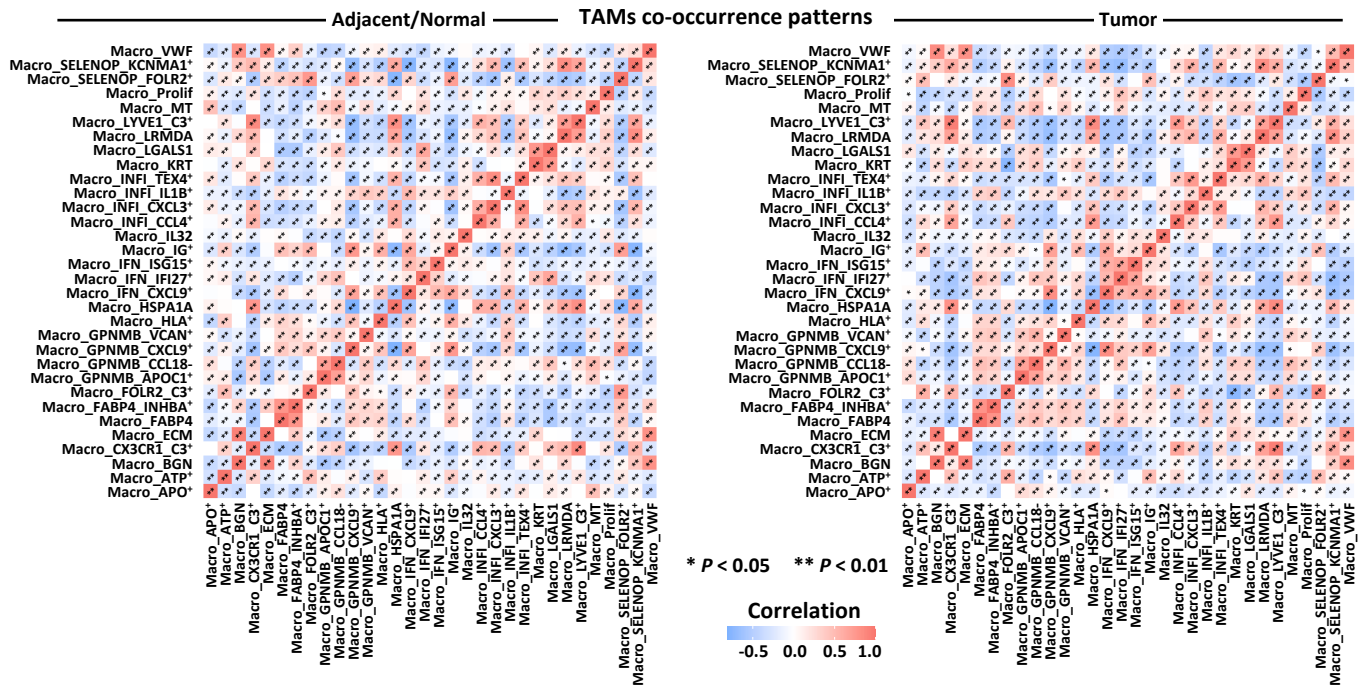
a UMAP plot displaying malignant cells inferred using CopyKAT, where red represents cells with malignant features and gray represents cells without malignant tendencies. **b, c** Heatmap shows the interaction strength between macrophages and other microenvironmental cell types (y-axis) across different cancer types (x-axis) under adjacent or normal (b) and tumor tissue conditions (c), inferred using CellChat. **d** Enrichment analysis of macrophages across different cancer types using clusterProfiler, with pathways sourced from KEGG. **e, f** Separate enrichment analysis of macrophages in LUAD (e) and LIHC (f) using clusterProfiler, with pathways sourced from Gene Ontology (GO) and Hallmark.



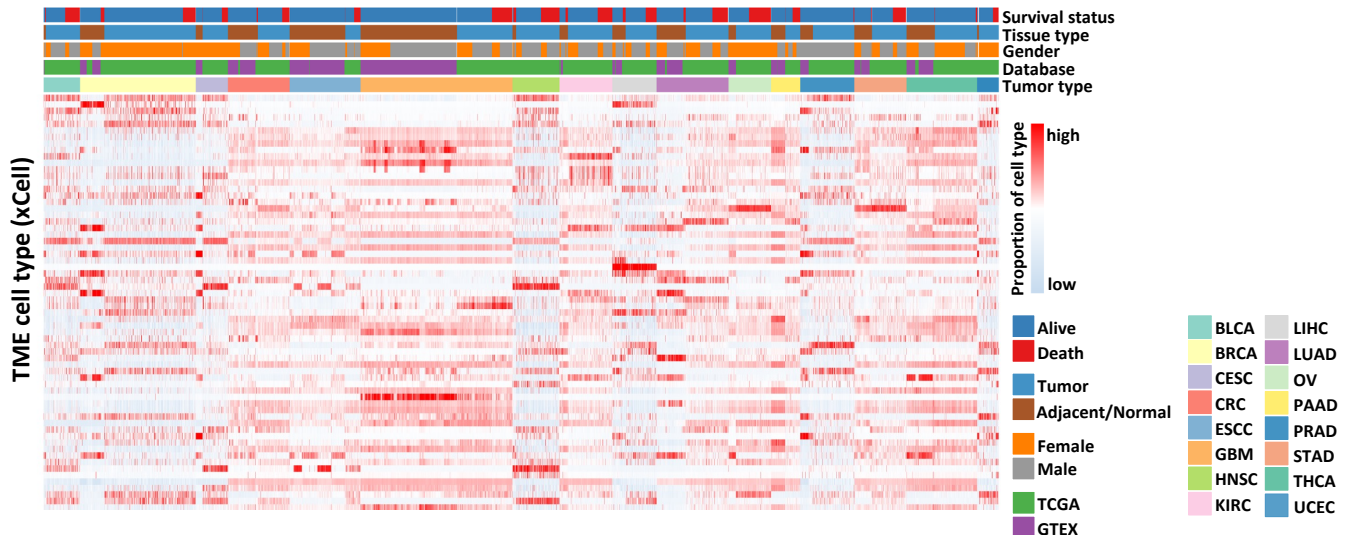
Supplementary Fig. S4. Characterization of TAM features and biological functions at a pan-cancer level, related to Fig. 2.

a UMAP plot showing the mixing level of cells from tumor tissues and normal or adjacent tissues within the myeloid cell population. Blue represents cells from tumor tissues, and brown represents cells from adjacent normal or healthy tissues. **b** Use scIB framework to evaluate the effect of cross-tissue data integration before and after Harmony batch correction. **c** Boxplots showing cell purity for each TAM subset across samples by ROGUE index. **d** Bar plot showing the proportion of different TAM subtypes across various cancer types. **e** Heatmap showing the association between different TAM subtypes and Hallmark pathways inferred using the ssGSEA algorithm. **f** UMAP plot displaying the developmental trajectory of macrophages inferred using Monocle. **g** Ridge plot showing the abundance of pan-cancer subtypes at different time points along the developmental trajectory. **h** Heatmap showing gene expression changes along the pseudotime trajectory, with GO and ridge plots. **i** Bubble plot displaying the functional enrichment analysis results for different clusters along the developmental trajectory.

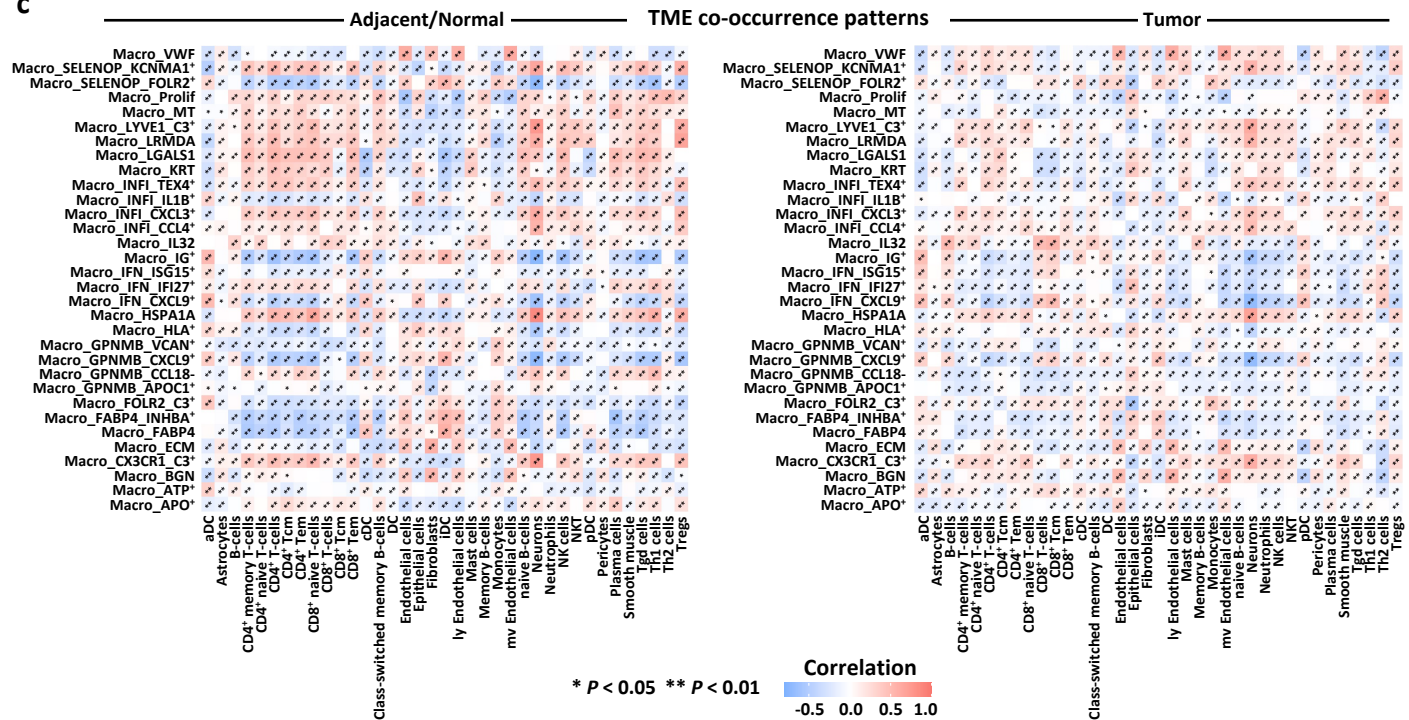
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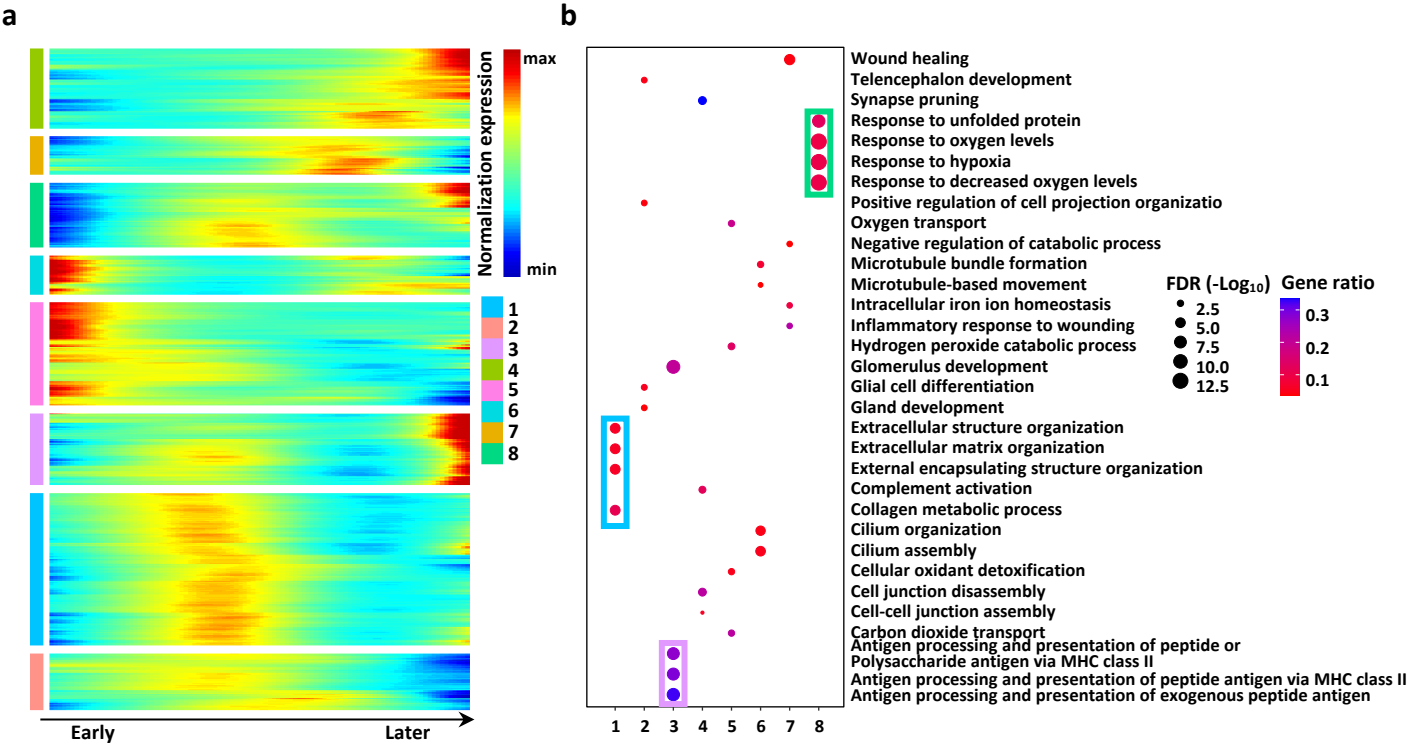


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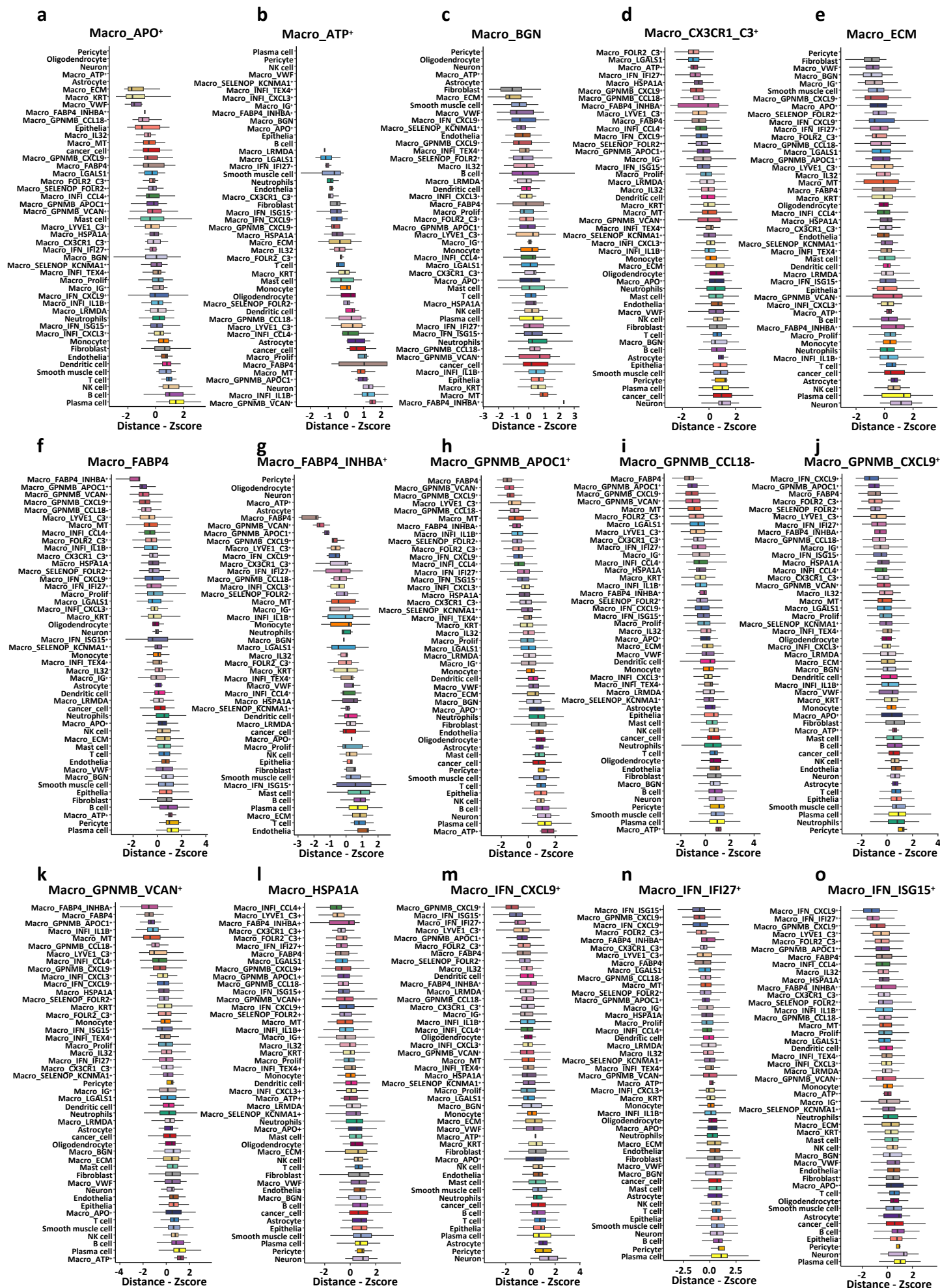
Supplementary Fig. S5. Inference of cell type proportions in bulk RNA-seq using xCell, related to Fig. 3.

a Pearson correlation analysis of the co-occurrence patterns of different TAM subtypes in normal or adjacent tissues (left) and tumor tissues (right) (* $P < 0.05$, ** $P < 0.01$). **b** Heatmap displaying the proportions of different TME components in TCGA and GTEx bulk RNA-seq samples inferred using the xCell algorithm. The left side represents all cell types included in xCell (Supplementary Table S9). **c** Pearson correlation analysis of the co-occurrence patterns between different hub TAM subtypes and TME components (inferred by Xcell) in normal or adjacent tissues (left) and tumor tissues (right) (* $P < 0.05$, ** $P < 0.01$).



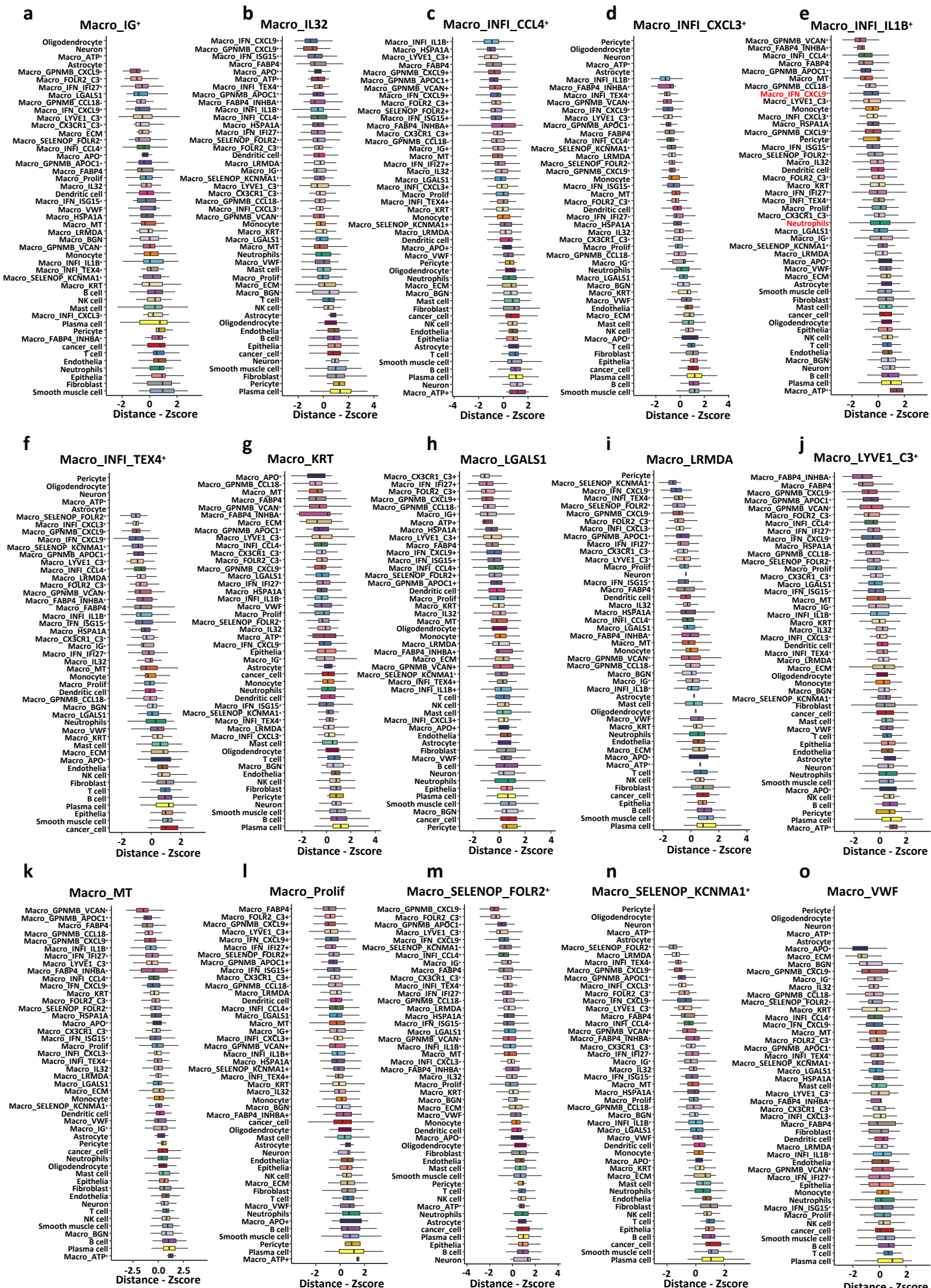
Supplementary Fig. S6. Developmental features of TAMs and activation of specific biological functions at the spatial level, related to Fig. 4.

a Heatmap showing gene expression across different clusters along the developmental trajectory axis (left to right: late development). **b** Bubble plot displaying GO enrichment analysis for different clusters along the developmental trajectory axis (clusters 1, 3 and 8 are primarily concentrated in late development).



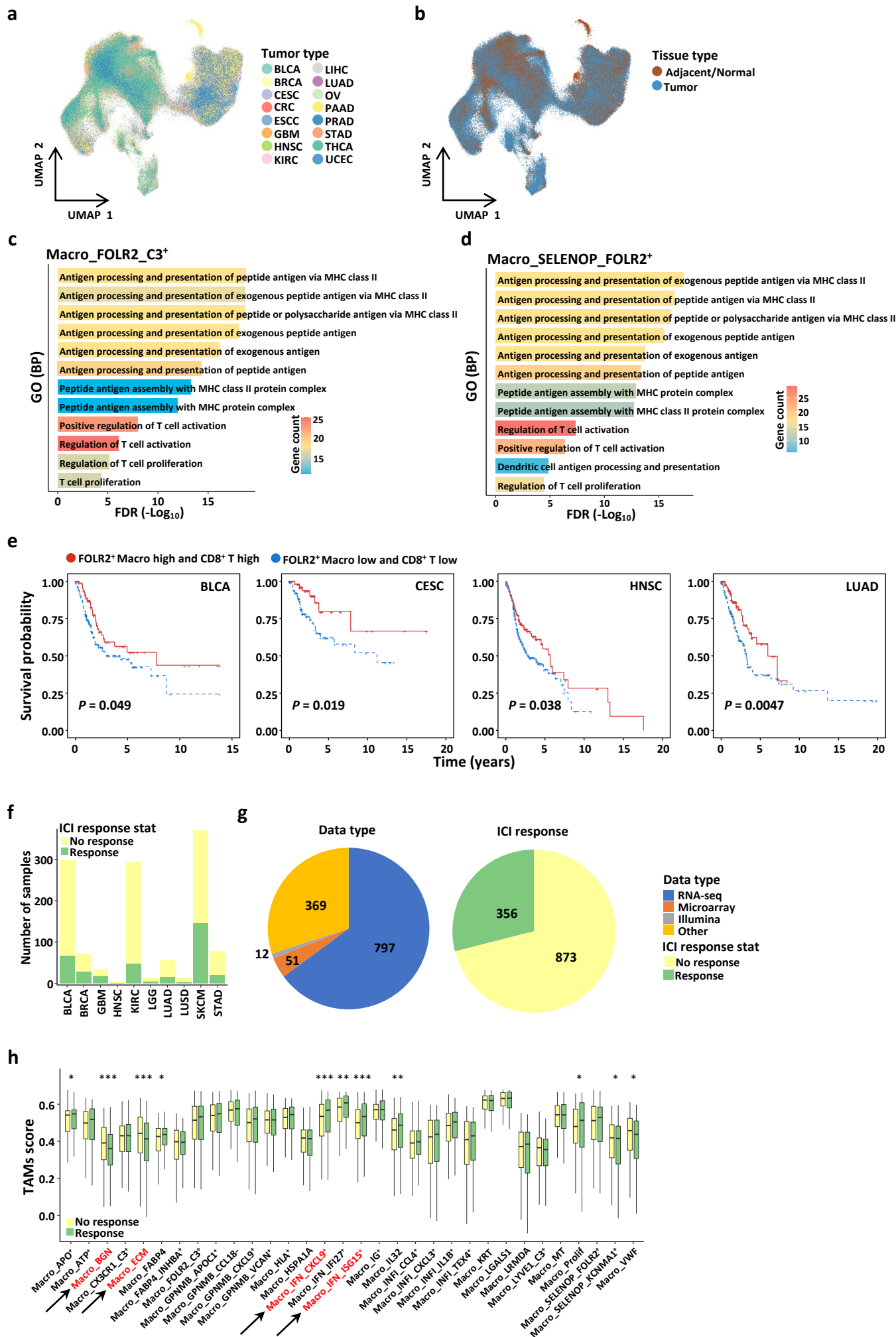
Supplementary Fig. S7. Spatial relationships between TAM subtypes and other cell types at a pan-cancer level, related to Fig. 4.

a-o Boxplots showing the average spatial distances between different TAM subtypes and other TME components, with spatial distances sorted in descending order based on mean distance.



Supplementary Fig. S8. Spatial relationships between TAM subtypes and other cell types at a pan-cancer level, related to Fig. 4.

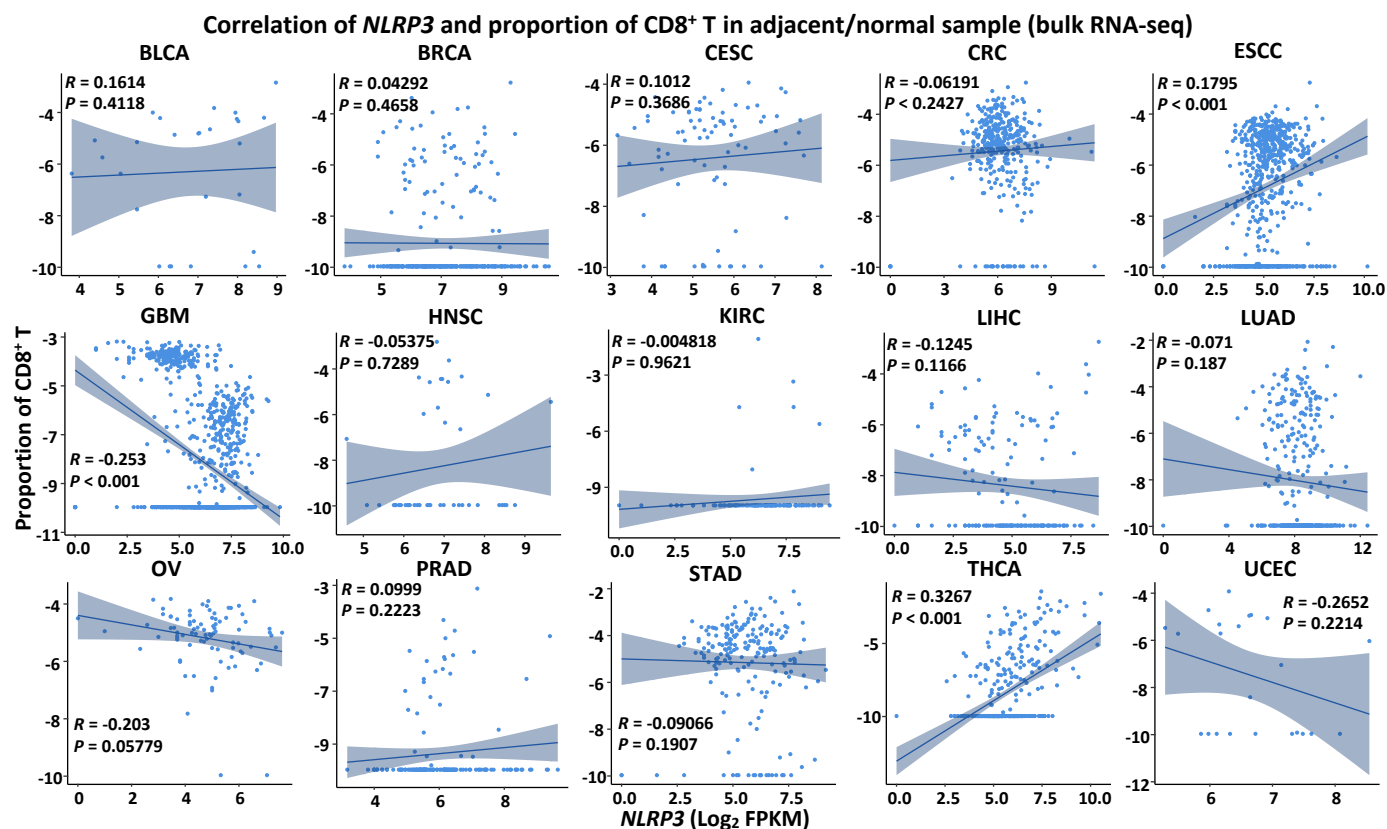
a-o Boxplots showing the average spatial distances between different TAM subtypes and other TME components, with spatial distances sorted in descending order based on mean distance.



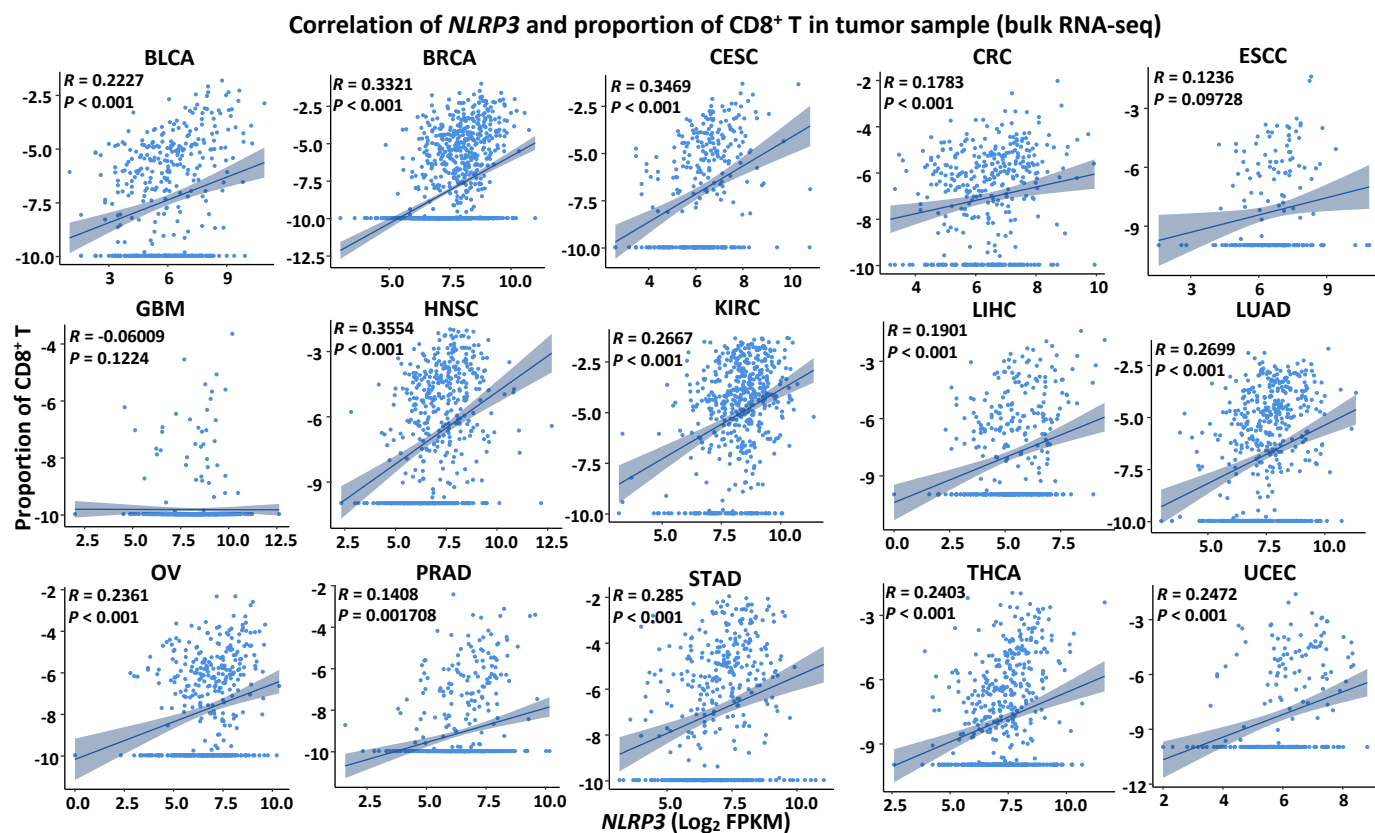
Supplementary Fig. S9. Potential interactions between TAMs and CD8⁺ T cells and association with immunotherapy, related to Fig. 5.

a UMAP plot showing the mixing level of different cancer types within T cells. **b** UMAP plot showing the mixing of tumor tissues and normal or adjacent tissues within T cells. **c, d** Bar plots displaying the functional enrichment analysis results for Macro_FOLR2_C3⁺ and Macro_SELENOP_FOLR2⁺. **e** Kaplan-Meier curves showing the prognostic value of high abundance of FLOR3⁺ Macro and CD8⁺ T cells in immunotherapy cohorts, with *P*-values calculated using the log-rank test. **f** Bar plot displaying the number of patients receiving ICI treatment. **g** Left: Pie chart showing the data sources of patients receiving ICI treatment; Right: Pie chart showing the number of responders and non-responders to ICI treatment, with yellow representing non-responders and green representing responders. **h** Boxplots showing the abundance of different TAM subtypes in immunotherapy cohorts, with yellow representing non-responders and green representing responders (**P* < 0.05, ***P* < 0.01, ****P* < 0.001).

a

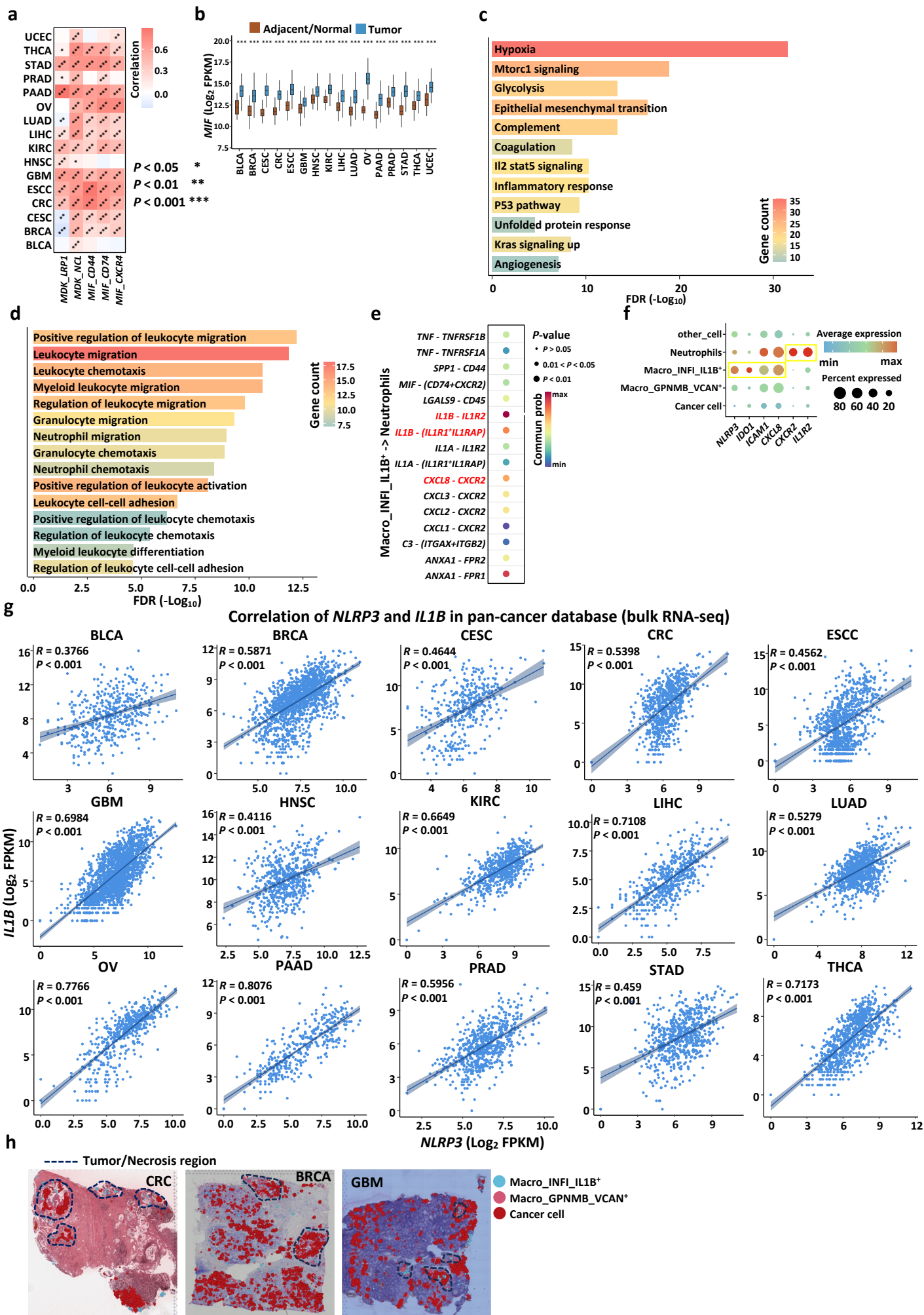


b



Supplementary Fig. S10. Correlation between inflammasome (*NLRP3*) and CD8⁺ T cell abundance, related to Fig. 5.

a Correlation between CD8⁺ T cell abundance and *NLRP3* expression in normal or adjacent tissues. **b** Correlation between CD8⁺ T cell abundance and *NLRP3* expression in tumor tissues.

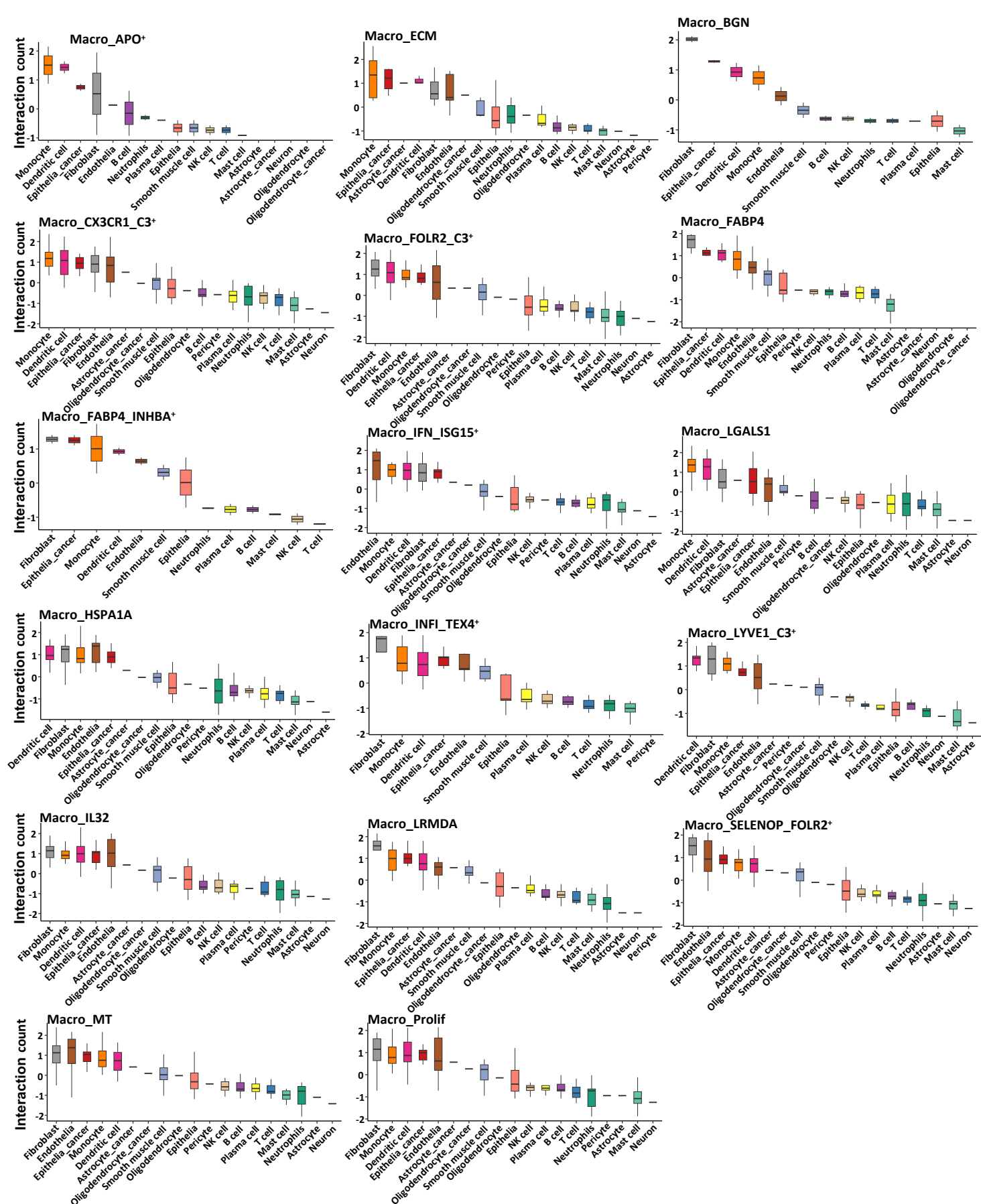


Supplementary Fig. S11. Interaction features between TAMs localized in the tumor core and cancer cells, related to Fig. 6.

a Heatmap showing the correlation between the expression of *MDK* and *MIF* ligands and their corresponding receptor genes ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$). **b** Boxplot showing the differential expression of *MIF* at a pan-cancer level ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$). **c** Hallmark enrichment analysis results for signature genes of Macro_GPNMB_VCAN⁺. **d** GO enrichment analysis results for signature genes of Macro_INFI_IL1B⁺. **e** Bubble plot showing the interaction ligands and receptors between Macro_INFI_IL1B⁺ and neutrophils. **f** Bubble plot displaying the expression of key signature genes (*NLRP3*, *IDO1*, and *ICAM1*) and ligand-receptor pairs (*CXCL8*, *CXCR2*, and *IL1R2*) in Macro_INFI_IL1B⁺ and neutrophils. **g** Correlation between *NLRP3* and *IL1B* gene expression at a pan-cancer level. **h** Annotation of necrotic regions on spatial slices, with red dashed lines marking the regions, the purple dashed area represents the tumor necrosis region.

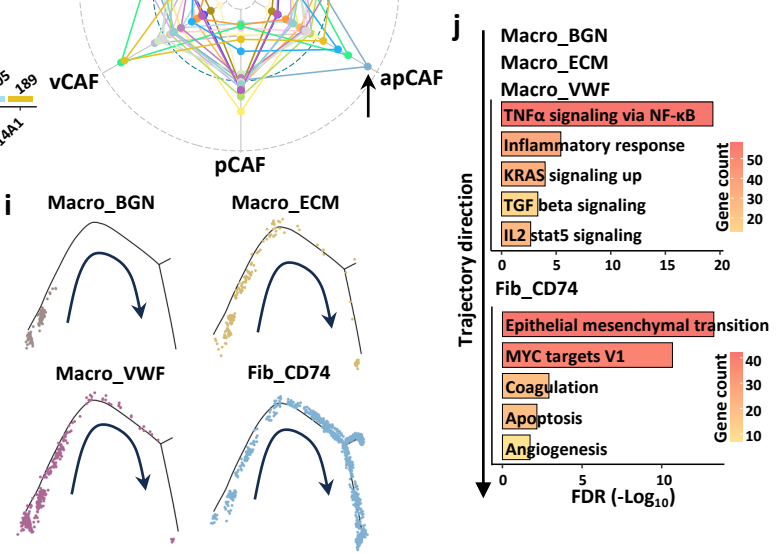
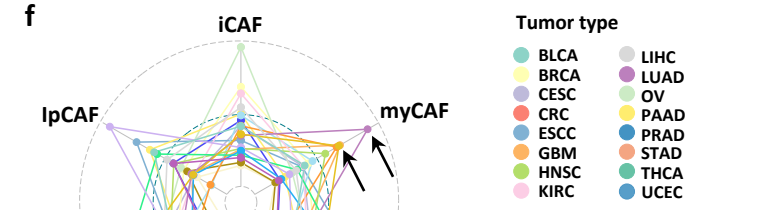
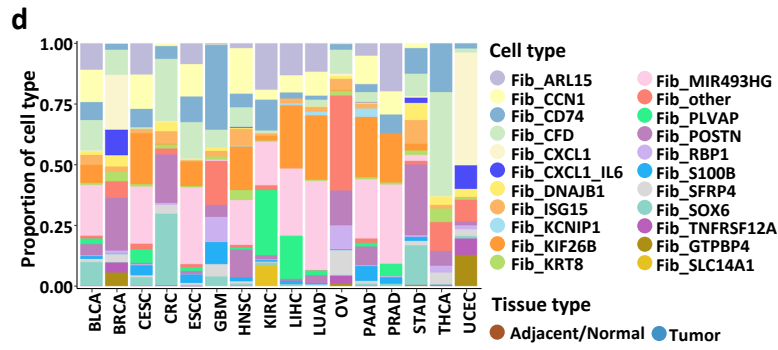
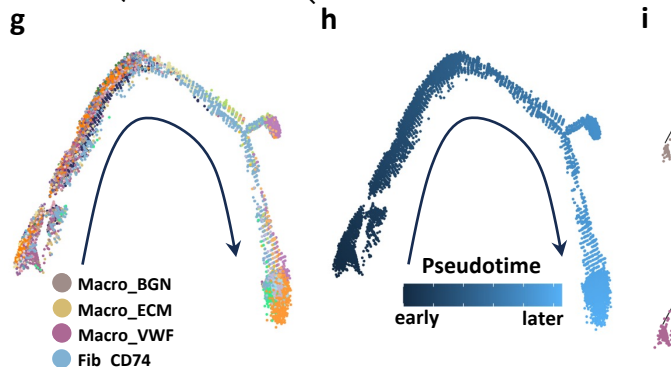
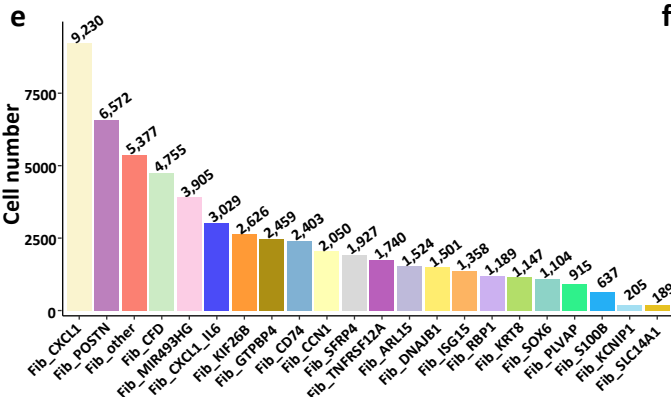
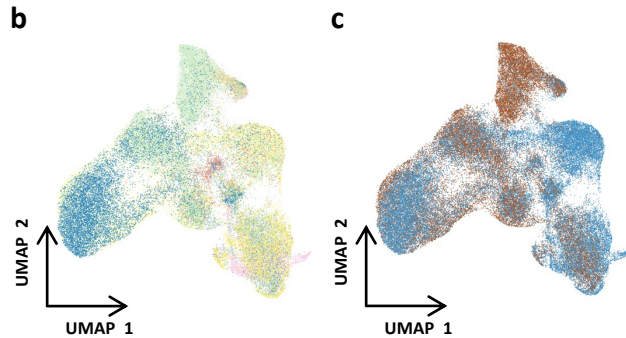
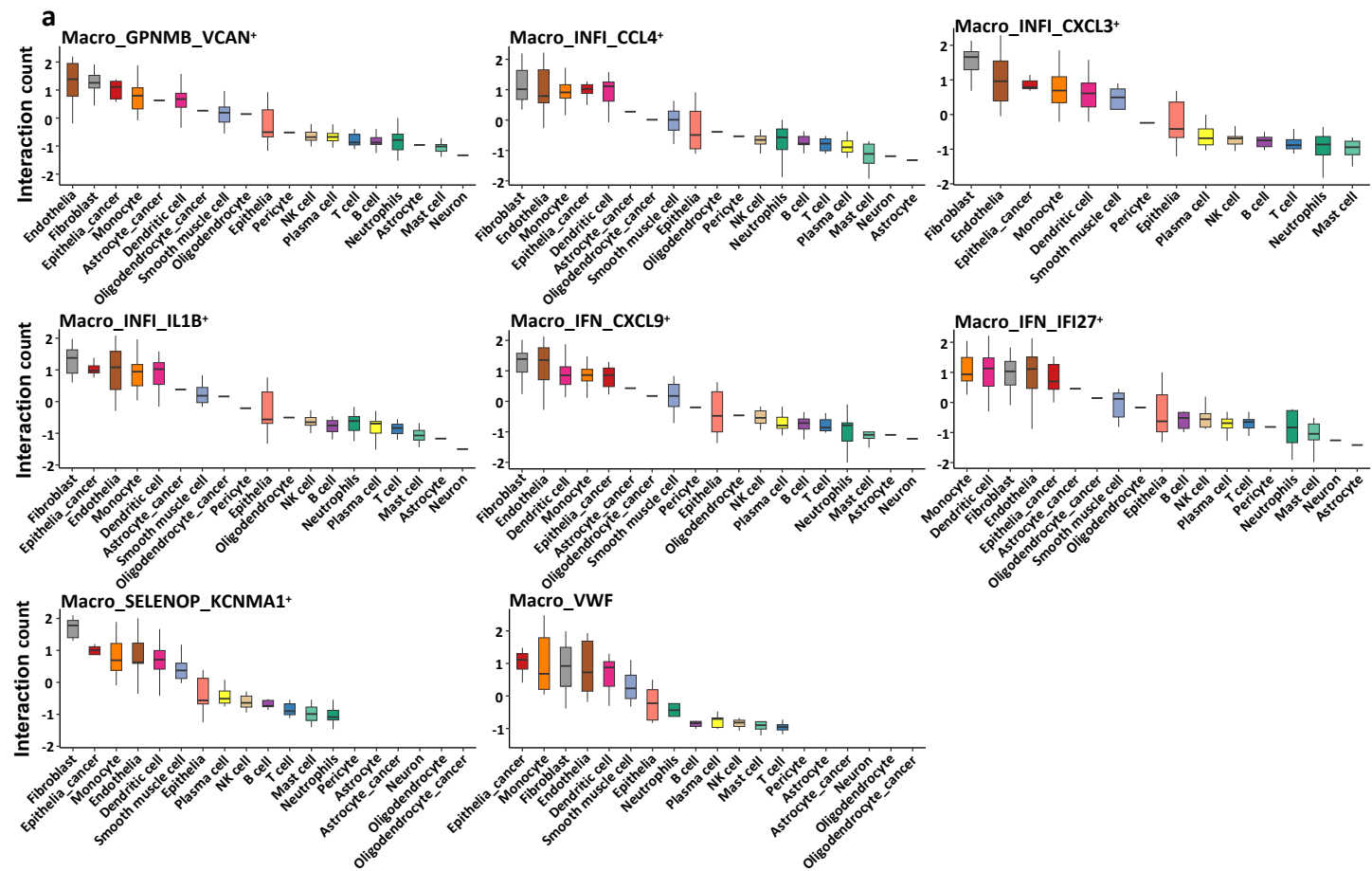


Supplementary Fig. S12. Involvement of TAMs in metabolic pathway activity, related to Fig. 6. Bubble plot displaying the metabolic pathway activity of different TAM subtypes analyzed using scMetabolism.



Supplementary Fig. S13. Interaction strength between TAM subtypes and TME components, related to Fig. 7.

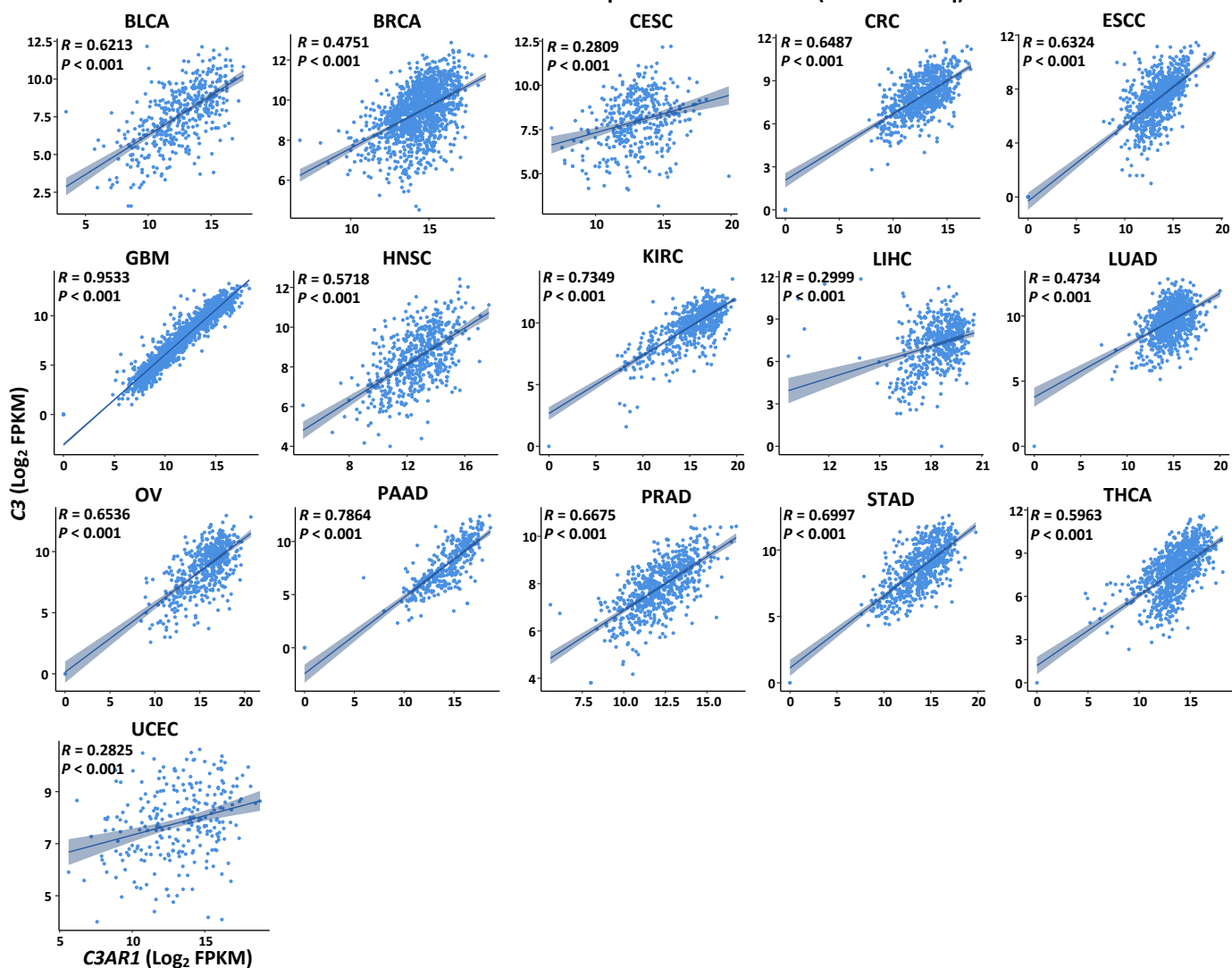
Boxplot showing the interaction strength between different TAM subtypes and other TME components inferred using CellChat.



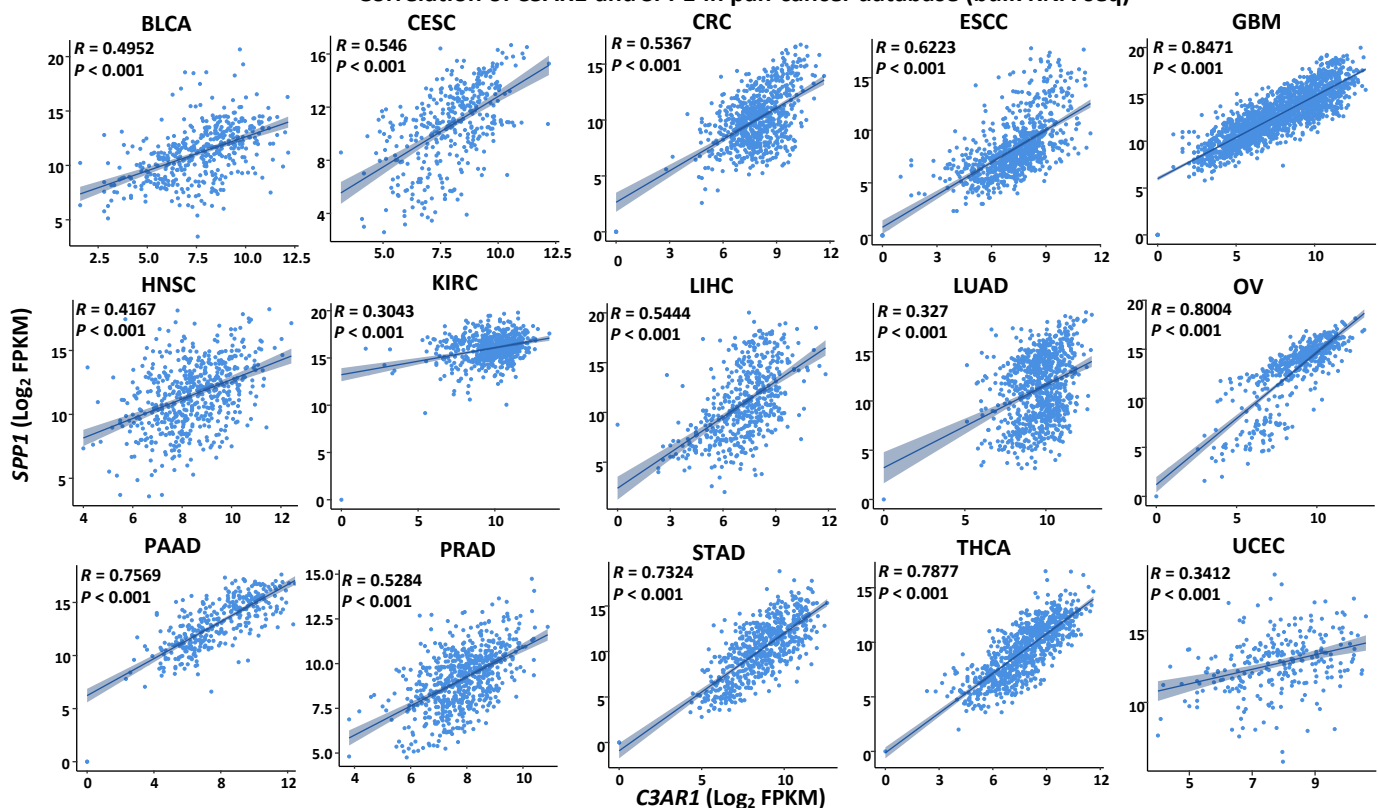
Supplementary Fig. S14. Characterization and developmental states of CAFs, related to Fig. 7.

a Boxplot showing the interaction strength between different TAM subtypes and other TME components inferred using CellChat. **b** UMAP plot showing the mixing level of different cancer types within fibroblasts. **c** UMAP plot showing the mixing of tumor tissues and normal or adjacent tissues within fibroblasts. **d** Bar plot displaying the proportion of different fibroblast subtypes across various cancer types. **e** Bar plot showing the number of different fibroblast subtypes across various cancer types. **f** Radar plot showing the similarity between fibroblast subtypes defined in this study and the six traditionally defined subtypes. **g, h** Distribution of different cell types (left) and developmental direction (right) along the trajectory inferred using Monocle2. **i** Distribution of Macro_BGN, Macro_ECM, Macro_VWF, and Fib_CD74 along the developmental trajectory. **j** Enrichment analysis results of signature genes during the development of Macro_BGN, Macro_ECM, Macro_VWF and Fib_CD74.

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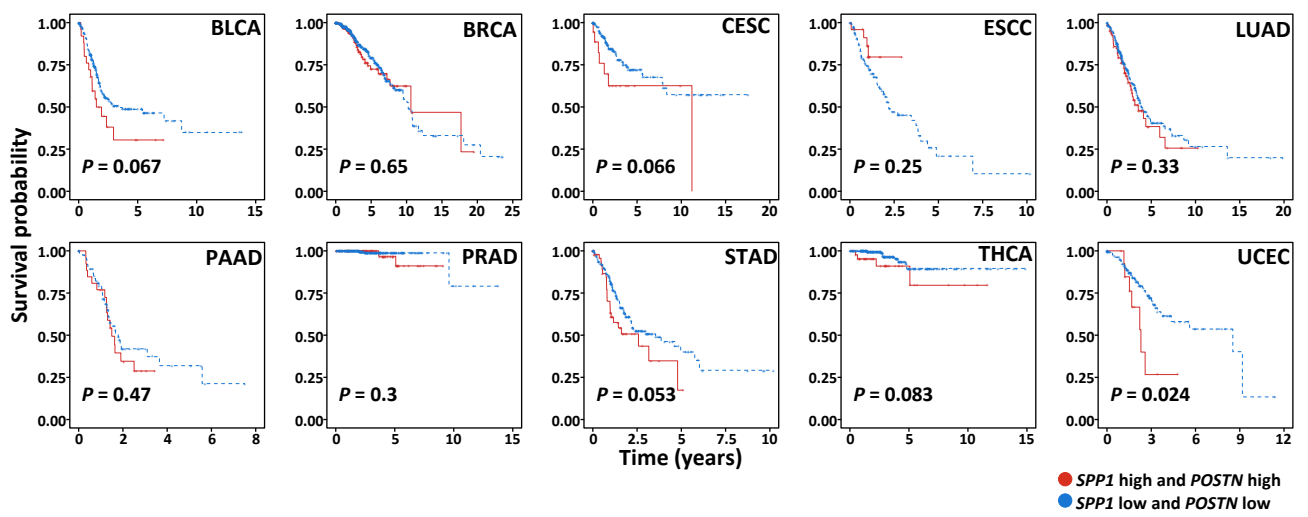
Correlation of *C3* and *C3AR1* in pan-cancer database (bulk RNA-seq)

b

Correlation of *C3AR1* and *SPP1* in pan-cancer database (bulk RNA-seq)

Supplementary Fig. S15. Pan-cancer correlation of key interaction proteins, related to Fig. 7.

a Correlation between *C3* and *C3AR1* gene expression at a pan-cancer level. **b** Correlation between *SPP1* and *C3AR1* gene expression at a pan-cancer level.



Supplementary Fig. S16. Prognostic correlation of *SPP1* and *POSTN* co-expression, related to Fig. 7.

Kaplan–Meier curves showing the association between co-expression of *SPP1* and *POSTN* and prognosis, with *P*-value calculated using the log-rank test.

Supplementary Table legends

Supplementary Table S1. Sample information of public single cell RNA-seq data.

Supplementary Table S2. Sample information of public spatial transcriptomics data.

Supplementary Table S3. Sample information of public bulk RNA-seq data.

Supplementary Table S4. Bulk RNA-seq survival data (TCGA).

Supplementary Table S5. Bulk RNA-seq tumor mutation burden (TMB) data (TCGA).

Supplementary Table S6. The differentially expressed genes in each Macrophages subtype.

Supplementary Table S7. M1 - M2 marker genes.

Supplementary Table S8. Bulk RNA-seq GSVA result.

Supplementary Table S9. Bulk RNA-seq xCell result.

Supplementary Table S10. Immunotherapy Sample information of public bulk RNA-seq data.

Supplementary Table S11. Mutation-associated neoantigen (MANA) marker genes.

Supplementary Table S12. SCPA metabolic pathways.