

Fig. S1, related to Fig 1; Single-cell immune landscapes of ESCA tumor ecosystems

- (A)** CyTOF gating strategy. Each dot represents one cell.
- (B)** Heatmap of the normalized median expression of markers that were significantly differentially expressed between normal and tumor tissues. (* $p < 0.05$, ** $p < 0.01$, *** $P < 0.001$, **** $p < 0.0001$, ns (no significance)). Red indicates high expression; green indicates low expression.
- (C)** t-SNE plots of twenty pairs of samples clustered according to the Phenograph algorithm. Each dot represents one cell, and each color represents one cell cluster.
- (D)** The average proportions of infiltrating immune cell clusters in normal and tumor tissues from patients with ESCA. Each color represents one cell cluster.
- (E)** Heatmap of marker expression for classical subsets of immune cells from normal and tumor tissues. Different colors represent different expression levels. Red indicates high expression; purple indicates low expression; yellow indicates medium expression.
- (F)** The t-SNE plots of CyTOF immune molecules expression. Different colors represent different expression levels. Red indicates high expression; purple indicates low expression; yellow indicates medium expression.
- (G)** The proportions of immune cell subsets in normal and tumor tissues from each patient. Each bar represents one patient. Each color represents one cell cluster.

Fig S2 Detailed statistical analysis of T-cells clusters in ESCA

- (A and B)** Plot showing the frequencies of CD4⁺ T cell clusters **(A)** and CD8⁺ T cell clusters **(B)** in paracancerous (Normal) and tumor tissues. Each point represents a sample ($n=20$); tumors are indicated in red, and paracancerous (Normal) tissues are indicated in black. The error bars represent the mean \pm SD. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns (no significance)).
- (C and D)** The average proportions of CD4⁺ T cell clusters **(C)** and CD8⁺ T cell clusters **(D)** in tumor (Tumor) and paracancerous (Normal) tissues. Each color represents one cell cluster.

(E and F) Kaplan–Meier overall survival curves of ESCA patients grouped by T cell **(E)** and CD8⁺ T cell proportions **(F)**. The median proportion of infiltrating cells was used to define the groups with high and low infiltration of T cells and CD8⁺ T cells. The low-infiltration group is indicated in blue, and the high-infiltration group is indicated in red.

Fig. S3 The CD8⁺ TRMs is the main antitumor cell in ESCA

(A) UMAP plot of CD8⁺ T cell clusters. Each point represents a single cell, colored according to the respective cell cluster. Each dot represents one cell, and each color represents one cell cluster.

(B) Heatmap showing the top ten differentially expressed genes in each CD8⁺ T cell cluster. Each color represents one cell cluster. The heat map color represents the gene expression level. Red indicates higher gene expression; blue indicates lower gene expression.

(C) Dot plot illustrating showing the Z-score-normalized mean expression (color scale) and percentage of expressing cells (size scale) for specified genes expression levels in each CD8⁺ T-cells cluster.

(D) Dot plot of naivety, tissue residency, exhaustion and cytotoxicity signatures in ESCA CD8⁺ T cell clusters. The color scale represents the mean expression and the size of the dots indicates the percentage of expressing cells.

(E) CD8⁺ T cell pseudotime trajectories were constructed with Monocle2. Each point represents a single cell, with different colors representing different cell clusters.

(F) Dot plot showing the expression levels of cytotoxicity-related genes (IFNG, GZMB, GZMH, PRF1, and NKG7) in each CD8⁺ T cell cluster. The color scale represents the mean expression and the size of the dots indicates specified genes expression levels.

(G) Violin plot of the immune checkpoint expression in different CD8⁺ T cell cluster. Different colors represent different CD8⁺ TRMs clusters.

Fig. S4, related to Fig 3 tTRMs is the main CD8⁺ TRMs mediating antitumor immunity

(A-D) Relationships between distinct CD8⁺ TRMs clusters (C19 **(A)**, C20 **(B)**, C26 **(C)**,

C27 (D) and clinicopathological parameters in ESCA. Each point represents a sample. The error bars represent the mean \pm SD.

Fig. S5, related to Fig 4 Transcriptional and TCR profiles of heterogeneous CD8⁺ TRMs

(A) UMAP plot of CD3D, CD3E, CD8A, CD69, ITGAE and ITGA1 expression levels in different clusters. Each point represents one cell.

(B) Heatmap of marker gene expression in individual cells of each cell type cluster. The heat map color represents the gene expression level. Red indicates higher gene expression; blue indicates lower gene expression.

(C) Violin plot of immune checkpoint expression of distinct CD8⁺ TRMs clusters. Different colors represent different CD8⁺ TRMs clusters.

(D and E) Dot plot of proliferative scores (D), tissue residency and exhaustion scores (E) in distinct ESCA CD8⁺ TRMs clusters. The color scale represents the mean expression and the size of the dots indicates the percentage of expressing cells.

Fig. S6 GO biological process analysis of SCENIC modules

(A-F) Bubble plot of the results of GO biological process analysis of SCENIC modules: module 1 (A), module 2 (B), module 3 (C), module 4 (D), module 5 (E), and module 6 (F). The x-axis represents Fold Enrichment. The y-axis represents the cellular biological process. The size of the point represents the number of genes. The color of the point represents the significance of enriched genes. The depth of color reflects the degree of significance.

Fig. S7, related to Fig 4 Transcriptional and TCR profiles of heterogeneous CD8⁺ TRMs

(A) The relationship between the number of clones of CD8⁺ TRMs and the number of clonotype cells. The light blue line separates nonclonal and clonal cells.

(B) The proportions of nonclonal and clonal cells in CD8⁺ TRMs. Clonal cells contained at least two cells of the same clonotype. Blue indicates nonclonal cells; dark yellow indicates clonal cells.

(C) TCR distribution of CD8⁺ TRMs in different patient samples: unique (n=1) and clonal (n=2, n≥3). Different colors represent different clonotypes. Clonotypes were divided into unique (n = 1) and clonal (n = 2 and n ≥ 3) categories based on cell number. Clonal cells contained at least two cells of the same clonotype.

(D) Pie charts showing the proportions of shared clonotypes between different CD8⁺ TRMs clusters. Blue indicates proportions of shared clonotypes; dark yellow indicates proportions of unshared clonotypes. Numbers indicate the specific proportion of shared clonotypes.

Fig. S8 Penotypic and functional characteristics of myeloid immune cells

(A) Heatmap showing differentially expressed genes of each myeloid cell cluster. The heat map color represents the gene expression level. Red indicates higher gene expression; blue indicates lower gene expression.

(B and C) GO biological process analysis of the cDC2 **(F)** and iMo **(G)** clusters. The x-axis represents Fold Enrichment. The y-axis represents the cellular biological process. The size of the point represents the number of genes. The color of the point represents the significance of enriched genes. The depth of color reflects the degree of significance

Fig. S9, related to Fig 6 The iMo/cDC2/CD8⁺ TRMs axis sustains the generation of CD8⁺ TRMs in the TME

(A) Flow cytometry gating strategy related to Fig. 6D **(B)**Flow cytometry gating strategy related to Fig 6E. **(C)**Direct comparison of HLA-DR expression in iMo between normal and tumor tissues. Each point represents a sample; tumor samples are indicated in red, and normal samples are indicated in green. The error bars represent the mean ± SD.

(D)Flow cytometry gating strategy related to Fig 6F

(E)Flow cytometry gating strategy related to Fig 6J.

Fig. S10, related to Fig 7 Hypoxia disrupts the iMo/cDC2/CD8⁺ TRMs axis in ESCA

(A) Flow cytometry gating strategy related to Fig 7D.

(B) Flow cytometry gating strategy related to Fig 7E.