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Analysis of Macrophage Spatial Distribution and Tumor Microenvironment in Esophageal Squamous Cell Carcinoma Using Stereo-CITE-seq

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INTRODUCTION

Esophageal cancer ranks as the sixth leading cause of cancer-related mortality worldwide, with 90% diagnosed as esophageal squamous cell carcinoma (ESCC). The tumor microenvironment (TME) in esophageal cancer is crucially linked to treatment outcomes and patient survival. Macrophages, key immune cells within the TME, are classified into M1 and M2 phenotypes. M1 macrophages are involved in antitumor activity, while M2 macrophages contribute to tumor progression. High densities of tumor-associated macrophages (TAMs) in esophageal cancer correlate with shorter survival. This study employs spatial multi-omics (Stereo-CITE-seq) to investigate macrophage phenotypes in the ESCC tumor invasive margin.

METHODS & MATERIALS

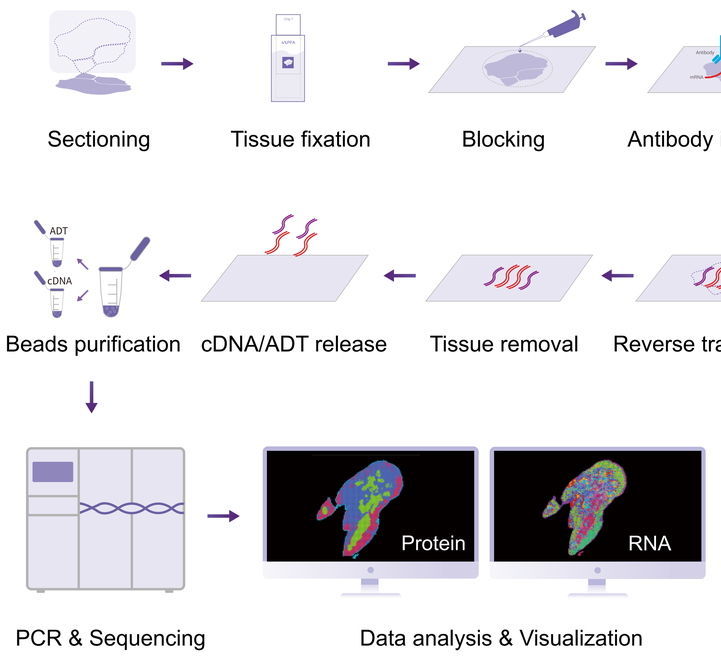
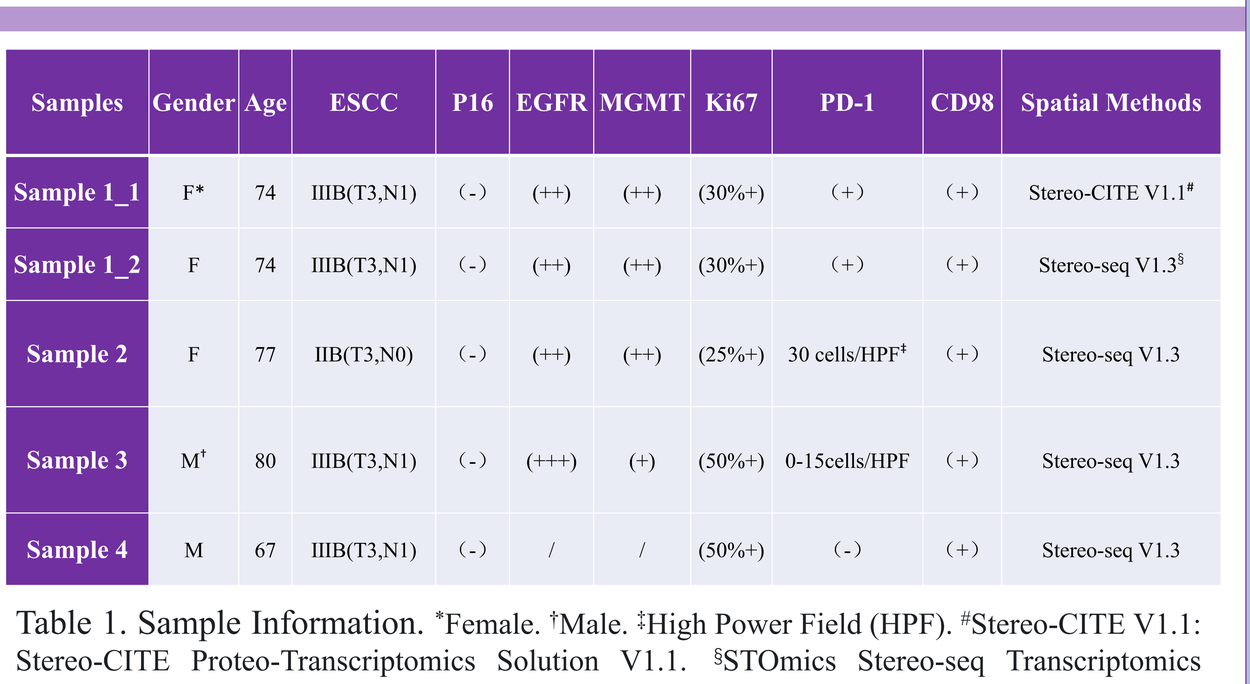


Figure 1. Workflow of Stereo-CITE-seq.

Samples	Gender	Age	ESCC	P16	EGFR	MGMT	Ki67	PD-1	CD98	Spatial Methods
Sample 1_1	F*	74	IIIB(T3,N1)	(-)	(++)	(++)	(30%+)	(+)	(+)	Stereo-CITE V1.1#
Sample 1_2	F	74	IIIB(T3,N1)	(-)	(++)	(++)	(30%+)	(+)	(+)	Stereo-seq V1.3§
Sample 2	F	77	IIB(T3,N0)	(-)	(++)	(++)	(25%+)	30 cells/HPF‡	(+)	Stereo-seq V1.3
Sample 3	M†	80	IIIB(T3,N1)	(-)	(+++)	(+)	(50%+)	0-15cells/HPF	(+)	Stereo-seq V1.3
Sample 4	M	67	IIIB(T3,N1)	(-)	/	/	(50%+)	(-)	(+)	Stereo-seq V1.3

Table 1. Sample Information. *Female. †Male. ‡High Power Field (HPF). #Stereo-CITE V1.1: Stereo-CITE Proteo-Transcriptomics Solution V1.1. §STOmics Stereo-seq Transcriptomics Solution V1.3.

RESULTS



Sample 1_1			
	Modal	Transcript	Protein
	Total Molecular Types	44,374	163
	Total Reads	3.26 G	0.72 G
	Saturation	86.9%	34.8%
Bin50	Count	87,472	
	Median MID	1,954	3,962
	Median Genes	1,200	

Figure 2. Multiple spatial omics signatures of the invasive margin in ESCC revealed by Stereo-CITE-seq. a. Tissue imaging, transcriptomics, and proteomics data on the same frozen section. The black dashed line denotes the tumor invasion margin. **b.** Cell annotations based on transcriptomics. **c.** Confidence scores of macrophages annotated by *cell2location*. **d.** Summary of multi-omics data at bin50 resolution. **e.** Spatial distribution of $CD4^+$ (red) and $CD8^+$ (green) bins. **f.** Spatial distribution of $CD19^+$ (dark blue) and $CD274^+$ ($PD\text{-}L1^+$, purple) bins. **g.** Median UMI counts(bin50) in the **outer tumor area** (12.5 μm, light yellow mark) and **inner tumor area** (12.5 μm, purple mark) of the tumor invasion margin.

RESULTS

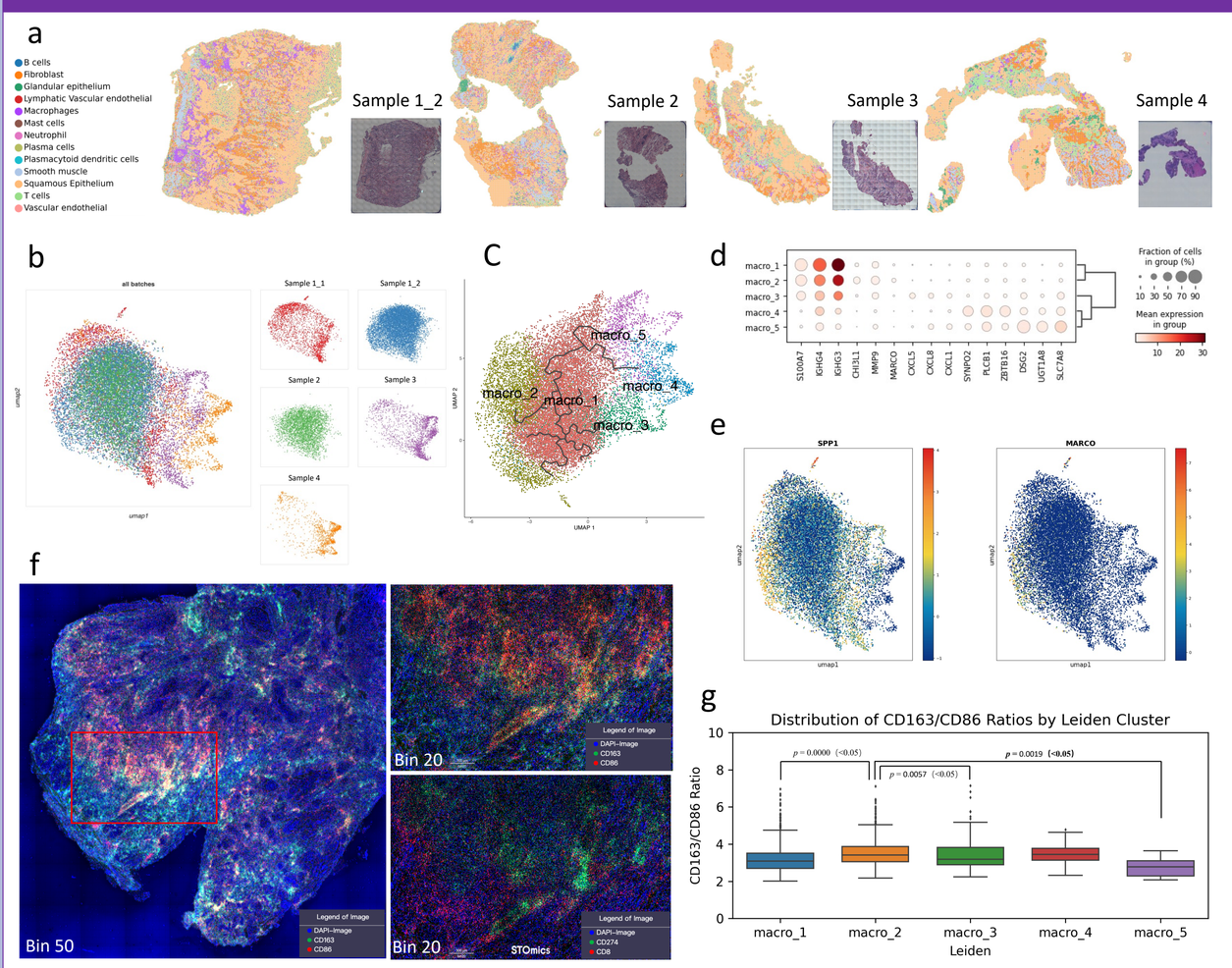


Figure 3. Spatial distribution of macrophage subclusters. a. Annotations of Samples 1–4. **b.** UMAP of macrophages across multiple samples after batch effect removal. **c.** Macrophage populations and trajectory analysis (root node: Macro_5). **d.** Dot plot showing marker genes average expression of differential macrophage populations. *CHI3L1*, *MMP9*, and *MARCO* are specifically expressed in Macro_2 (M2-like). **e.** Expression patterns of *SPP1* and *MARCO* on the UMAP. **f.** Spatial distribution of $CD86^+$ (red)/$CD163^+$ (green) and $CD8^+$ (red)/$CD274^+$ ($PD\text{-}L1^+$, green) bins at bin50 and bin20 scales. **g.** Distribution of CD163/CD86 ratios across differential macrophage populations. The median CD163/CD86 ratio in Macro_2 was significantly higher than that in Macro_1, Macro_3, and Macro_5 ($p < 0.05$, Mann-Whitney U test).

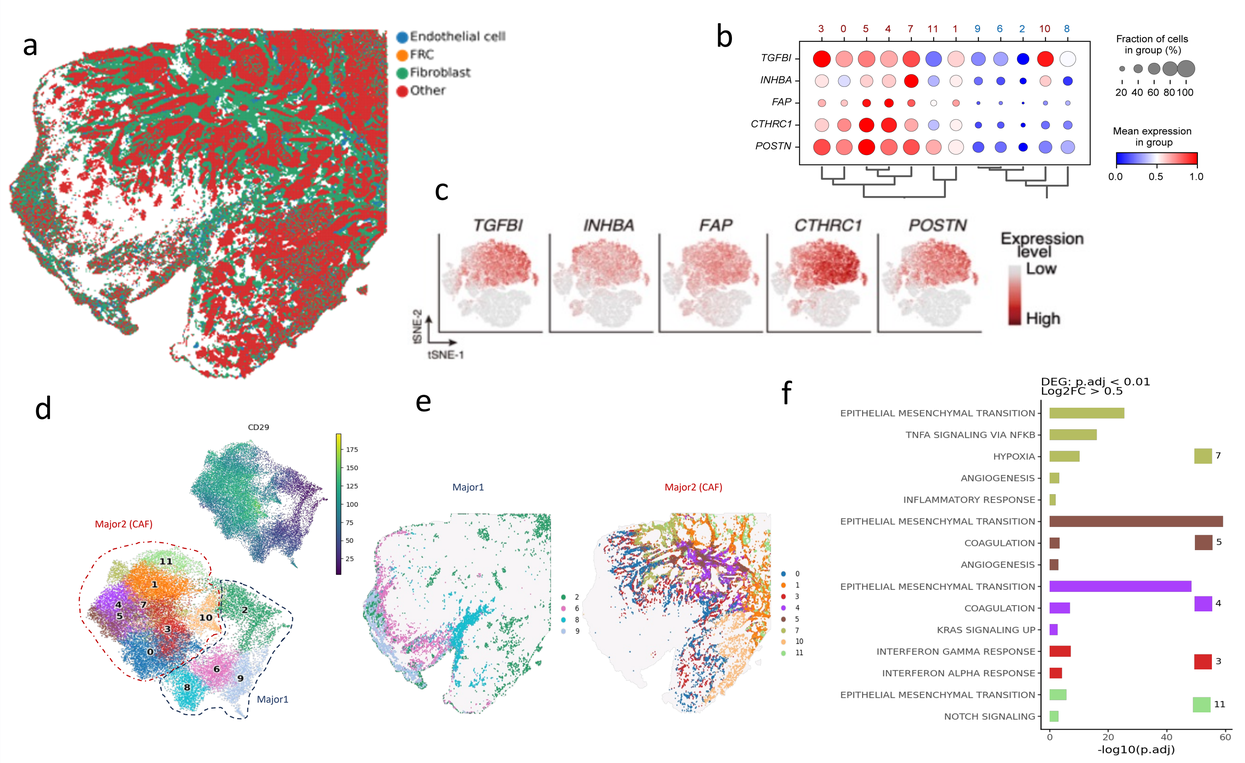


Figure 4. Identification of cancer-associated fibroblast (CAF) subclusters using Stereo-CITE-seq. a. Annotations of bin50 units across the tissue section. **b.** Dot plot showing average expression of marker genes in distinct fibroblast populations. **c.** UMAP visualization of CAF marker expression (*TGFBI*, *INHBA*, *FAP*, *CTHRC1*, *POSTN*). **d.** $CD29^+$ Major2 bins classified as cancer-associated fibroblasts (CAFs). **e.** Spatial distribution of CAF (Major2) and non-CAF (Major1) cell populations. **f.** Hallmark pathway enrichment analysis revealed that C3 clusters with invasive margin distribution are enriched in *interferon gamma (IFN-γ)* and *interferon alpha (IFN-α)* signaling pathways, indicating these CAFs may modulate immune responses via these pathways.

FUNDING AND DECLARATION OF COMPETING INTERESTS

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CONCLUSION

Stereo-CITE-seq reveals spatial distribution of macrophage phenotypes at the ESCC invasive margin, highlighting their role in TME and immune suppression. These findings suggest TAMs may serve as potential prognostic indicators and therapeutic targets for ESCC.