

An Interferon-Responsive Cancer-Associated Fibroblast Subtype Shapes an Immune-Active Tumor Microenvironment and Favors Survival in Ovarian Cancer

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Abstract

Background

Accumulating studies have demonstrated that discrete subsets of cancer-associated fibroblast (CAF) can potentiate antitumor immunity and are associated with favorable clinical outcomes across multiple malignancies. However, the role of CAFs within the ovarian cancer immune microenvironment remains poorly understood, and their prognostic relevance has not been fully elucidated.

Methods

Comprehensive transcriptomic analyses were conducted using datasets from The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) to identify a prognosis-favorable subtype of CAF in high grade serous ovarian cancer (HGSOC). Single-cell and spatial transcriptomic analyses revealed intimate crosstalk between this CAF subtype and intratumoral immune cells, with these fibroblasts preferentially aggregated in tumor immune-infiltrated parenchyma. Furthermore, in vitro transcriptomic and proteomic profiling validated that type I interferon drives the differentiation of this subtype. Finally, an optimal prognostic model was established using 117 machine learning algorithms based on subtype-specific marker genes.

Results

An interferon-hyperresponsive CAF subpopulation was identified, named MP4-high-CAF in HGSOC, which recruits immune cells and predicts superior overall survival. Besides, a prognostic signature based on MP4-high-CAF marker genes that reliably predicts clinical outcomes in HGSOC patients was constructed and validated using a combinatorial machine-learning strategy.

Conclusion

This study established MP4-high-CAF as a central orchestrator of immune remodeling and a positive prognosis biomarker in HGSOC. The identified eight-genes derived from MP4-high-CAF signature exhibited promising translational value for risk stratification in HGSOC, pending additional clinical validation. Exogenous interferon stimulation may be beneficial for improving the immune microenvironment of ovarian cancer.

1.Introduction

Ovarian cancer ranks sixth in mortality among female malignant tumours, imposing a severe burden on women's physical and psychological health[1]. High-grade serous ovarian carcinoma (HGSOC) is the most common histological subtype, accounting for over 70% of all ovarian cancer diagnoses[2]. Current standard therapeutic regimens for HGSOC mainly rely on platinum-based chemotherapy and targeted therapies[3]. While the introduction of poly(ADP-ribose) polymerase inhibitors (PARPi) has yielded durable clinical response in a fraction of HGSOC patients[4], the overall survival benefit remains modest, highlighting the urgent demand for novel therapeutic strategies.

Immunotherapy has emerged as a transformative research frontier for solid tumors, yet its clinical efficacy in HGSOC is largely limited, suggesting that intrinsic structural and molecular features of the ovarian tumor immune microenvironment may constrain antitumor immune responses[5]. Therefore, a deeper understanding of the cellular and molecular mechanisms governing the ovarian tumor immune microenvironment (TME) is essential for identifying actionable therapeutic targets, refining patient stratification and developing more effective treatment strategies.

CAFs constitute the dominant stromal compartment of TME and have attracted intensive research attention over the past decades[6]. CAF regulates numerous paracrine signals within the TME that modulate tumor cell proliferation, extracellular remodeling and the function[7]. Recent advances in single-cell sequencing (scRNA-seq) technologies have enabled the characterization of fibroblast differentiation trajectories across multiple solid tumors, revealing substantial heterogeneity within the CAF compartment[8–10]. Distinct CAF subpopulations with diverse molecular and functional characteristics have been identified across cancer types, and emerging evidence suggests that these subsets may influence tumor progression, treatment response, and clinical outcomes[9, 11, 12]. Specifically, CAF subsets with oncogenic or ECM-remodeling traits, such as LRR15⁺ and MMP1⁺ CAF, create immune-excluded and immunosuppressive microenvironments by recruiting regulatory T cells and pro-tumor macrophages, correlating with poor prognosis[13]. In contrast, proinflammatory CAF subsets like PI16⁺ progenitors and CD74⁺ antigen-presenting CAF promote immune cell infiltration and activation, and are tightly linked to prolonged patient survival[13]. However, the spectrum of CAF subpopulations in HGSOC, along with their transcriptional signature and immunomodulatory functions, have not been fully clarified.

In this study, we analyzed public scRNA-seq to characterize CAF heterogeneity in treatment-naïve HGSOC. We identified a previously unrecognized chemokine-expressing CAF subpopulation that could be induced by interferon (IFN) signaling and shared transcriptional features with the interferon-responsive CAFs (ifnCAFs) reported in other epithelial malignancies[9]. This subset exhibited enhanced JAK-STAT pathway activity compared with other CAF populations and showed distinct interactions with intratumoral immune cells, providing new insights into CAF-mediated immune regulation in ovarian cancer. Through the development of a scoring system based on the marker genes of this CAF subset, we successfully stratified HGSOC patients into distinct prognostic groups and identified key genes associated with clinical outcomes.

2.Materials and methods

2.1. Public Data Collection

Database	Dataset ID	Data type	Sample number	Purpose
GSA-Human	PRJCA005422[14]	Single cell transcriptome	14 (HGSOC)	Single cell data analysis
GEO	GSE184880[15]	Single cell transcriptome	7 (HGSOC)	Single cell data analysis
10x Genomics	CytAssist_11mm_FFPE_Human_Ovarian_Carcinoma[16]	Spatial transcriptome	1 (HGSOC)	Spatial transcriptome data analysis
GEO	GSE189843[17]	Spatial transcriptome	12(HGSOC)	Spatial transcriptome data analysis
TCGA	TCGA-OV[18]	Bulk transcriptome	380 (HGSOC)	Prognostic analysis; model construction
GEO	GSE49997[19]	Bulk transcriptome	204 (HGSOC)	Model validation
GEO	GSE26712[20]	Bulk transcriptome	185 (HGSOC)	Model validation
GEO	GSE26193[21]	Bulk transcriptome	107 (HGSOC)	Model validation
GEO	GSE156699[22]	Bulk transcriptome	38/50 (NonResponders/Responders)	Investigating the Contribution of MP4_high_CAF to Chemotherapy Response
GEO	GSE143897[23]	Bulk transcriptome	18/18 (Before neoadjuvant chemotherapy/After neoadjuvant chemotherapy)	Investigating the Effects of Neoadjuvant Chemotherapy on MP4_high_CAF
GEO	GSE164088[24]	Bulk transcriptome	3/3 (Before Niraparib stimulation/After Niraparib stimulation)	Investigating the Inductive Role of PARPi in Human-Derived CAFs
GEO	GSE83848[25]	Bulk transcriptome	3/3 (Before interferon stimulation/After interferon stimulation)	Investigating the Inductive Effects of Interferon on Mouse Tumor Models
GEO	GSE3400[26]	Bulk transcriptome	3/3 (Before interferon stimulation/After interferon stimulation)	Investigating the Inductive Effects of Interferon on Mouse Fibroblasts
2.2.Statistical Analysis				

Current research was carried by R software (version 4.5.1) for all statistical analyses. For data that followed a normal distribution, we used the Student's t-test to compare two groups. For data that did not follow a normal distribution, we used the Wilcoxon rank-sum test. When comparing multiple groups, one-way ANOVA was used for normally distributed data and the Kruskal-Wallis test for non-normally distributed data. The relationship between continuous variables was assessed using Spearman's rank correlation. Kaplan-Meier analysis was applied to show survival outcomes and the log-rank test to compare them. A *P*-value less than 0.05 was considered statistically significant.

2.3. Analysis of single-cell sequencing data

To increase the sample size, we integrated and analyzed the two single-cell data samples. Quality control of the single-cell dataset samples was conducted utilizing the R package Seurat (v5.3.1)[27]. The threshold for excluding low-quality cells and low-expressed genes was established as follows: The feature count per cell ranged from 200 to 8000, while the count of observations per cell varied between 200 and 75000. The percentage of mitochondrial genes in each cell was below 10%. Secondly, the NormalizeData function was employed for normalization, while the “FindVariableFeatures” function (nfeatures = 2000) was utilized to identify hypervariable genes. Batch correction among samples was conducted using the R package Harmony (v1.2.4) to mitigate the influence of batch effects on subsequent analyses. The data were subsequently scaled, linearly transformed via ScaleData, and subjected to dimensionality reduction through RunPCA. The elbow plot was generated using ElbowPlot to assess the dimensionality reduction of the data. Thirty principal components were chosen for subsequent analysis, utilizing the FindNeighbors and FindClusters functions for cell clustering (dims = 1:30, resolution = 1). Cell annotations from the original dataset authors and relevant reference were utilized in this analysis to further differentiate annotated different cells. After extracting the CAF, the aforementioned analysis was repeated, yielding 15 CAF subclusters. Among these, cluster10 and cluster14 were merged into cluster5 due to similarities in their feature genes and cluster5. This resulted in a final set of 13 clusters. Differential expression analysis was performed with the “FindAllMarkers” function in the Seurat package with default parameters. Genes with an adjusted min.pct = 0.5 and log2FC > 1 were considered cell signature Genes.

2.4. Consensus non-negative matrix factorization (cNMF) analysis

GeneNMF package (v0.9.2) was used to identify potential expression programs in all subclusters of CAF[28]. By systematically testing k values from 4 to 10, and based on the stability of the consensus matrix and the inflection point of reconstruction error, k = 9 was finally determined as the optimal number of clusters. After calculating the Pearson correlation coefficients of these programs, they were classified into corresponding metaprograms.

2.5. BayesPrism

We estimated cell type proportions in TCGA bulk RNA-seq data using BayesPrism (v2.2.3)[29], a Bayesian deconvolution method that employs single-cell RNA-seq data as an informative prior. Our pre-classified scRNA-seq reference included major tumor microenvironment cell types. Raw count matrices of both scRNA-seq and bulk data were input into BayesPrism, which performed Gibbs sampling to obtain posterior distributions of cell-type fractions. The resulting cell-type proportions were used for downstream integrative analyses.

2.6. Functional enrichment analysis

The GSVA package (v2.2.1) was employed to perform GO and KEGG pathway enrichment analysis on the nine gene sets for constructing metaprograms and cell signature genes of each CAF subcluster[30].

2.7. GSEA enrichment analysis

In our previous analysis, we obtained two gene sets. One consists of marker genes extracted from the MP4_high_CAF subgroup using “FindAllMarker”, while the other comprises genes forming the MP4_metaprogram identified through cNMF analysis. We performed gene set enrichment analysis (GSEA) on the TCGA_OV cohort and expression matrices GSE156699, GSE143897, GSE164088, and GSE3400 using the GSEA function within the clusterProfiler package (v4.16.0)[31]. Prior to gene set enrichment, we had already ranked differentially expressed genes between groups using the limma package (v3.64.3).

2.8. Cell communication analysis

Cellular communication between two types of CAF and other cells was analyzed using CellChat (v2.2.0)[32]. Normalized single-cell expression matrices were imported into CellChat, and the standard analysis workflow was executed with default parameters. Ligand–receptor pairs and signaling pathways were annotated using the built-in CellChat database. The netAnalysis_contribution function was used to quantify the contribution of individual ligand-receptor pairs to overall signaling. The netAnalysis_signallingRole function was applied to define sender and receiver roles for each cell population. Inferred communication networks were visualized using the netVisual_aggregate function for integrated pathway-level communication patterns.

2.9. Processing of spatial transcriptome sequencing data

The data is also analyzed using Seurat in R. The UMI counts are normalized, scaled, and the most variable features are determined by the “SCTransform” function. Dimensionality reduction is then performed using “RunPCA” for unsupervised clustering analysis. The “FindNeighbors” and “FindClusters” functions were executed with default parameters and the 30 most significant principal components. The “SpatialFeaturePlot” function was utilized for visualizing subgroups and genes.

2.10. Define the proportion of cell types at spatial locations

The Visium V2 platform provides a spatial resolution of 55 µm, allowing individual barcodes to capture transcripts from multiple cell types. To resolve cellular composition within each spatial barcode, deconvolution analysis was performed using the RCTD algorithm in the spacexr

package (v2.2.1), integrating single-cell transcriptomic data as a reference[33]. A spot is considered an MP4_high_CAF cluster if the MP4_high_CAF weight values within that spot rank among the top two weights assigned to any cell.

2.11. PROGENy analysis

PROGENy (v1.30.0) builds models of pathway responsive gene expression from publicly available inferences pathway activity using a linear model[34]. Correlation analysis was performed between pathway activity scores for each single cell, spatial spot, and sample within the TCGA_OV cohort, and scores obtained using the “AddModuleScore” function for single-cell and spatial spot CAF signature gene sets, or ssGSEA scores for CAF signature gene sets in the TCGA expression matrix.

2.12. Immune cell analysis

CIBERSORT algorithm (v0.1.0) was used for the immune cell analysis of the TCGA_OV gene expression matrix[35]. CIBERSORT will automatically choose the record with the highest mean expression across the mixtures during analysis. The created gene expression file with the 379 cases was uploaded to CIBERSORT as a mixture file, and CIBERSORT was run with the following options: relative and absolute modes together, LM22 signature gene file. At the same time, we used the TIDE algorithm to calculate immune dysfunction score and immune rejection score[36].

2.13. Semla

We profiled gradual changes in gene expression along radial axes originating from MP4-high-CAF enriched regions of interest (ROIs). Spatially discontinuous subregions within the same category were partitioned using the DisconnectRegions function in the semla package[37]. Radial distance metrics for all ROI-containing tissue sections were subsequently extracted via the RadialDistance function implemented, with isolated singleton spots removed from downstream analysis. Smoothed curves illustrating radial gene expression patterns were generated using the geom_smooth function in the ggplot2 R package.

2.14. Cell cultures and Reagents

Immortalized Human Ovarian Tumor-Associated Fibroblasts (i-hOTAFs) were purchased from Shanghai Meiyuan Biotechnology Co., Ltd. All cell lines were tested for mycoplasma contamination and authenticated by short tandem repeat (STR) analysis before experimentation. Cells were cultured in DMEM, supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. The cultures were maintained at 37°C in a humidified atmosphere containing 5% CO₂.

2.15. Bulk RNA-seq

Total RNA was extracted from triplicate experimental and control samples with TRIzol reagent. mRNA was enriched, fragmented and reverse-transcribed into cDNA. After end repair, A-tailing and adapter ligation, target-sized fragments were purified, followed by PCR amplification for library construction. Qualified libraries were sequenced on Illumina Novaseq X Plus platform.

2.16. Proteomics sequencing

Three biological replicates each of experimental and control protein samples were lysed, homogenized and centrifuged, and quantified via BCA assay. Proteins were denatured, reduced, alkylated and digested with trypsin using commercial kits, followed by peptide purification and lyophilization. Peptides were separated by liquid chromatography and analyzed with timsTOF Pro2 mass spectrometer, and DIA data were collected under diaPASEF mode.

2.17. Single-Cell Identification of Subpopulations with bulk Sample phenotype correlation (Scissor)

Scissor analysis was performed to integrate bulk RNA-seq data from IFN-treated and control CAFs with single-cell RNA-seq data. Scissor analysis was performed using the “Scissor” function with ‘family = binomial’, ‘alpha = 0.1’. Scissor-positive and -negative cells were identified based on the resulting ‘Scissor_pos’ and ‘Scissor_neg’ indices. Cell-level Scissor classifications were incorporated into the Seurat object using the “AddMetaData” function and visualized using the “DimPlot” function. The distribution of Scissor-positive cells across the two CAF subtypes was subsequently evaluated.

2.18. Multiple models construction and validation

The Mime package (v0.0.0.9000) was employed to construct prognostic models[38]. The TCGA_OV cohort was utilized for model development, and we additionally collected three clinical cohorts with prognostic information for model validation. A total of 117 combinations were integrated into the computational framework with K-fold cross-validation for model construction on the training dataset. All the parameters are using the default values.

Result

Identification of an Immune-Promoting Metaprogram (MP4) associated with Favorable Prognosis in HGSOC

To explore heterogeneity and TME regulatory functions of CAF in HGSC and identify CAF subpopulations associated with favorable clinical outcomes, we integrated two single-cell RNA-seq datasets from untreated primary HGSOC patients [14, 15]. Following standard Seurat workflow, a total of 5,968 CAFs were extracted for downstream analysis (Fig. 1B, C). Unsupervised clustering paired with cNMF transcriptional decomposition resolved nine distinct CAF metaprograms (MPs). Among all identified MPs, MP4 (defined by a set of 18 genes, including CXCL10, IFIT1, ISG20), showed strong positive correlations with prolonged overall survival and enhanced chemotherapy response in the TCGA_OV cohort (Fig. 1D, E, F).

Functional annotation of each metaprogram revealed divergent core biological functions: MP1 mediated extracellular matrix remodeling; MP2 regulated angiogenesis; MP3 controlled general stimulus-responsive transcription; MP4 drove immune defense; MP5 modulated growth factor signaling; MP6 coordinated inflammatory cascades; MP7 mediated cellular stress adaptation; MP8 governed hypoxia and apoptotic pathways; MP9 recapitulated developmental transcriptional programs (Supplementary Fig. 1). Notably, only MP4-related genes were enriched in biological processes associated with immune defense and immune surveillance. Based on these observations, we hypothesized that MP4-high-CAF may remodel the ovarian tumor microenvironment by promoting anti-tumor immune responses, thereby contributing to improved clinical outcomes.

MP4-High-CAF Represents a Unique IFN-Responsive CAF Subtype Predicting Favorable Prognosis in HGSOC.

To dissect CAF heterogeneity in depth, we performed subclustering analysis on purified fibroblasts and uncovered distinct subclusters. We confirmed MP4 signature are selectively upregulated in a CAF subcluster whose transcriptional landscape overlaps extensively with interferon-response CAFs (ifnCAFs) identified in other solid tumor types, expressing ifnCAF markers (CXCL10, IFIT1, IFIT2, IFIT3, RSAD2, IRF7, CMPK2, MX2, ISG20) (Fig. 2A, B, C) [8, 39, 40]. To evaluate molecular discrepancies associated with the MP4 signature within CAFs, we defined the subcluster 8 as MP4-high-CAF, while the remaining cells are collectively referred to as MP4-low-CAF in this study (Fig. 2D).

Across multiple human solid malignancies, intratumoral ifnCAF abundance acts as a robust positive prognostic biomarker [8–10]. These fibroblasts foster immune-inflamed, immunogenic TMEs and correlate with prolonged patient survival. However, the clinical and biological relevance of IFN-CAFs remains poorly defined in ovarian cancer. We applied the BayesPrism deconvolution algorithm to estimate the MP4-high-CAF fractions for each TCGA-OV patient, stratifying the entire cohort by median MP4-high-CAF abundance. Patients with higher MP4-high-CAF abundance showed significantly better prognosis relative to low-abundance counterparts, confirming that MP4-high-CAF recapitulates the protective ifnCAF phenotype observed in other epithelial cancers (Fig. 2E).

We next systematically compared molecular profiles between MP4-high-CAF and MP4-low-CAF. Differential gene expression analysis revealed profound upregulation of canonical interferon-stimulated genes (ISGs) in MP4-high-CAF, encompassing BST2, IFI27, multiple IFI family members, and IFI6 (Fig. 2F). GO biological process and KEGG pathway enrichment analyses of MP4-high-CAF signature genes further demonstrated striking enrichment of interferon response cascades (Fig. 2G, H). Specifically, core ISGs including ISG15 which mediates protein ISGylation to potentiate anti-tumor signaling and the JAK-STAT signaling cascade, the major downstream effector pathway triggered by IFN receptor engagement, were markedly overexpressed in this subset [41]. Moreover, chemokine and cytokine biosynthesis pathways (eg, Cytokine production, Chemokine signaling pathway, Cytokine-cytokine receptor interaction) were strongly hyperactivated within MP4-high-CAF, endowing these fibroblasts with an intrinsic ability to recruit immune cells and shape an immune-inflamed tumor microenvironment.

To our knowledge, this study provides the first description of a unique CAF subpopulation in HGSOC that undergoes IFN-dependent transcriptional reprogramming to secrete immunomodulatory chemokines, ultimately contributing to favorable clinical outcomes. We filtered CAF subtype-specific markers, identifying 247-genes for MP4-high-CAF signature deployed for all downstream analytical workflows (Fig. 2I).

MP4-high-CAF Promotes Intratumoral Immune Cell Infiltration by Producing Chemokines

To dissect the immunomodulatory functions of MP4-high-CAF within the ovarian TME, we performed single-cell cell-cell communication analysis (Fig. 3A). MP4-high-CAF established extensive, high-magnitude ligand-receptor crosstalk with all major TME cell compartments, with the CXCL chemokine axis representing the dominant intercellular signaling module (Fig. 3B, C). MP4-high-CAF robustly secrete CXCL10 and CXCL11; these ligands bind CXCR3 receptors expressed on CD8⁺ cytotoxic T lymphocytes and natural killer (NK) cells to facilitate their intratumoral trafficking and proliferation. Collectively, this signaling circuit suggests that tumor regions enriched for MP4-high-CAF are accompanied by abundant tumor-infiltrating immune effector cells. Intriguingly, despite this immune-active microenvironment, MP4-high-CAF simultaneously exhibited enhanced LGALS9-mediated signaling to immune cells via the HAVCR2 (TIM-3) and CD45 receptors, suggesting that the recruited immune cells may be restrained by immune checkpoint signaling [42]. This coexistence of immune activation and immune suppression implies that MP4-high-CAF-rich tumors may be particularly amenable to therapeutic strategies targeting the LGALS9/TIM3 immune checkpoint pathway.

Spatial transcriptomics combined with RCTD spot deconvolution further verified this spatial co-localization pattern. Unlike other CAF subsets largely restricted to the peripheral stroma, MP4-high-CAF preferentially accumulate within tumor parenchymal cores. Spot-level correlation analyses identified a strong positive correlation between the fractional abundance of MP4-high-CAF and total immune cell proportion in individual spatial capture regions (Fig. 4A, B. Positive correlation between MP4-high-CAF and T/NK cells: Spearman's correlation coefficient = 0.41, $P < 0.001$). We further quantified chemokine transcript gradients within three MP4-high-CAF dense aggregates CXCL chemokine expression peaked at the center of MP4-high-CAF aggregates and gradually declined toward the periphery, forming a directional chemotactic gradient that recruits circulating immune effector cells (Fig. 4C, D, E).

To rule out slice-specific spatial heterogeneity artifacts, we further validated these findings using 12 pre-treatment HGSOC tissue sections from Stur study. These samples were derived from patients with distinct clinical responses to neoadjuvant chemotherapy, including 6 excellent responders (ER) and 6 poor responders (PR). Pretreatment ER tumors exhibited substantially higher MP4-high-CAF density, with MP4-high-CAF aggregates tightly co-localized with immune cell-dense stromal regions; this immune-favorable spatial signature was markedly diminished in tissue from PR patients (Supplementary Fig. 2, Supplementary Fig. 3). Simultaneously, CIBERSORT immune deconvolution and TIDE immune scoring analyses revealed that tumors from patients with high expression of the MP4-high-CAF signature in the TCGA-OV cohort exhibited more pronounced immune cell infiltration (eg, Plasma cells, CD8 T cell, NK cells activated and Macrophages M1) and higher immune response scores (eg, T-cell infiltration increased, IFN- γ signaling enhanced, M2 macrophage infiltration reduced and Microsatellite instability increased) (Fig. 5A, B).

HGSOC is widely regarded as an immune “cold” tumor characterized by sparse spontaneous immune infiltration[5]. Nevertheless, abundant intratumoral MP4-high-CAF constitute a powerful biomarker for elevated intratumoral immune activity and superior therapeutic response. We further validated this association using a paired neoadjuvant chemotherapy (NACT) bulk transcriptomic cohort, as studies have reported that NACT can improve the immune microenvironment in ovarian cancer[23, 43]. GSEA analyses of paired pre- and post-NACT bulk transcriptomic datasets demonstrated significant upregulation of the MP4-high-CAF signature following chemotherapy (Fig. 5D). This partially demonstrates that immune activation within the ovarian cancer immune microenvironment correlates positively with increased MP4-high-CAF proportion. Besides, we divided the Bosquet's study into chemotherapy-responsive and non-responsive groups for differential expression analysis[22]. The MP4-high-CAF signature tended to align with genes highly expressed in the chemotherapy-responsive population, suggesting that the accumulation improves therapeutic outcomes in HGSOC (Fig. 5C). PARP inhibitor (PARPi) treatment also triggered transcriptional activation of the MP4 metaprogram in patient-derived primary CAFs, implying potential combinatorial synergy between PARPi and IFN-dependent stromal immunomodulation (Fig. 5E).

The JAK-STAT Pathway is Activated within MP4-high-CAF in Response to IFN Stimulation to Produce Chemokines.

The cellular communication analysis results above indicate that both chemokines CXCL10 and CXCL11 are regulated by transcription factors from the STAT family (Fig. 3). Additionally, GSEA demonstrated that the genes upregulated in MP4-high CAFs were significantly enriched in the JAK–STAT signaling pathway. (Fig. 2H). We hypothesize that upon IFN stimulation, activation of the JAK-STAT pathway in CAFs triggers chemokine secretion, leading to differentiation into the MP4-high-CAF subtype.

To demonstrate this, we first validated the specificity of JAK-STAT hyperactivation within MP4-high-CAF. The UMAP plot reveals that JAK-STAT activation is distinctly concentrated solely within areas exhibiting MP4-high-CAF distribution (Fig. 6A, B). Violin plots demonstrate that MP4-high-CAF regions relatively overexpress key molecules across numerous JAK-STAT pathways (Fig. 6C). Furthermore, activation of the JAK-STAT pathway within individual cells correlates with high expression of MP4-related genes. Spatially, spots with a high proportion of MP4-high-CAF exhibit correspondingly higher activity of the JAK-STAT pathway (Fig. 6D, E, F). A trend recapitulated across the TCGA-OV cohort. These results provide preliminary evidence that activation of the JAK-STAT signaling pathway characterizes the MP4-high-CAF subtype (Fig. 6G).

We next performed orthogonal in vitro and in vivo validation to confirm IFN directly drives fibroblast reprogramming toward the MP4-high-CAF transcriptional state. Published bulk transcriptomics from IFN-treated mouse tumor tissue and murine embryonic fibroblasts showed robust enrichment of the MP4-high-CAF signature among IFN-upregulated transcripts by GSEA analysis (Fig. 7A, B). For human functional validation, we selected IFN- β as the stimulus, consistent with murine experimental regimens and supported by the pronounced upregulation of type I IFN response pathways in MP4-high-CAF (Fig. 7C). Patient-derived primary HGSOC CAFs were cultured to 70–80% confluence and treated with IFN- β or vehicle control for 5 h ($n = 3$ biological replicates per group), followed by bulk RNA-seq and DIA proteomic profiling. Differential expression analysis demonstrated IFN- β treatment induced significant transcriptional upregulation of MP4 signature genes, with full enrichment of the MP4-high-CAF transcriptional program (Fig. 7D, E). Scissor multi-omics integration was applied to integrate IFN-treated bulk CAF transcriptomic perturbations with scRNA-seq profiles; nearly 90% of annotated scRNA-seq MP4-high CAFs were classified as Scissor-positive, whose transcriptional profiles closely recapitulated IFN- β -stimulated primary human CAFs, definitively establishing IFN signaling as the upstream driver of this CAF subtype. (Fig. 7F). Proteomic profiling corroborated robust upregulation of the MP4 signature in IFN- β -stimulated fibroblasts (Fig. 7G, H). Complementary transcriptomic and proteomic datasets further verified striking hyperactivation of the JAK–STAT signaling cascade following IFN- β treatment (Fig. 7I, J).

Together, these findings indicate selective JAK–STAT pathway activation within MP4-high CAFs and a tight causal association between IFN- β exposure, JAK–STAT signaling, and differentiation into immunostimulatory MP4-high-CAF, supporting an IFN-dependent ontogeny for this protective CAF subtype.

Key Genes Identified in the MP4-high-CAF Feature were Selected to Construct a Prognostic Model that Effectively Distinguishes High-risk from Low-risk Populations.

To translate the prognostic significance of the MP4-high-CAF signature into a clinically useful predictive framework, we established a prognostic risk model using MP4-high-CAF-associated genes in the TCGA cohort and validated its performance across three independent HGSOC datasets (GSE49997, GSE26712, and GSE26193). Univariate Cox regression identified 19 prognostically relevant genes, including CDC42EP2, CXCL10, DNAJA1, DUSP1, EMP1, FAS, GMPR, HLA-F, IFI27, ISG20, PSMA4, PSMB9, PTGER3, RELB, STAT1, TAP1, USP18, WTAP, and ZFP36L1. We applied 117 combinations of algorithms to both the training and validation cohorts to identify the optimal model and mitigate bias from algorithm selection (Fig. 8A). The optimal model - StepCox[both] + Lasso - included 8 genes: DNAJA1, EMP1, GMPR, IFI27, ISG20, RELB, TAP1, and WTAP, which were used to estimate overall survival (OS) in HGSOC patients. The four KM curves demonstrate that this model effectively categorizes individuals into high-risk and low-risk groups (Fig. 8B, C, D, E), while also confirming that MP4-high-CAF correlates with improved prognosis in HGSOC.

Discussion

In this study, we used single-cell RNA sequencing, spatial transcriptomics, and bulk transcriptome data to systematically characterize CAF heterogeneity in treatment-naïve primary HGSOC. Previous studies have characterized the CAF landscape in HGSOC as comprising myofibroblastic CAFs (myCAF) and inflammatory CAFs (iCAF)[13, 44]. To our knowledge, this study provides the first evidence for an IFN-responsive CAF population in HGSOC by identifying and characterizing a distinct CAF subtype, termed MP4-high-CAF, whose transcriptional program closely resembles the interferon-responsive CAF (ifnCAF) state previously described in other cancer types. This subset responds robustly to IFN stimulation, secretes abundant chemokines that recruit cytotoxic immune cells and remodel the immunosuppressive ovarian TME. Importantly, its intratumoral abundance independently predicts favorable clinical outcomes in patients with HGSOC. We further demonstrated that MP4-high-CAF can be differentiated from other CAFs upon interferon induction. Using a combinatorial machine-learning strategy, we constructed and validated a prognostic signature based on MP4-high-CAF marker genes, which reliably predicts clinical outcomes in HGSOC patients. Collectively, our findings expand the understanding of CAF subpopulations in ovarian cancer and highlight the translational potential of interferon-related strategies in HGSOC therapy.

Accumulating studies have revealed remarkable CAF heterogeneity across cancer types, with distinct subsets exerting either pro-tumor or anti-tumor functions in the TME. Recent studies have proposed that CAF can be broadly categorized into three conserved molecular phenotypes: myofibroblastic CAFs (myCAF), inflammatory CAFs (iCAF), and antigen-presenting CAFs (apCAF)[45]. Among these, myCAF are primarily associated with extracellular matrix remodeling, whereas iCAF and apCAF have been implicated in regulating anti-tumor immunity. iCAF are characterized by the secretion of inflammatory cytokines and chemokines, including IL6, LIF, CXCL1, and CXCL2, and are predominantly localized within tumor-distal inflammatory stroma, where they regulate immune responses and myeloid cell recruitment. In contrast, apCAF constitutively express MHC-II antigen-presentation machinery and are enriched in immune-associated niches, including regions adjacent to tumor cells and tertiary lymphoid structures (TLSs). In addition, scholars have proposed that ifnCAF represent a context-specific, interferon-responsive subtype within the iCAF spectrum. However, the microenvironmental factors that drive this phenotypic transition remain largely undefined. MP4-high-CAF identified in our study shares striking molecular similarities with ifnCAF described in other solid tumors, as evidenced by high expression of interferon-stimulated genes. Compared with iCAF, which primarily produce inflammatory cytokines and recruit myeloid cells, ifnCAF exhibit a specialized interferon-driven transcriptional program that preferentially promotes T/NK cell recruitment through CXCR3 ligands, including CXCL10, and CXCL11. Notably, unlike the tumor-distal localization typically described for iCAF, our spatial transcriptomic analyses demonstrated that ifnCAF may predominantly distributed within the tumor parenchyma, where they colocalize with infiltrating immune cells. This distinct spatial organization may enable ifnCAF to establish localized immune-permissive niches within the tumor core, thereby facilitating lymphocyte infiltration and enhancing anti-tumor immune surveillance. Taken together, our findings highlight a distinct CAF subtype in the HGSOC tumor microenvironment and demonstrate its role in promoting a more immunologically favorable tumor microenvironment.

In recent years, prospective clinical trials and preclinical model studies have collectively confirmed the therapeutic benefits of type I interferon in HGSOC: multi-omics data indicate that high baseline expression of the IFN-I pathway predicts sensitivity to platinum-based chemotherapy, and supplemental IFN- α can reverse chemotherapy resistance caused by pathway silencing[46]; Transient IFN-I signaling can remodel the tumor microenvironment and drive the infiltration of CD8⁺ T cells and NK cells, synergistically enhancing the efficacy of PARP inhibitors and immune checkpoint inhibitors[47]. Meanwhile, ovarian-specific IFN- ϵ can suppress the stem cell-like properties of HGSOC and peritoneal metastasis, exerting multidimensional antitumor effects[48]. Despite these compelling clinical and preclinical observations, the exact

molecular events and cellular interactions through which interferon reconfigures the tumor microenvironment to achieve therapeutic gains in HGSOC are not fully defined. A key mechanistic finding of this study is that MP4-high-CAF can be directly induced and differentiated from other CAFs by interferon stimulation. Transcriptional profiling and pathway analyses confirmed that interferon exposure upregulates signature genes of MP4-high-CAF and activates the JAK-STAT signaling cascade, which further promotes chemokine expression and immune cell recruitment. These results experimentally validate the inducibility of this beneficial CAF phenotype and support the potential value of interferon in ovarian cancer treatment by reshaping the TME toward an immune-active state. Our data provide a biological rationale for combining interferon-based interventions with conventional or targeted therapies to overcome immune suppression in HGSOC.

Beyond its potential therapeutic implications, our findings also provide insights into how treatment-induced immune remodeling may be mediated by TME components. Although the impact of NACT on immune alterations in HGSOC has been increasingly recognized[49, 50], the contribution of interferon-responsive CAFs remains poorly understood. Here, we observed that MP4-high-CAFs were preferentially enriched in tumors from patients with favorable responses to NACT and were spatially located adjacent to immune infiltrates, particularly T/NK cells. Moreover, MP4-high-CAF signature genes showed increased enrichment after NACT, suggesting that chemotherapy-induced inflammatory signals may promote the expansion of this immune-positive CAF state. These findings raise the possibility that MP4-high-CAF abundance may serve as a predictive biomarker for NACT response and provide a rationale for exploring strategies that enhance IFN-driven reprogramming in combination with chemotherapy.

To translate our findings into clinical application, we employed a comprehensive panel of 117 machine-learning algorithm combinations to construct a prognostic model using MP4-high-CAF-related genes. The StepCox[both] + Lasso model showed optimal performance in the TCGA-OV training cohort and was validated in multiple independent datasets. The model effectively stratified patients into high-risk and low-risk groups with significantly distinct survival outcomes, confirming that our target gene set can robustly predict HGSOC prognosis. This CAF subset-based signature complements traditional clinicopathological factors and offers a novel tool for risk stratification and personalized management of ovarian cancer patients.

Despite these novel findings, several limitations should be acknowledged. First, the sample size of the single-cell sequencing data is relatively small, which may limit the detection of rare CAF subpopulations and reduce the generalizability of subtype identification. Second, we defined the signature of MP4-high-CAF using 247 genes filtered by $\log_2FC > 2$ and $P < 0.05$; the stringency of this threshold may affect the comprehensiveness and accuracy of the subtype definition. Third, only interferon-beta was used in vitro experiments, the effects of type I interferon subtypes (e.g., interferon-alpha) remain to be explored, and the source of interferon within the tumor microenvironment responsible for the formation of this CAF subtype has not been elucidated. Fourth, we lack in vivo animal experiments to directly verify that interferon-induced MP4-high-CAF promotes immune cell infiltration and suppresses tumor growth in living organisms. Finally, the datasets used for model construction and validation are limited in number and ethnic diversity, which may restrict the model's applicability across different populations.

Future research directions include exploring combination strategies of interferon induction with immune checkpoint inhibitors or chemotherapy to enhance anti-tumor immunity in HGSOC. Preclinical studies using patient-derived xenografts or immunocompetent mouse models are needed to validate the in vivo function of MP4-high-CAF and the therapeutic efficacy of interferon-based regimens. Additional single-cell datasets from multi-center cohorts will help refine the CAF subtype definition and improve the robustness of the prognostic model. Elucidating the crosstalk between MP4-high-CAF and other TME components will further uncover regulatory mechanisms and identify actionable targets for ovarian cancer immunotherapy.

Conclusion

In conclusion, our study identified a novel interferon-responsive MP4-high-CAF subtype in HGSOC, which can mediate immune cell chemotaxis and is associated with favorable prognosis. We confirmed that this subtype can be induced by interferon, suggesting the potential value of interferon in ovarian cancer treatment. Meanwhile, the prognostic model constructed based on MP4-high-CAF signature genes shows good predictive efficiency. This study expands the cognition of CAF heterogeneity in ovarian cancer, provides new biomarkers and therapeutic targets, and offers new ideas for improving the prognosis and treatment of HGSOC patients.

Abbreviations

CAF cancer-associated fibroblasts

HGSOC high-grade serous ovarian carcinoma

IFN interferon

ifnCAF interferon response CAF

ISGs interferon-stimulated genes

NACT neoadjuvant chemotherapy

PARPi Poly ADP-ribose polymerase inhibitors

TME tumor microenvironment

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors agree to publish.

Competing interest

The authors declare that they have no competing interest for this work.

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Author contributions

YS designed experiments; TH, and RX curated and analyzed data curation; YS, and WS did data analysis and wrote the original draft; WP, BX, and WJ oversaw conceptualization, supervision and funding acquisition.

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Not available.

Data availability

All datasets involved in this study have been made publicly available. The TCGA-OV sequencing data used in this article can be accessed openly via the TCGA database. Datasets with accession numbers GSE49997, GSE26712, GSE26193, GSE156699, GSE143897, GSE164088, GSE83848 and GSE3400 are openly available through the GEO database. The single-cell RNA sequencing (scRNA-seq) data were obtained from the GEO database and GSA-Human database (accession numbers: GSE184880, PRJCA005422). The spatial transcriptomics data involved in this study were derived from 10x Genomics accession CytAssist_11mm_FFPE_Human_Ovarian_Carcinoma and GSE189843. All data used in the study are fully accessible within this article.

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Figures

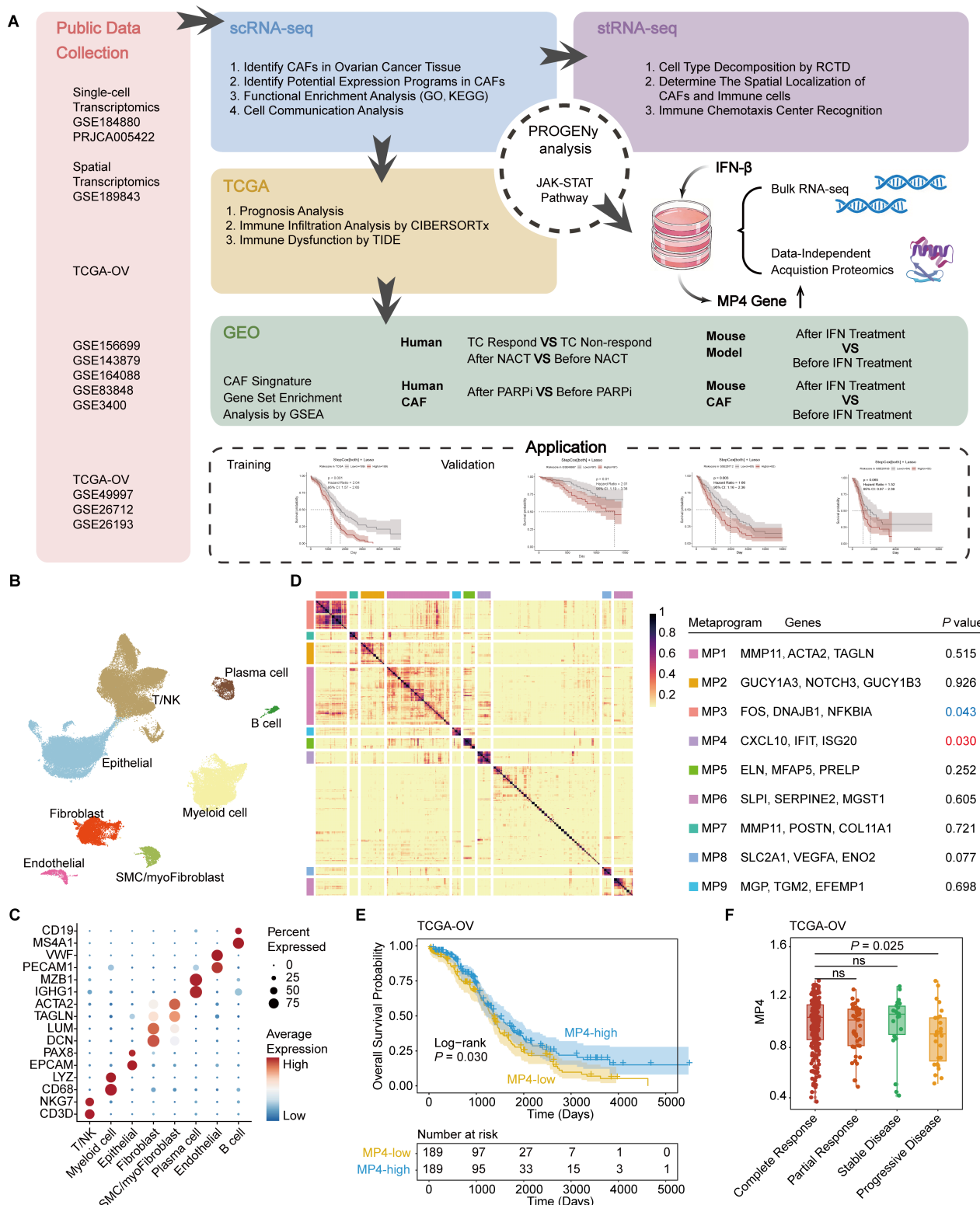


Figure 1

Identification of Recurrent Gene Modules in CAFs.

(A) A flowchart of this study, illustrating the research design and process.

(B) UMAP plot depicting the major cell types in HGSOV from two scRNA-seq datasets.

(C) Dot plots showing the expression levels of canonical cell-type marker genes in each cell type. The size of dots indicates the proportion of cells expressing the particular marker gene. The spectrum of color represents the mean expression levels of the marker genes.

(D) The heatmap on the left displays the NMF analysis of CAF to identify gene modules. The right bar indicates the significance of the overlap with consensus modules (hypergeometric test P value). The table on the right list representative genes within each metaprogram (MP1-MP9). P values reflect prognostic differences between patient subgroups stratified by median enrichment scores of the nine gene modules in the TCGA-OV transcriptomic cohort. High expression of MP3 or MP4-related genes is associated with favorable and poor prognosis, respectively.

(E) Kaplan-Meier survival curves stratified by MP4 signature in TCGA-OV cohort.

(F) The box plot comparing MP4 signature expression between patients achieving complete remission versus those experiencing disease progression following standard chemotherapy.

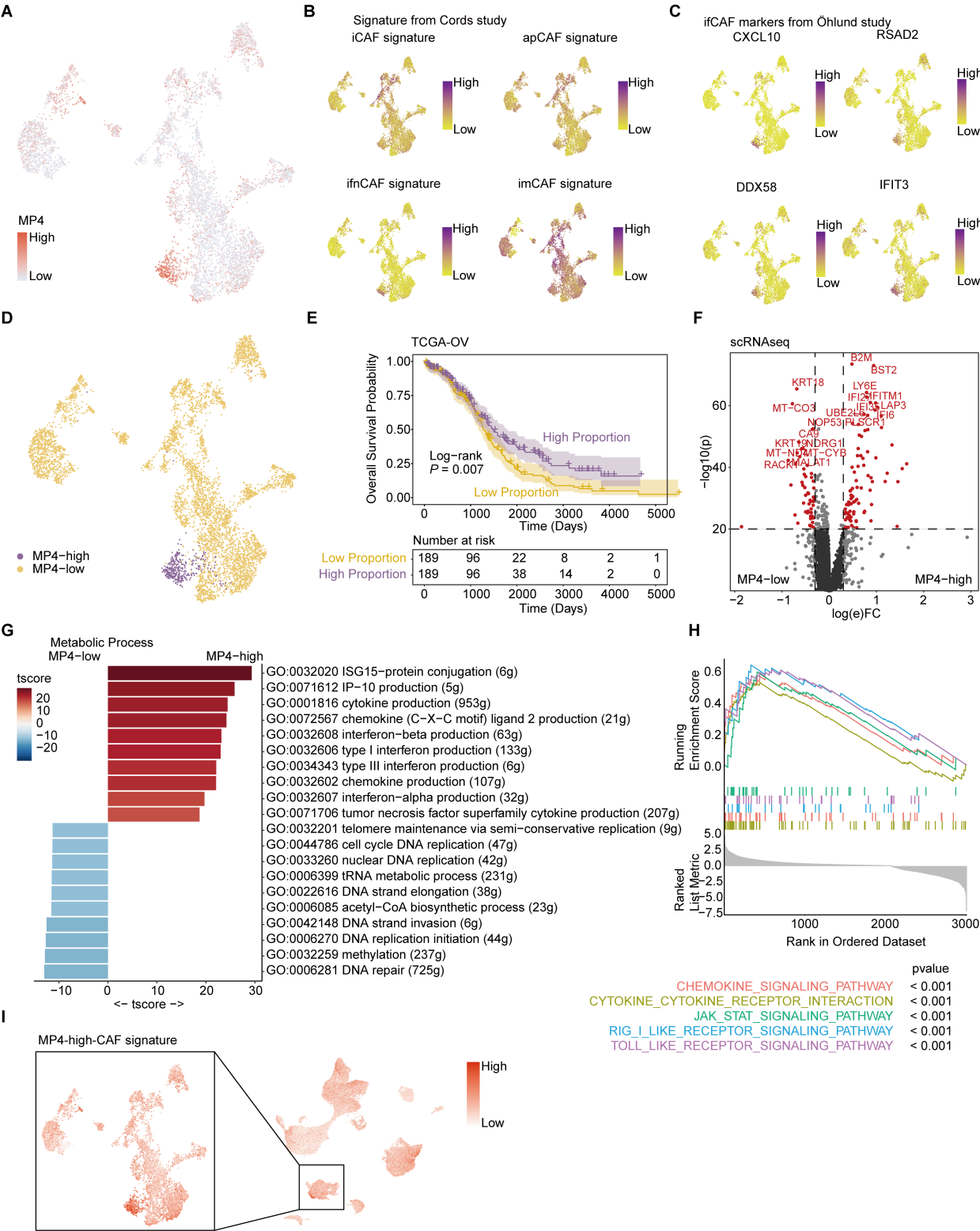


Figure 2

Characteristics of CAFs with High MP4 Signature Expression.

- (A) UMAP visualization showing the value of MP4 signature.
- (B) UMAP visualization showing the value of CAF subtype signature, linked to immune response processes, as reported in previous studies on other types of cancer.
- (C) UMAP visualization showing the expression of marker genes for interferon-responsive CAFs identified in the Daniel Öhlund et al.'s study.
- (D) UMAP distribution of MP4-high-CAF and MP4-low-CAF in CAF subcluster.
- (E) The Kaplan-Meier curve comparing the probability of survival according to MP4-high-CAF proportion in TCGA-OV cohort. Patients were dichotomized into two groups based on the score distribution: the high-score group comprised the top half of patients with the highest scores, while the low-score group included the remaining. *P* values were calculated with log-rank test.
- (F) Volcano plot displays the top ten different expression gene between two sets of CAF. The FindAllMarker method was used to extract marker genes for MP4-high-CAF across all cell types.
- (G) The activities in regulation of vasculature development in between MP4-High-CAF and MP4-Low-CAF cells. Color indicates the T score scaled value.
- (H) GSEA enrichment in MP4-high-CAF compared to MP4-low-CAF. *P* values respective to each pathway are shown bottom.
- (I) UMAP visualization showing the value of MP4-high-CAF signature in all scRNA cluster (right) and CAF subcluter (left).

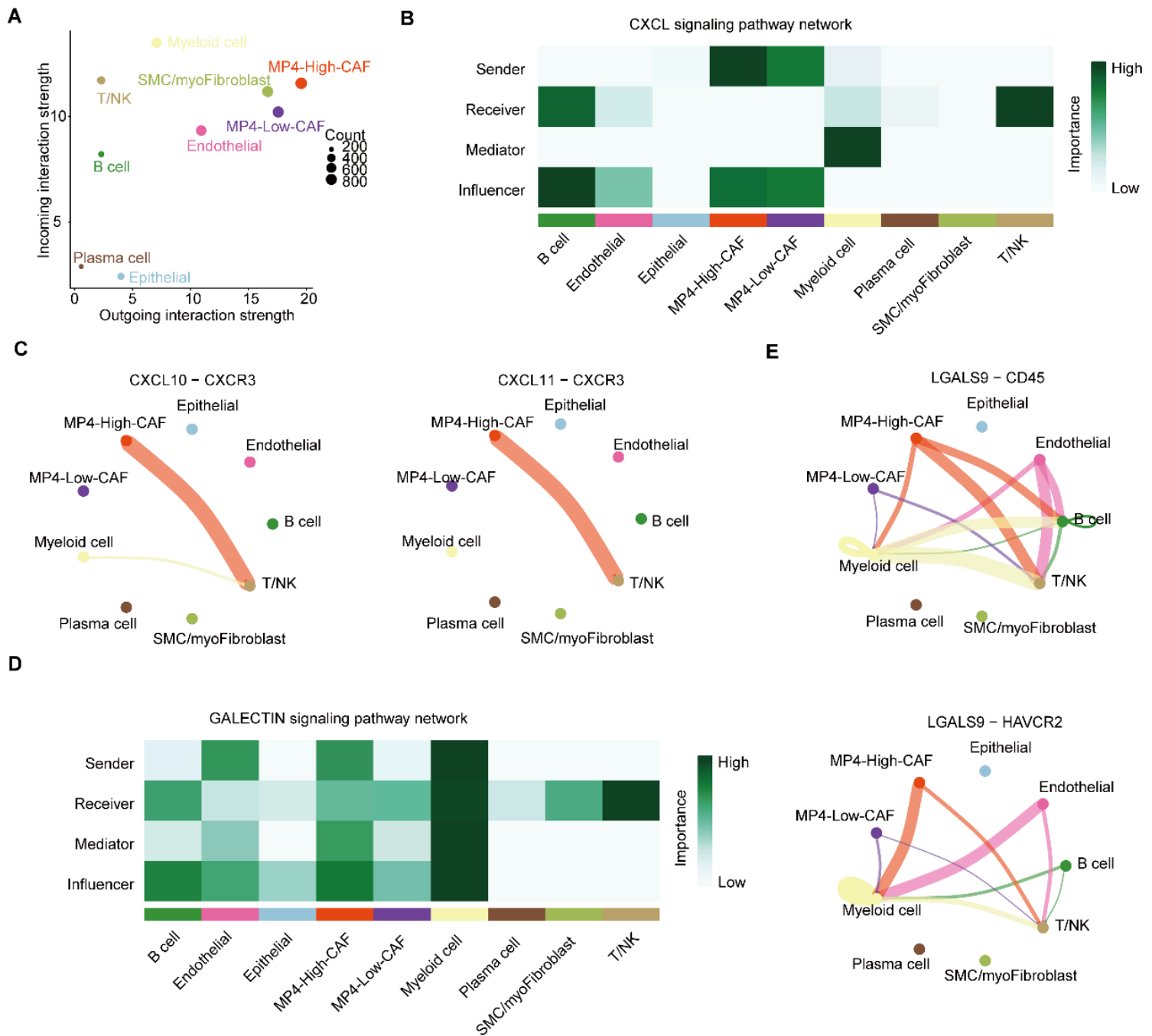


Figure 3

MP4-high-CAF exhibits stronger communication with immune cells than MP4-low-CAF.

(A) Scatter plots showing interaction intensity with other cells in the tumor microenvironment, both as signal senders and receivers.

(B) Overview of intercellular communication network for CXCL signaling pathway. Relative importance of each cell group based on four network centrality measures are shown.

(C) Cellular communication network of CXCL10-CXCR3 (left) and CXCL11-CXCR3 (right) signaling pathway among TME.

(D) Overview of intercellular communication network for GALECTIN signaling pathway. Relative importance of each cell group based on four network centrality measures are shown.

(E) Cellular communication network of LGALS9-CD45 (up) and LGALS9-HAVCR2 (bottom) signaling pathway among TME.

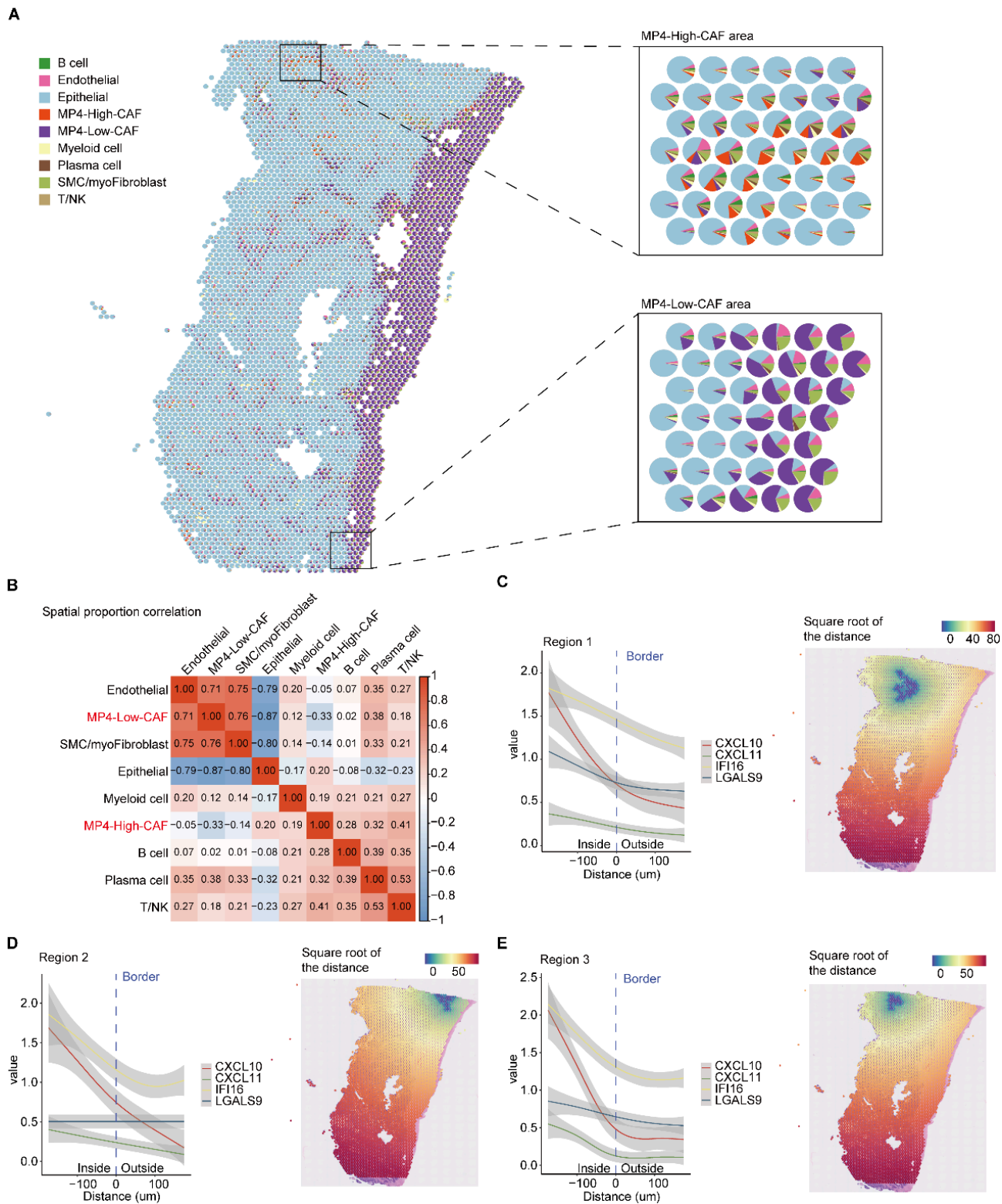


Figure 4

MP4-high-CAF tends to localize within the tumor.

(A) Spatial transcriptomics deconvolution annotations showing cellular composition. Two representative regions of MP4-high-CAF (up) and MP4-low-CAF (bottom) enriched area are shown.

(B) Heatmap showing Pearson's correlation coefficients among cell type proportion in each spatial spot within slide.

(C–E) Spatial expression pattern (left) of chemokines genes and spatial distribution of distance (right) from regions (C: region 1, D: region 2, E: region 3) within slide.

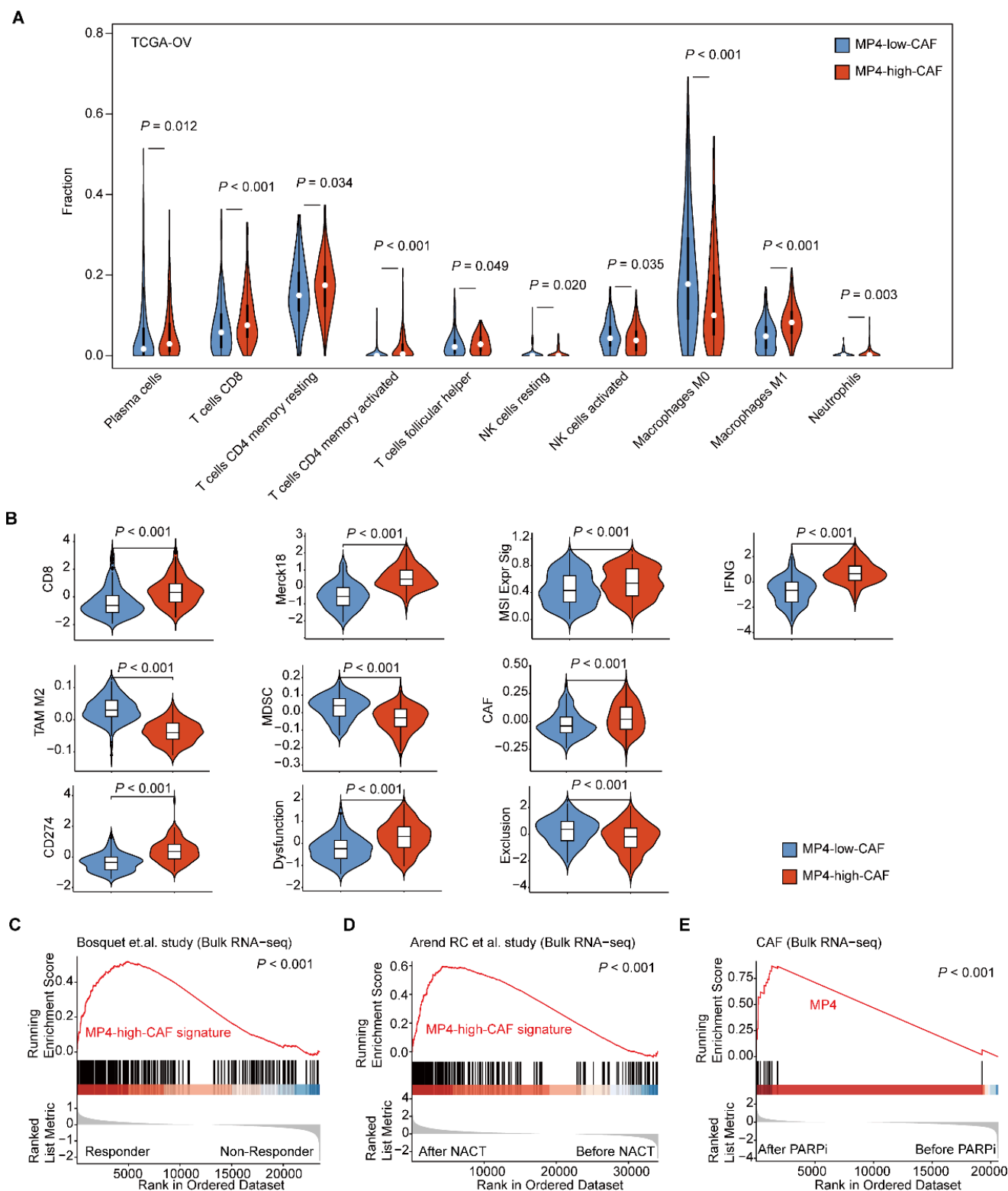


Figure 5

Patients with high MP4-high-CAF signature display more prominent immune infiltration.

(A) Violin plots of CIBERSORT tumor microenvironment signature scores derived from bulk RNA-seq profiling of TCGA-OV cohort. Intergroup comparisons between MP4-high-CAF and MP4-low-CAF patients were performed using wilcoxTest.

(B) Violin plots of TIDE tumor microenvironment signature scores derived from bulk RNA-seq profiling of TCGA-OV cohort. Intergroup comparisons between MP4-high-CAF and MP4-low-CAF patients were performed using wilcoxTest.

(C) MP4-high-CAF transcriptional signature GSEA enrichment in responder compared to non-responder.

(D) MP4-high-CAF transcriptional signature GSEA enrichment in post-NACT patients compared to pre-NACT patients.

(E) MP4 transcriptional signature GSEA enrichment in post-PARPi compared to pre-PARPi CAF cell line.

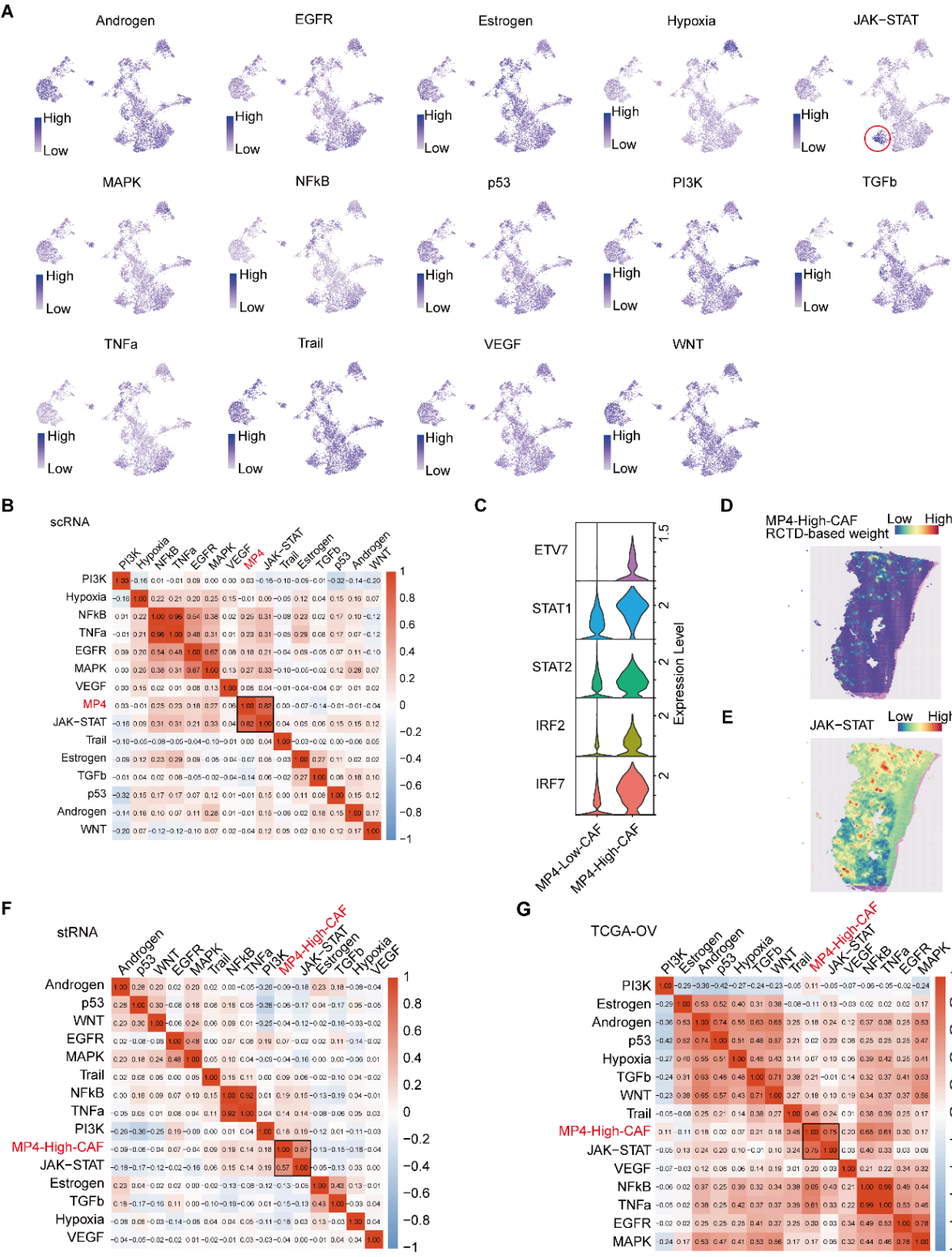


Figure 6

MP4-high-CAF shows enhanced JAK-STAT pathway activity.

(A) UMAP visualization showing the activity of PROGENy pathway signatures across CAF subclusters. Red circle indicates cell populations with high JAK-STAT pathway activity.

(B) Heatmap showing Pearson's correlation coefficients among PROGENy pathway signatures and MP4 score in single-cell dataset of CAF.

(C) Violin plots comparing expression of core JAK-STAT pathway genes in MP4-high-CAF and MP4-low-CAF.

(D-E) Spatial expression pattern of MP4-high-CAF proportion (D) and JAK-STAT (E) activity within slide.

(F) Heatmap showing Pearson's correlation coefficients among MP4-high-CAF proportion and PROGENy pathway signatures in each spatial spot within slide.

(G) Heatmap showing Pearson's correlation coefficients among MP4-high-CAF proportion and PROGENy pathway signatures in each patient of TCGA-OV cohort.

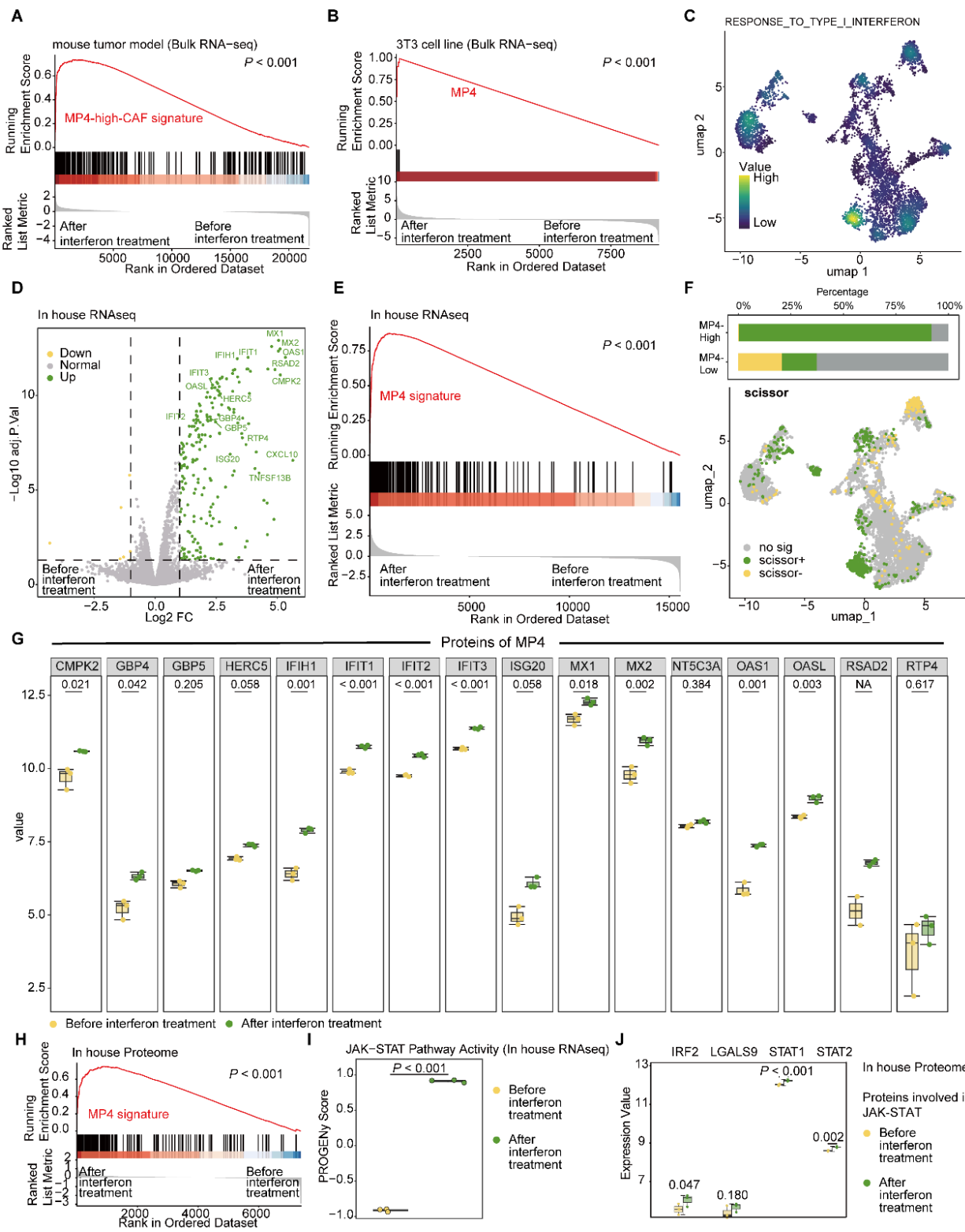


Figure 7

Type I interferon induces the MP4-high-CAF signature and activates the JAK-STAT pathway in fibroblast.

- (A) MP4-high-CAF transcriptional signature GSEA enrichment in post-interferon mouse models compared to pre-interferon.
- (B) MP4 transcriptional signature GSEA enrichment in post-interferon mouse fibroblast cell line compared to pre-interferon.
- (C) UMAP visualization showing the activity of response-to-type-I-interferon signatures across CAF subclusters.
- (D) Volcano plot of transcriptional data showing differentially expressed genes between pre- and post-interferon CAF cell line, with up-regulated genes annotated by green color.
- (E) MP4 transcriptional signature GSEA enrichment in post-interferon CAF cell line compared to pre-interferon.
- (F) UMAP distribution of scissor-annotated cluster in CAF subcluster. Composition of scissor+ and scissor- cells in MP4-high-CAFs and MP4-low-CAFs (up).
- (G) Box-and-scatter plots of expression in MP4 proteins derived from proteomic profiling of CAF cell line. Intergroup comparisons between pre- and post-interferon CAF were performed using empirical Bayes moderated t-test.
- (H) MP4 proteomic signature GSEA enrichment in post-interferon CAF cell line compared to pre-interferon.
- (I, J) Box-and-scatter plots of expression in JAK-STAT genes (I) and proteins (J) of CAF cell line. Intergroup comparisons between pre- and post-interferon CAF were performed using empirical Bayes moderated t-test.

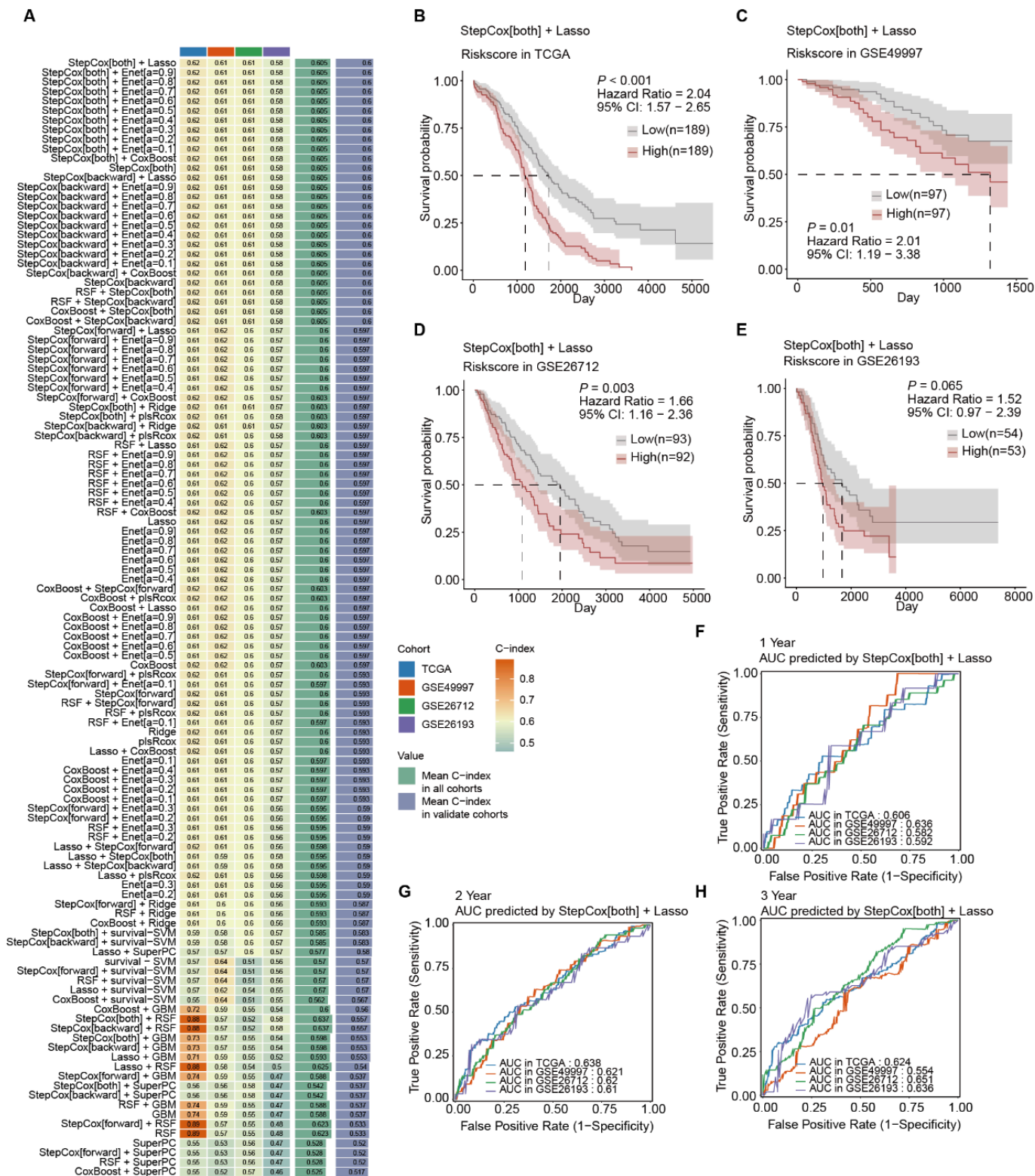


Figure 8

Prognosis Model Development.

(A) Four epithelial ovarian cancer cohorts were utilized for constructing and validating the prognostic model, with the StepCox[both] + Lasso model demonstrating the most superior performance among numerous models.

(B, C, D, E) The StepCox[both] + Lasso model can accurately classify patients across TCGA_OV(B), GSE49997(C), GSE26712(D), GSE26193(E) into high-risk and low-risk groups, with patients in the high-risk group exhibiting poor prognosis.

(F, G, H) Three ROC curves show the AUC values predicted by the StepCox[both] + Lasso model for the four cohorts at 1-year(F), 2-year(G), and 3-year(H) survival times.

Supplementary Files

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