

Supplementary Materials for
Single-cell analysis reveals the stromal dynamics and tumor-specific
characteristics in the microenvironment of ovarian cancer

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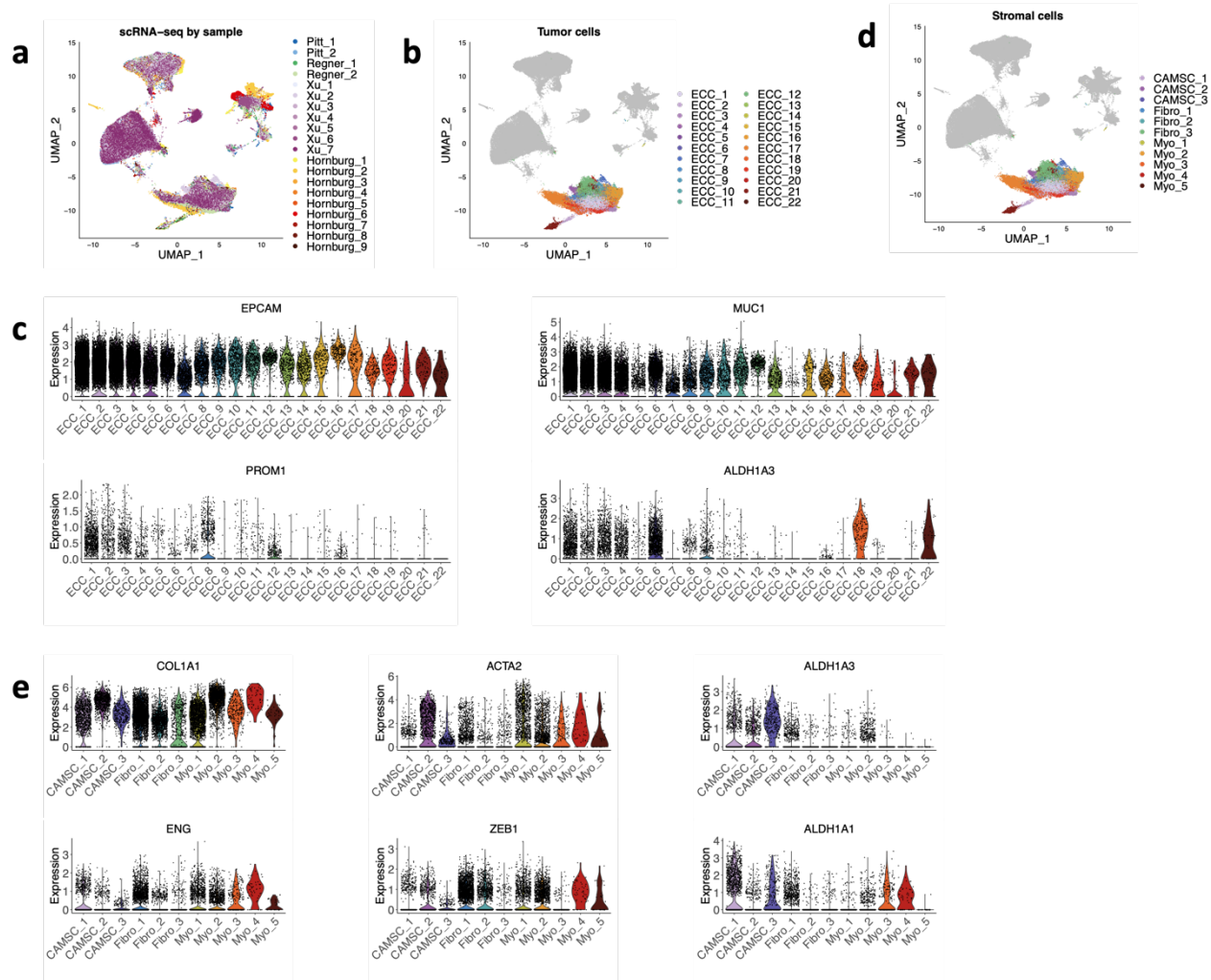


Figure S1. Extended view of HGSOc samples. **a** Uniform manifold approximation and projection (UMAP) clustering of the integrated scRNA-seq data, colored by sample. Each dot represents a single cell. **b** UMAP plot of subclusters of tumor cells. **c** Violin plots showing the expression of *EPCAM*, *MUC1*, *PROM1*, and *ALDH1A3* in tumor cells by subcluster. **d** UMAP plot of subclusters of stromal cells. **e** Violin plots showing the expression of *COL1A1*, *ACTA2*, *ALDH1A3*, *ENG*, *ZEB1*, and *ALDH1A1* in stromal cells by subcluster.

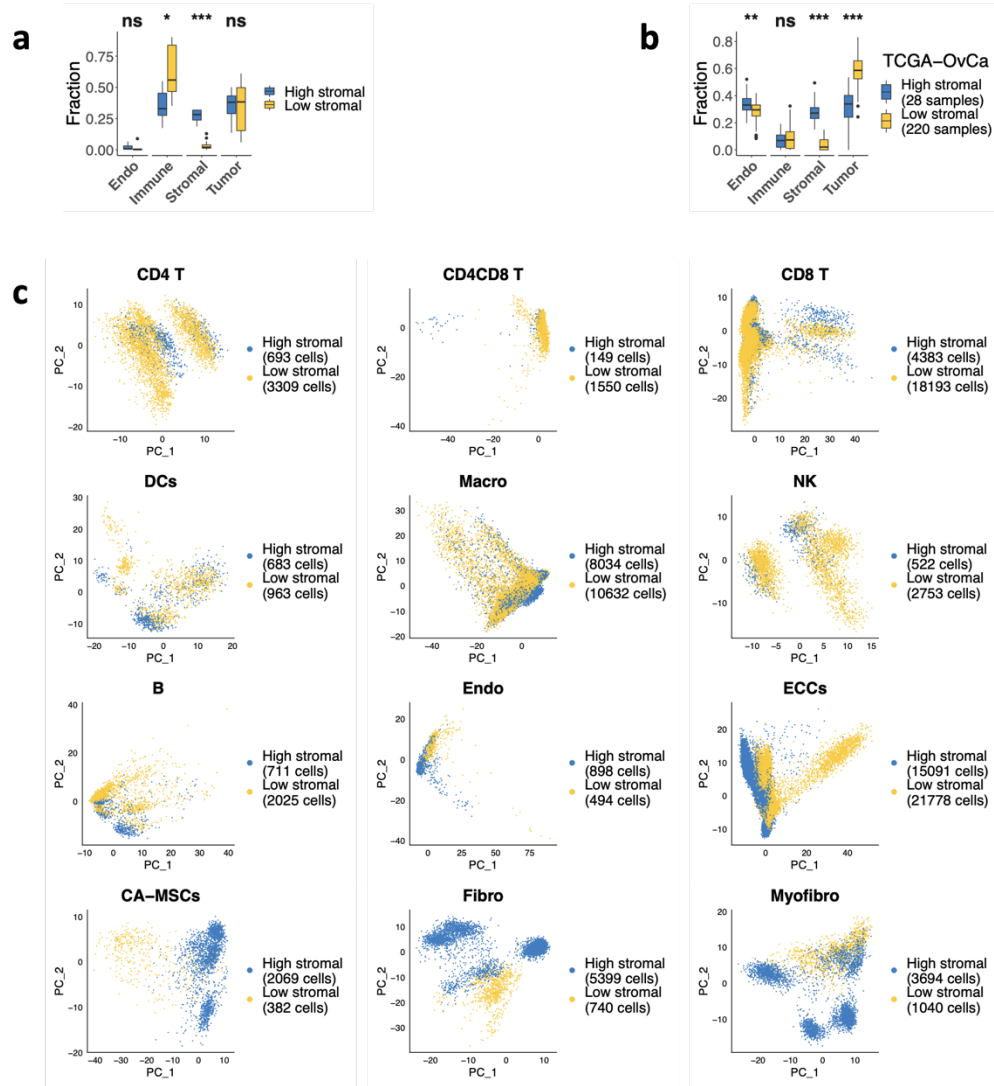


Figure S2. Different landscapes between high- and low-stromal tumors. **a** Box plot showing the fractions of endothelial, immune, stromal, and tumor cells in each sample, colored by tumor group (high-/low-stromal groups). The p -values are computed from the two-sided Wilcoxon signed-rank test between the two groups for each cell type. Statistical significance is coded by the following symbols: . p -value < 0.1 , * p -value < 0.05 , ** p -value < 0.01 , and *** p -value < 0.001 . **b** Principal component analysis (PCA) and scatter plots along the top 2 PCs for each cell type. Each dot represents a single cell. **c** Box plot showing the inferred cell type fractions in each sample of the TCGA-OvCa dataset, colored by tumor group.

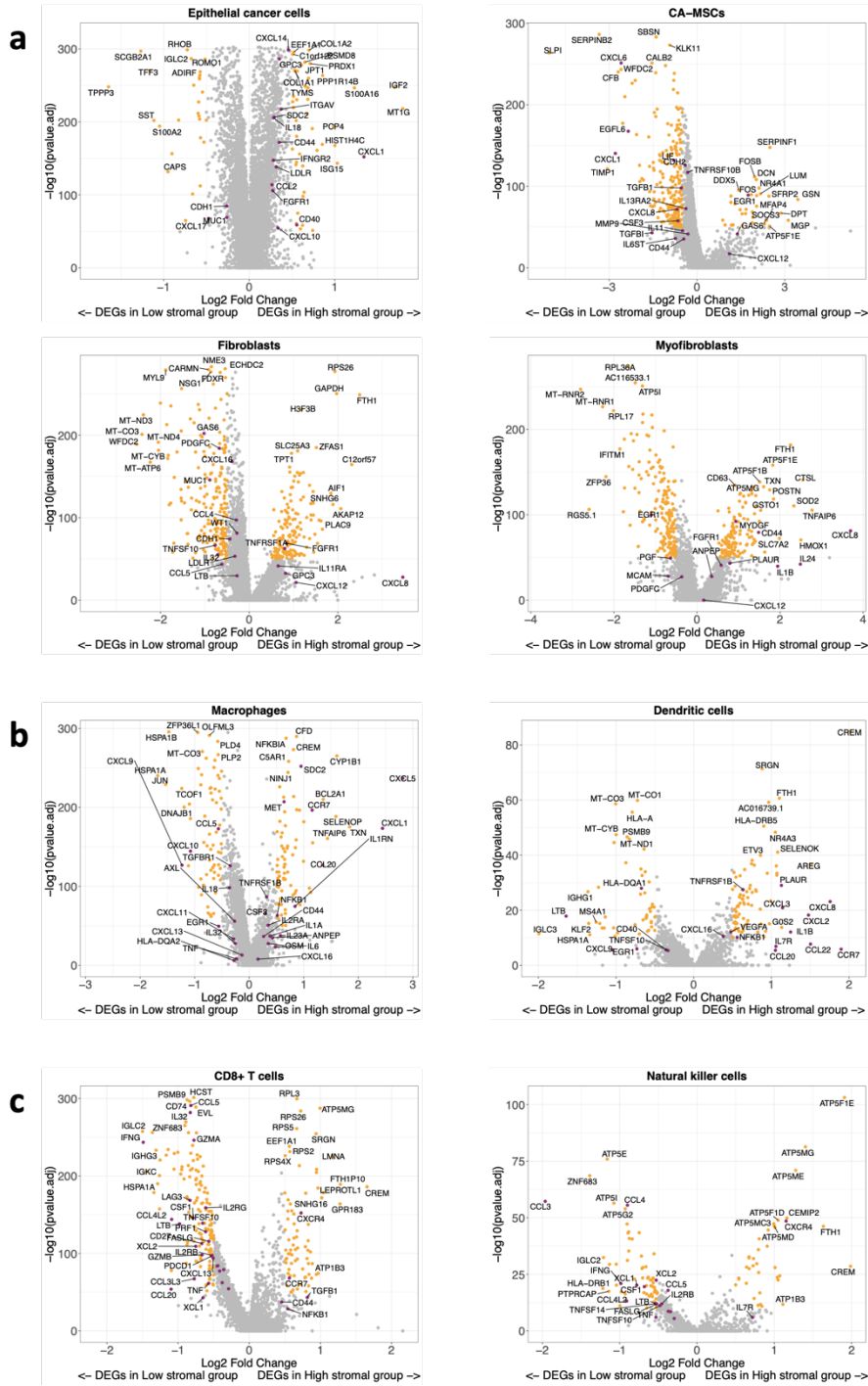


Figure S3. Differentially expressed genes. **a** Volcano plots showing differentially expressed genes (DEGs) in epithelial cancer cells, CA-MSCs, fibroblasts, and myofibroblasts between high- and low-stromal groups. DEGs (colored in orange) were identified using the Seurat's *FindMarkers* function. In particular, differentially expressed cytokines and surface proteins are colored in purple. Genes with positive log2 fold change are upregulated in the high-stromal group, and vice versa. **b** Volcano plots showing DEGs in macrophages and dendritic cells. **c** Volcano plots showing DEGs in CD8⁺ T cells and natural killer cells.

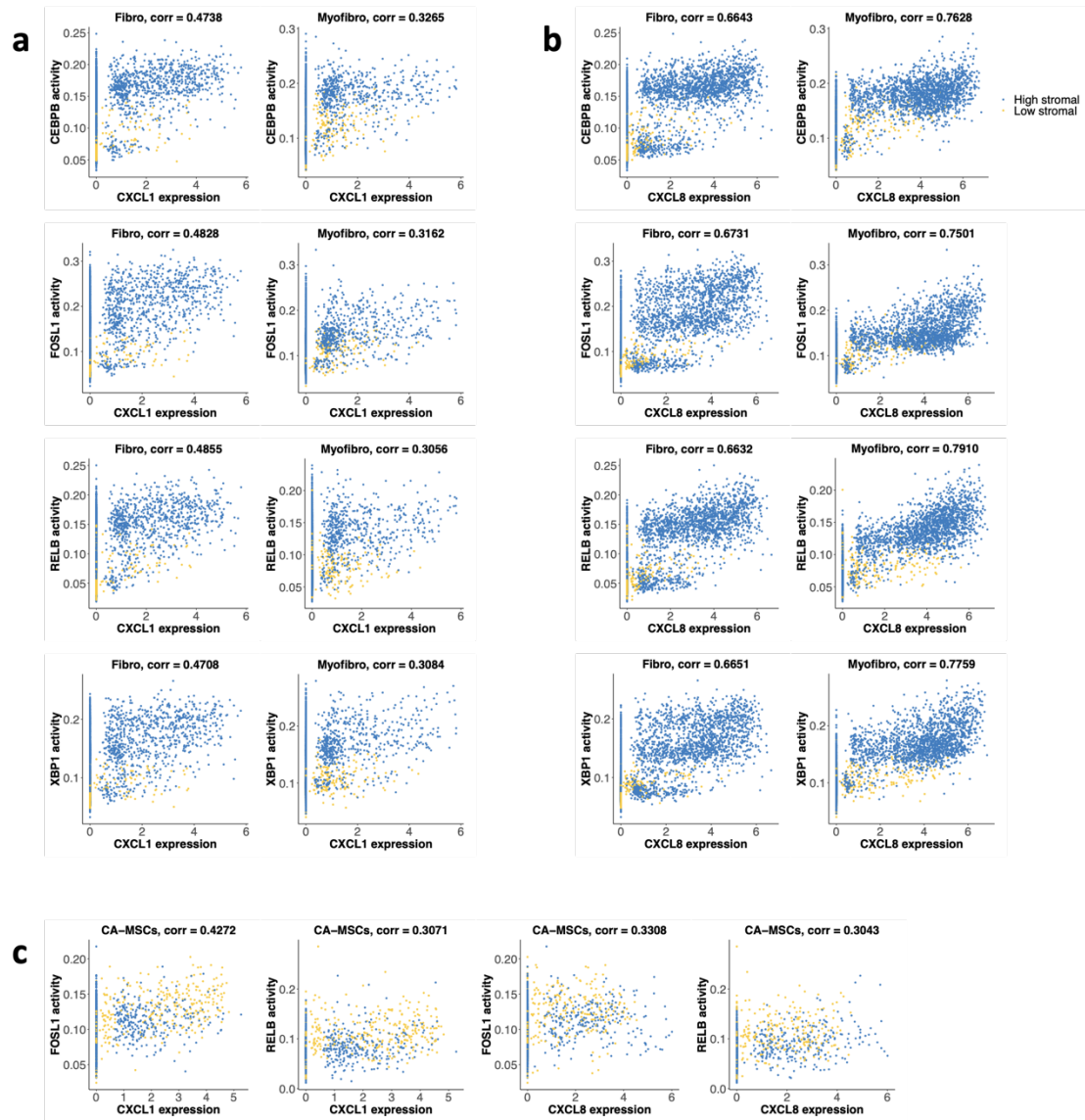


Figure S4. Highly correlated gene-TF pairs in stromal population. **a** Scatter plots of selected highly correlated *CXCL1*-TF pairs and **b** *CXCL8*-TF for fibroblasts and myofibroblasts. Each dot represents a cell, colored by the tumor group. **c** Scatter plots of selected highly correlated *CXCL1*-TF and *CXCL8*-TF pairs for CA-MSCs.

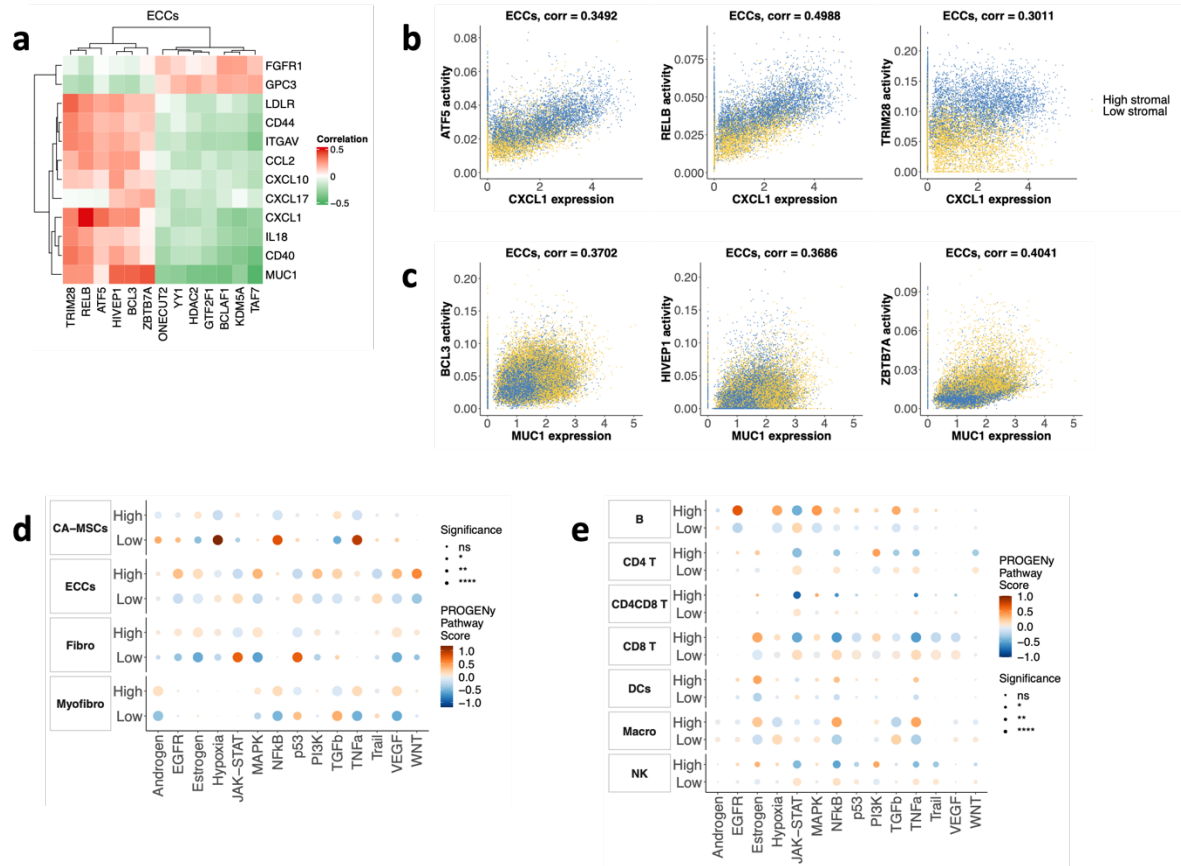


Figure S5. Transcription factor activity analysis and pathway analysis. **a** Heatmap revealing correlations between inferred TF activities (columns) and surface protein (SP)/cytokine expression (rows) in epithelial cancer cells (ECCs). For clarity, highly correlated SPs/cytokines-TF pairs were selected (**Table S4**). **b** Scatter plots of selected highly correlated *CXCL1*-TF pairs and **c** *MUC1*-TF pairs for ECCs. Each dot represents a cell, colored by the tumor group. **d** **e** PROGENy pathway score by cell type. For each pathway signaled in each cell type, the two-sided Wilcoxon test is applied between the high- and low-stromal groups; the resulting *p*-values are adjusted based on Bonferroni correction using all pathways in the dataset. Adjusted *p*-values are indicated by circle size.

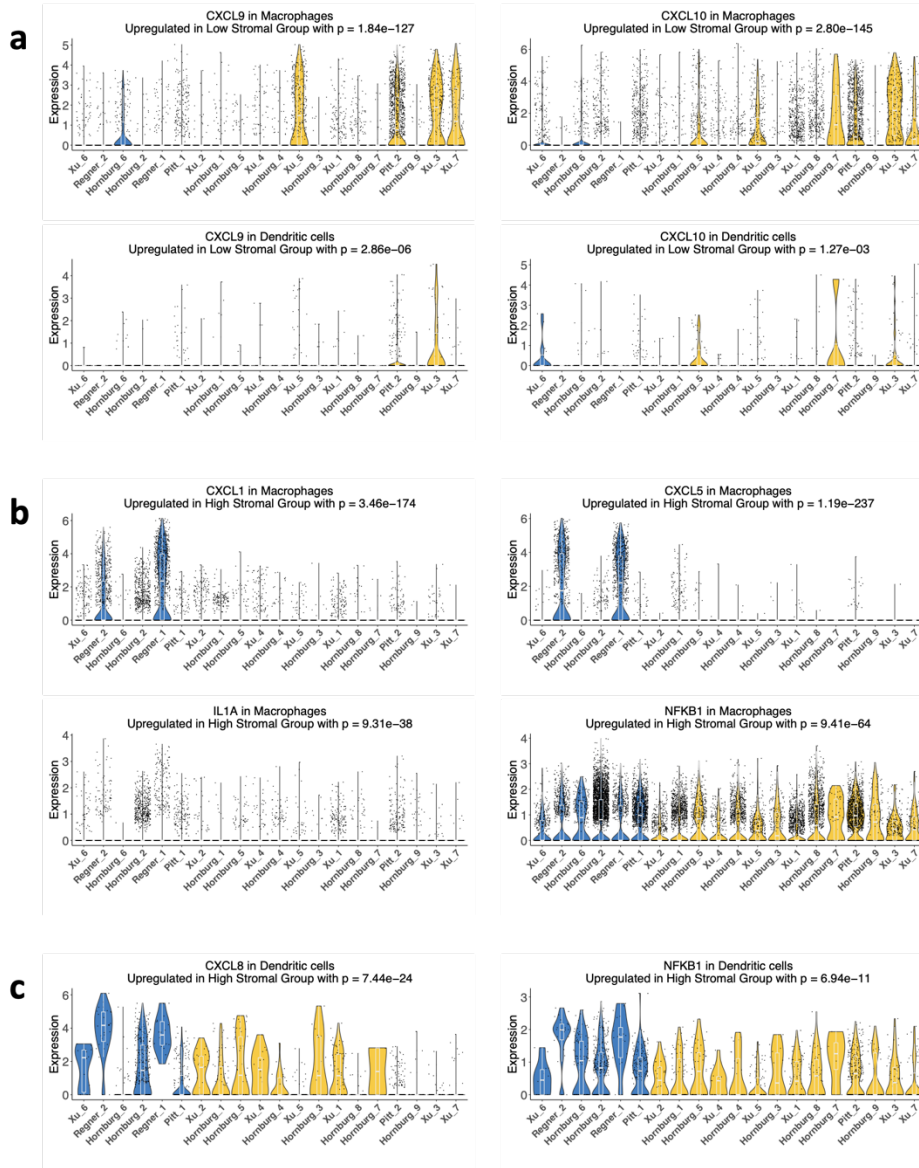
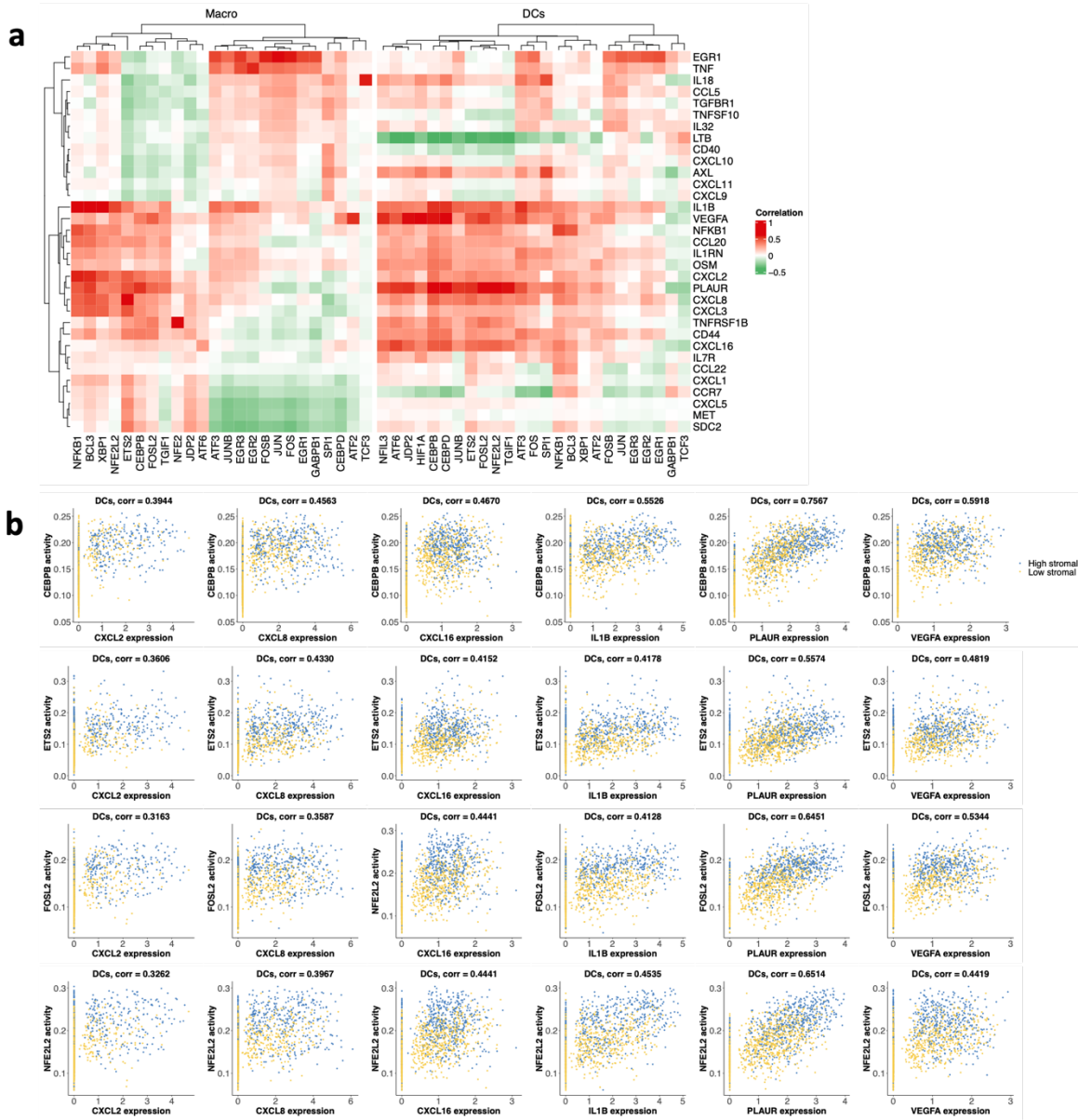


Figure S6. Differentially expressed genes of macrophages and dendritic cells. **a** Violin plots showing the expression of *CXCL9* and *CXCL10* in macrophages and dendritic cells, **b** the expression of *CXCL1*, *CXCL5*, *IL1A*, and *NFKB1* in macrophages, and **c** the expression of *CXCL8* and *NFKB1* in dendritic cells by sample. Each sample is color-coded by high- (blue) or low- (yellow) stromal group. The p -values are computed from the two-sided Wilcoxon tests, adjusted based on Bonferroni correction using all genes in the dataset. Statistical significance is coded by the following symbols: . p -value < 0.1, * p -value < 0.05, ** p -value < 0.01, and *** p -value < 0.001.



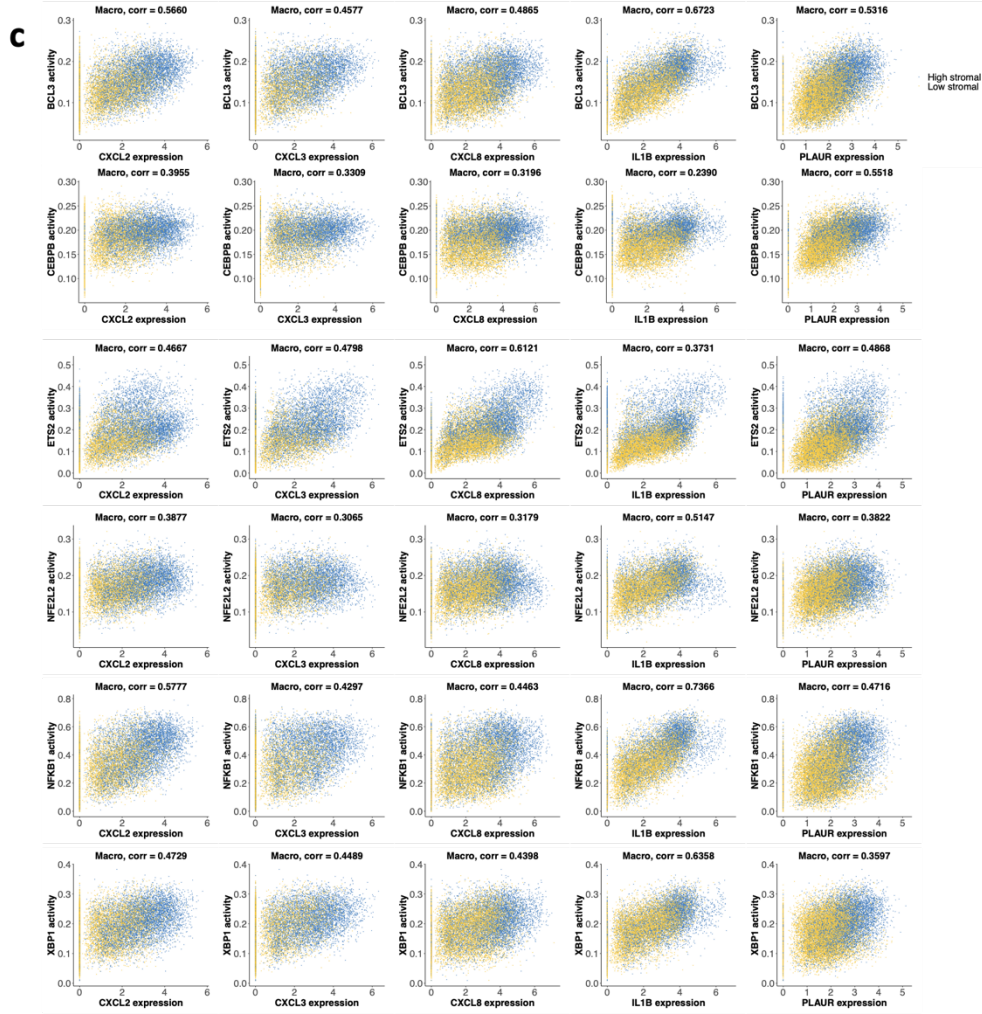
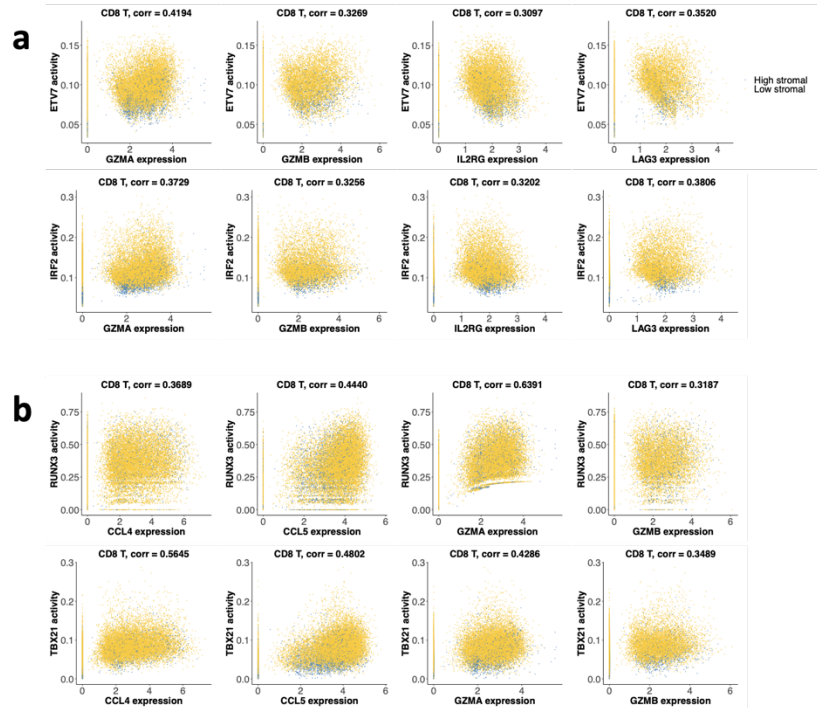


Figure S7. Highly correlated gene-TF pairs in macrophages and dendritic cells. **a** Heatmap revealing correlations between inferred TF activities (columns) and SP/cytokine expression (rows) in macrophages and dendritic cells (DCs). For clarity, we selected highly correlated SP/cytokine-TF pairs (**Table S4**) and used the union of the selected genes. The correlation coefficients of the top correlated TFs with each SP/cytokine are shown for each cell type. **b c** Scatter plots of selected highly correlated gene-TF pairs for each cell type. Each dot represents a cell, colored by the tumor group.



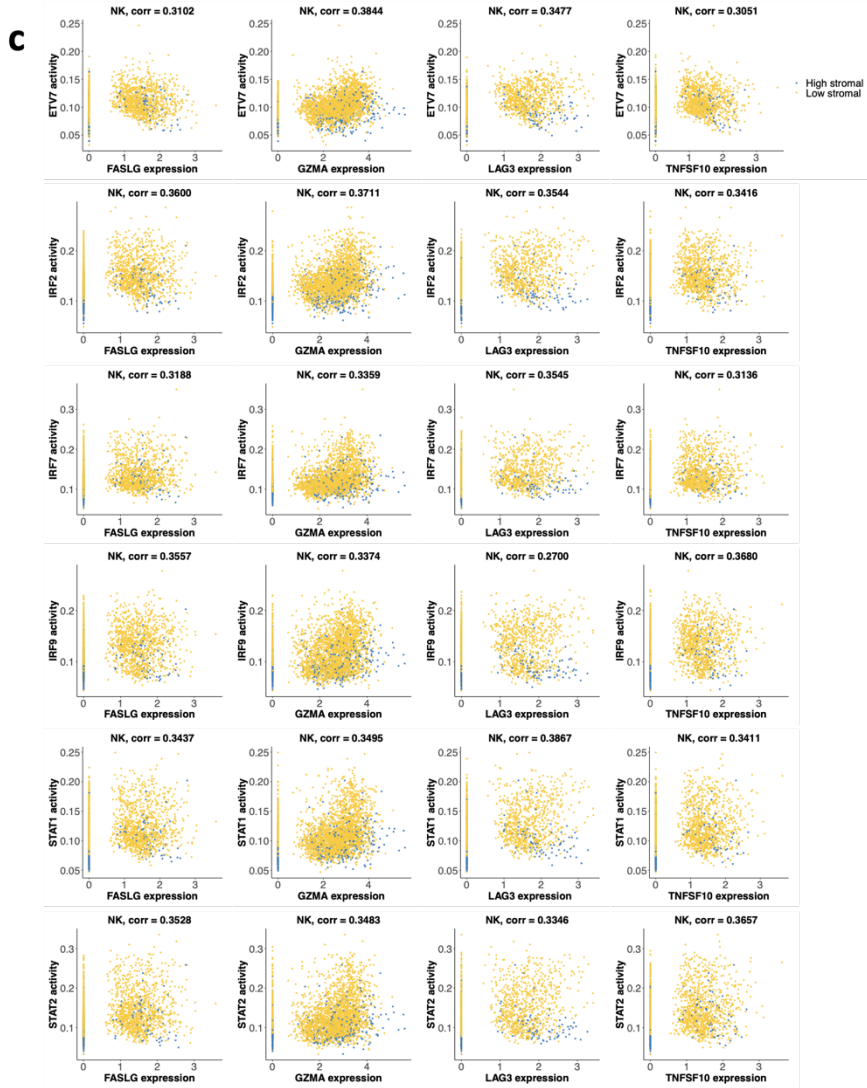


Figure S8. Highly correlated gene-TF pairs in CD8⁺ T and NK cells. **a b** Scatter plots of selected highly correlated gene-TF pairs for CD8⁺ T cells and **c** for natural killer cells (NK). Each dot represents a cell, colored by the tumor group.

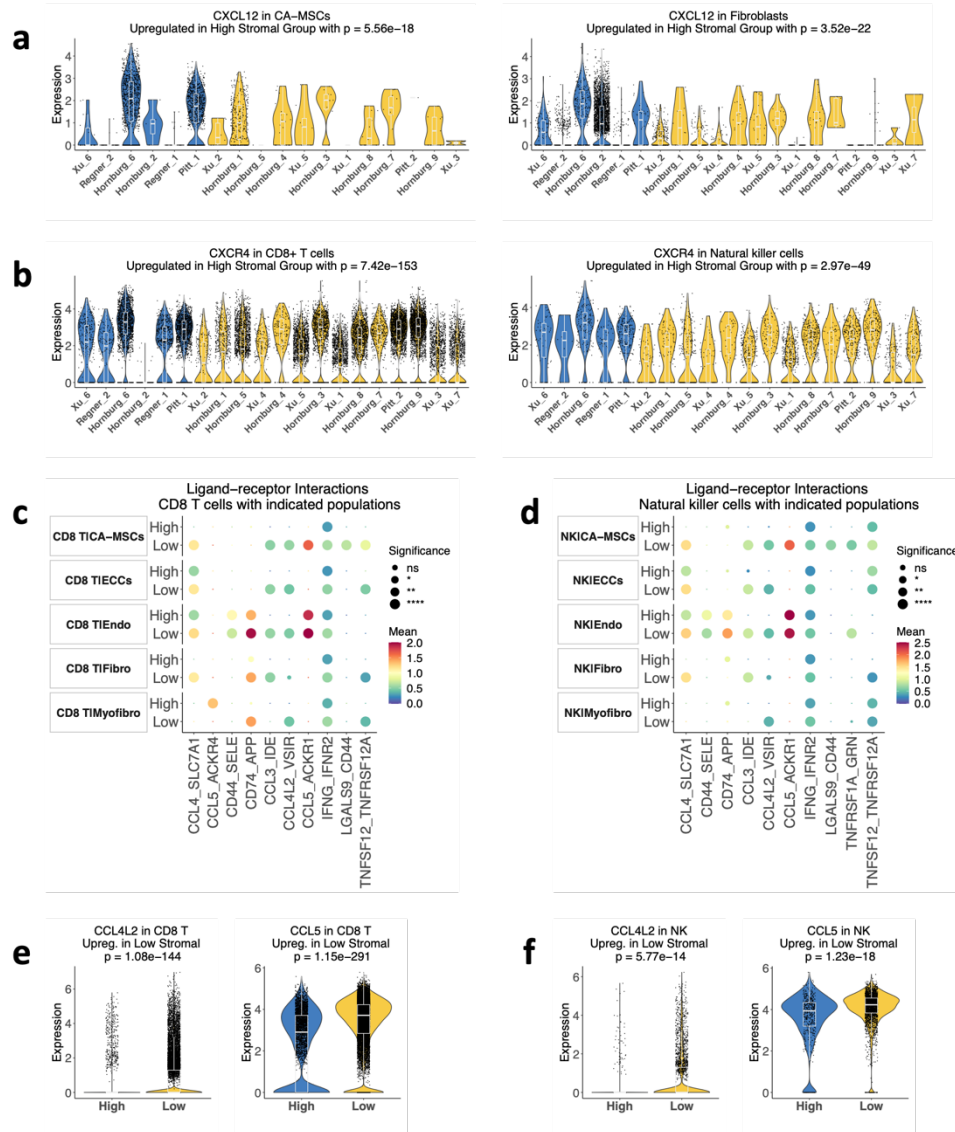


Figure S9. Cell-cell integrations between CA-MSCs/fibroblasts and CD8⁺ T/NK cells. a Violin plots showing the expression of *CXCL12* in CA-MSCs and fibroblasts by sample. Each sample is color-coded by high- (blue) or low- (yellow) stromal group. **b** Violin plots showing the expression of *CXCR4* in CD8⁺ T and natural killer (NK) cells by sample. **c d** Statistically significant interactions between CD8⁺ T/NK cells and other cell types using the CellPhoneDB pipeline. Size indicates p -values, and color indicates the means of the receptor-ligand pairs between the two tumor groups. **e f** Violin plots showing the expression of *CCL4L2* and *CCL5* in CD8⁺ T and NK cells by tumor group.

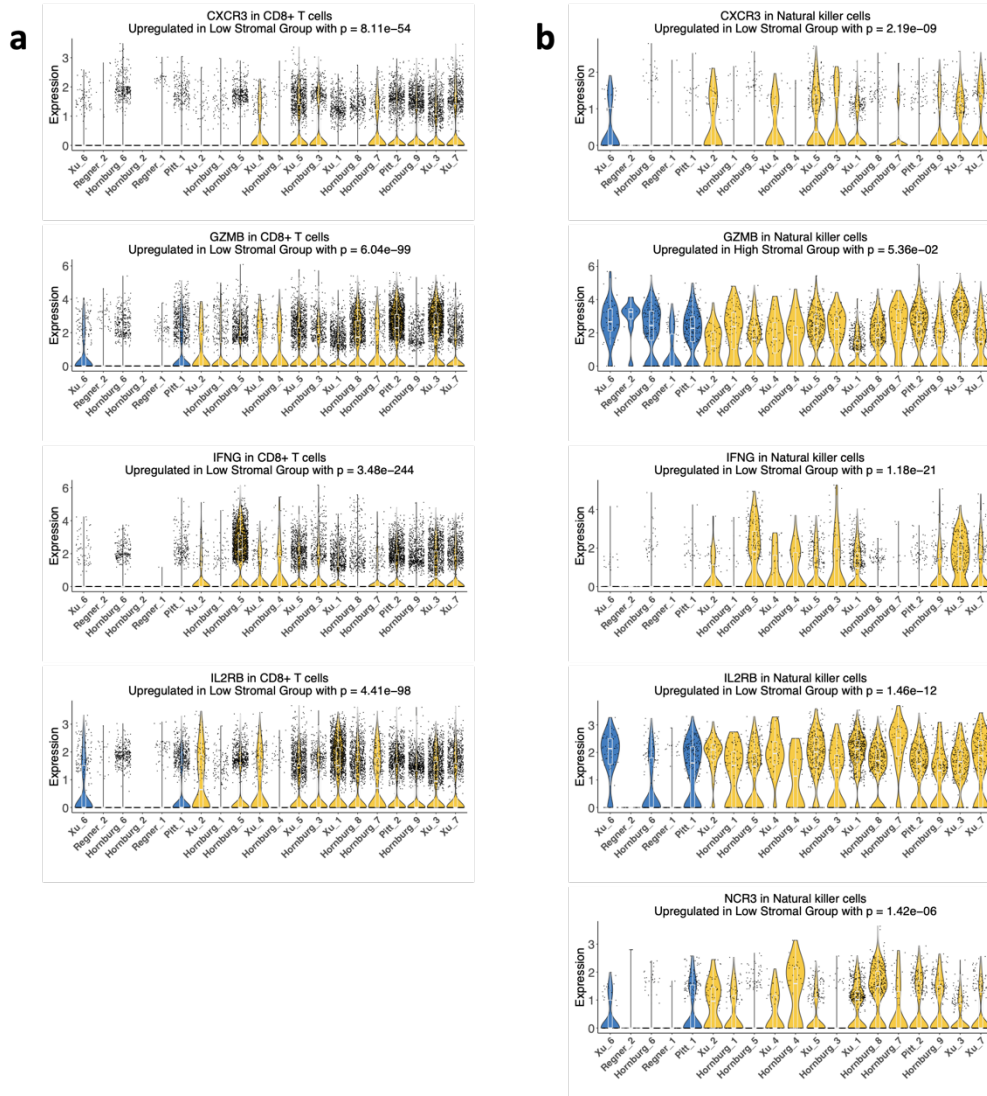
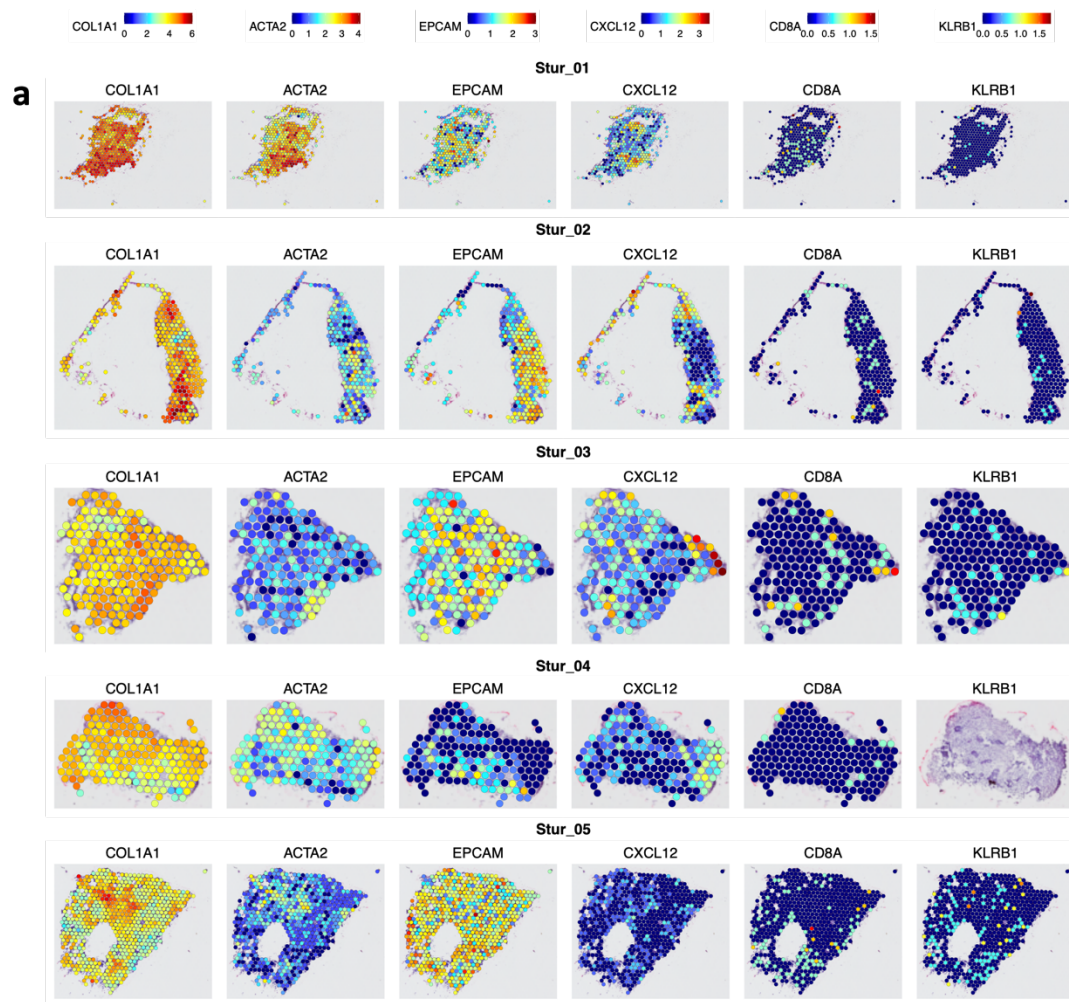


Figure S10. Expression of marker genes associated with activated CD8⁺ T and natural killer cells. a Violin plots showing the expression of *CXCR3*, *GZMB*, *IFNG*, and *IL2RB* in CD8⁺ T cells by sample. Each sample is color-coded by high- (blue) or low- (yellow) stromal group. **b** Violin plots showing the expression of *CXCR3*, *GZMB*, *IFNG*, *IL2RB*, and *NCR3* in natural killer cells by sample.



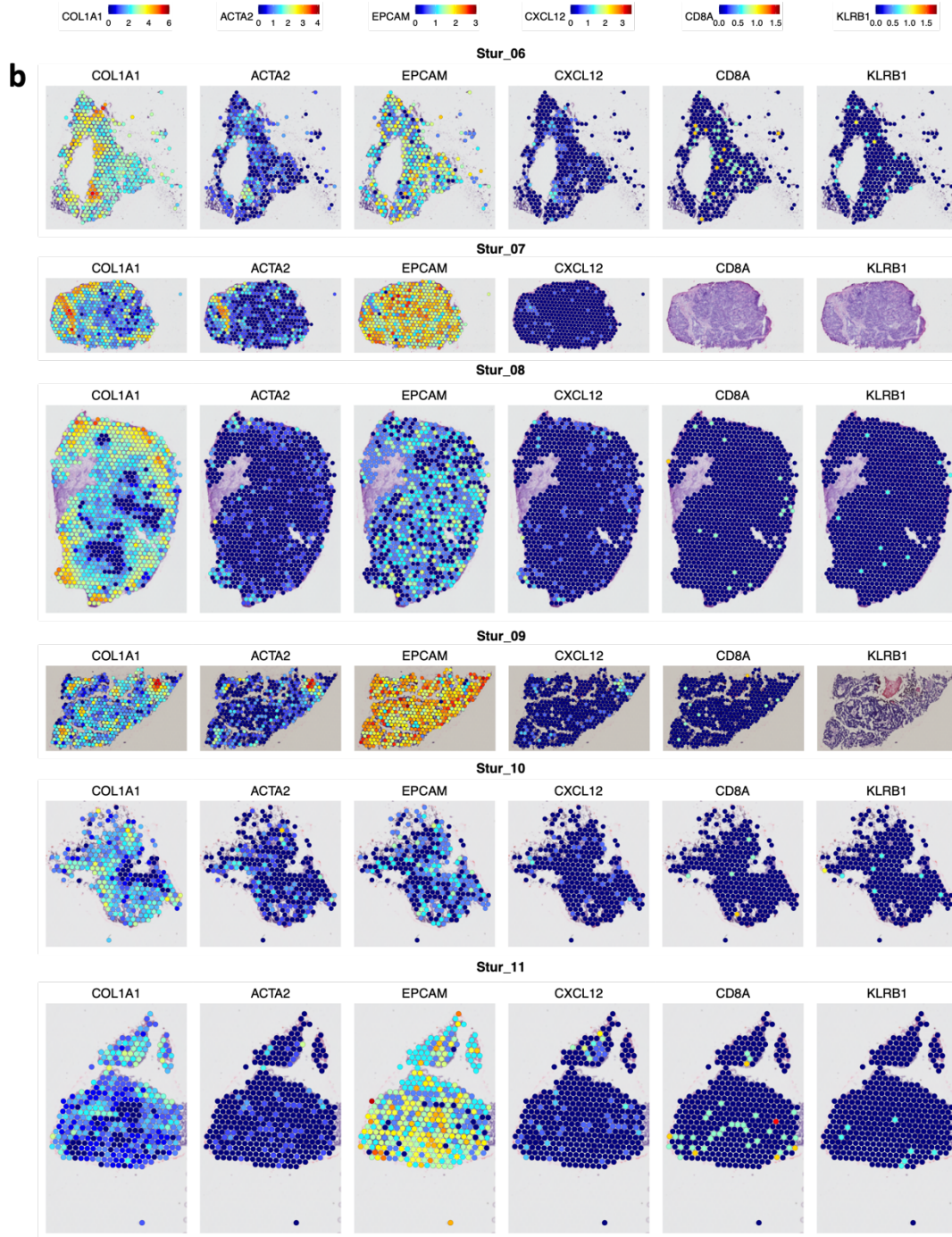


Figure S11. Spatial transcriptomics. **a** Spatial feature plots of selected marker genes of high-stromal samples and **b** low-stromal samples from the ST dataset. The samples are ordered based the average expression of COL1A1 from high (first) to low (last). Expression of the same marker across all samples shares a common color scale (tops).

Table S1. Sample and Data Information

In this study, we combined inhouse data with 3 deposited single-cell RNA sequencing (scRNA-seq) dataset. The integrated scRNA-seq dataset consists of high-grade serous ovarian carcinoma (HGSOC) samples from 20 treatment-naïve patients. We also analyzed a spatial transcriptomics (ST) dataset which consists of HGSOC samples from 11 treatment-naïve patients, and the TCGA-OV dataset (n=248).

Sample ID	Dataset Identifier and Data Type	Original Patient ID	Histology and Tumor Stage	#cells/spots used in this study
Pitt_1	Inhouse	TP18-M419	HGSOC IIIB	7,612
Pitt_2	GSE232314, scRNA-seq	TP18-M509	HGSOC IVB	10,780
Regner_1	Regner et al. ¹	Patient 8	HGSOC IIB	6,147
Regner_2	GSE173682 scRNA-seq	Patient 9	HGSOC IIIC	5,966
Xu_1	Xu et al. ² GSE184880 scRNA-seq	Cancer1	HGSOC IIIB	6,421
Xu_2		Cancer2	HGSOC IIB	3,026
Xu_3		Cancer3	HGSOC IC2	3,537
Xu_4		Cancer4	HGSOC IC2	1,604
Xu_5		Cancer5	HGSOC IIB	4,422
Xu_6		Cancer6	HGSOC IIIC	3,525
Xu_7		Cancer7	HGSOC IC2	3,890
Hornburg_1	Hornburg et al. ³ EGAS00001004935 scRNA-seq	des3	Serous IIIB	7,022
Hornburg_2		des4	HGSOC IIIC	14,589
Hornburg_3		exc2	HGSOC IIIC	1,864
Hornburg_4		exc3	HGSOC IVB	3,660
Hornburg_5		inf1	HGSOC IC	3,711
Hornburg_6		inf2	HGSOC IIIC	4,823
Hornburg_7		inf3	HGSOC, unknown	1,429
Hornburg_8		inf4	HGSOC, unknown	5,618
Hornburg_9		inf5	HGSOC IIIA2	6,875
Stur_01	Stur et al. ⁴ GSE189843 ST data	PR_4	HGSOC, unknown	428
Stur_02		ER_6		285
Stur_03		ER_3		172
Stur_04		PR_5		170
Stur_05		ER_5		596
Stur_06		ER_4		347
Stur_07		PR_2		499
Stur_08		PR_3		949
Stur_09		ER_1		436
Stur_10		PR_1		290
Stur_11		ER_2		381

Table S2. Differentially Expressed Genes

The *FindMarkers* function from Seurat was used to identify differentially expressed genes (DEGs) between high- and low-stromal groups for each cell type. From the outputs of *FindMarkers*, we set cutoffs for DEGs.

Cell type	p val adj	avg logFC
B cells, Endothelial cells	< 1e-25	Absolute value > 0.5
CA-MSCs, Fibroblasts, Myofibroblasts	< 1e-50	
CD4 ⁺ T cells, Dendritic cells, Natural killer cells	< 1e-10	
CD8 ⁺ T cells, Macrophages	< 1e-50	
CD4 ⁺ CD8 ⁺ T cells	< 1e-5	
Epithelial cancer cells	< 1e-50	

Table S3: Cytokines and Surface Proteins

Category	Gene Symbol
Cytokines 5 6 7	AXL, BMP2, BMP4, CCL1 – CCL5, CCL7, CCL8, CCL11, CCL13 – CCL28, CCL3L1, CCL3L3, CCL4L1, CCL4L2, CCR2, CCR4, CCR5, CCR7, CCR8, CCR10, CD27, CD40, CD40LG, CD86, CSF1- CSF3, CX3CL1, CXCL1 – CXCL3, CXCL5, CXCL6, CXCL8 – CXCL14, CXCL16, CXCL17, CXCR1 – CXCR4, CXCR6, EBI3, EGFL6, EGR1, FAS, FASLG, FGF2, FGFR1 – FGFR4, FLT1, FLT3, FOXP3, GAS6, GZMA, GZMB, ICOSLG, IFNA1, IFNA2, IFNA4 – IFNA8, IFNA10, IFNA13, IFNA14, IFNA16, IFNA17, IFNA21, IFNAR1, IFNAR2, IFNB1, IFNE, IFNG, IFNGR1, IFNGR2, IFNK, IFNL1 – IFNL4, IL2 – IL7, IL9 – IL11, IL13, IL15, IL16, IL18 – IL22, IL24 – IL27, IL31 – IL34, IL37, IL1A, IL1B, IL1RN, IL2RA, IL2RB, IL2RG, IL4R, IL6R, IL6ST, IL7R, IL10RA, IL10RB, IL12A, IL12B, IL12RB1, IL12RB2, IL15RA, IL17A – IL17F, IL18R1, IL18RAP, IL21R, IL23A, IL23R, IL36A, IL36B, IL36G, ITGAV, KITLG, LAG3, LIF, LTA, LTB, MYDGF, NFKB1, NRP1, OSM, PDCD1, PDGFC, PDGFRA, PF4V1, PGF, PPBP, PRF1, TGFB1 – TGFB3, TGFB1, TGFB1 – TGFB3, TNF, TNFRSF1A, TNFRSF1B, TNFSF4, TNFSF8–TNFSF15, TNFSF18, TNFRSF10B, TNFSF13B, VEGFA, WT1, XCL1, XCL2
Surface Proteins 8	ACKR1, ACVR2B, ANPEP, APP, CD163, CD274, CD44, CD93, CDH1, CDH2, CR2, EGFR, EPHA2, EPHA4, EPHB1 – EPHB4, ERBB2, FLT4, FOLH1, GNRHR, GPC3, GPR55, GRPR, IL11RA, IL13RA2, KDR, LDLR, LEPR, LRP1, MC1R, MC4R, MCAM, MET, MMP9, MRC1, MUC1, PDGFRB, PLAUR, PROM1, PTPRJ, SDC2, SELE, TACR1, TEK, VIPR1, VIPR2

Table S4: Gene-TF correlations

Within each cell type, we computed the Spearman correlation coefficient of the expression of each differentially expressed *surface protein* or *cytokine* (determined using the cutoffs in Table S2) and the activity of each transcription factor (TF, inferred via pySCENIC ⁹). From the results, we set the following cutoffs to identify highly correlated gene-TF pairs. For visualization (Figures 2B, 4B, S5A, S7A), we took the union of the filtered highly correlated genes of the indicated cell types.

Cell type	To keep a gene, there is at least one TF such that	To keep a TF, there is at least one gene such that
CA-MSCs	cor > 0.4	cor > 0.5
Fibroblasts	cor > 0.4	cor > 0.7
Myofibroblasts	cor > 0.4	cor > 0.75
CD8 ⁺ T cells	cor > 0.4	cor > 0.35
Natural killer cells	cor > 0.35	cor > 0.35
Dendritic cells	cor > 0.5	cor > 0.3
Macrophages	cor > 0.5	cor > 0.3

Table S5: Ligand-receptor interactions

CellPhoneDB ¹⁰ was used to identify the potential ligand-receptor for each cell type based on the raw count matrices of the high- and low-stromal groups separately. From the outputs of CellPhoneDB, we set cutoffs for ligand-receptor pairs of interest. Here “Fold change” is not a direct output of CellPhoneDB, which computes the difference of the means from the high- and low-stromal group.

Sender population	Receiver population	Means	Pvalues	Fold change
CA-MSCs	An immune population	> 0.5 in high-stromal AND	< 0.01 in high-stromal AND < 0.01 in low-stromal	> 0.1
Fibroblasts	An immune population	> 0.5 in low-stromal		> 0.14
CD8 ⁺ T cells	A nonimmune population	> 0.5 in high-stromal OR		> 0.32
Natural killer cells	A nonimmune population	> 0.5 in low-stromal		> 0.25

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