Supplementary Figure

Figure S1. Heterogeneity of cell composition among patients.

A) UMAP plot colored by patient identity number. Localized patients: CC1, CC3, and CC6. Regional patients: CC4, CC7, CC10 and CC11. Metastatic patients: CC2, CC5, CC8 and CC9.

B) Sankey plot illustrating the proportion of 5 major cell types in 11 CC patients. The cell cluster colors are identified in Fig.1B.

Figure S2. Analysis of TME landscapes, Related to Figure 1.

A) UMAP plots showing the TME cell clusters and cell densities across different tissue groups with all cells included.

B) Down-sampling analysis showing the results are reproducible and not influenced by the total number of cells from each group.

Figure S3. Characterization of epithelial cell subsets.

A) UMAP plot showing average z-score expression of cell cycle gene signatures.

B) Heatmap shows CNVs for tumor subsets with the reference of fibroblasts. The results were inferred by inferCNVpy.

C) Dot plots showing the MHC class I and II gene expression of epithelial cell subsets.

D) Boxplots showing the pEMT score of epithelial cells in 3 groups in external validation dataset. Statistical significance was analyzed using Kruskal-Wallis test. The boxplots display the median, upper quartile, and lower quartile.

E) Dot plots showing the chemokine gene expression of epithelial cell in 3 groups in external validation dataset.

Figure S4. The phenotype diversity of macrophage.

A) UMAP plot of the macrophage (denoted by colors).

B) Dot plot showing the expression of marker genes of each macrophage subsets. Dot size represents the fraction of cells expressing the given gene, with colors indicating the normalized expression levels.

C) Expression levels and frequencies of genes composing the M1-like, M2-like, angiogenesis and checkpoint genes across myeloid cell clusters.

D) Heatmap showing the ORs of myeloid-derived cell subsets occurring in each group. *OR > 1.5 indicates significant enrichment of the subset in the corresponding tissue.

E) Violin plot showing the expression of cytokines for each macrophage subset. Colors represent different cell subsets.

F) GSEA enrichment plot showing the upregulated pathways (blue) and downregulated pathways (green) of Mac_CCL20 compared to other macrophage subsets.

G) GSEA analysis of Mac_CCL20 subset.

H) Circle plots show cell-cell interaction number differences of macrophage and neutrophils in localized, regional and metastatic CC samples.

Figure S5. Endothelial cell transcriptomes altered during CC metastasis.

A) The enriched GO terms of EC subclusters. a. lymph vessel morphogenesis.

B) GSEA analysis showing the upregulated pathways of capEC2 compared to other EC subsets.

C) GSEA analysis showing the downregulated pathways of capEC2 compared to other EC subsets.

D) Differentially expressed genes of capEC2.

E) Circle plots showing the VEGF signaling inferred by CellChat among epithelial cell and ECs.

F) Dot plot depicting ligand-receptor interactions between epithelial cell and ECs. Communication probability and *P* values were calculated by CellChat, and were indicated by circle colour and size, respectively.

Figure S6. Additional analysis of figure 6.

A) Violin plot showing the expression of OSM for each cell subset. Colors represent different cell subsets.

B) Violin plot showing the expression of OSMR for each cell subset. Colors represent different cell subsets.

C-D) Correlation of OSM (C) and OSMR (D) expression with infiltration level of indicated cell types in CC samples. Spearman's correlation coefficients and *P* values are shown. TPM, transcript count per million reads.

E) Correlation of TGFBR1 and TGFBR2 levels with CXCL1, CXCL2 and CXCL3 expression in CC samples. Spearman's correlation coefficients and *P* values are shown. TPM, transcript count per million reads.

Figure S7. Additional analysis of figure 7.

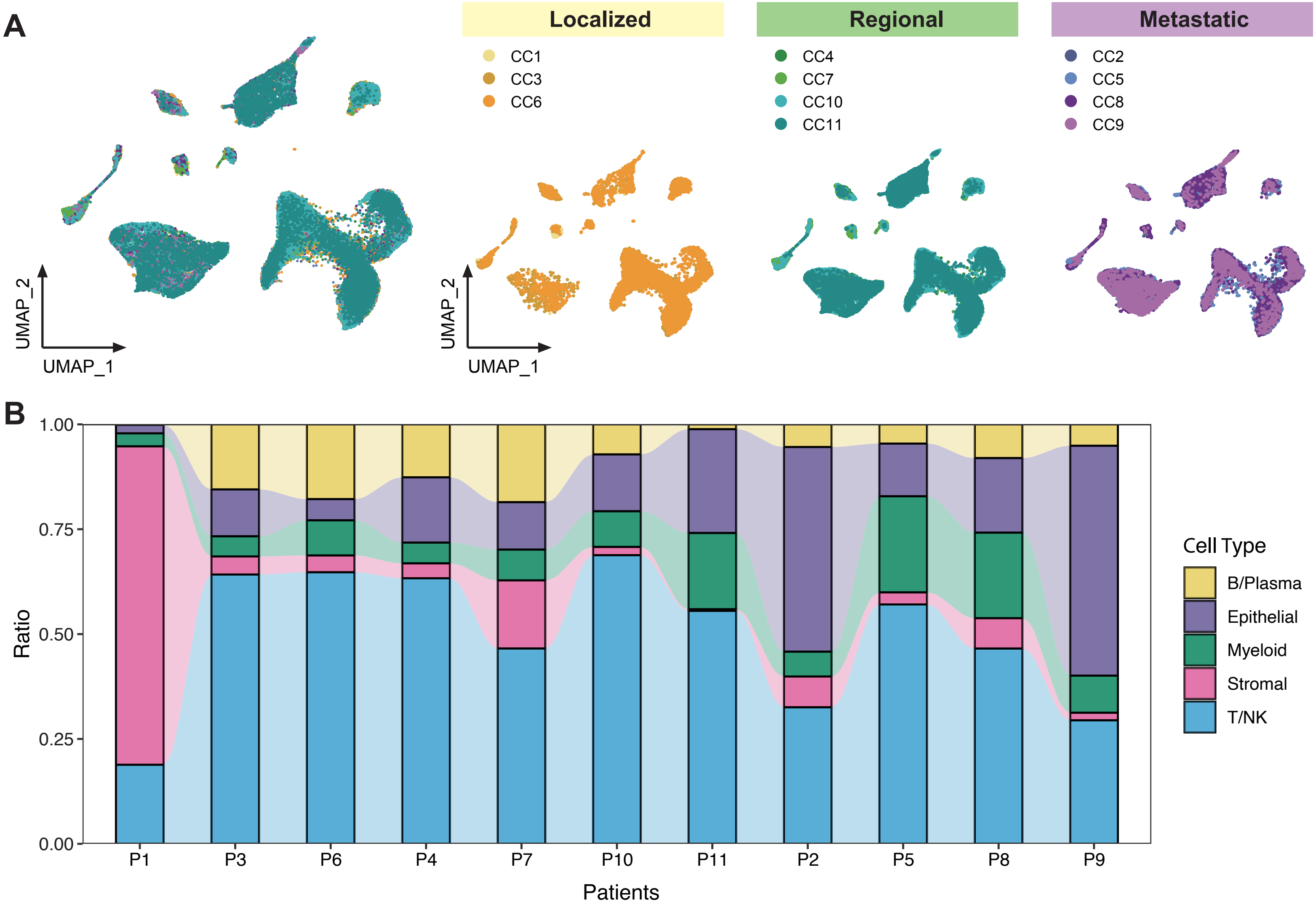
A) Circle plots depicting cell-cell interaction strength from CellChat analysis of scRNA-seq dataset.

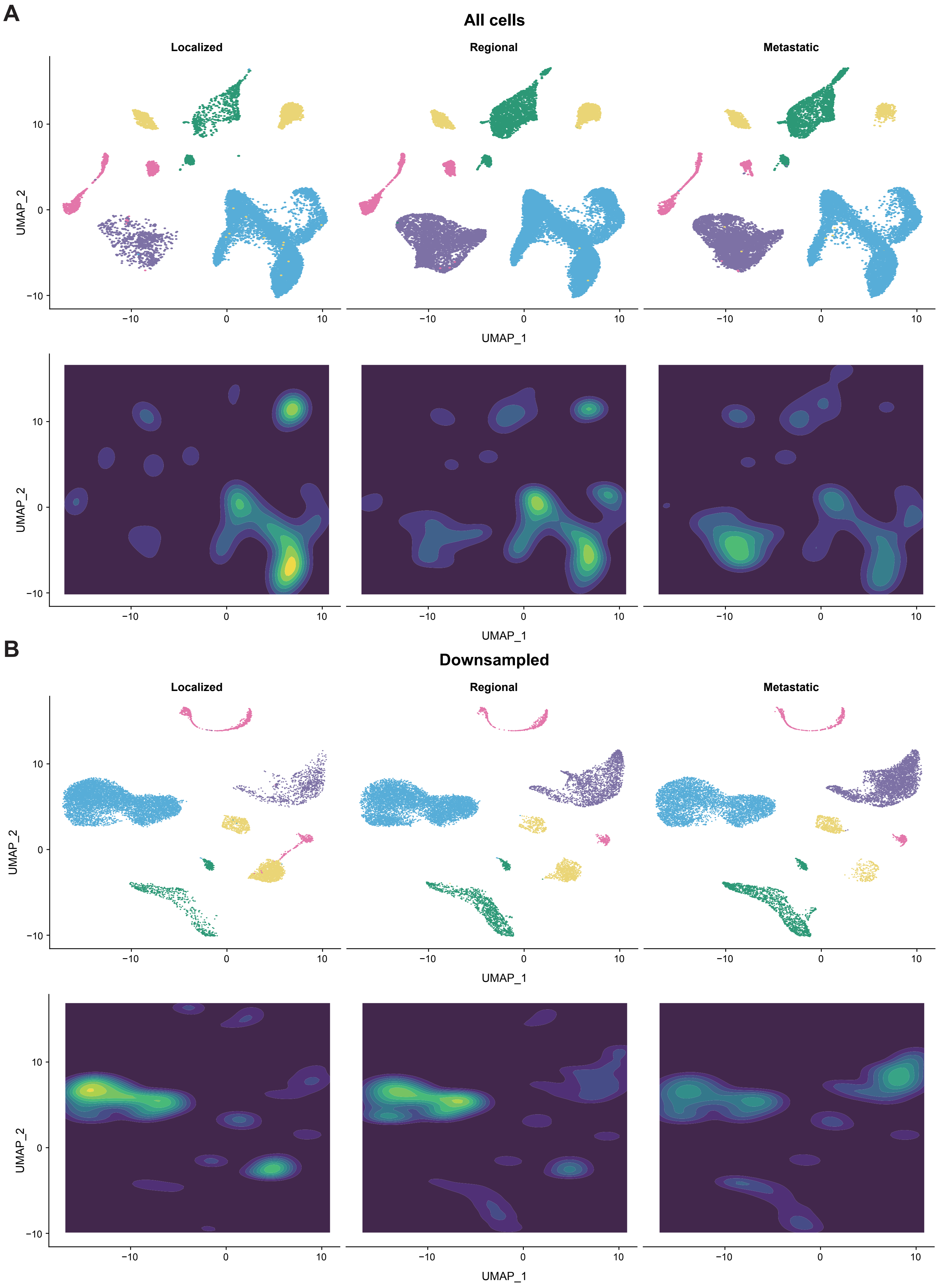
B) Bubble plots of conserved ligand-receptor pairs shared between scRNA-seq modalities. Bubble size indicates interaction probability.

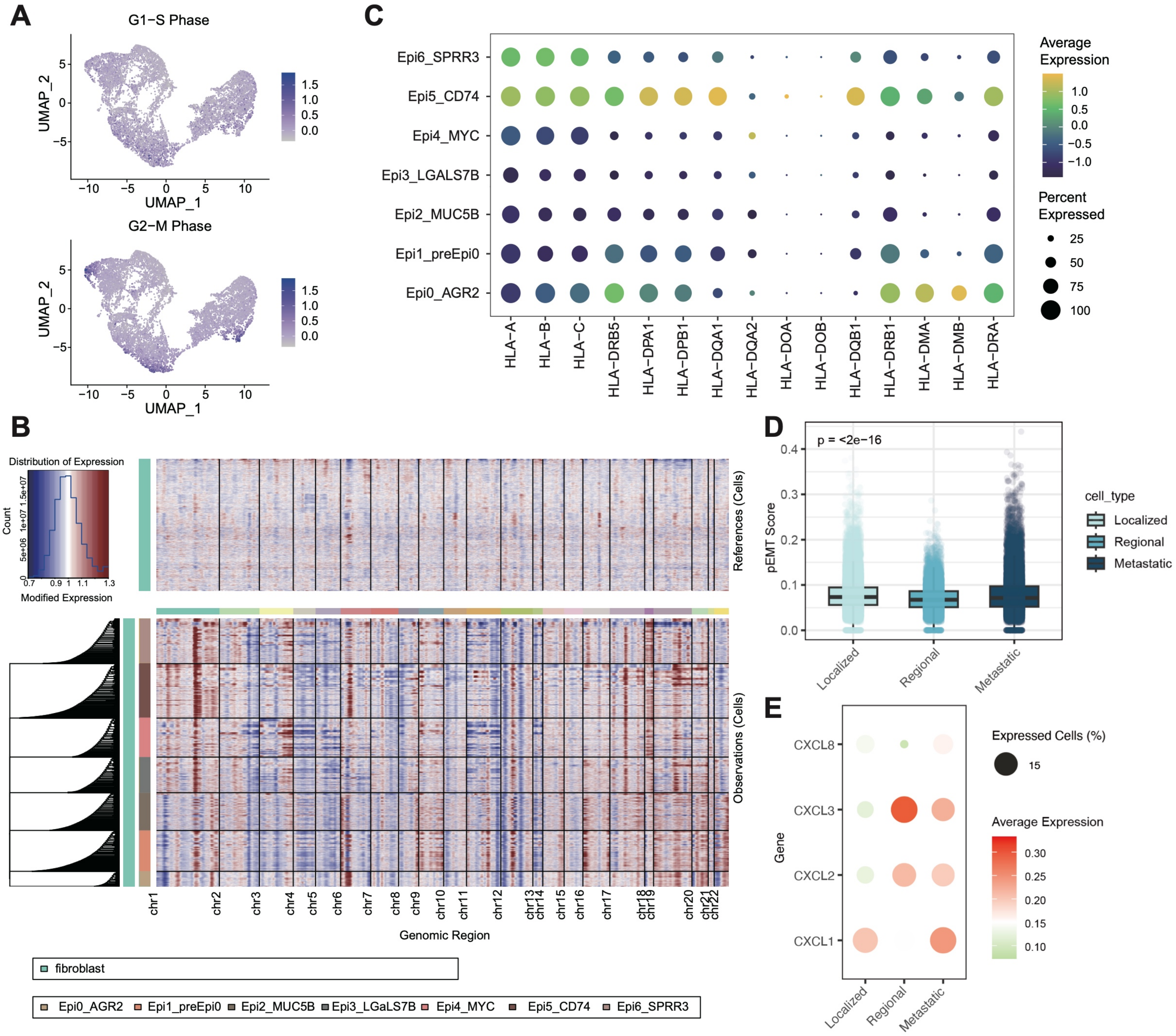
C) Circle plots depicting cell-cell interaction strength from CellChat analysis of ST dataset.

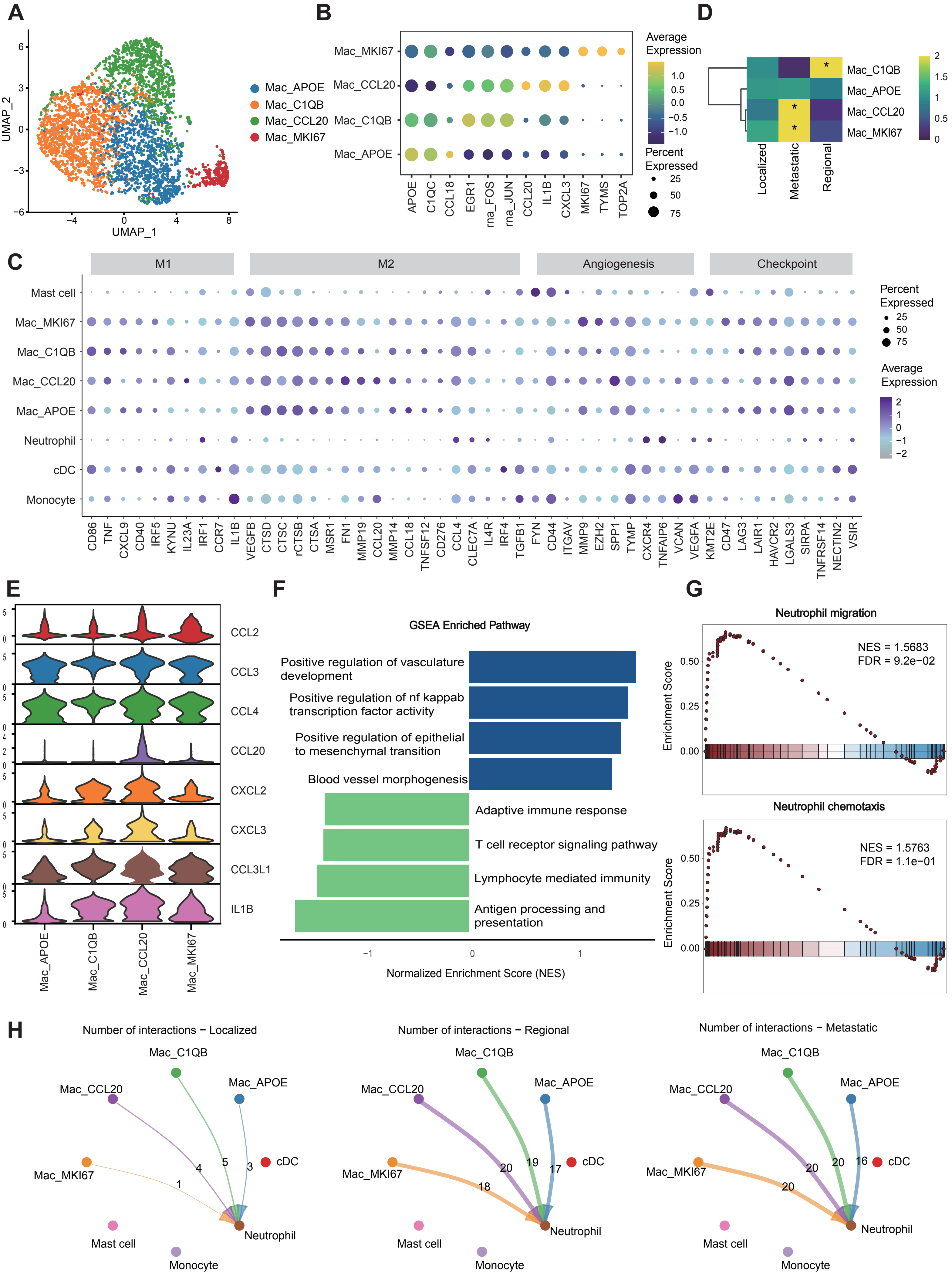
D) Bubble plots of conserved ligand-receptor pairs shared between ST modalities. Bubble size indicates interaction probability.

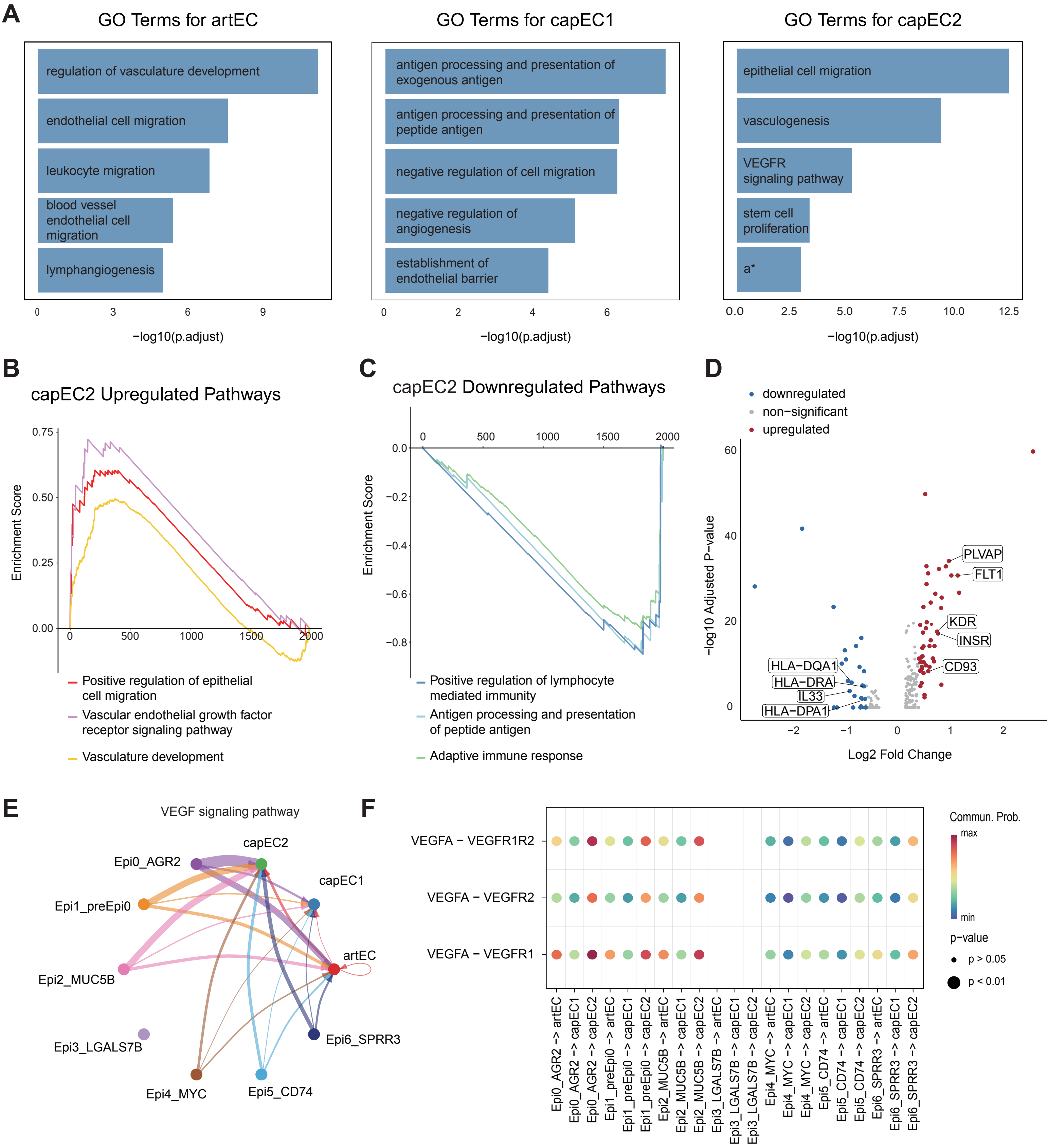
E-F) Heatmaps comparing TGFβ signaling interaction strengths in (E) scRNA-seq and (F) ST data.

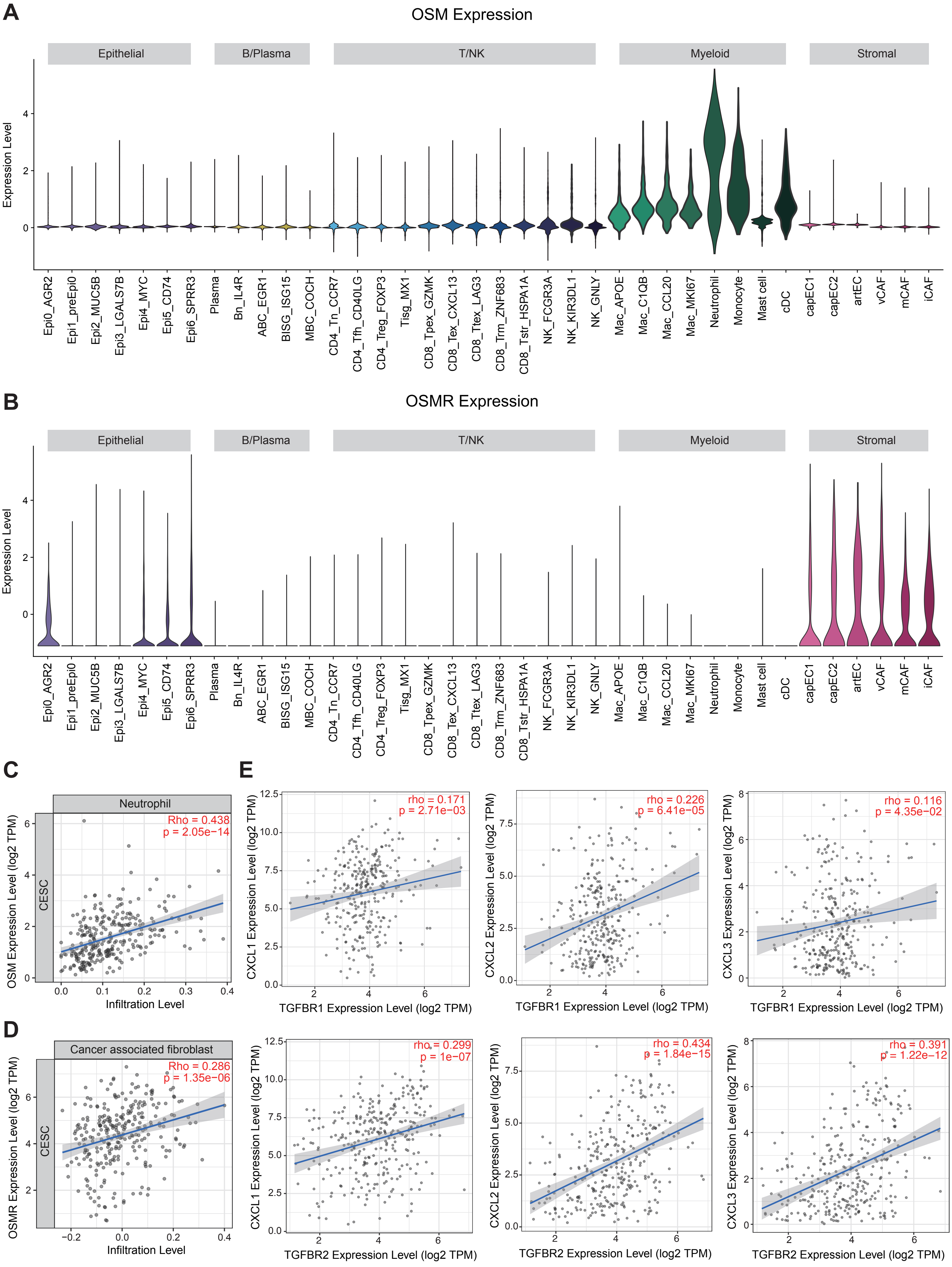

A
Localized
CC1
CC3
CC6
Regional
CC4
CC7
CC10
CC11
Metastatic
CC2
CC5
CC8
CC9
UMAP_2
UMAP_1
UMAP_2
UMAP_1
B
1.00
0.75
0.50
0.25
0.00
Ratio
P1
P3
P6
P4
P7
P10
P11
P2
P5
P8
P9
Patients
Cell Type
B/Plasma
Epithelial
Myeloid
Stromal
T/NK

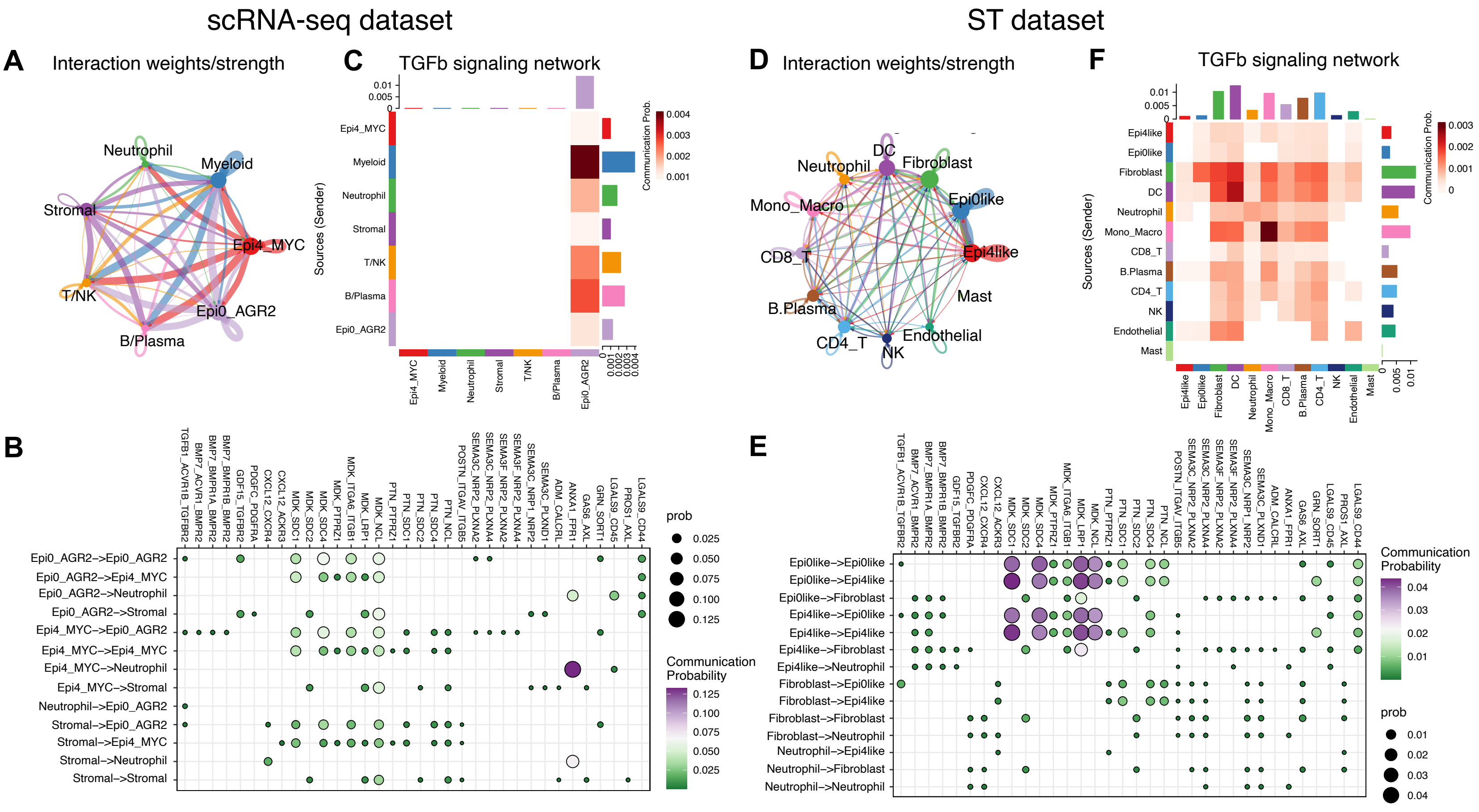

A
All cells
Localized
Regional
Metastatic
UMAP_2
10
0
−10
−10
0
10
UMAP_1
B
Downsampled
Localized
Regional
Metastatic
UMAP_2
10
0
−10
−10
0
10
UMAP_1


A
G1−S Phase
UMAP_2
UMAP_1
G2−M Phase
1.5
1.0
0.5
0.0
C
Epi6_SPRR3
Epi5_CD74
Epi4_MYC
Epi3_LGALS7B
Epi2_MUC5B
Epi1_preEpi0
Epi0_AGR2
HLA−A
HLA−B
HLA−C
HLA−DRB5
HLA−DPA1
HLA−DPB1
HLA−DQA1
HLA−DQA2
HLA−DOA
HLA−DOB
HLA−DQB1
HLA−DRB1
HLA−DMA
HLA−DMB
HLA−DRA
Average Expression
Percent Expressed
25
50
75
100
B
Distribution of Expression
Count
Modified Expression
References (Cells)
Observations (Cells)
Genomic Region
chr1
chr2
chr3
chr4
chr5
chr6
chr7
chr8
chr9
chr10
chr11
chr12
chr13
chr14
chr15
chr16
chr17
chr18
chr19
chr20
chr21
chr22
fibroblast
Epi0_AGR2
Epi1_preEpi0
Epi2_MUC5B
Epi3_LGaLS7B
Epi4_MYC
Epi5_CD74
Epi6_SPRR3
D
p = <2e−16
pEMT Score
cell_type
Localized
Regional
Metastatic
E
Gene
CXCL8
CXCL3
CXCL2
CXCL1
Expressed Cells (%)
15
Average Expression
0.30
0.25
0.20
0.15
0.10


A
UMAP_2
UMAP_1
Mac_APOE
Mac_C1QB
Mac_CCL20
Mac_MKI67
B
Mac_MKI67
Mac_CCL20
Mac_C1QB
Mac_APOE
APOE
C1QC
CCL18
EGR1
rna_FOS
rna_JUN
CCL20
IL1B
CXCL3
MKI67
TYMS
TOP2A
Average Expression
Percent Expressed
D
Mac_C1QB
Mac_APOE
Mac_CCL20
Mac_MKI67
Localized
Metastatic
Regional
*
C
M1
M2
Angiogenesis
Checkpoint
Mast cell
Mac_MKI67
Mac_C1QB
Mac_CCL20
Mac_APOE
Neutrophil
cDC
Monocyte
CD86
TNF
CXCL9
CD40
IRF5
KYNU
IL23A
IRF1
CCR7
IL1B
VEGFB
CTSD
CTSC
rCTSB
CTSA
MSR1
FN1
MMP19
CCL20
MMP14
CCL18
TNFSF12
CD276
CCL4
CLEC7A
IL4R
IRF4
TGFB1
FYN
CD44
ITGAV
MMP9
EZH2
SPP1
TYMP
CXCR4
TNFAIP6
VCAN
VEGFA
KMT2E
CD47
LAG3
LAIR1
HAVCR2
LGALS3
SIRPA
TNFRSF14
NECTIN2
VSIR
Percent Expressed
Average Expression
E
CCL2
CCL3
CCL4
CCL20
CXCL2
CXCL3
CCL3L1
IL1B
Mac_APOE
Mac_C1QB
Mac_CCL20
Mac_MKI67
F
GSEA Enriched Pathway
Positive regulation of vasculature development
Positive regulation of nf kappab transcription factor activity
Positive regulation of epithelial to mesenchymal transition
Blood vessel morphogenesis
Adaptive immune response
T cell receptor signaling pathway
Lymphocyte mediated immunity
Antigen processing and presentation
Normalized Enrichment Score (NES)
G
Neutrophil migration
NES = 1.5683
FDR = 9.2e−02
Neutrophil chemotaxis
NES = 1.5763
FDR = 1.1e−01
Enrichment Score
H
Number of interactions − Localized
Number of interactions − Regional
Number of interactions − Metastatic
Mac_C1QB
Mac_APOE
Mac_CCL20
Mac_MKI67
cDC
Mast cell
Monocyte
Neutrophil
4
5
3
1
20
19
17
18
20
20
16
20


A
GO Terms for artEC
regulation of vasculature development
endothelial cell migration
leukocyte migration
blood vessel endothelial cell migration
lymphangiogenesis
0
3
6
9
−log10(p.adjust)
GO Terms for capEC1
antigen processing and presentation of exogenous antigen
antigen processing and presentation of peptide antigen
negative regulation of cell migration
negative regulation of angiogenesis
establishment of endothelial barrier
0
2
4
6
−log10(p.adjust)
GO Terms for capEC2
epithelial cell migration
vasculogenesis
VEGFR signaling pathway
stem cell proliferation
a*
0.0
2.5
5.0
7.5
10.0
12.5
−log10(p.adjust)
B
capEC2 Upregulated Pathways
Enrichment Score
0.75
0.50
0.25
0.00
0
500
1000
1500
2000
Positive regulation of epithelial cell migration
Vascular endothelial growth factor receptor signaling pathway
Vasculature development
C
capEC2 Downregulated Pathways
Enrichment Score
0.0
−0.2
−0.4
−0.6
−0.8
0
500
1000
1500
2000
Positive regulation of lymphocyte mediated immunity
Antigen processing and presentation of peptide antigen
Adaptive immune response
D
downregulated
non−significant
upregulated
−log10 Adjusted P−value
60
40
20
0
−2
−1
0
1
2
Log2 Fold Change
PLVAP
FLT1
KDR
INSR
CD93
HLA−DQA1
HLA−DRA
IL33
HLA−DPA1
E
VEGF signaling pathway
capEC2
Epi0_AGR2
capEC1
Epi1_preEpi0
artEC
Epi2_MUC5B
Epi3_LGALS7B
Epi6_SPRR3
Epi4_MYC
Epi5_CD74
F
VEGFA − VEGFR1R2
VEGFA − VEGFR2
VEGFA − VEGFR1
Epi0_AGR2 −> artEC
Epi0_AGR2 −> capEC1
Epi0_AGR2 −> capEC2
Epi1_preEpi0 −> artEC
Epi1_preEpi0 −> capEC1
Epi1_preEpi0 −> capEC2
Epi2_MUC5B −> artEC
Epi2_MUC5B −> capEC1
Epi2_MUC5B −> capEC2
Epi3_LGALS7B −> artEC
Epi3_LGALS7B −> capEC1
Epi3_LGALS7B −> capEC2
Epi4_MYC −> artEC
Epi4_MYC −> capEC1
Epi4_MYC −> capEC2
Epi5_CD74 −> artEC
Epi5_CD74 −> capEC1
Epi5_CD74 −> capEC2
Epi6_SPRR3 −> artEC
Epi6_SPRR3 −> capEC1
Epi6_SPRR3 −> capEC2
Commun. Prob.
max
min
p−value
p > 0.05
p < 0.01


A
OSM Expression
Epithelial
B/Plasma
T/NK
Myeloid
Stromal
Expression Level
0
2
4
Epi0_AGR2
Epi1_preEpi0
Epi2_MUC5B
Epi3_LGALS7B
Epi4_MYC
Epi5_CD74
Epi6_SPRR3
Plasma
Bn_IL4R
ABC_EGR1
BISG_ISG15
MBC_COCH
CD4_Tn_CCR7
CD4_Tfh_CD40LG
CD4_Treg_FOXP3
Tisg_MX1
CD8_Tpex_GZMK
CD8_Tex_CXCL13
CD8_Ttex_LAG3
CD8_Trm_ZNF683
CD8_Tstr_HSPA1A
NK_FCGR3A
NK_KIR3DL1
NK_GNLY
Mac_APOE
Mac_C1QB
Mac_CCL20
Mac_MKI67
Neutrophil
Monocyte
Mast cell
cDC
capEC1
capEC2
artEC
vCAF
mCAF
iCAF
B
OSMR Expression
Epithelial
B/Plasma
T/NK
Myeloid
Stromal
Expression Level
0
2
4
Epi0_AGR2
Epi1_preEpi0
Epi2_MUC5B
Epi3_LGALS7B
Epi4_MYC
Epi5_CD74
Epi6_SPRR3
Plasma
Bn_IL4R
ABC_EGR1
BISG_ISG15
MBC_COCH
CD4_Tn_CCR7
CD4_Tfh_CD40LG
CD4_Treg_FOXP3
Tisg_MX1
CD8_Tpex_GZMK
CD8_Tex_CXCL13
CD8_Ttex_LAG3
CD8_Trm_ZNF683
CD8_Tstr_HSPA1A
NK_FCGR3A
NK_KIR3DL1
NK_GNLY
Mac_APOE
Mac_C1QB
Mac_CCL20
Mac_MKI67
Neutrophil
Monocyte
Mast cell
cDC
capEC1
capEC2
artEC
vCAF
mCAF
iCAF
C
Neutrophil
CESC
Rho = 0.438
p = 2.05e−14
OSM Expression Level (log2 TPM)
0
2
4
6
0.0
0.1
0.2
0.3
0.4
Infiltration Level
E
rho = 0.171
p = 2.71e−03
CXCL1 Expression Level (log2 TPM)
0.0
2.5
5.0
7.5
10.0
12.5
2
4
6
TGFBR1 Expression Level (log2 TPM)
rho = 0.226
p = 6.41e−05
CXCL2 Expression Level (log2 TPM)
0.0
2.5
5.0
7.5
2
4
6
TGFBR1 Expression Level (log2 TPM)
rho = 0.116
p = 4.35e−02
CXCL3 Expression Level (log2 TPM)
0
2
4
6
8
2
4
6
TGFBR1 Expression Level (log2 TPM)
D
Cancer associated fibroblast
CESC
Rho = 0.286
p = 1.35e−06
OSMR Expression Level (log2 TPM)
2
4
6
−0.2
0.0
0.2
0.4
Infiltration Level
rho = 0.299
p = 1e−07
CXCL1 Expression Level (log2 TPM)
0.0
2.5
5.0
7.5
10.0
12.5
2
4
6
TGFBR2 Expression Level (log2 TPM)
rho = 0.434
p = 1.84e−15
CXCL2 Expression Level (log2 TPM)
0.0
2.5
5.0
7.5
2
4
6
TGFBR2 Expression Level (log2 TPM)
rho = 0.391
p = 1.22e−12
CXCL3 Expression Level (log2 TPM)
0
2
4
6
8
2
4
6
TGFBR2 Expression Level (log2 TPM)


scRNA-seq dataset
ST dataset
A
Interaction weights/strength
Neutrophil
Myeloid
Stromal
Epi4_MYC
T/NK
Epi0_AGR2
B/Plasma
C
TGFb signaling network
Sources (Sender)
Communication Prob.
Epi4_MYC
Myeloid
Neutrophil
Stromal
T/NK
B/Plasma
Epi0_AGR2
0.004
0.003
0.002
0.001
0.01
0.005
0
D
Interaction weights/strength
DC
Neutrophil
Fibroblast
Mono_Macro
Epi0like
CD8_T
Epi4like
Mast
B.Plasma
Endothelial
CD4_T
NK
F
TGFb signaling network
Sources (Sender)
Communication Prob.
Epi4like
Epi0like
Fibroblast
DC
Neutrophil
Mono_Macro
CD8_T
B.Plasma
CD4_T
NK
Endothelial
Mast
0.003
0.002
0.001
0
B
TGFB1_ACVR1B_TGFBR2
BMP7_ACVR1_BMPR2
BMP7_BMPR1A_BMPR2
BMP7_BMPR1B_BMPR2
GDF15_TGFBR2
PDGFC_PDGFRA
CXCL12_CXCR4
CXCL12_ACKR3
MDK_SDC1
MDK_SDC2
MDK_SDC4
MDK_PTPRZ1
MDK_ITGA6_ITGB1
MDK_LRP1
MDK_NCL
PTN_PTPRZ1
PTN_SDC1
PTN_SDC2
PTN_SDC4
PTN_NCL
POSTN_ITGAV_ITGB5
SEMA3C_NRP2_PLXNA2
SEMA3C_NRP2_PLXNA4
SEMA3F_NRP2_PLXNA2
SEMA3F_NRP2_PLXNA4
SEMA3C_NRP1_NRP2
SEMA3C_PLXND1
ADM_CALCRL
ANXA1_FPR1
GAS6_AXL
GRN_SORT1
LGALS9_CD45
PROS1_AXL
LGALS9_CD44
Epi0_AGR2->Epi0_AGR2
Epi0_AGR2->Epi4_MYC
Epi0_AGR2->Neutrophil
Epi0_AGR2->Stromal
Epi4_MYC->Epi0_AGR2
Epi4_MYC->Epi4_MYC
Epi4_MYC->Neutrophil
Epi4_MYC->Stromal
Neutrophil->Epi0_AGR2
Stromal->Epi0_AGR2
Stromal->Epi4_MYC
Stromal->Neutrophil
Stromal->Stromal
prob
0.025
0.050
0.075
0.100
0.125
Communication Probability
0.125
0.100
0.075
0.050
0.025
E
TGFB1_ACVR1B_TGFBR2
BMP7_ACVR1_BMPR2
BMP7_BMPR1A_BMPR2
BMP7_BMPR1B_BMPR2
GDF15_TGFBR2
PDGFC_PDGFRA
CXCL12_CXCR4
CXCL12_ACKR3
MDK_SDC1
MDK_SDC2
MDK_SDC4
MDK_PTPRZ1
MDK_ITGA6_ITGB1
MDK_LRP1
MDK_NCL
PTN_PTPRZ1
PTN_SDC1
PTN_SDC2
PTN_SDC4
PTN_NCL
POSTN_ITGAV_ITGB5
SEMA3C_NRP2_PLXNA2
SEMA3C_NRP2_PLXNA4
SEMA3F_NRP2_PLXNA2
SEMA3F_NRP2_PLXNA4
SEMA3C_NRP1_NRP2
SEMA3C_PLXND1
ADM_CALCRL
ANXA1_FPR1
GAS6_AXL
GRN_SORT1
LGALS9_CD45
PROS1_AXL
LGALS9_CD44
Epi0like->Epi0like
Epi0like->Epi4like
Epi0like->Fibroblast
Epi4like->Epi0like
Epi4like->Epi4like
Epi4like->Fibroblast
Epi4like->Neutrophil
Fibroblast->Epi0like
Fibroblast->Epi4like
Fibroblast->Fibroblast
Fibroblast->Neutrophil
Neutrophil->Epi4like
Neutrophil->Fibroblast
Neutrophil->Neutrophil
Communication Probability
0.04
0.03
0.02
0.01
prob
0.01
0.02
0.03
0.04

Supplementary Table 1. Clinical information of patients enrolled in this study

Sample	**Patient**	**Data source**	**Site**	**Histology**	**Stage**	**Age (years)**	**HPV**	**scRNA cells**
1	CC1	In house	CA	SCC	IB2	44	16, 66	743
2	CC2	In house	CA	SCC	IIIC1	39	45	3829
3	CC3	In house	CA	SCC	IB1	51	16	3944
4	CC4	In house	CA	SCC	IIA1	52	16	4502
5	CC5	In house	CA	SCC	IIIC2	32	18	4600
6	CC6	In house	CA	SCC	IB1	56	31	7888
7	CC7	In house	CA	SCC	IIB	59	16	5479
8	CC8	In house	CA	SCC	IVB	49	16	3927
9	CC9	In house	CA	AC	IIIC2	57	+	3370
10	CC10	In house	CA	SCC	IIB	51	+	5507
11	CC11	In house	CA	SCC	IIA2	77	+	9095

CA: cancer; SCC: Squamous cell carcinoma, AC: Adenocarcinoma;

Supplementary Table 2. Signature score genes for survival analysis

Epi0_AGR2	**Neutrophils**	**CAFs**
SERPINA1	OSM	ACTA2
AGR2	CXCR2	MCAM
CCL20	CSF3R	RGS5
CXCL2	FCGR3B	MT1A
CXCL3	IL1B	FAP
WFDC2	CXCL8	DCN
CLDN10	S100A9	CFD
MMP7	PLAUR	TAGLN
CXCL1		
KLF2		
NR4A1		

Supplementary Table 3. Information on single-cell RNA sequencing

Sample	Number of Reads	Number of Mean_Genes	Number of Cells
CC1	3,719,641	1708	743
CC2	17,909,403	1296	3829
CC3	22,305,001	1387	3944
CC4	16,152,610	1257	4502
CC5	42,074,586	2169	4600
CC6	49,664,215	1981	7888
CC7	46,023,285	2365	5479
CC8	17,900,865	1619	3927
CC9	30,196,421	2702	3370
CC10	30,778,771	1977	5507
CC11	58,039,936	2234	9095