

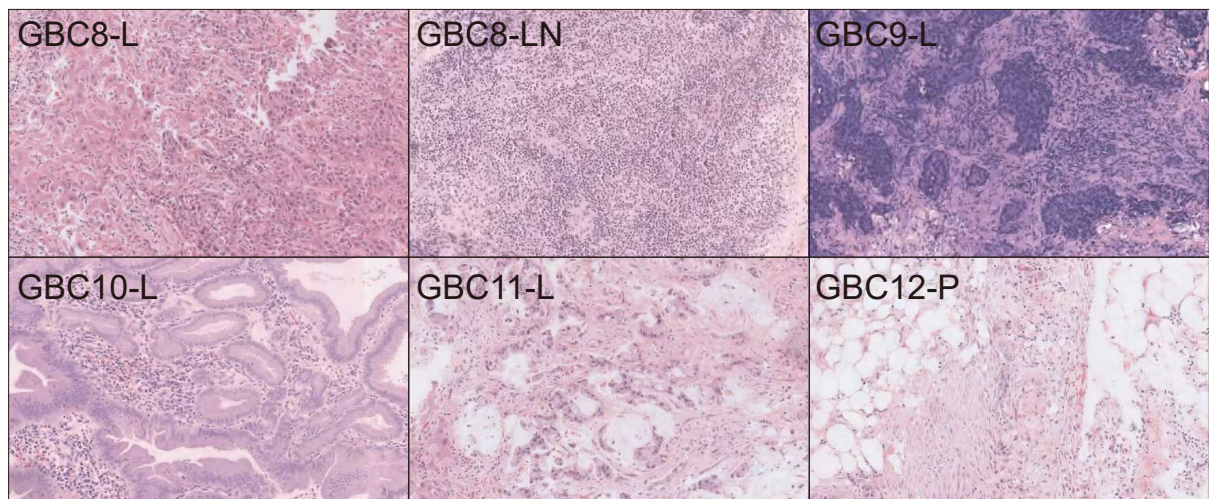
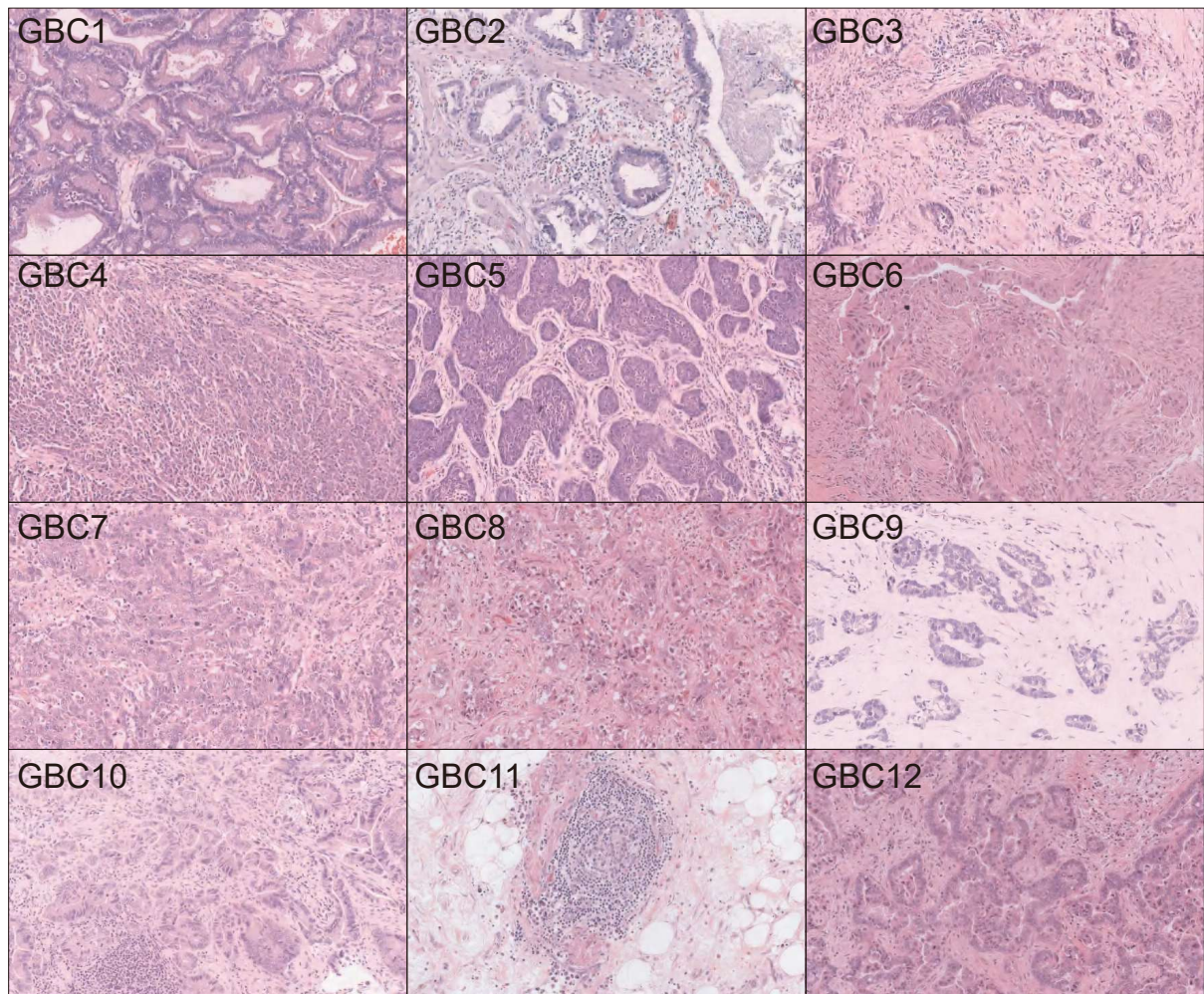
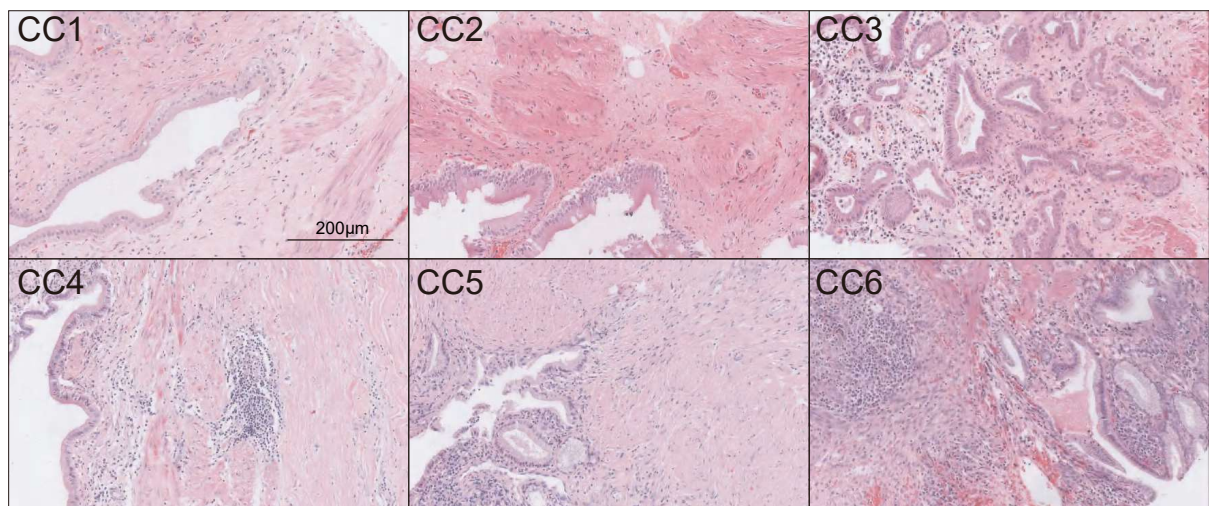
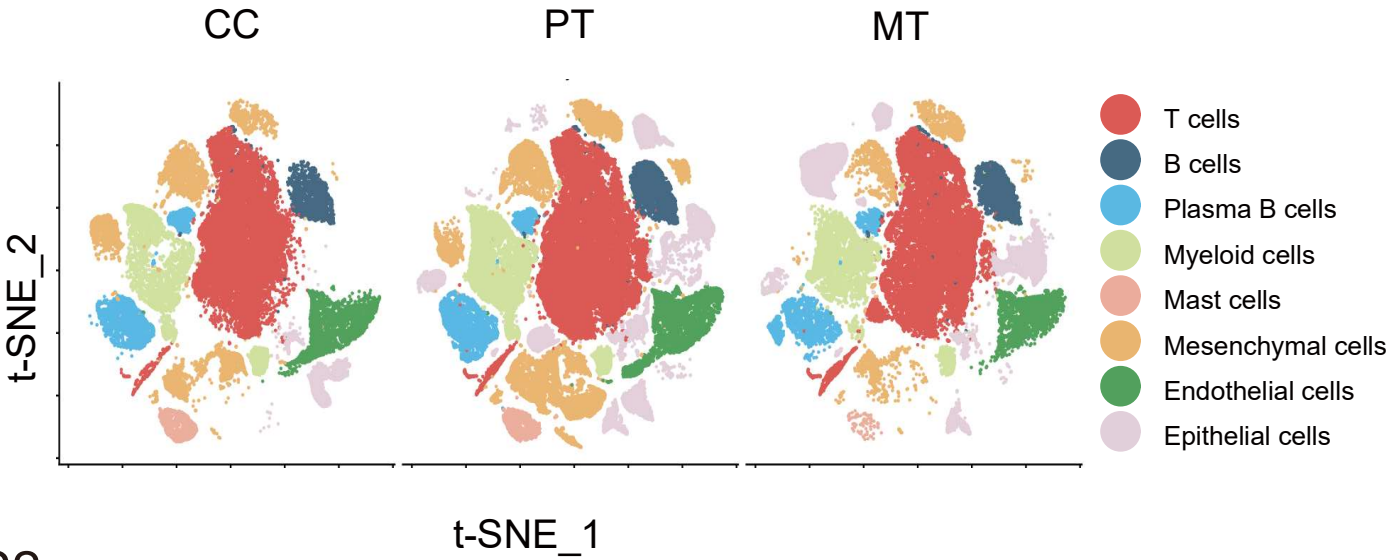


Fig.S1

**Supplementary Fig. S1. Hematoxylin and eosin (H&E) staining of all samples in our scRNA-seq cohort (n = 24). Scale bars, 200  $\mu$ m. CC, chronic cholecystitis; GBC, gallbladder carcinoma.**

a



b

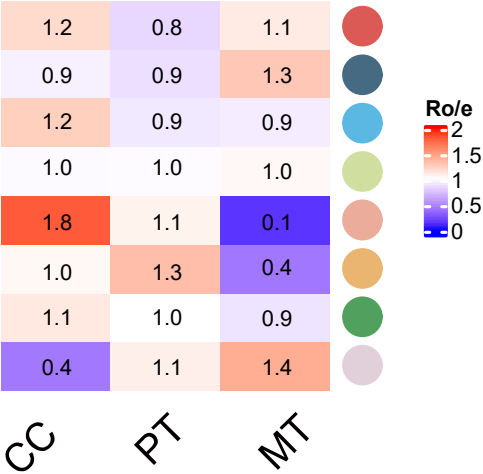


Fig.S2

**Supplementary Fig. S2. Comparison of cellular composition between CCs, PTs, and MTs.** **a** t-SNE plots visualizing the distribution of cell types across CCs, PTs, and MTs. **b** Heatmap showing the preferential enrichment of various cell types across CCs, PTs, and MTs. Cell types are colored in line with **a**.  $Ro/e > 1$  indicates significant enrichment.

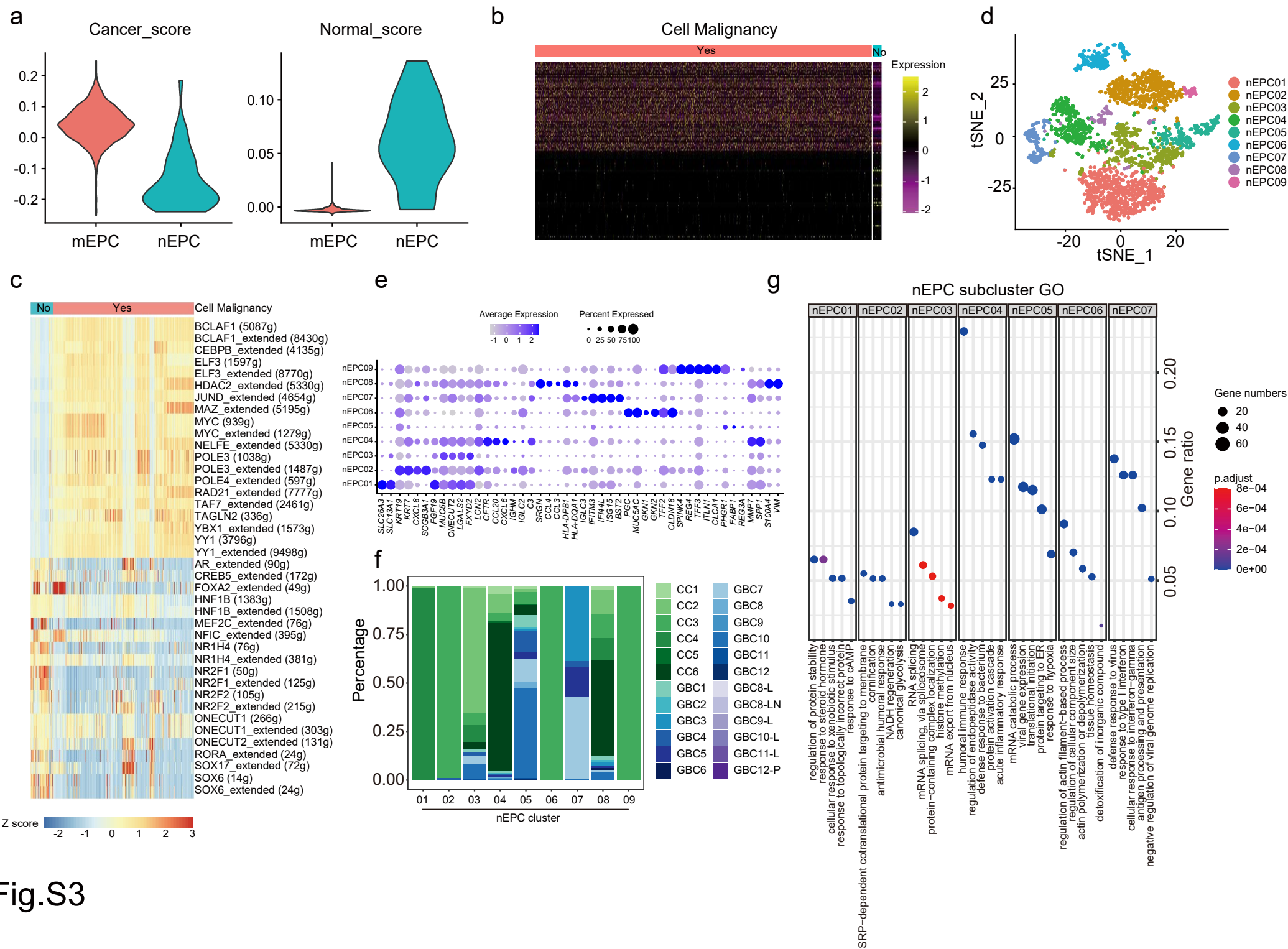


Fig.S3

**Supplementary Fig. S3. Discrimination of mEPCs and nEPCs, and characterization of nEPCs.** **a** Violin plots comparing tumor gene scores and normal gene set scores between CNV-inferred mEPCs and nEPCs in GBC7, respectively. The scoring system was defined by the top 50 DEGs based on bulk RNA-seq data (paired tumoral and peritumoral tissues from GBC7). **b** Heatmap showing the top 50 DEGs identified by bulk RNA-seq analyses of GBC7 sample and its paired peritumoral sample. **c** Heatmap comparing activities of transcription factors between mEPCs and nEPCs, based on SCENIC analysis. **d** t-SNE plot visualizing color-coded nEPC clusters (n = 9). **e** Bubble plots showing expressions of phenotype-specific gene signatures per nEPC cluster. **f** Distribution of samples across different nEPC clusters. **g** Dot plots showing typically significantly enriched GO pathways among nEPC clusters.

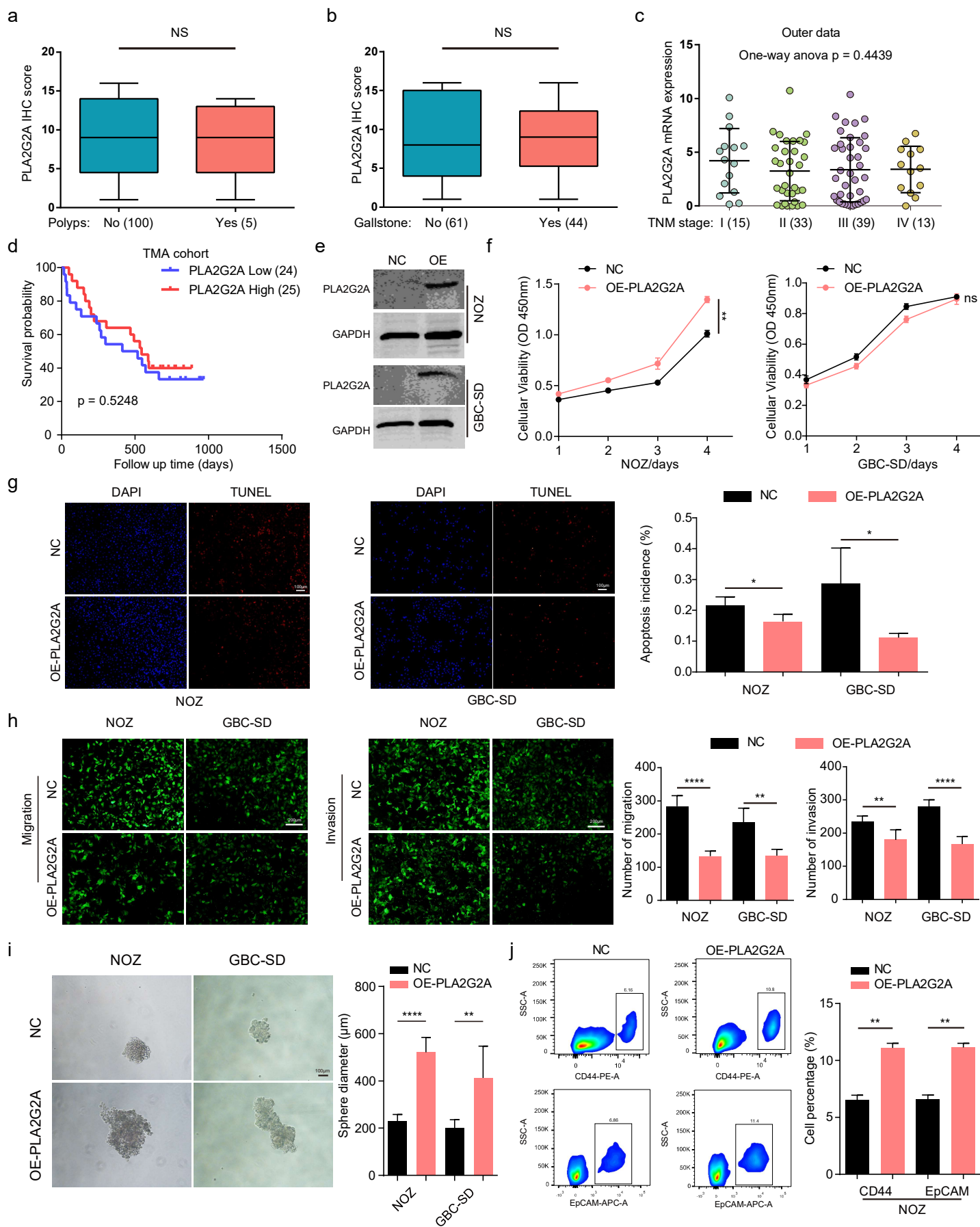
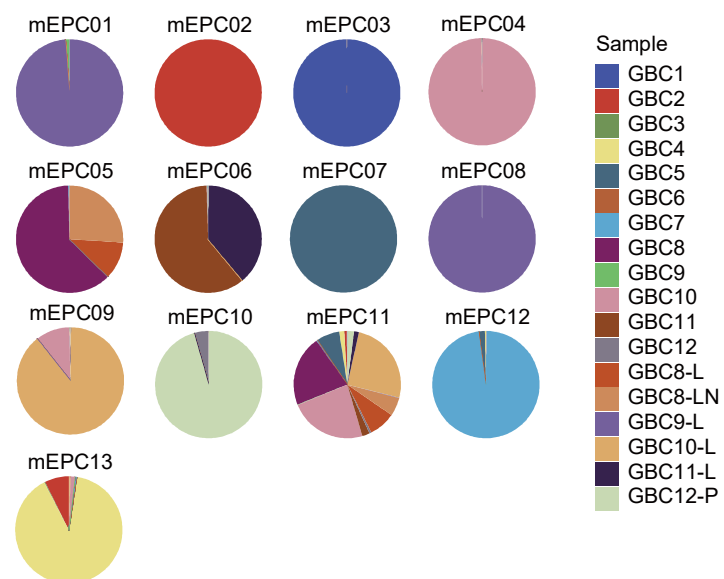


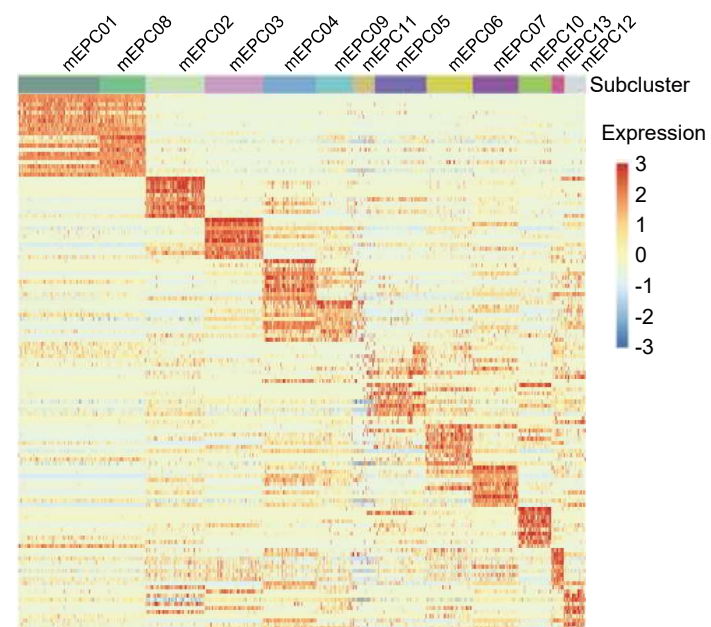
Fig.S4

**Supplementary Fig. S4. Association between PLA2G2A and clinico-biological features of gallbladder carcinoma.** **a** Boxplots comparing the expression of PLA2G2A between polyps-related GBCs (n = 5) and GBCs without polyps (n = 100) based on IHC staining. Wilcoxon rank-sum test; NS, not significant. **b** Boxplots comparing the expression of PLA2G2A between gallstone-related GBCs (n = 44) and GBCs without gallstones (n = 61) based on IHC staining. Wilcoxon rank-sum test; NS, not significant. **c** Beeswarm plots displaying the transcription level of PLA2G2A across different TNM stages of GBCs based on bulk RNA-seq data from an external GBC cohort (n = 100). One-way ANOVA test;  $P = 0.44$ . **d** Kaplan-Meier curves of overall survival when stratifying patients by high or low expression of PLA2G2A, based on our GBC tissue microarray (n = 49). Log-rank test,  $P = 0.52$ . TMA, tissue microarray. **e** Western blot analysis verifying increased PLA2G2A expression in *PLA2G2A*-overexpressing GBC cell lines (NOZ, GBC-SD) as compared with control cells. NC, normal control; OE, overexpression. **f** CCK8 assays comparing cell growth capacity between *PLA2G2A*-overexpressing cells and control cells. Left panel: NOZ cells,  $**P < 0.01$ ; right panel: GBC-SD cells; ns, no significance.  $P$  value was calculated using two-sided unpaired Student's  $t$  test. **g** Representative images and quantification of TUNEL assays (marking apoptotic bodies) in *PLA2G2A*-overexpressing GBC cell lines versus control cells. DAPI: blue, TUNEL: red. Scale bars, 100  $\mu\text{m}$ . Left panel: TUNEL staining in NOZ cells; middle panel: TUNEL staining in GBC-SD cells; right panel: boxplots of apoptosis index. Two-sided unpaired Student's  $t$  test.  $*P < 0.05$ . **h** Representative images and quantitative boxplots showing transwell migration and invasion assays in *PLA2G2A*-overexpressing GBC cell lines (NOZ, GBC-SD) versus control cells. From left to right: migration assay, invasion assay, boxplots quantifying migration capacity, boxplots quantifying invasion capacity. Two-sided unpaired Student's  $t$  test.  $**P < 0.01$ ;  $****P < 0.0001$ . Scale bars, 200  $\mu\text{m}$ . **i** Representative images and quantification of colony formation assay in *PLA2G2A*-overexpressing GBC cell lines (NOZ, GBC-SD) versus control cells. Scale bars, 100  $\mu\text{m}$ . Two-sided unpaired Student's  $t$  test.  $**P < 0.01$ ;  $****P < 0.0001$ . **j** Representative images and quantification based on flow cytometry analysis of stem cell markers (CD44, EpCAM) in *PLA2G2A*-overexpressing GBC cell line (NOZ) versus control cells. Left: markers in control cells; middle: markers in *PLA2G2A*-overexpressing cells; right: boxplots quantifying positive cells. Two-sided unpaired Student's  $t$  test.  $**P < 0.01$ .

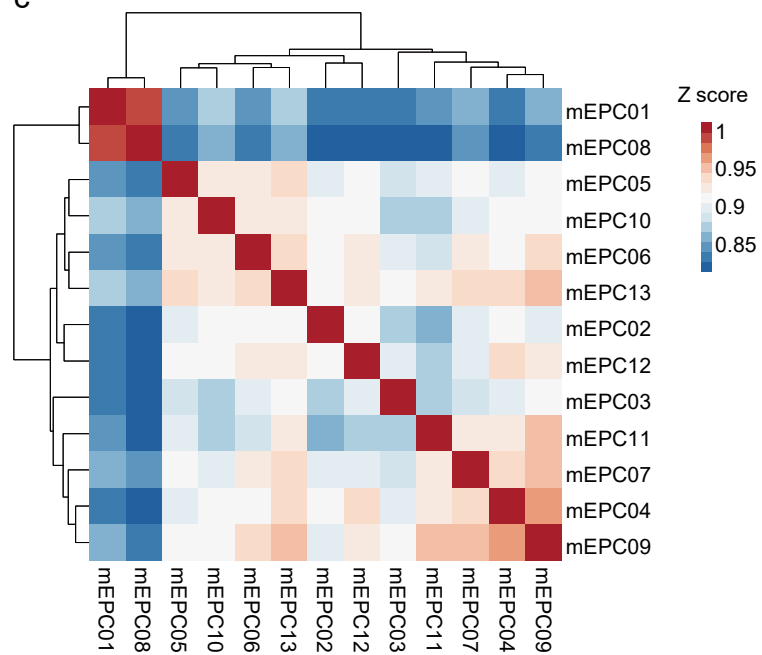
a



b



c



d

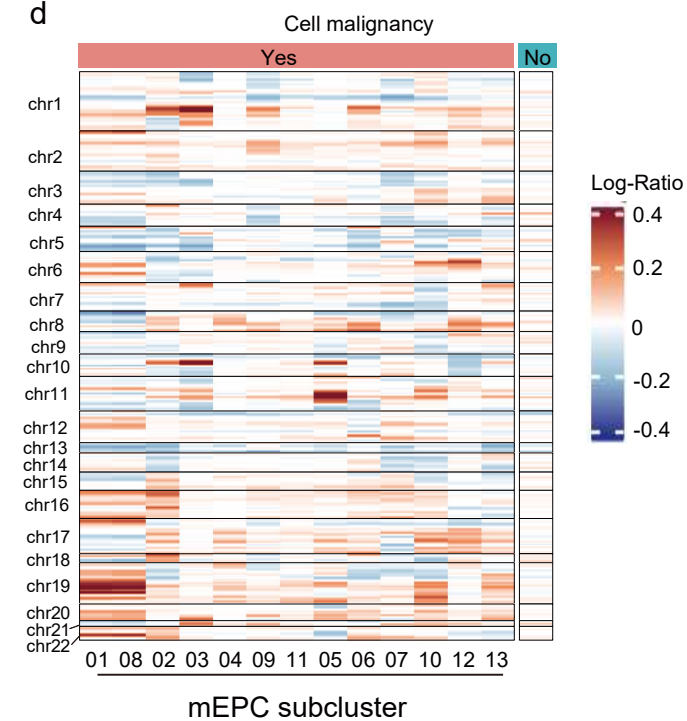


Fig.S5

**Supplementary Fig. S5. Comparison of transcriptional profiles between malignant epithelial clusters.** **a** Pie charts showing the relative abundance of tumor samples ( $n = 18$ ) across different malignant epithelial clusters (mEPC01-13). **b** Gene expression heatmap showing top-ranking DEGs for malignant epithelial clusters ( $n = 12$ ). **c** Correlation heatmap displaying similarities of transcriptional profiles between mEPC subsets, color-coded by Z scores. **d** Heatmap showing distributions of inferred CNV hotspots (copy number gains or losses) across the chromosomal regions within different mEPC subsets.

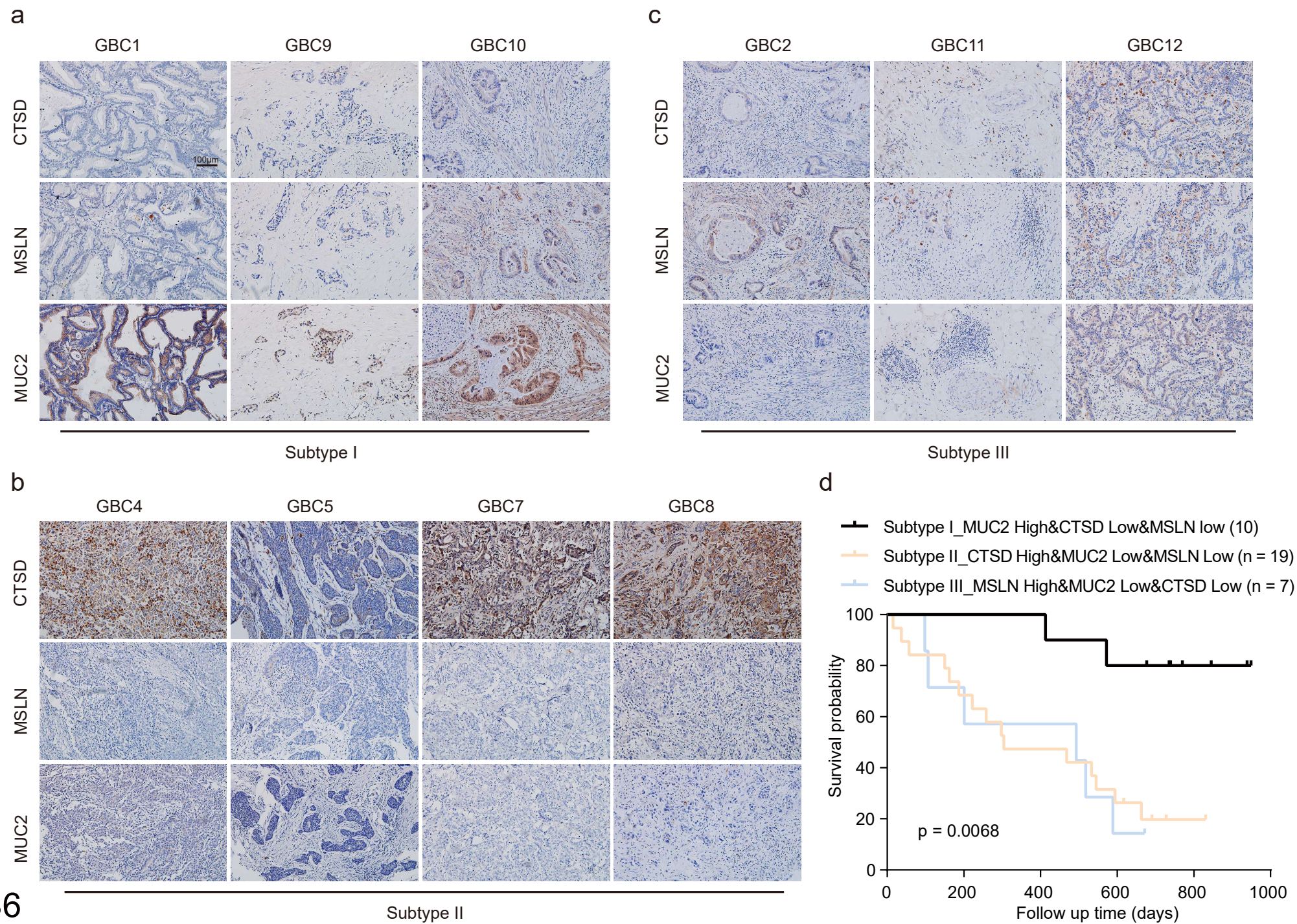


Fig.S6

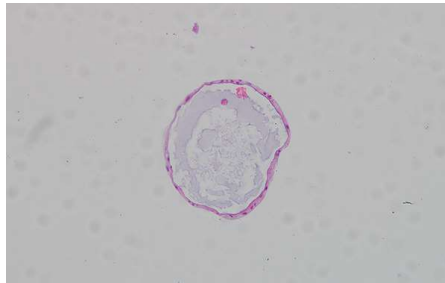
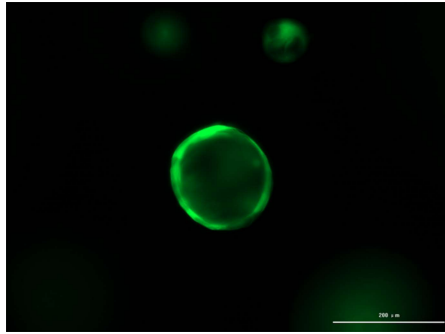
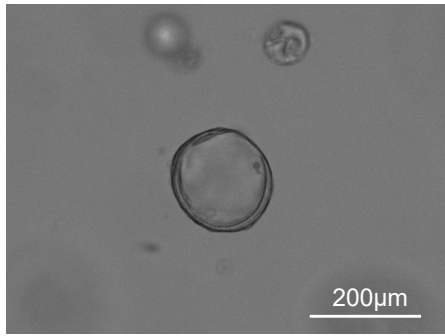
**Supplementary Fig. S6. IHC staining images of subtype-specific markers (subtype I: MUC2; subtype II: CTSD; subtype III: MSLN) across GBC samples in our scRNA-seq cohort. a** Images showing MUC2<sup>+</sup>CTSD<sup>-</sup>MSLN<sup>-</sup> staining among samples of GBC1, GBC9, and GBC10, respectively. **b** Images showing CTSD<sup>+</sup>MUC2<sup>-</sup>MSLN<sup>-</sup> staining among samples of GBC4, GBC5, GBC7, and GBC8, respectively. **c** Images showing MSLN<sup>+</sup>MUC2<sup>-</sup>CTSD<sup>-</sup> staining among samples of GBC2, GBC11, and GBC12, respectively. **d.** Kaplan-Meier curves comparing overall survival between three subtypes of GBC patients. Log-rank test,  $P = 0.007$ .



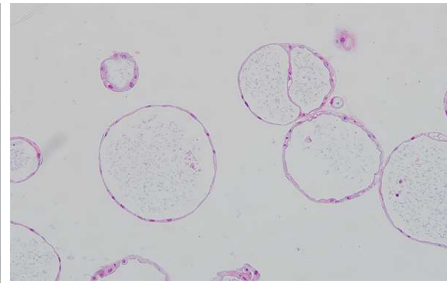
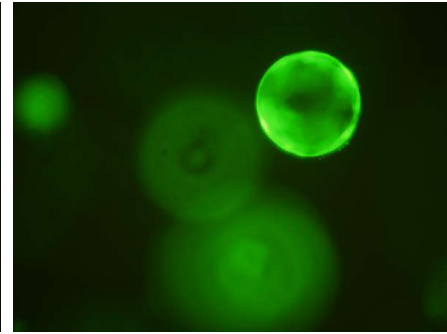
**Supplementary Fig. S7. Genomic alterations of GBC samples in our scRNA-seq cohort and the establishment of gallbladder organoids from patients with chronic cholecystitis. a** Whole-exome sequencing (WES) revealing distributions of most frequently altered (> 10% of the cohort) oncogenes and tumor suppressor genes (mutations or copy number variations), together with tumor mutation burden (TMB) levels, among eight GBC patients of different mEPC subtypes. Top histograms, frequency of genomic alterations per patient; left column, frequencies of somatic alterations; right column, gene names. **b** Establishment of MUC2<sup>-</sup>REG4<sup>-</sup> GBO-819 and MUC2<sup>+</sup>REG4<sup>+</sup> GBO-831 gallbladder organoids. First row: bright-field images; second to last rows: H&E and immunohistochemical staining images showing cystic morphology and positive CK7 staining for both organoids. GBO-831 distinctly displayed positive staining of MUC2 and REG4. Scale bars, 200  $\mu$ m.

GBO-819

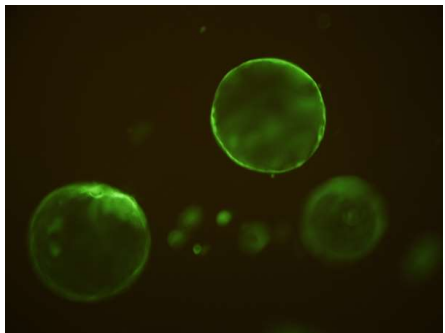
*GFP*



*GFP*



*KRAS*<sup>G12D</sup>



*KRAS*<sup>G12D</sup>



Fig.S8

**Supplementary Fig. S8. MUC2<sup>-</sup>REG4<sup>-</sup> patient-derived gallbladder organoid (GBO-819) transfected with lentivirus encoding *GFP* (control), or with lentivirus encoding *KRAS*<sup>G12D</sup>.** **a** First to third rows: bright-field, fluorescence, and H&E staining images showing microscopic appearance after two weeks of lenti-*GFP* transfection. Scale bars, 200  $\mu$ m. **b** First to third rows: bright-field, fluorescence, and H&E staining images showing microscopic appearance after two weeks of lenti-*KRAS*<sup>G12D</sup> transfection. Scale bars, 200  $\mu$ m.

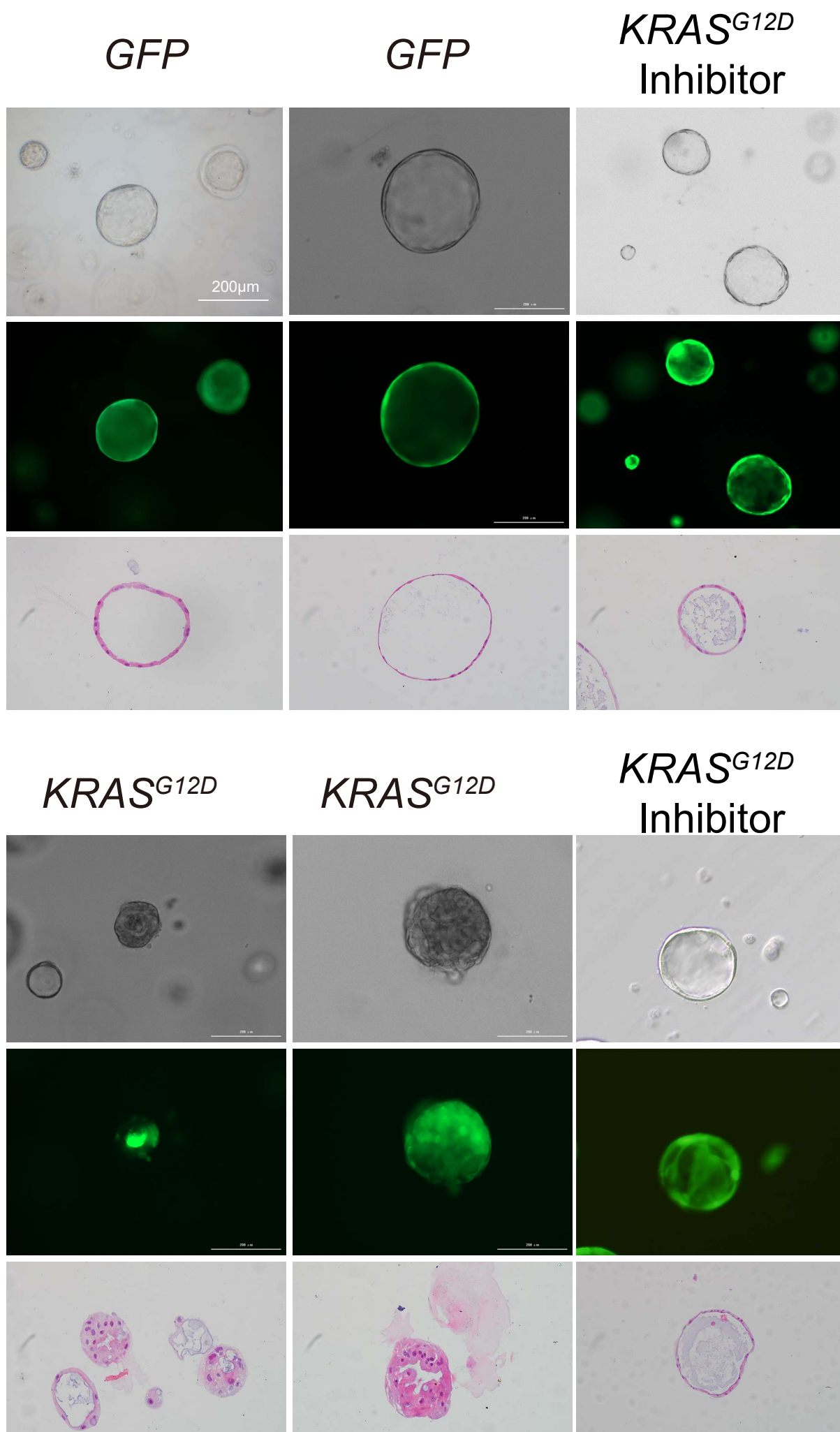
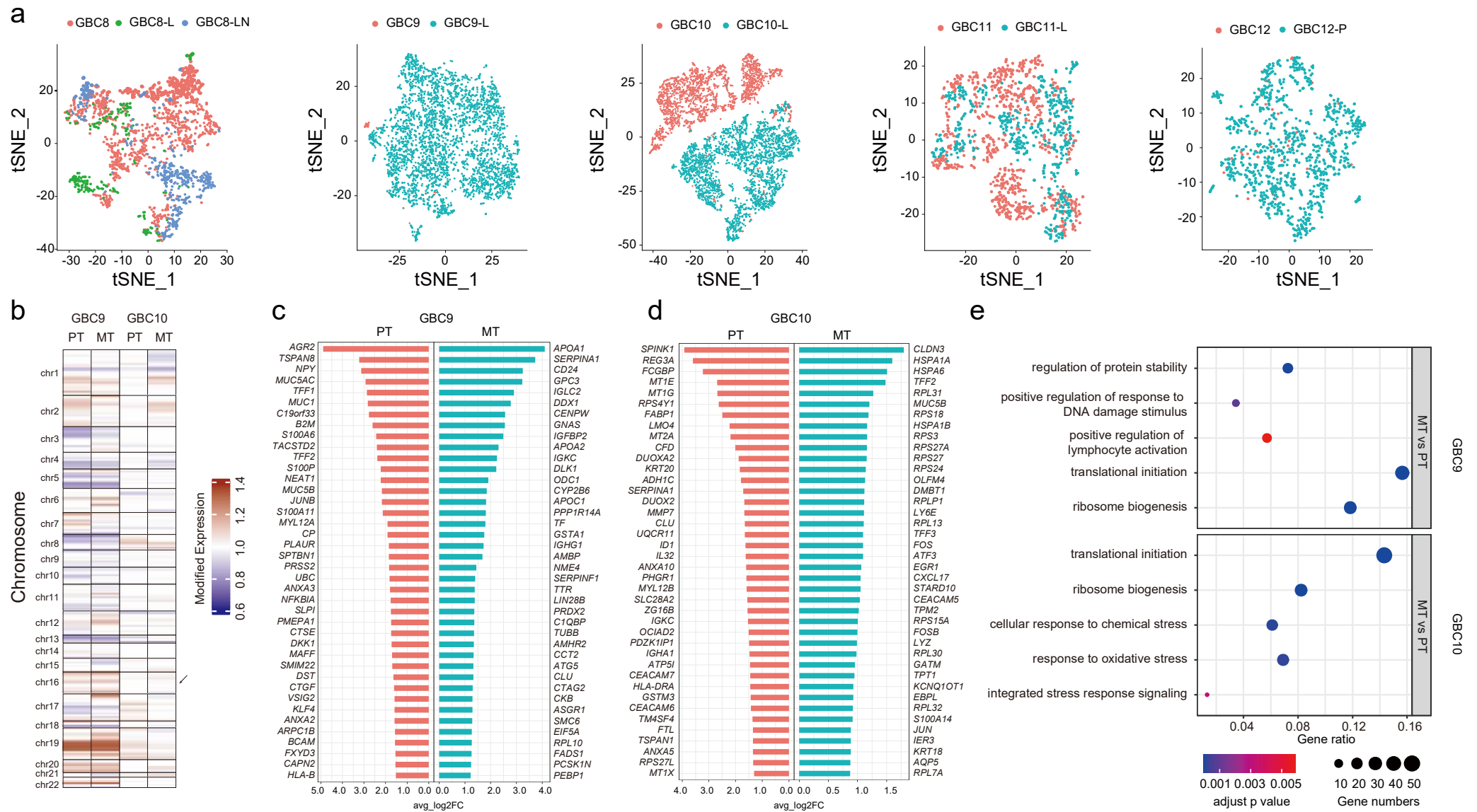


Fig.S9

**Supplementary Fig. S9. MUC2<sup>+</sup>REG4<sup>+</sup> patient-derived gallbladder organoid (GBO-831) transfected with lentivirus encoding *GFP*, or with lentivirus encoding *KRAS*<sup>G12D</sup>. **a** First to third rows: bright-field, fluorescence, and H&E staining images showing microscopic appearance after 2 weeks of lenti-*GFP* transfection, with or without treatment of *KRAS*<sup>G12D</sup> inhibitor 14 (MedChemExpress LLC, 2  $\mu$ M). Scale bars, 200  $\mu$ m. **b** First to third rows: bright-field, fluorescence, and H&E staining images showing microscopic appearance after 2 weeks of lenti-*KRAS*<sup>G12D</sup> transfection, with or without treatment of *KRAS*<sup>G12D</sup> inhibitor 14 (MedChemExpress LLC, 2  $\mu$ M). Scale bars, 200  $\mu$ m.**



**Supplementary Fig. S10. Comparison of transcriptional profiles between paired primary and metastatic GBC samples.** **a** t-SNE plots showing clustering of primary and metastatic cancer cells across paired samples from GBC patients (n = 5). **B** Heatmap comparing inferred CNV landscapes between PTs and MTs from two patients (GBC9-10). **c-d** Bar plots comparing transcription levels of top-ranking DEGs between PTs and MTs from GBC9 (**c**) and GBC10 (**d**). **e** Dot plots showing significantly enriched GO pathways for PTs versus paired MTs from two patients (GBC9-10).

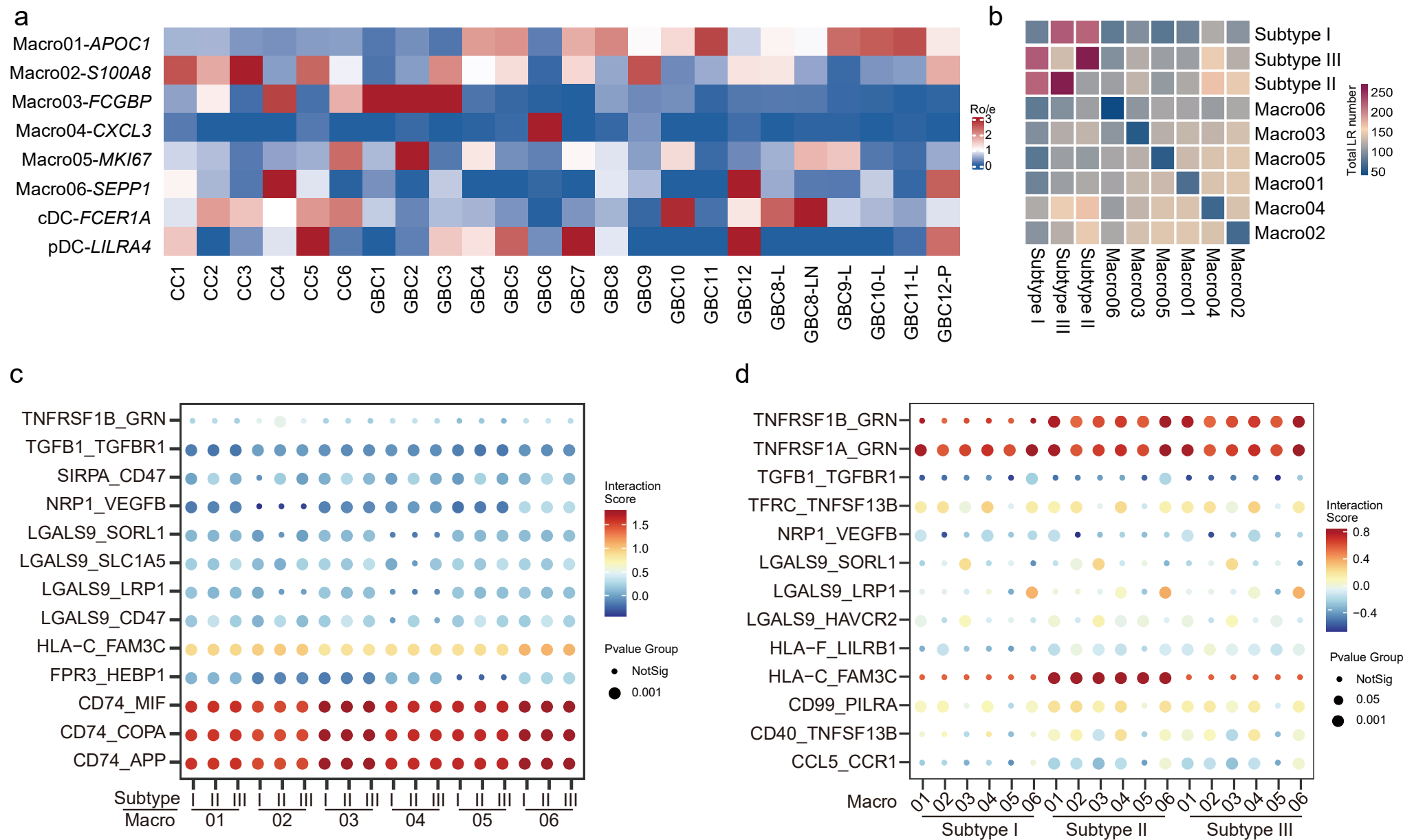


Fig.S11

**Supplementary Fig. S11. Distributions of myeloid cell clusters and interactions between macrophage clusters and mEPC subsets.** **a** Heatmap showing the relative abundance of different myeloid cell clusters in each sample.  $Ro/e > 1$  indicates significant enrichment. **b** Heatmap showing the total number of ligand-receptor interactions between macrophage clusters and mEPC subsets (Subtype I-III). **c-d** Bubble plots showing representative ligand-receptor pairs between macrophage clusters and mEPC subsets (Subtype I-III). Dot size indicates  $P$  value, colored by interaction score.

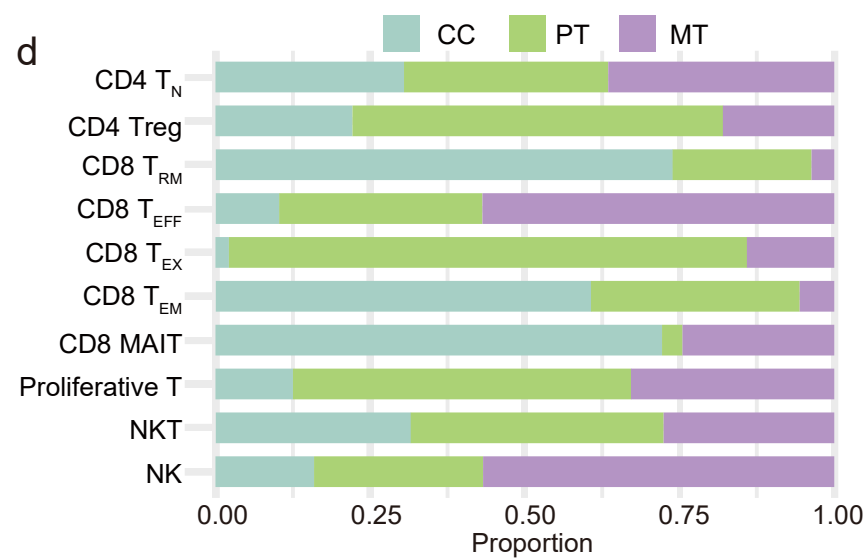
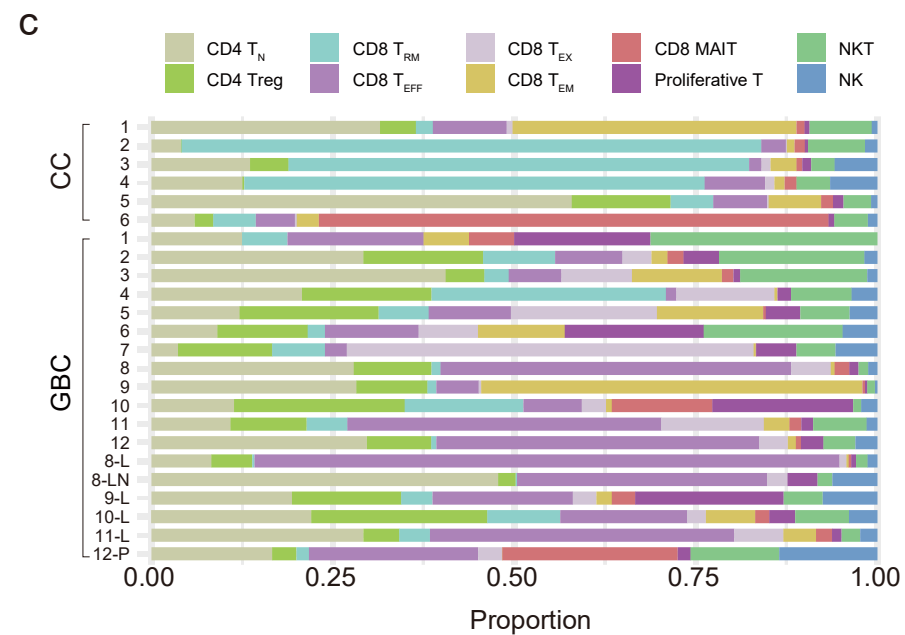
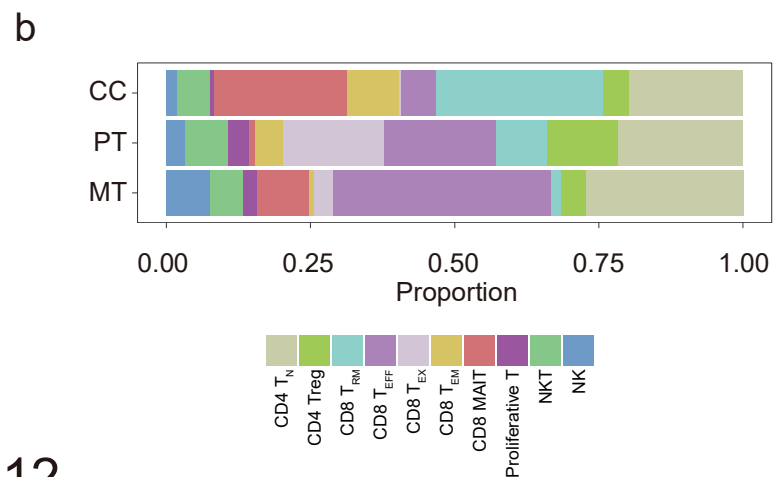
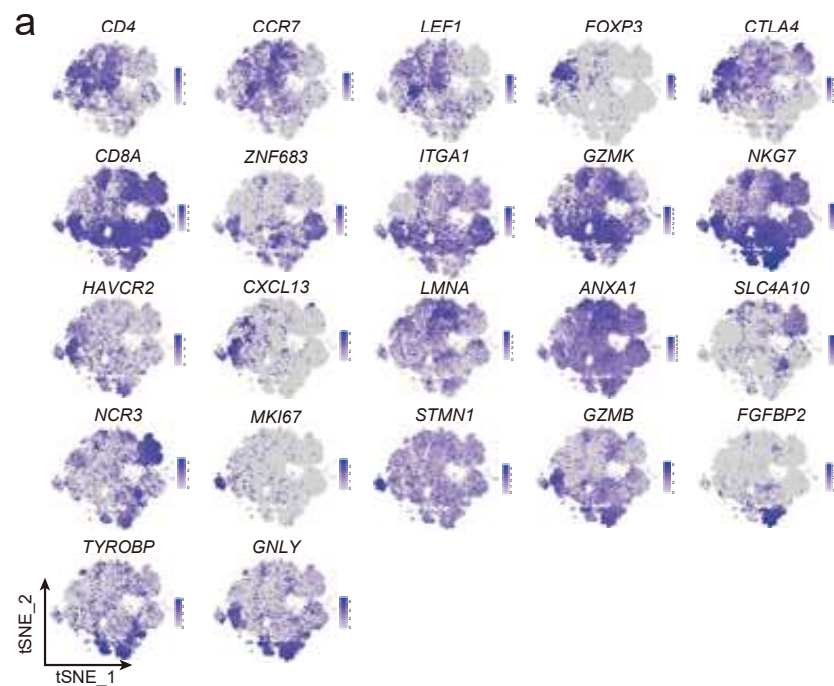


Fig.S12

**Supplementary Fig. S12. Gene signatures and distributions of T Cells and NK cells.** **a** t-SNE plots visualizing color-coded distributions of marker genes for T cells and NK cells. **b** Horizontal bar plots showing the relative abundance of T cell and NK cell subsets across CCs, PTs, and MTs. **c** Horizontal bar plots showing fractions of T cell and NK cell clusters in each sample. **d** Horizontal bar plots showing tissue sources of each T cell and NK cell cluster.

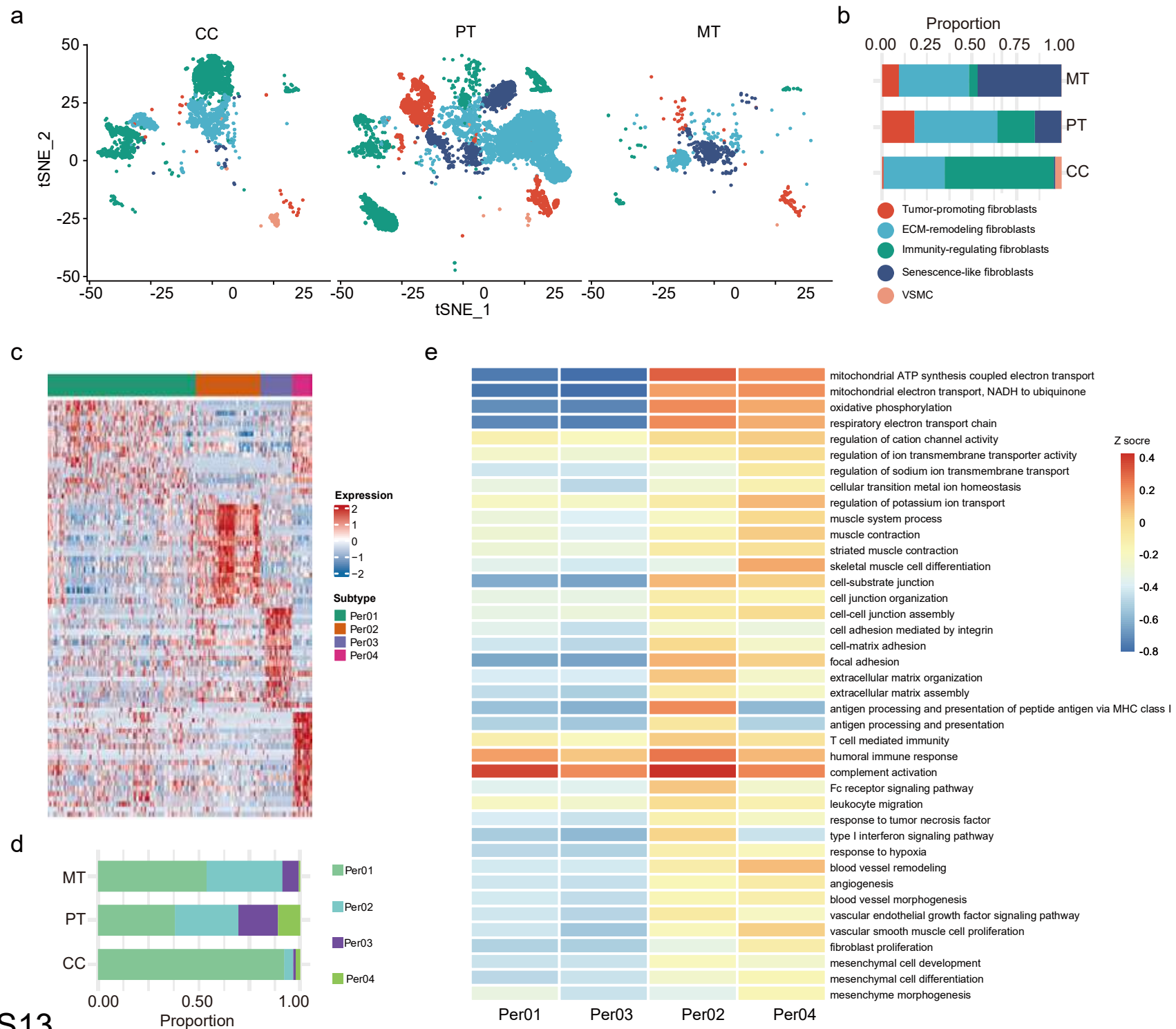
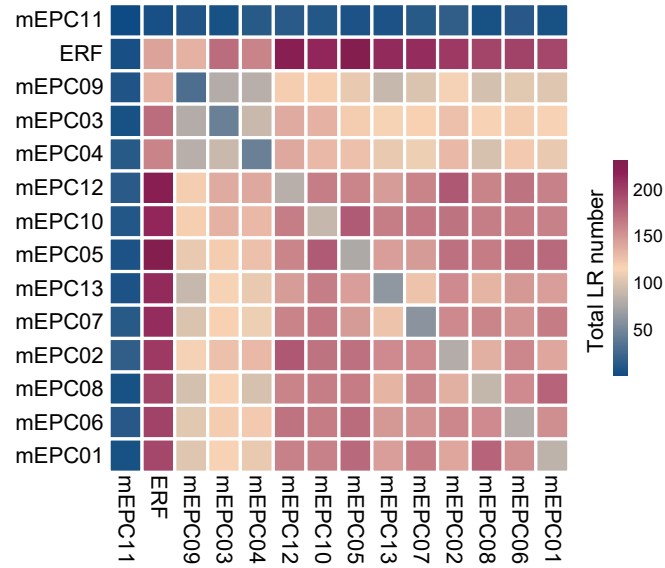


Fig.S13

**Supplementary Fig. S13. Distribution of fibroblast subtypes and characterization of pericytes.** **a** t-SNE plot visualizing the distribution of fibroblast subsets across CCs, PTs, and MTs. Five subsets were colored in line with **b**. **b** Horizontal bar plots showing fractions of different fibroblast subsets across CCs, PTs, and MTs. **c** Heatmap showing specific expression profiles for each pericyte cluster. **d** The relative abundance of pericytes subsets across CCs, PTs, and MTs. **e** Heatmap showing differentially enriched GO terms across four pericyte clusters, based on GSVA analysis.

a



b

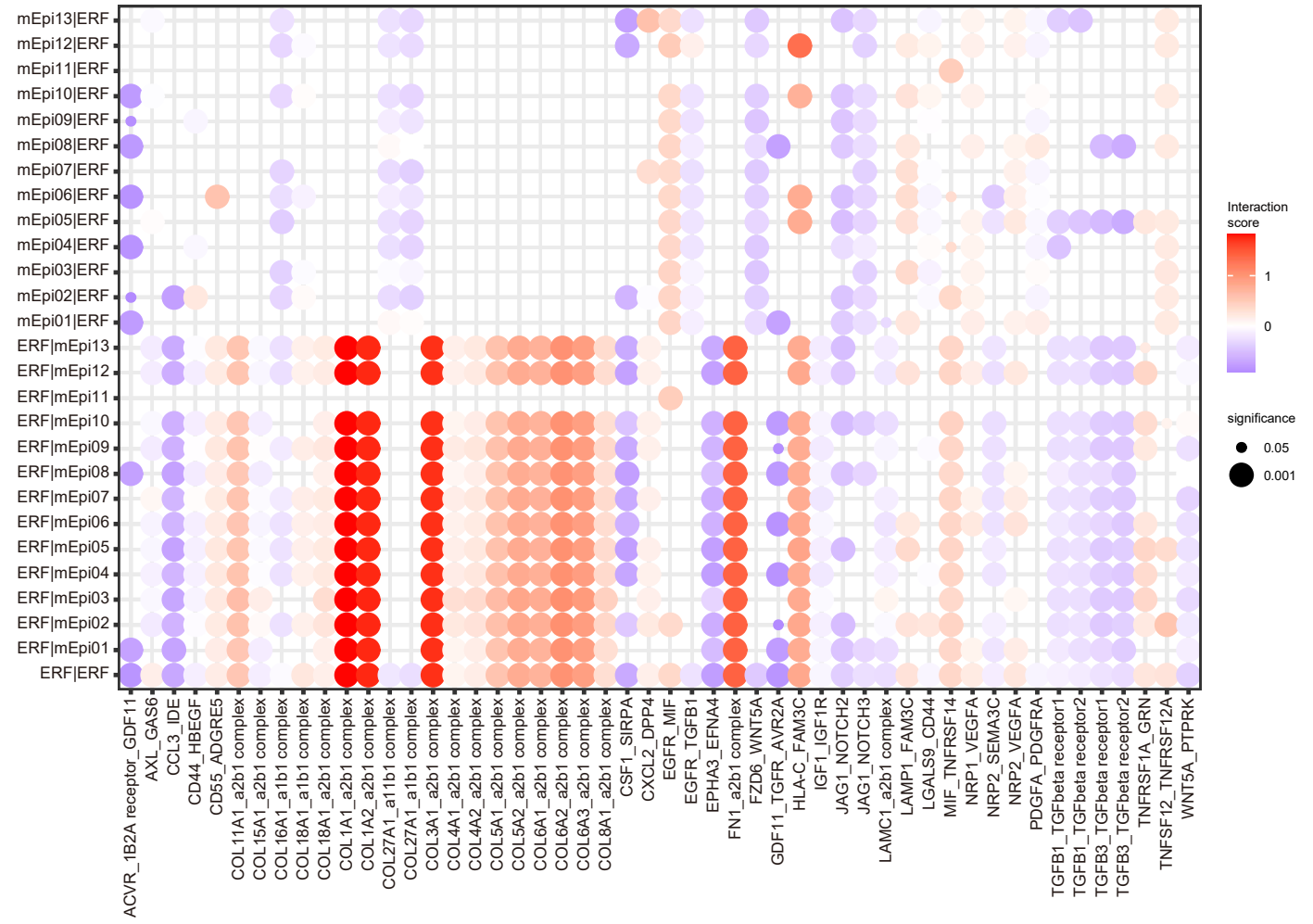
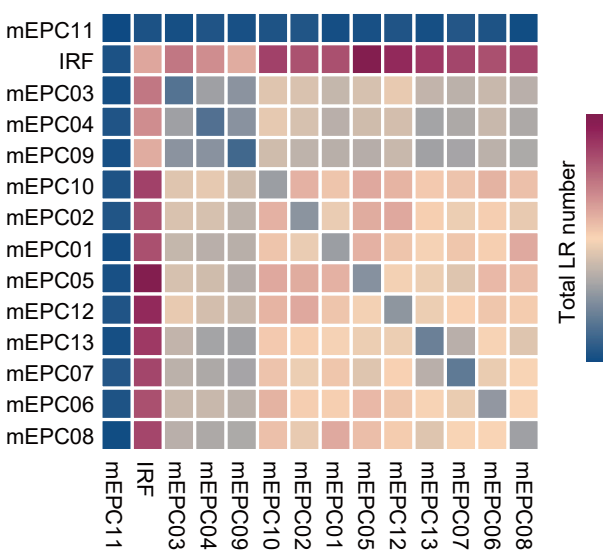


Fig.S14

**Supplementary Fig. S14. Ligand-receptor interactions between malignant epithelial clusters and ECM-remolding fibroblasts (ERF) based on CellphoneDB analysis.** **a** Heatmap showing the total number of ligand-receptor interactions between malignant epithelial clusters and ERF. **b** Bubble plot showing significant ligand-receptor pairs between malignant epithelial clusters and ERF. Dot size represents  $P$  value and dot color intensity indicates interaction score.

Fig.S15



significance

● 0.05

● 0.001

Interaction score

1.0

0.5

0.0

-0.5

**Supplementary Fig. S15. Ligand-receptor interactions between malignant epithelial clusters and immunity-regulating fibroblasts (IRF) based on CellphoneDB analysis.** **a** Heatmap showing the total number of ligand-receptor interactions between malignant epithelial clusters and IRF. **b** Bubble plot showing significant ligand-receptor pairs between malignant epithelial clusters and IRF. Dot size represents  $P$  value and dot color intensity indicates interaction score.

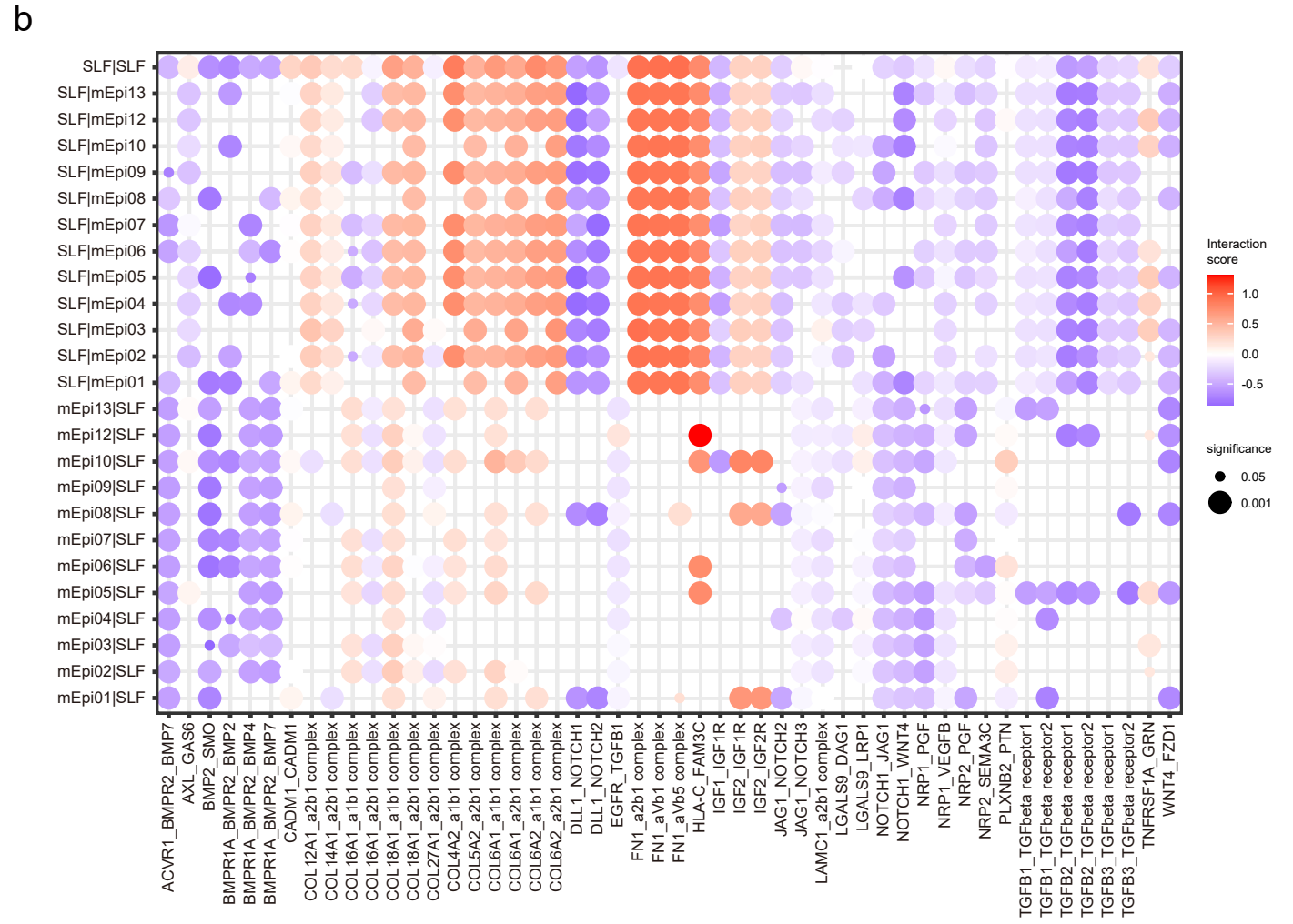
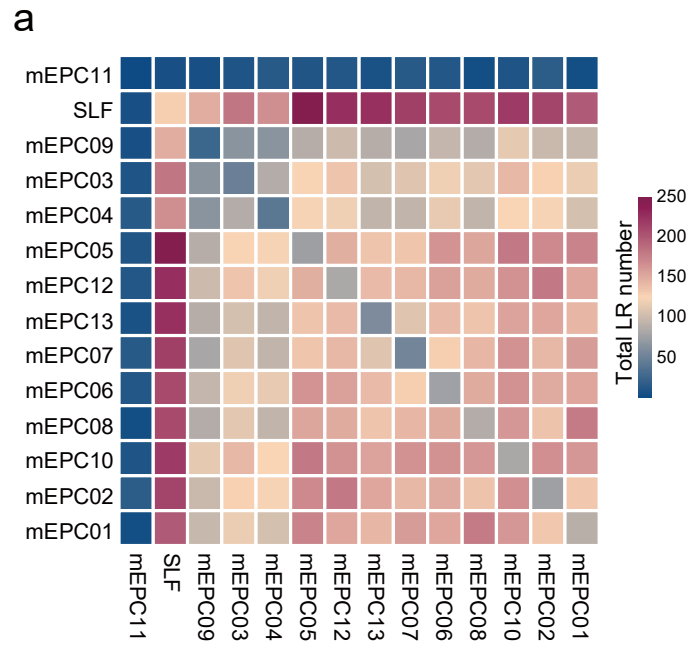
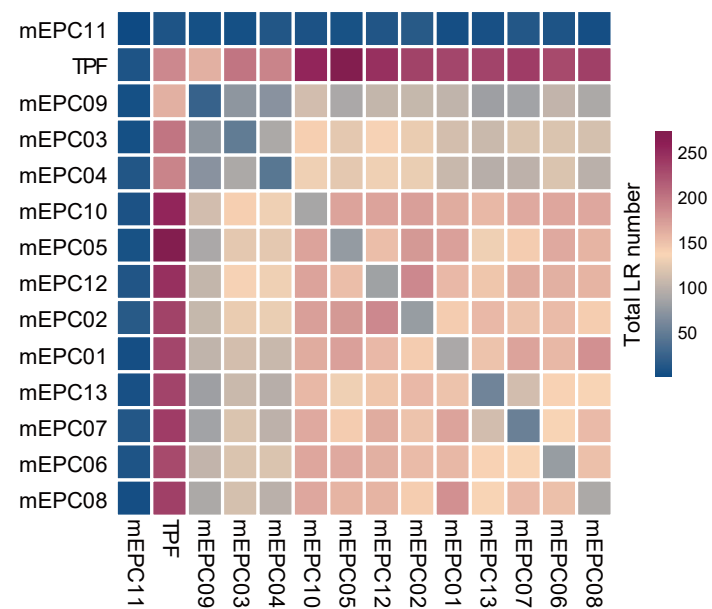


Fig.S16

**Supplementary Fig. S16. Ligand-receptor interactions between malignant epithelial clusters and senescence-like fibroblasts (SLF) based on CellphoneDB analysis.** **a** Heatmap showing the total number of ligand-receptor interactions between malignant epithelial clusters and SLF. **b** Bubble plot showing significant ligand-receptor pairs between malignant epithelial clusters and SLF. Dot size represents  $P$  value and dot color intensity indicates interaction score.

a



**b**

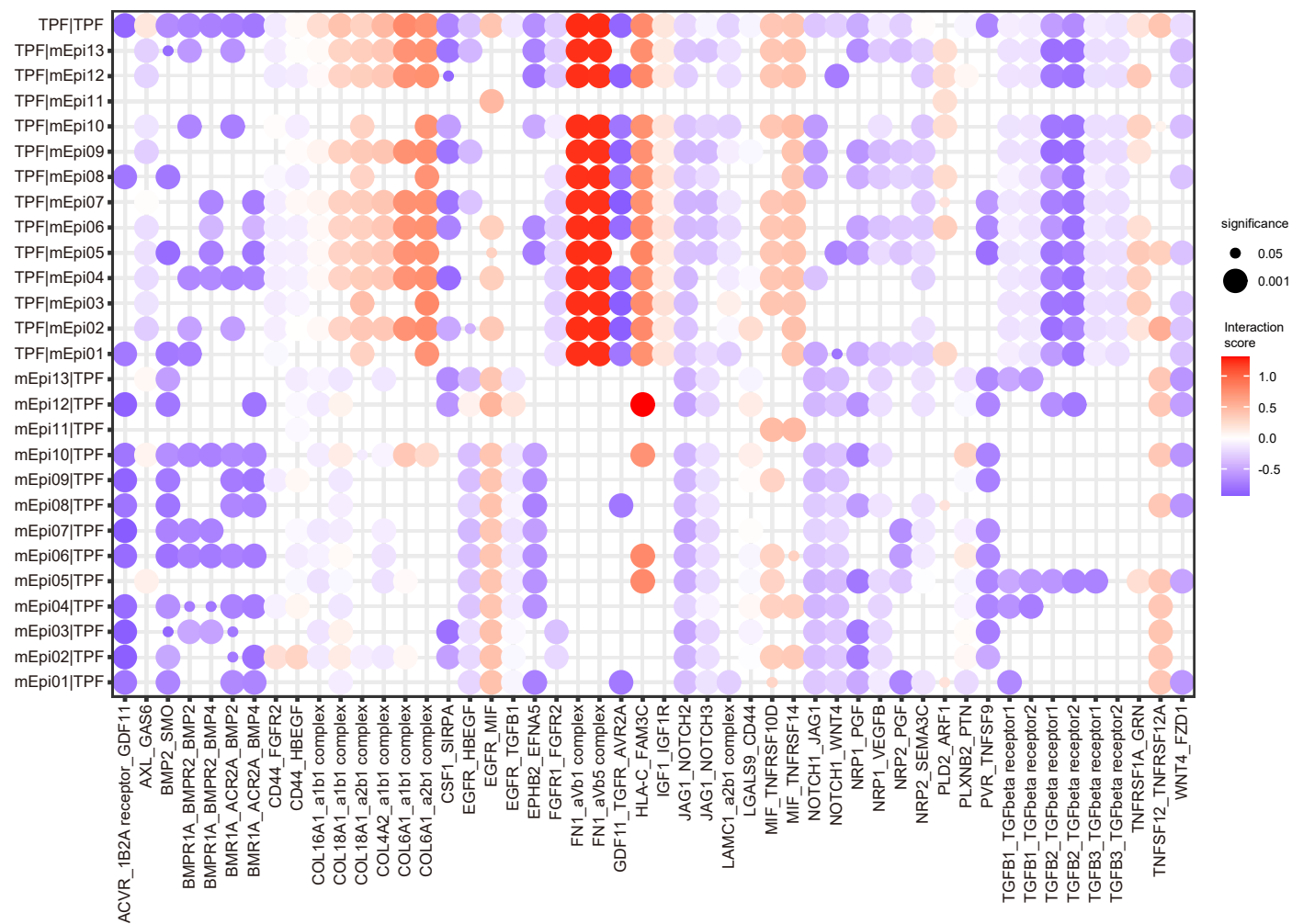


Fig.S17

**Supplementary Fig. S17. Ligand-receptor interactions between malignant epithelial clusters and tumor-promoting fibroblasts (TPF) based on CellphoneDB analysis.** **a** Heatmap showing the total number of ligand-receptor interactions between malignant epithelial clusters and TPF. **b** Bubble plot showing significant ligand-receptor pairs between malignant epithelial clusters and TPF. Dot size represents  $P$  value and dot color intensity indicates interaction score.