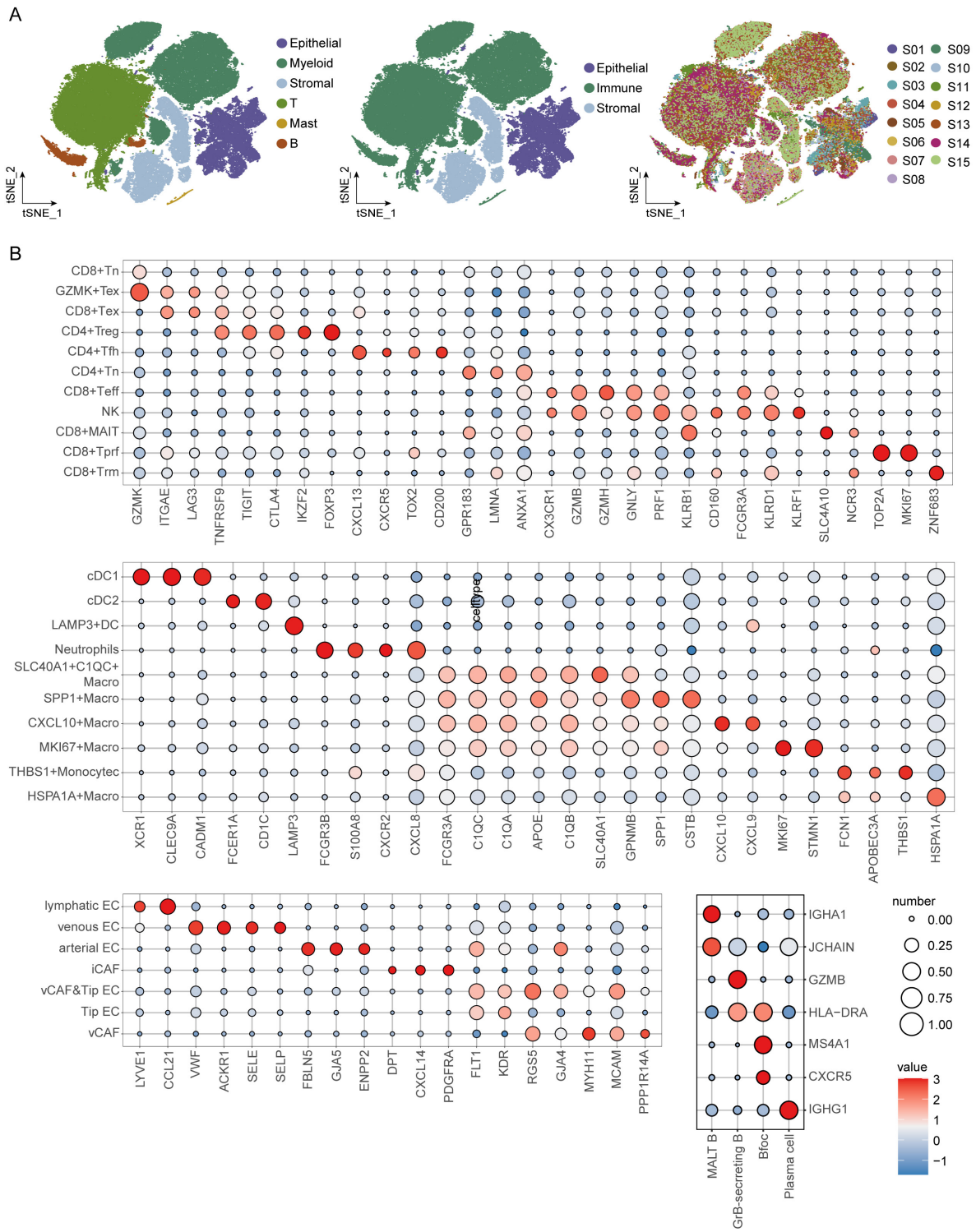

Figure S1 A comprehensive atlas of H-ChC, HCC, and iCCA. (A) t-SNE plot of all cells coloured by identity. Left: cell type, epithelial cells, myeloid cells, stromal cells, T cells, mast cells, B cells. Middle: cellular identity, epithelial cells, immune cells, stromal cells. Right: sample identity. (B) Marker genes used to identify various cell types. H-ChC, hepatocholangiocarcinoma; HCC, hepatocellular carcinoma; iCCA, intrahepatic cholangiocarcinoma; t-SNE, T-distribution stochastic neighbour embedding.

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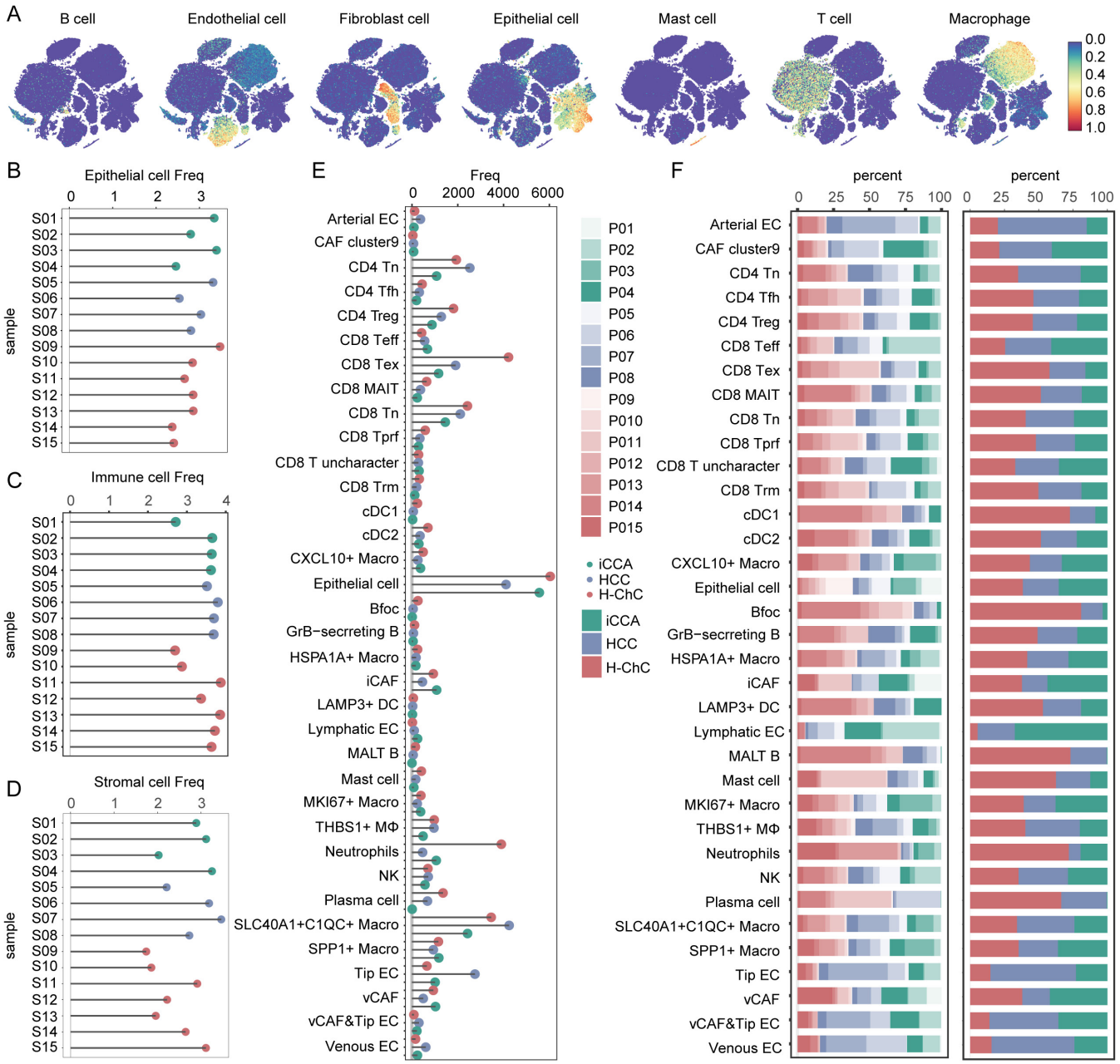

Figure S2 Different cell subpopulations and immune components of H-ChC, HCC, and iCCA. (A) Epithelial and nonepithelial cells were grouped by known cell lineage-specific marker genes. (B) Number of epithelial cells per specimen. (C) Number of immune cells per specimen. (D) Number of stromal cells per specimen. (E) Number of cell subpopulations in different cell types per specimen. (F) Fraction of cells from the different specimens and tumours within each cell type. H-ChC, hepatocholangiocarcinoma; HCC, hepatocellular carcinoma; iCCA, intrahepatic cholangiocarcinoma.

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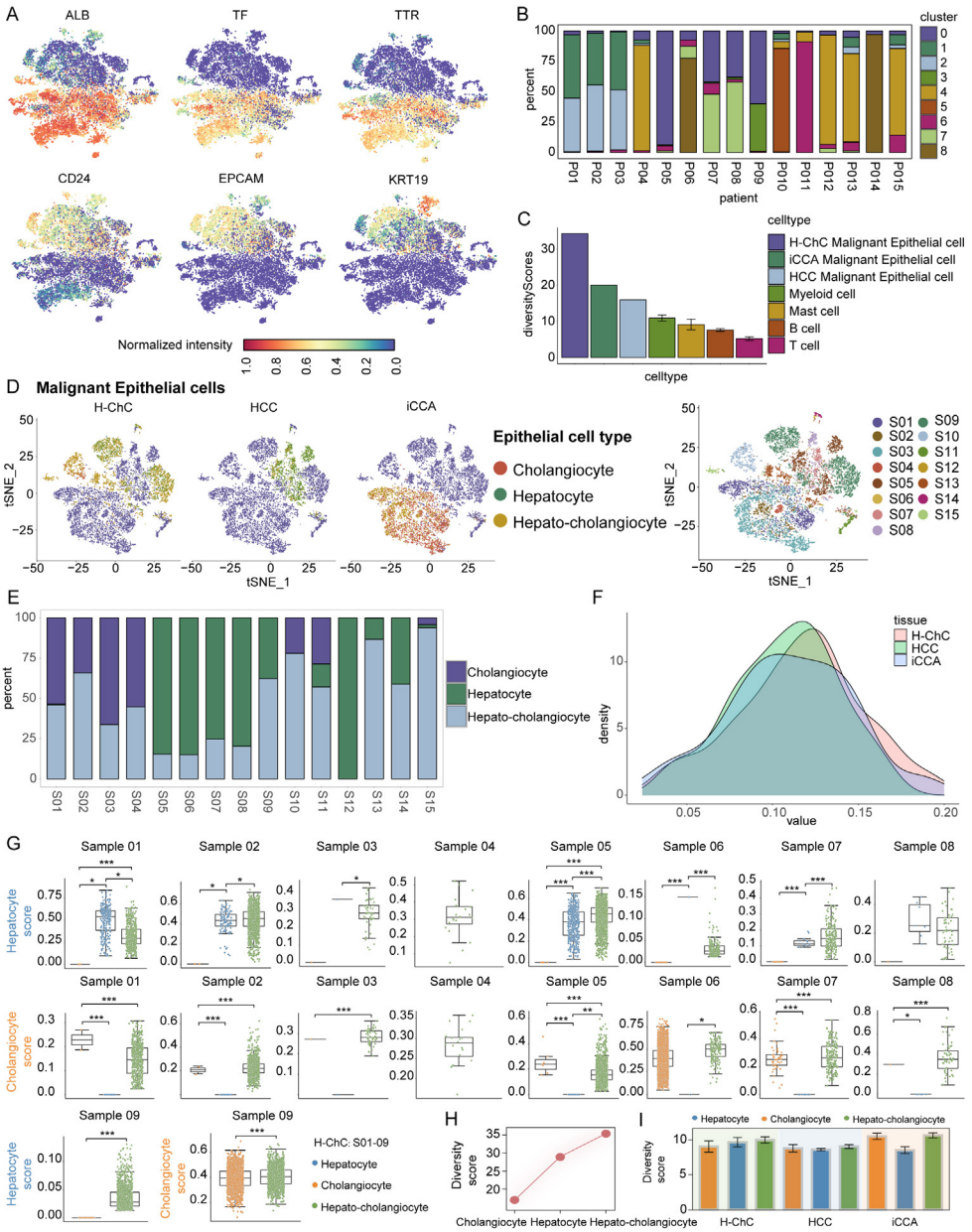

Figure S3 H-ChC is more heterogeneous than HCC and iCCA. (A) Epithelial cells were marked by known cell lineage-specific marker genes. (B) The proportion of epithelial cell clusters in different patients. Each bar is coloured by cluster. (C) Intratumoral heterogeneity within each malignant epithelial cell type and immune cell type (myeloid cells, mast cells, T cells and B cells) as measured by diversity score for treatment-naïve samples in H-ChC, HCC and iCCA. (D) t-SNE plot of the malignant epithelial cell partition coloured according to identify. Left: cellular identity, malignant hepatocholangiocytes, malignant hepatocytes, malignant cholangiocytes. Right: sample identity. (E) The proportion of malignant epithelial cell clusters in different patients. Each bar is coloured by cluster. (F) Single-cell CNV burden of malignant cells in H-ChC, HCC and iCCA. The single-cell CNV burden was estimated by CopyKAT. (G) Boxplots showing the hepatocyte score and cholangiocyte score alterations of malignant epithelial cell clusters in different specimens in the data of Xue *et al*. (H) Intratumoural heterogeneity within different malignant cell types as measured by diversity score for cholangiocyte, hepatocyte and hepato-cholangiocyte cells. (I) Intratumoural heterogeneity within different malignant cell types as measured by diversity score for cholangiocyte, hepatocyte and hepato-cholangiocyte cells in the data of Xue *et al*. H-ChC, hepatocholangiocarcinoma; HCC, hepatocellular carcinoma; iCCA, intrahepatic cholangiocarcinoma; t-SNE, T-distribution stochastic neighbour embedding; CNV, copy number variant.

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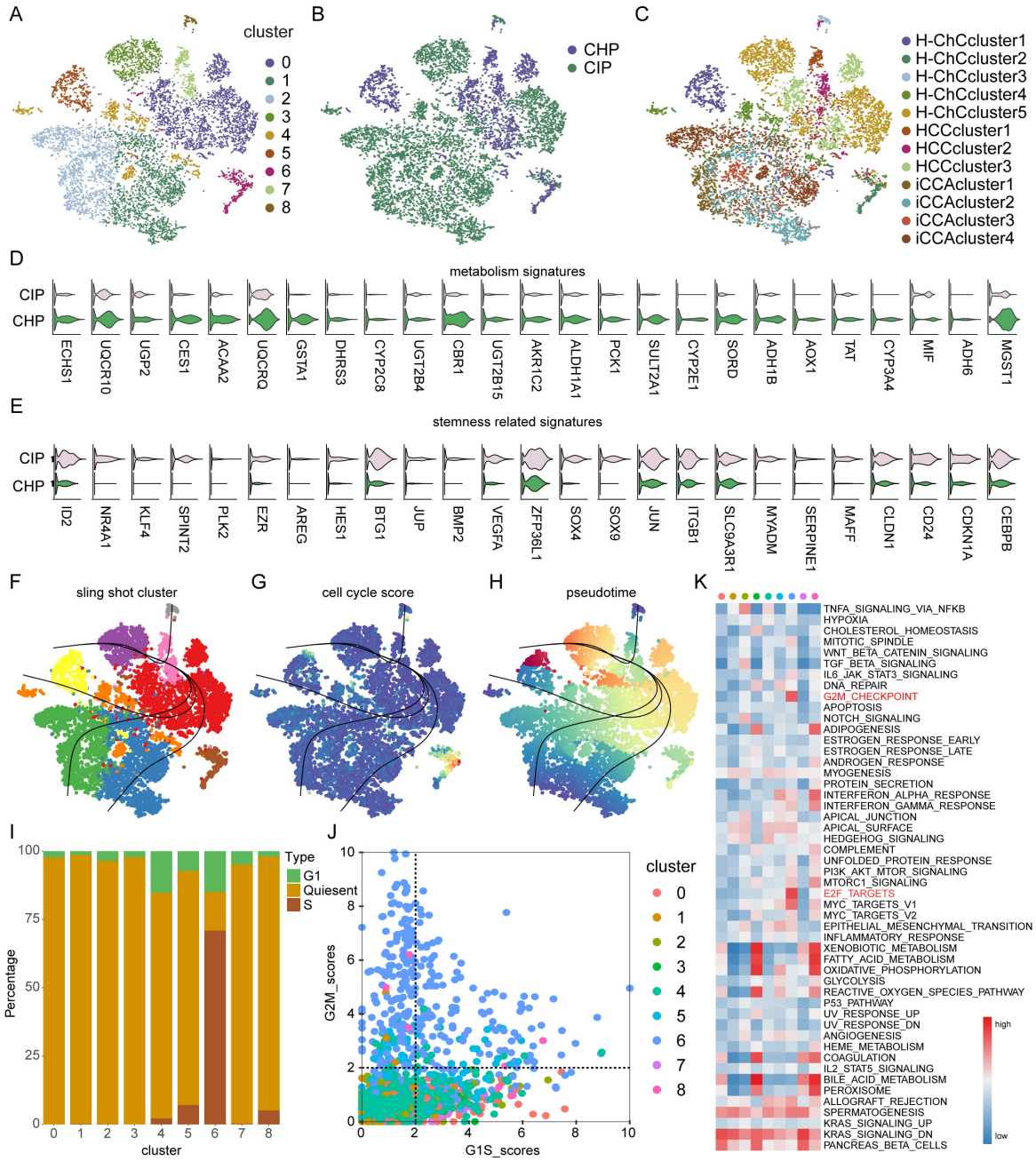

Figure S4 H-ChC can be defined as a separate tumour and has two phenotypes: CHP and CIP. (A) t-SNE plot of malignant epithelial cells coloured by cluster. (B) t-SNE plot of malignant epithelial cells coloured by phenotype. (C) t-SNE plot of malignant epithelial cells coloured by NMF cluster. (D) Violin plot showing selected DEGs associated with metabolism between CHP and CIP. (E) Violin plot showing selected DEGs associated with epithelial proliferation between CHP and CIP. (F) Trajectory of malignant epithelial cells coloured according to the cluster of malignant epithelial cells. (G) Trajectory of malignant epithelial cells coloured according to the cell cycle score of malignant epithelial cells. (H) Trajectory of malignant epithelial cells coloured according to the pseudotime of malignant epithelial cells. (I) The distribution of cell cycle status in malignant epithelial cells from different clusters. Each bar is coloured according to the cell cycle state. (J) Cell cycle scores for each cluster of malignant epithelial cells. (K) The enriched HALLMARK gene pathways of different clusters in malignant epithelial cells. H-ChC, hepatocholangiocarcinoma; t-SNE, T-distribution stochastic neighbour embedding; CNV, copy number variant.

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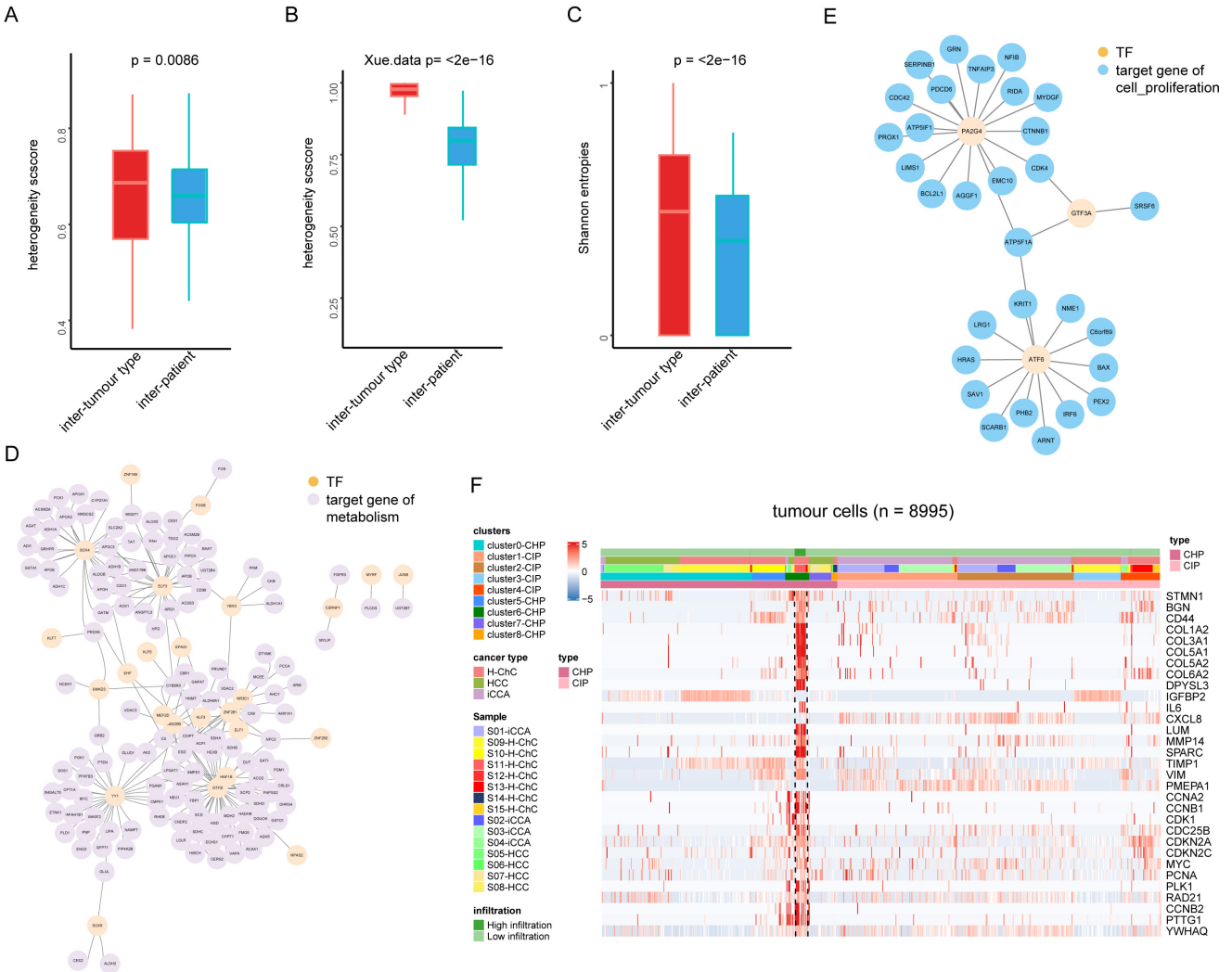

Figure S5 (A,B) The heterogeneity between patients is significantly lower than that between cancer types in our cohort (A) and Xue's data (B). (C) The heterogeneity (Shannon entropies measure) between patients is significantly lower than that between cancer types in our cohort. (D) TF target network created from malignant cell, organized into super-regulons for CHP. (E) TF target network created from malignant cell, organized into super-regulons for CIP. (F) Complex heatmap showing the expression patterns of cell cycle genes in malignant epithelial cells.

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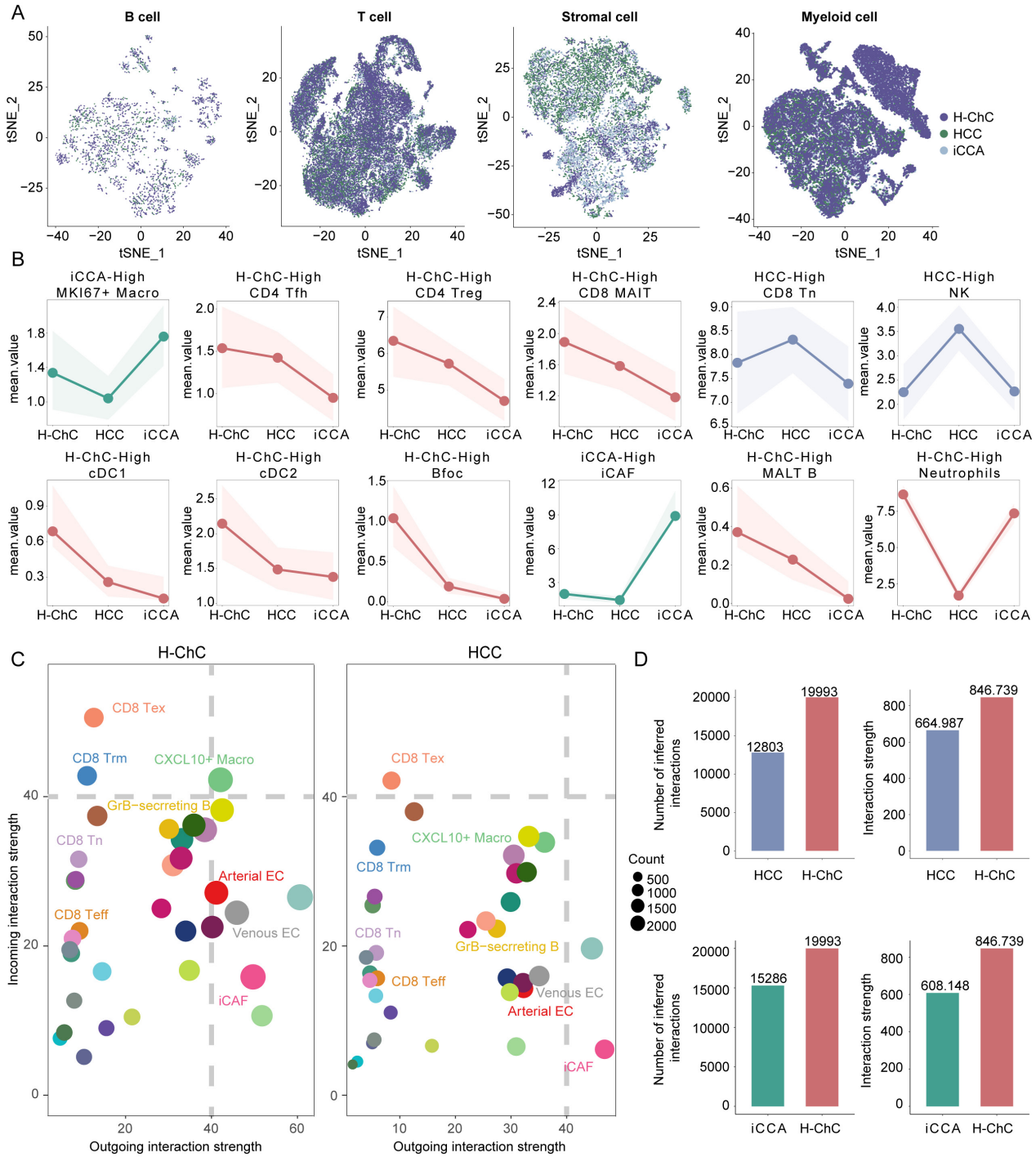

Figure S6 H-ChC shows greater exhausted CD8+ T-cell dysfunction than HCC and iCCA. (A) t-SNE projections of the nonepithelial subsets of H-ChC, HCC and iCCA coloured by tumour type. (B) The proportions of the other nonepithelial cell subsets. The y-axis represents the percentage (bootstrap), and the x-axis represents different tumours. The shaded areas represent the upper and lower quantile bootstrap cell proportions. (C) The incoming interaction and outgoing interaction strengths of different cell types in H-ChC (left) and HCC (right). (D) The total number and intensity of interactions in H-ChC, HCC and ICC. The total number and intensity of interactions were both highest in H-ChC. H-ChC, hepatocholangiocarcinoma; HCC, hepatocellular carcinoma; iCCA, intrahepatic cholangiocarcinoma; t-SNE, T-distribution stochastic neighbour embedding.

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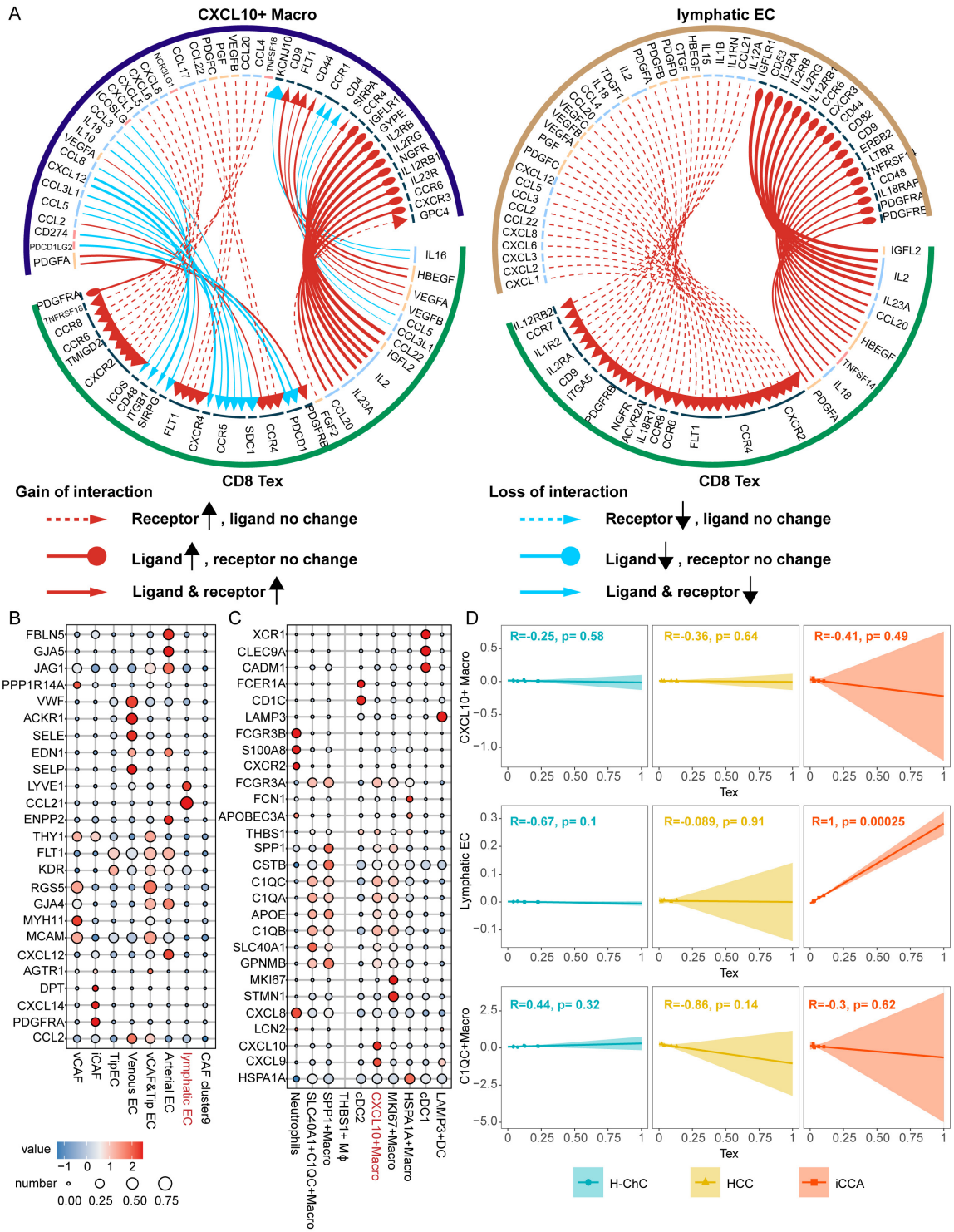

Figure S7 The interaction relationship of CD8⁺ Tex cells. (A) iTALK analysis showing the interaction of CD8⁺ Tex cells with CXCL10⁺ Macro- and lymphatic ECs. (B) Bubble heatmap showing the expression patterns of selected marker genes in distinct stromal cell subtypes. (C) Bubble heatmap showing the expression patterns of selected marker genes in distinct myeloid cell subtypes. (D) Scatterplots showing correlations of CD8⁺ Tex cells with CXCL10⁺ Macro, lymphatic ECs, and C1QC⁺ Macro in H-ChC (left), HCC (middle) and iCCA (right). EC, endothelial cell; H-ChC, hepatocholangiocarcinoma; HCC, hepatocellular carcinoma; iCCA, intrahepatic cholangiocarcinoma.

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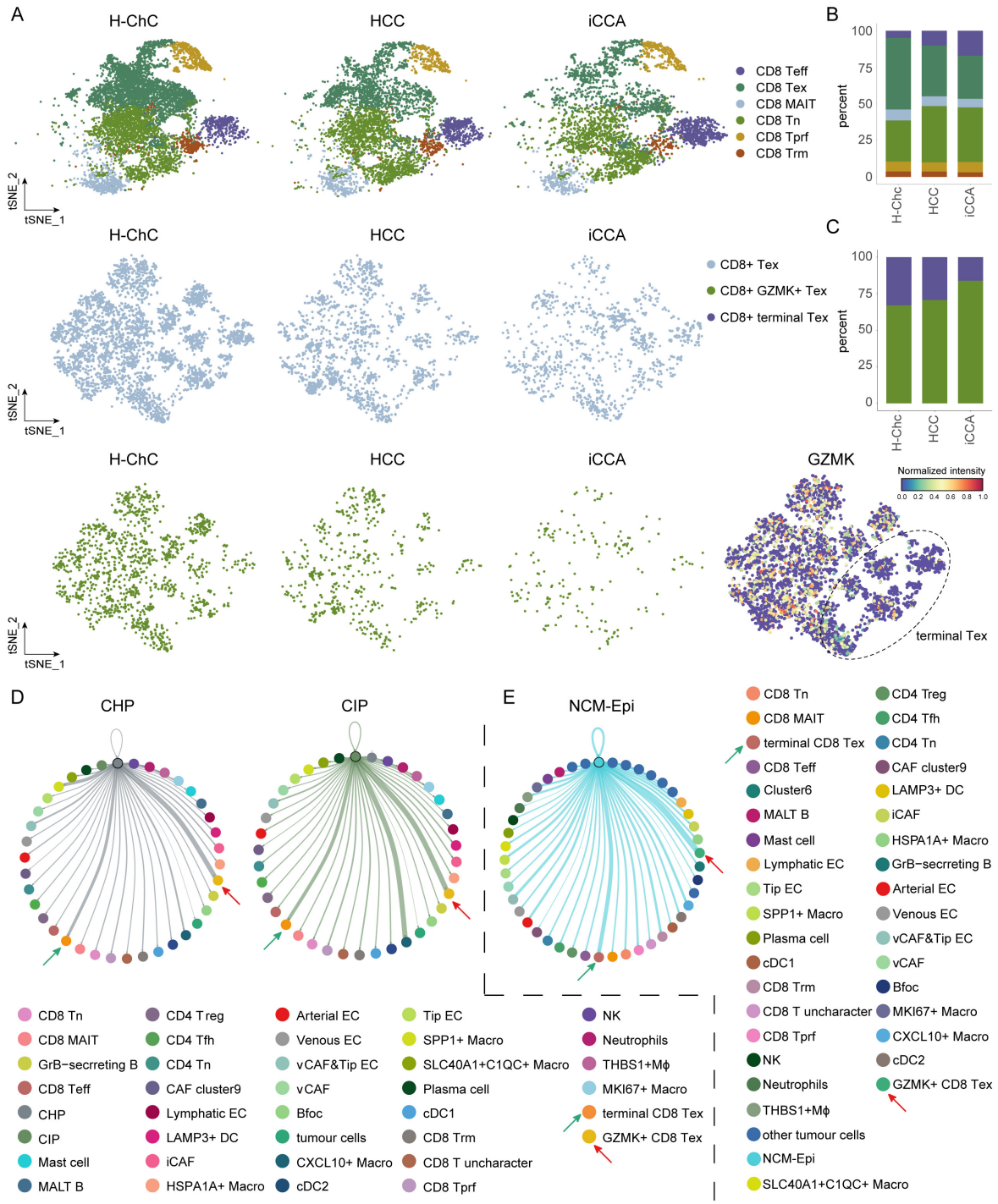

Figure S8 NCM-Epi was associated with immunosuppression and CD8⁺ T-cell exhaustion. (A) t-SNE plot of T cells coloured according to their cell type in H-ChC, HCC and iCCA (top). t-SNE plot of Tex cells coloured according to their cell type in H-ChC, HCC and iCCA (middle and bottom). (B) The proportions of selected T-cell subsets. The percentage is presented along the y-axis, and the different tumours are presented along the x-axis. (C) The proportions of selected Tex-cell subsets. The percentage is presented along the y-axis, and the different tumours are presented along the x-axis. (D) The differential crosstalk of CHP and CIP with other cells. (E) The differential crosstalk of cluster 6 with other cells. t-SNE, T-distribution stochastic neighbour embedding; H-ChC, hepatocholangiocarcinoma; HCC, hepatocellular carcinoma; iCCA, intrahepatic cholangiocarcinoma;.

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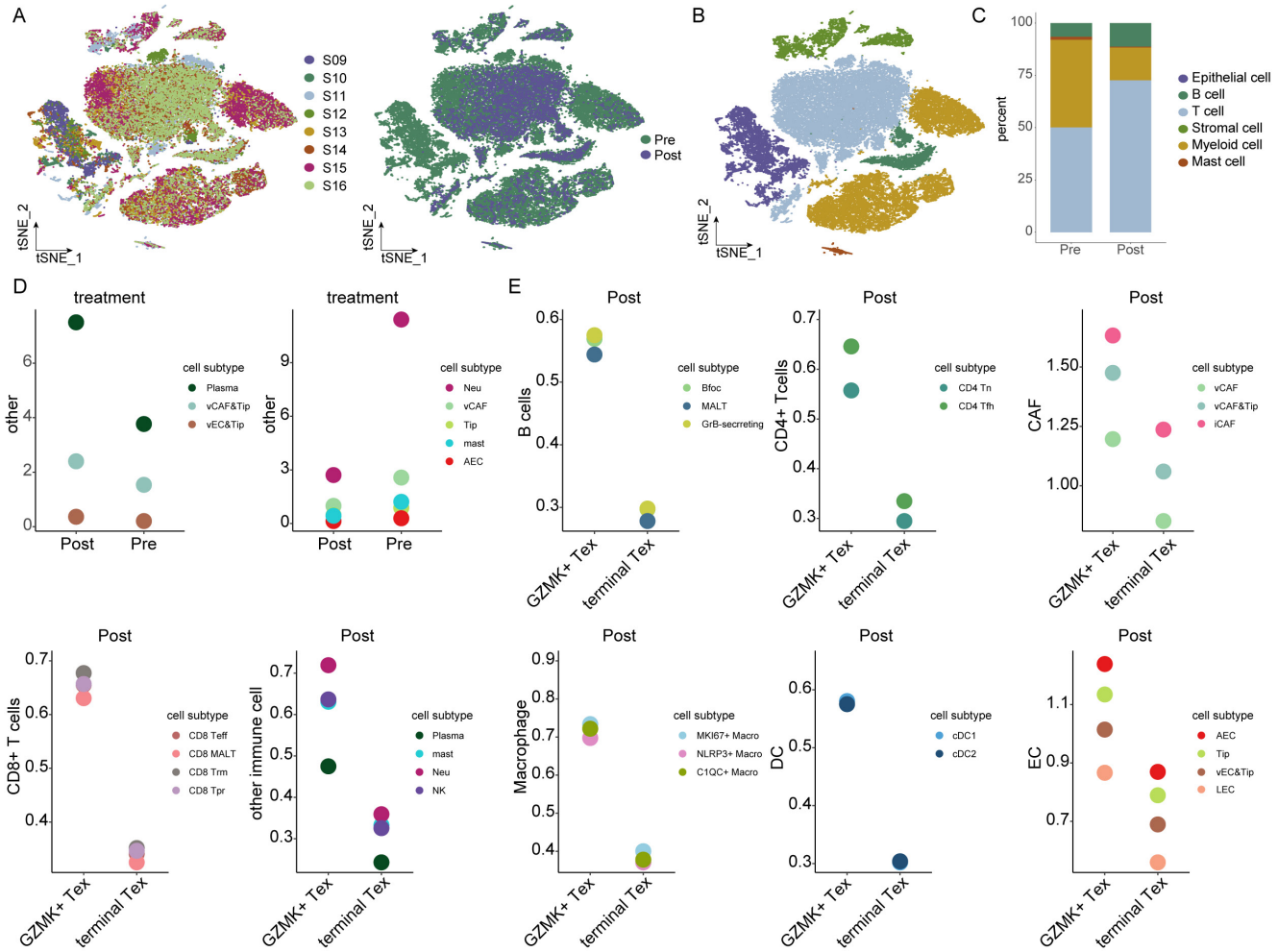

Figure S9 CD8⁺ Tex cells may play a key role in clinical immunotherapy. (A) t-SNE plot of all cells coloured by samples and treatment option. (B) t-SNE plot of all cells coloured by cell type. (C) The cellular compositions of major immune cell types in H-ChC tumours pre- and posttreatment. (D,E) Interaction strengths between selected cell types and CD8⁺ Tex subpopulations in H-ChC tumours (pre- *vs*. posttreatment). t-SNE, T-distribution stochastic neighbour embedding; H-ChC, hepatocholangiocarcinoma; HCC, hepatocellular carcinoma; iCCA, intrahepatic cholangiocarcinoma.

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Table S1 Clinical features and follow-up information of this study

Patient	Sample	Sex	Age, years	HBV	HCV	Liver cirrhosis	Alcohol	ECOG score	Child score	Pathology	Differentiation	Tumor number	BCLC stage
P01	S01	Male	67	0	0	0	0	2	A	iCCA	L-M	2	B
P02	S02	Male	59	0	0	0	0	0	A	iCCA	M	3	B
P03	S03	Female	57	0	0	0	0	0	A	iCCA	M	3	B
P04	S04	Male	53	0	0	0	1	1	A	iCCA	L	4	B
P05	S05	Male	56	1	0	1	1	0	A	HCC	M-H	3	C
P06	S06	Male	46	1	0	1	1	1	A	HCC	M	6	C
P07	S07	Male	78	0	1	0	0	1	A	HCC	M-H	1	A
P08	S08	Male	64	1	0	1	1	0	A	HCC	M	2	B
P09	S09, S10	Male	37	1	0	1	0	0	A	H-ChC	M	1	A
P10	S11, S12	Male	40	1	0	1	1	0	A	H-ChC	L-M	1	A
P11	S13, S14	Female	52	1	0	1	0	0	A	H-ChC	NA	2	B
P12	S15	Male	60	1	0	1	0	1	A	H-ChC	NA	1	A
P13	S16	Male	53	1	0	1	0	0	A	H-ChC	L-M	1	A

HBV: HBV infection status of patients, 1 indicates HBV infection. HCV: HCV infection status of patients, 1 indicates HCV infection. Liver cirrhosis: liver cirrhosis or not of patients; 1 indicates liver cirrhosis. Alcohol: degree of liver cirrhosis, 1 indicates patient drinking alcohol. ECOG score: 0 points, the activity ability of patient is completely normal; 1 points, able to move freely and engage in light physical activities, including general household or office work, but unable to engage in heavy physical activities; 2 points, patients can be able to move freely and take care of oneself, but has lost the ability to work, and can wake up and exercise at least half of the day. Child score: the lowest score for the total is 5 points, and the highest score is 15 points. Liver reserve function is divided into three levels: A, B, and C, indicating three different degrees of liver damage (the higher the score, the worse the liver reserve function). Pathology: pathological indications of cancer types in patients. Differentiation: L, Low differentiation; L-M, low medium differentiation; M, medium differentiation; M-H, medium high differentiation. BCLC stage, A, liver cancer is still limited to the liver, but it is over 5 centimeters in size or has small cancer lesions. Liver function is normal or slightly damaged, and there are no symptoms or only mild symptoms. B, liver cancer has invaded liver blood vessels or lymph nodes, or liver function is moderately damaged, with symptoms such as abdominal pain and fatigue. C, liver cancer has spread to surrounding tissues or organs, or liver function is severely damaged, with serious symptoms such as ascites, jaundice. ECOG, Eastern Cooperative Oncology Group; HCC, hepatocellular carcinoma; iCCA, intrahepatic cholangiocarcinoma; H-ChC, hepatocholangiocarcinoma; BCLC, Barcelona Clinic Liver Cancer.

Table S2 Clustering results of epithelial cell subpopulations

Cluster	Cholangiocyte	Hepatocyte	Hepato-cholangiocyte	Non-hepatobiliary
0	46	1406	1,057	3
1	1403	16	497	78
2	738	0	1,188	0
3	0	351	429	0
4	80	71	480	18
5	117	0	431	0
6	21	75	122	177
7	0	297	72	0
8	0	54	44	0

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Table S3 The results of pathway analysis for CHP and CIP

ID	Description	GeneRatio	BgRatio	P value	Padjust	Q value
GO:0072575	epithelial cell proliferation involved in liver morphogenesis	6/366	21/18862	2.17E−06	0.000101936	6.99E−05
GO:1904019	epithelial cell apoptotic process	7/366	116/18862	0.007499678	0.039408852	0.027041118
GO:0030856	regulation of epithelial cell differentiation	11/366	156/18862	0.000239539	0.003483129	0.002390014
GO:0002064	epithelial cell development	14/366	207/18862	5.54E−05	0.001199403	0.000822993
GO:0050679	positive regulation of epithelial cell proliferation	16/366	203/18862	2.35E−06	0.000108556	7.45E−05
GO:0010631	epithelial cell migration	17/366	357/18862	0.000649938	0.007099692	0.004871586
GO:0050673	epithelial cell proliferation	26/366	428/18862	3.15E−07	2.37E−05	1.62E−05
GO:0044262	cellular carbohydrate metabolic process	10/215	288/18862	0.001784299	0.013594191	0.010345719
GO:0019318	hexose metabolic process	12/215	250/18862	3.13E−05	0.000474842	0.000361374
GO:0006641	triglyceride metabolic process	13/215	108/18862	3.03E−10	2.32E−08	1.77E−08
GO:0033559	unsaturated fatty acid metabolic process	14/215	115/18862	5.28E−11	5.20E−09	3.96E−09
GO:1901605	alpha-amino acid metabolic process	14/215	191/18862	4.20E−08	1.61E−06	1.22E−06
GO:0006520	cellular amino acid metabolic process	15/215	331/18862	6.40E−06	0.000124268	9.46E−05
GO:0009259	ribonucleotide metabolic process	20/215	425/18862	9.53E−08	3.28E−06	2.50E−06
GO:0006163	purine nucleotide metabolic process	20/215	441/18862	1.73E−07	5.48E−06	4.17E−06
GO:0008202	steroid metabolic process	29/215	329/18862	1.10E−17	3.37E−15	2.57E−15
GO:0006631	fatty acid metabolic process	35/215	392/18862	2.21E−21	6.08E−18	4.63E−18
GO:0072575	epithelial cell proliferation involved in liver morphogenesis	6/366	21/18862	2.17E−06	0.000101936	6.99E−05

GeneRatio: The number of gene in CHP and CIP, which enriched in each GO term. BgRatio: gene background in pathway analysis. P value: P value of pathway analysis. Padjust: adjust P value of pathway analysis. Q value: q value of pathway analysis. CHP, an H-ChC component that is functionally similarity to HCC with high expression of iCCA epithelial markers (HCC component with the iCCA phenotype, CHP); CIP: an H-ChC component that is functionally similarity to iCCA with high levels of HCC epithelial markers (i.e., an iCCA component with the HCC phenotype, CIP).

Table S4 Sample distribution of malignant epithelial cells in each cluster

Sample	Cluster0 number	Cluster1 number	Cluster2 number	Cluster3 number	Cluster4 number	Cluster5 number	Cluster6 number	Cluster7 number	Cluster8 number
P01	46	783	661	0	0	3	6	1	0
P02	10	236	301	0	1	1	5	0	0
P03	15	888	920	0	2	0	38	0	0
P04	10	4	2	0	118	0	2	0	0
P05	954	1	0	8	0	0	43	14	0
P06	3	0	0	0	0	0	2	4	31
P07	140	0	1	3	0	0	30	161	0
P08	120	1	0	5	0	0	7	183	0
P09	1,168	0	0	763	0	0	18	0	0
P010	12	31	12	0	36	542	3	0	0
P011	2	0	0	0	16	0	184	0	0
P012	1	0	0	0	27	0	1	1	0
P013	24	36	24	1	330	2	32	5	1
P014	2	0	0	0	0	0	0	0	66
P015	5	14	5	0	119	0	24	0	0

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Table S5 The ligand-receptor pairs between lymphatic EC/CXCL10$^+$ Macro and CD8$^+$ Tex cells

Ligand	Receptor	Cell_from_mean	Cell_from	Cell_to_mean	Cell_to	Comm_type
CTGF	ITGA5	2.241534315	Lymphatic EC	2.051873601	CD8 exhausted T	Growth factor
CTGF	NTRK1	2.241534315	Lymphatic EC	1.53643783	CD8 exhausted T	Growth factor
HBEGF	CD9	2.12E+00	CD8 exhausted T	0.320861407	Lymphatic EC	Growth factor
CCL21	CCR7	–0.013880989	Lymphatic EC	1.946558075	CD8 exhausted T	Cytokine
TGFB1	TGFBR2	0.584443203	CD8 exhausted T	–1.006550986	Lymphatic EC	Growth factor
TGFB1	TGFBR3	0.584443203	CD8 exhausted T	–1.326855207	Lymphatic EC	Growth factor
VEGFA	NRP2	0.995247731	CD8 exhausted T	–1.078495717	Lymphatic EC	Growth factor
VEGFA	ITGA9	0.995247731	CD8 exhausted T	–1.200254612	Lymphatic EC	Growth factor
IGF1	INSR	–1.213266824	Lymphatic EC	1.410906948	CD8 exhausted T	Growth factor
VEGFA	KDR	0.995247731	CD8 exhausted T	–2.03562391	Lymphatic EC	Growth factor
PGF	NRP2	2.858365925	CD8 exhausted T	–1.078495717	Lymphatic EC	Growth factor
CTGF	ITGB2	2.241534315	Lymphatic EC	–1.854352123	CD8 exhausted T	Growth factor
CTGF	LRP1	2.241534315	Lymphatic EC	–1.99961507	CD8 exhausted T	Growth factor
CCL20	CXCR3	2.713026562	CD8 exhausted T	3.268075237	CXCL10$^+$ Macro	Cytokine
PDGFA	PDGFRB	2.44569463	CXCL10$^+$ Macro	2.199402843	CD8 exhausted T	Growth factor
CXCL13	CXCR3	1.559592496	CD8 exhausted T	3.268075237	CXCL10$^+$ Macro	Cytokine
PDCD1LG2	PDCD1	–2.153288341	CXCL10$^+$ Macro	–1.968315888	CD8 exhausted T	Checkpoint
PGF	FLT1	2.858365925	CD8 exhausted T	1.460720315	CXCL10$^+$ Macro	Growth factor
CCL2	CCR4	1.340128267	CXCL10$^+$ Macro	3.010369018	CD8 exhausted T	Cytokine
CD274	PDCD1	–1.760050494	CXCL10$^+$ Macro	–1.968315888	CD8 exhausted T	Checkpoint
HBEGF	CD9	2.117100193	CD8 exhausted T	1.311776193	CXCL10$^+$ Macro	Growth factor
CCL5	SDC1	–0.889546203	CXCL10$^+$ Macro	–2.752131668	CD8 exhausted T	Cytokine
CCL3L1	CCR5	–1.579205429	CXCL10$^+$ Macro	–1.451614317	CD8 exhausted T	Cytokine
CCL19	CCRL2	–2.098246978	CXCL10$^+$ Macro	–0.923788443	CD8 exhausted T	Cytokine
VEGFA	FLT1	0.623892524	CXCL10$^+$ Macro	2.751450721	CD8 exhausted T	Growth factor
IL16	KCNJ10	–0.523504711	CD8 exhausted T	–3.26174571	CXCL10$^+$ Macro	Cytokine
CD274	PDCD1	0.991632456	CD8 exhausted T	1.715534214	CXCL10$^+$ Macro	Checkpoint
CXCL12	CXCR4	–2.650115259	CXCL10$^+$ Macro	–0.631616657	CD8 exhausted T	Cytokine
VEGFA	FLT1	0.995247731	CD8 exhausted T	1.460720315	CXCL10$^+$ Macro	Growth factor
CCL5	CCR5	–0.889546203	CXCL10$^+$ Macro	–1.451614317	CD8 exhausted T	Cytokine
IL10	SIRPG	–1.313590966	CXCL10$^+$ Macro	–0.97249241	CD8 exhausted T	Cytokine
TGFB1	CD109	0.584443203	CD8 exhausted T	2.060182385	CXCL10$^+$ Macro	Growth factor
VEGFB	FLT1	0.784018583	CD8 exhausted T	1.460720315	CXCL10$^+$ Macro	Growth factor
CCL8	CCR5	–0.783271643	CXCL10$^+$ Macro	–1.451614317	CD8 exhausted T	Cytokine
VEGFB	FLT1	0.387632218	CXCL10$^+$ Macro	2.751450721	CD8 exhausted T	Growth factor
PDGFB	PDGFRB	0.475112377	CXCL10$^+$ Macro	2.199402843	CD8 exhausted T	Growth factor
TNFSF14	LTBR	–0.374220439	CXCL10$^+$ Macro	–2.048524671	CD8 exhausted T	Checkpoint
HBEGF	CD44	2.117100193	CD8 exhausted T	0.35278872	CXCL10$^+$ Macro	Growth factor
VEGFA	EPHB2	0.995247731	CD8 exhausted T	0.702710173	CXCL10$^+$ Macro	Growth factor
HBEGF	CD9	0.34170287	CXCL10$^+$ Macro	2.008261355	CD8 exhausted T	Growth factor
CCL3	CCR5	–0.469738204	CXCL10$^+$ Macro	–1.451614317	CD8 exhausted T	Cytokine
PDGFB	S1PR1	0.475112377	CXCL10$^+$ Macro	1.307673326	CD8 exhausted T	Growth factor
TGFB1	TGFBR1	0.584443203	CD8 exhausted T	1.060594525	CXCL10$^+$ Macro	Growth factor
CXCL12	ITGB1	–2.650115259	CXCL10$^+$ Macro	–0.198146096	CD8 exhausted T	Cytokine
IL18	CD48	–0.945599968	CXCL10$^+$ Macro	–0.506412474	CD8 exhausted T	Cytokine
CCL5	CCR1	–0.708723492	CD8 exhausted T	–0.608173888	CXCL10$^+$ Macro	Cytokine
CCL13	CCR5	–0.265356963	CXCL10$^+$ Macro	–1.451614317	CD8 exhausted T	Cytokine
IL16	CD4	–0.523504711	CD8 exhausted T	–0.673083462	CXCL10$^+$ Macro	Cytokine
TNFSF14	TNFRSF14	–0.374220439	CXCL10$^+$ Macro	–0.892274606	CD8 exhausted T	Checkpoint
VEGFA	SIRPA	0.995247731	CD8 exhausted T	0.320057055	CXCL10$^+$ Macro	Growth factor
VEGFA	ITGB1	0.995247731	CD8 exhausted T	0.2807948	CXCL10$^+$ Macro	Growth factor
IL3	CSF2RB	–0.311559077	CD8 exhausted T	–0.824224417	CXCL10$^+$ Macro	Cytokine
ICOSLG	ICOS	–0.619217922	CXCL10$^+$ Macro	–0.390355078	CD8 exhausted T	Checkpoint
IL1A	IL1R2	0.127680803	CXCL10$^+$ Macro	1.681488163	CD8 exhausted T	Cytokine
HBEGF	CD44	0.34170287	CXCL10$^+$ Macro	0.293561862	CD8 exhausted T	Growth factor
CD86	CD28	–0.090943957	CXCL10$^+$ Macro	–0.992057719	CD8 exhausted T	Checkpoint
IL15	IL2RG	–1.060111849	CXCL10$^+$ Macro	–0.06978574	CD8 exhausted T	Cytokine
CD86	CTLA4	–0.090943957	CXCL10$^+$ Macro	–0.435509566	CD8 exhausted T	Checkpoint
IL10	IL10RA	1.466048502	CD8 exhausted T	0.021637341	CXCL10$^+$ Macro	Cytokine
CCL3L1	CCR1	–0.014274102	CD8 exhausted T	–0.608173888	CXCL10$^+$ Macro	Cytokine
TNFSF9	TNFRSF9	0.042765332	CXCL10$^+$ Macro	–0.654331941	CD8 exhausted T	Checkpoint
IL15	IL2RB	–1.060111849	CXCL10$^+$ Macro	0.0795582	CD8 exhausted T	Cytokine
VEGFA	ITGB1	0.623892524	CXCL10$^+$ Macro	–0.198146096	CD8 exhausted T	Growth factor

ligand: ligand name. receptor: receptor name. cell_from_mean: the average input signal of cells. cell_from: cell which input the signal. cell_to_mean: the average output signal of cells. cell_to: cell which output the signal. comm_type: the type of different interactions between ligand and receptor.

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Table S6 The count of major cell types in posttreatment sample

Major cell type	Count
Epithelial cell	29
Myeloid cell	855
Stromal cell	357
T cell	3,957
Mast cell	25
B cell	615

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