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Supplemental information

Lineage and ecology define liver tumor evolution in response to treatment

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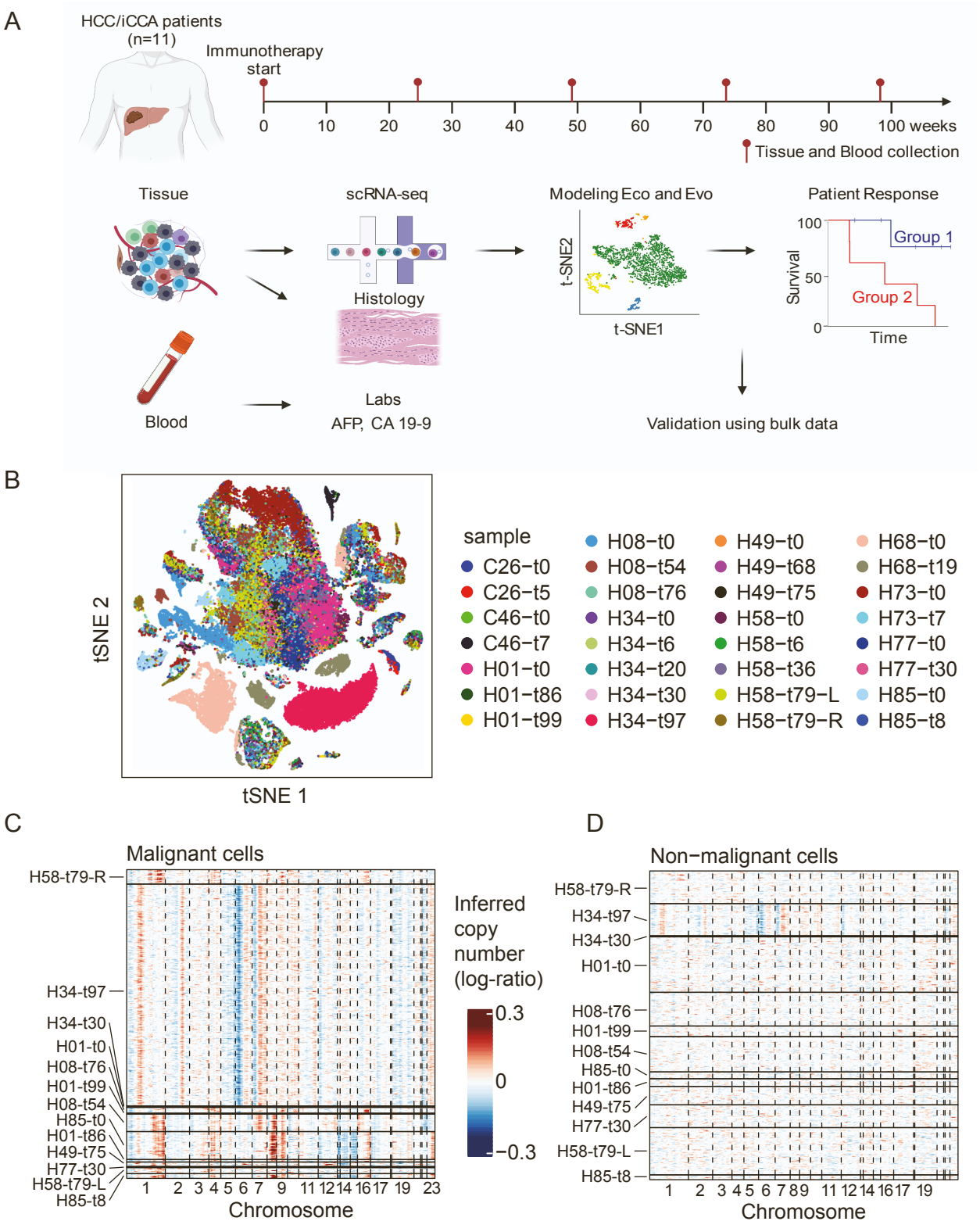

Figure S1. Dissection of primary liver tumor using scRNA-seq. Related to Figure 1.
(A) Workflow of this single-cell study of longitudinal samples from liver cancer patients.
(B) t-SNE plot of all 57,591 single cells from 11 patients. Sample names starting with H and C denote the clinical diagnoses of HCC and iCCA, respectively. Sample names with t0 indicate baseline samples while others are post-treatment samples. Cells are colored by sample.
(C and D) Inferred CNVs of malignant cells (C) and non-malignant cells (D) for newly collected samples in this cohort. Red, amplifications; blue, deletions.

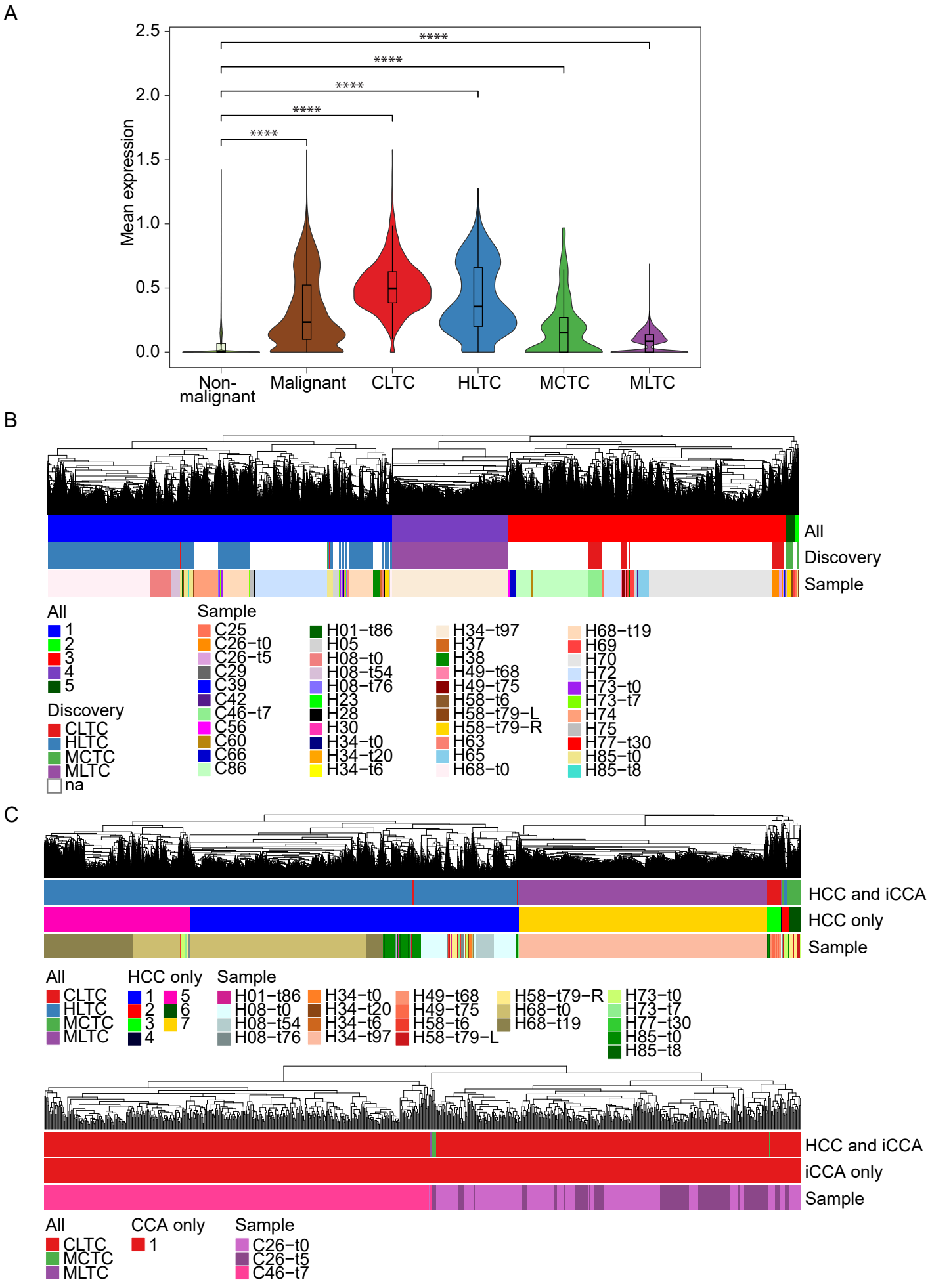
A
Mean expression
2.5
2.0
1.5
1.0
0.5
0.0
****
****
****
****
****
Non-malignant
Malignant
CLTC
HLTC
MCTC
MLTC
B
All
Discovery
Sample
All
1
2
3
4
5
Discovery
CLTC
HLTC
MCTC
MLTC
na
Sample
C25
C26−t0
C26−t5
C29
C39
C42
C46−t7
C56
C60
C66
C86
H01−t86
H05
H08−t0
H08−t54
H08−t76
H23
H28
H30
H34−t0
H34−t20
H34−t6
H34−t97
H37
H38
H49−t68
H49−t75
H58−t6
H58−t79−L
H58−t79−R
H63
H65
H68−t0
H68−t19
H69
H70
H72
H73−t0
H73−t7
H74
H75
H77−t30
H85−t0
H85−t8
C
HCC and iCCA
HCC only
Sample
All
CLTC
HLTC
MCTC
MLTC
HCC only
1
2
3
4
5
6
7
Sample
H01−t86
H08−t0
H08−t54
H08−t76
H34−t0
H34−t20
H34−t6
H34−t97
H49−t68
H49−t75
H58−t6
H58−t79−L
H58−t79−R
H68−t0
H68−t19
H73−t0
H73−t7
H77−t30
H85−t0
H85−t8
HCC and iCCA
iCCA only
Sample
All
CLTC
MCTC
MLTC
CCA only
1
Sample
C26−t0
C26−t5
C46−t7

Figure S2. Analysis of malignant cells. Related to Figure 1.

(A) Violin plots with embedded boxplots showing mean expression of key tumor markers in non-malignant cells (n = 44,525), malignant cells (n = 13,042), CLTCs (n = 1,152), HLTCs (n = 7,622), MCTCs (n = 258), and MLTCs (n = 4,010) from the longitudinal single-cell cohort (n = 11 patients). The tumor marker signature comprises 10 genes: AFP, ALDH1A1, ANPEP, EPCAM, GPC3, HNF4A, ICAM1, KRT19, PROM1, and SPP1. All groups were compared to non-malignant cells as a reference using a one-sided Wilcoxon test. ****, p value < 0.0001.

(B) Hierarchical cluster of malignant cells (n = 25,728 cells) from all available single-cell samples (n = 44 samples) across both the discovery and validation cohorts. Cells are grouped into the top 5 clades of the hierarchical tree, indicated by distinct colors on the top row. The lineage of cells from the longitudinal cohort are indicated by distinct colors on the second row, while those from the validation cohort are in white. The sample of origin for each cell is indicated on the bottom row. All clusters are comprised of multiple patients (cluster 1, n = 19 patients; cluster 2, n = 11; cluster 3, n = 25; cluster 4, n = 6; cluster 5, n = 10).

(C) Hierarchical clustering of HCC tumor cells (n = 12,135, top) and iCCA tumor cells (n = 907, bottom) from the longitudinal single-cell cohort (n = 11 patients). Cells are annotated using their lineage from the main analysis in the first row, their cluster from this analysis in the second row, and their sample in the third row. The tree was divided into the top 7 clades for HCC and only 1 clade for iCCA.

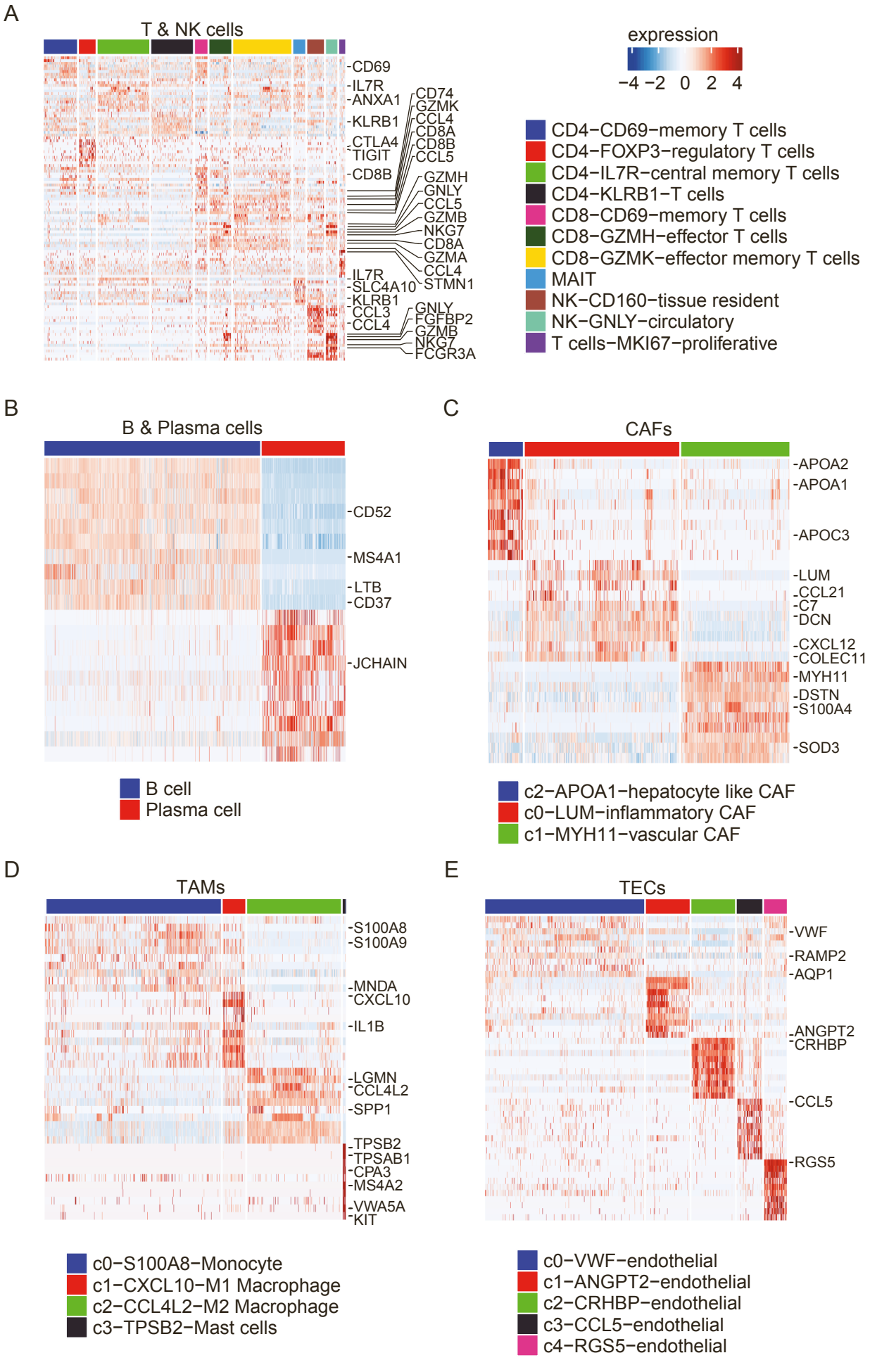
A
T & NK cells
expression
−4 −2 0 2 4
CD69
IL7R
ANXA1
KLRB1
CTLA4
TIGIT
CD8B
CD74
GZMK
CCL4
CD8A
CD8B
CCL5
GZMH
GNLY
CCL5
GZMB
NKG7
CD8A
GZMA
CCL4
STMN1
IL7R
SLC4A10
KLRB1
CCL3
CCL4
GNLY
FGFBP2
GZMB
NKG7
FCGR3A
CD4−CD69−memory T cells
CD4−FOXP3−regulatory T cells
CD4−IL7R−central memory T cells
CD4−KLRB1−T cells
CD8−CD69−memory T cells
CD8−GZMH−effector T cells
CD8−GZMK−effector memory T cells
MAIT
NK−CD160−tissue resident
NK−GNLY−circulatory
T cells−MKI67−proliferative
B
B & Plasma cells
CD52
MS4A1
LTB
CD37
JCHAIN
B cell
Plasma cell
C
CAFs
APOA2
APOA1
APOC3
LUM
CCL21
C7
DCN
CXCL12
COLEC11
MYH11
DSTN
S100A4
SOD3
c2−APOA1−hepatocyte like CAF
c0−LUM−inflammatory CAF
c1−MYH11−vascular CAF
D
TAMs
S100A8
S100A9
MNDA
CXCL10
IL1B
LGMN
CCL4L2
SPP1
TPSB2
TPSAB1
CPA3
MS4A2
VWA5A
KIT
c0−S100A8−Monocyte
c1−CXCL10−M1 Macrophage
c2−CCL4L2−M2 Macrophage
c3−TPSB2−Mast cells
E
TECs
VWF
RAMP2
AQP1
ANGPT2
CRHBP
CCL5
RGS5
c0−VWF−endothelial
c1−ANGPT2−endothelial
c2−CRHBP−endothelial
c3−CCL5−endothelial
c4−RGS5−endothelial

Figure S3. Immune cell subtype determination. Related to Figure 2.
(A-E) Expression of top differentially expressed genes (rows) in T & NK cells (A), B & plasma cells (B), CAFs (C), TAMs (D), TECs (E). Cell subtypes are indicated at the top with colors corresponding to accompanying legends. Key genes are labelled.

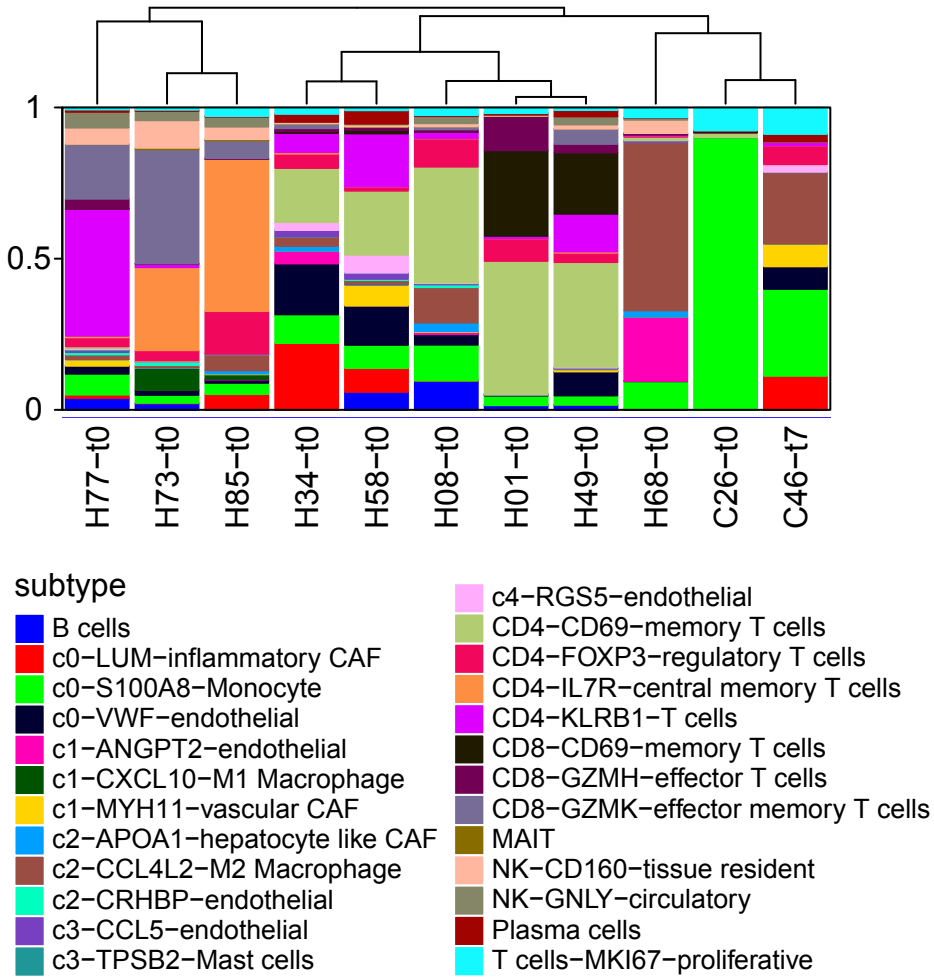

Figure S4. Hierarchical relationship of the TME composition. Related to Figure 2.
Hierarchical relationship of the TME composition in each baseline or other earliest tumor sample with at least 15 non-malignant cells detected. Subtypes of immune/stromal cells are indicated by colors.

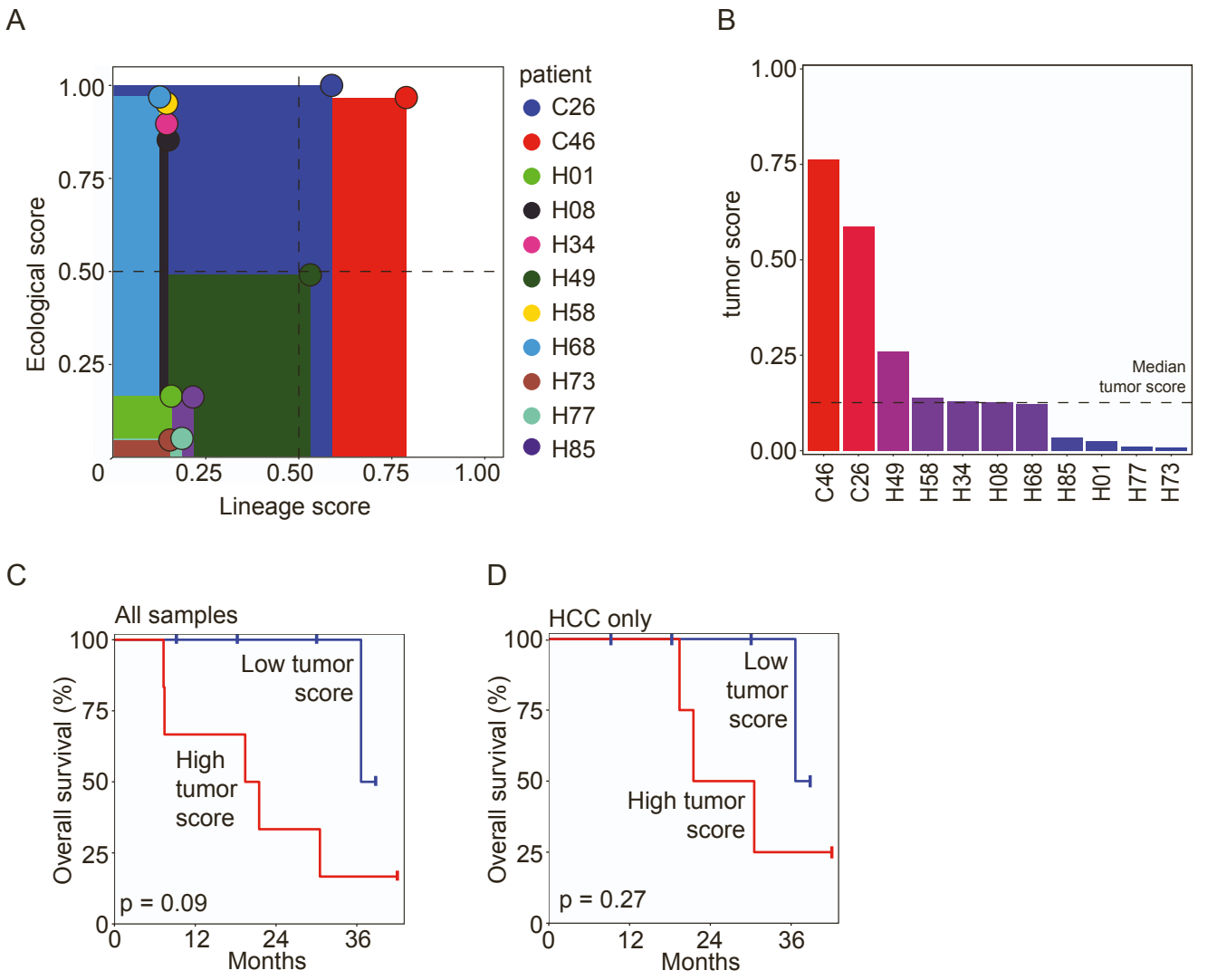

Figure S5. Characterizing a tumor ecosystem using a tumor score. Related to Figure 3.
(A) Projection of baseline samples from the 11 patients of our single-cell discovery cohort into the lineage-ecological space, with the area between each sample (points) and the origin shaded. These areas represent the tumor score for each sample. Samples and their accompanying areas are colored by patients.
(B) Tumor score for each baseline sample from the 11 patients, ordered from high to low. Median tumor score for the cohort is shown with a dashed line.
(C and D) Overall survival of the 11 HCC or iCCA patients (C) and HCC patients only (D). Samples were divided into high or low tumor score group by the median value as indicated in (B). The p value was calculated using the log-rank test.

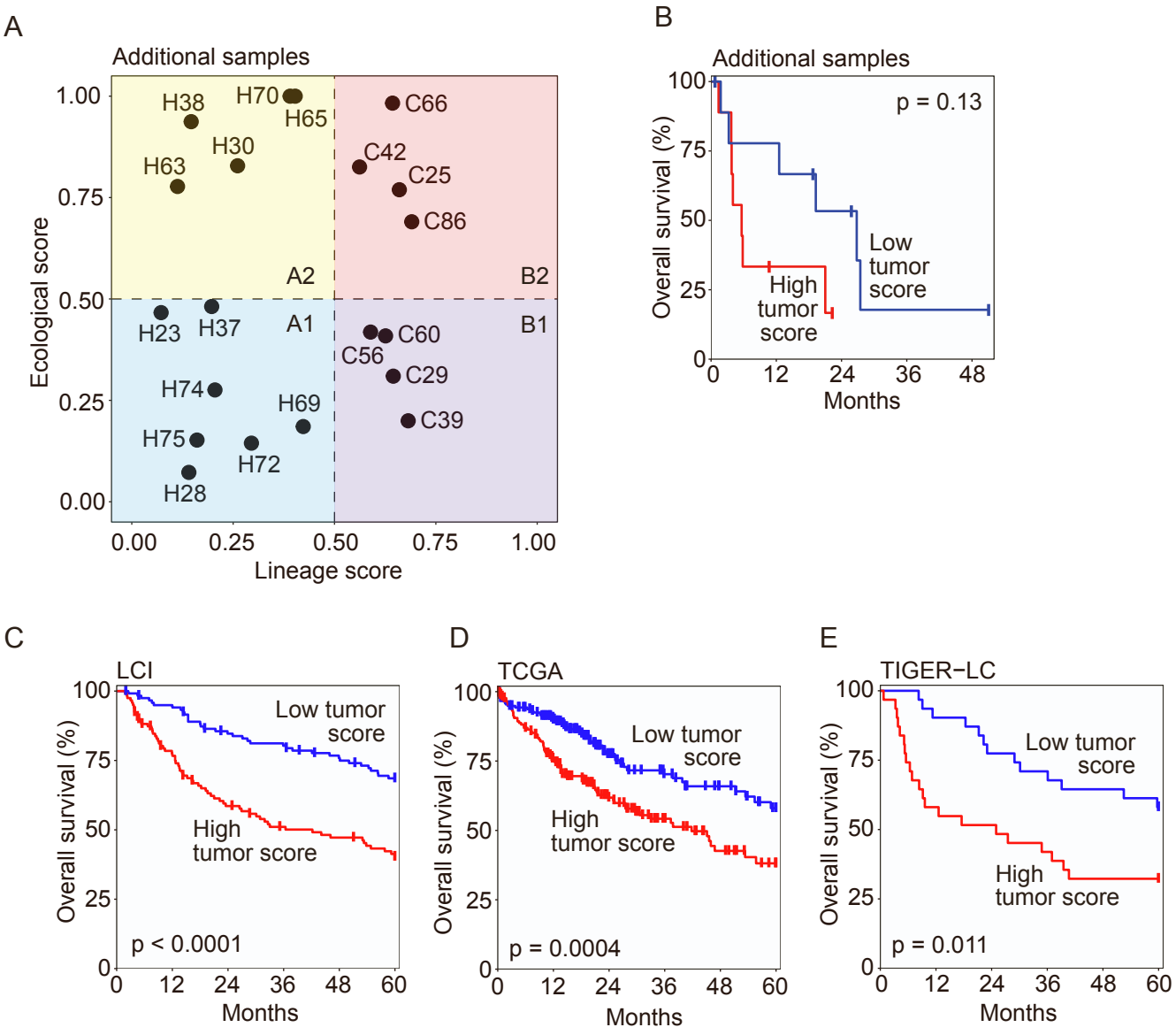

Figure S6. Validation of the tumor score in patient stratification. Related to Figure 3.
(A) Projection of additional samples (n = 20) from an independent single-cell cohort of HCC/iCCA patients into the lineage-ecological space. One of these samples does not have accompanying survival data and is therefore excluded from survival analyses in (B) and Fig. 3F.
(B) Overall survival of the patients in (A). Samples were divided into high or low tumor score group by the median value in this cohort. The p value was calculated using the log-rank test.
(C-E) Kaplan-Meier plots of HCC patients from the LCI (C, n = 239), TCGA (D, n = 363), and the TIGER-LC (E, n = 62) cohorts. Samples were divided into high or low tumor score group by the median value in each cohort. The p value was calculated using the log-rank test.

A

Variable	Multivariate		Univariate	
	Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value
Quadrants				
A1	Reference		Reference	
B2	4.78 (1.15—19.95)	0.03	4.34 (1.46—12.89)	0.008
Gender				
Male	Reference		Reference	
Female	3.86 (0.9—16.53)	0.07	2.57 (0.84—7.83)	0.1
Age (years)	1 (0.93—1.08)	0.95	0.96 (0.91—1.03)	0.26
Race				
Caucasian	Reference		Reference	
African American	0.65 (0.32—18.14)	0.63	0.28 (0.06—1.31)	0.11
Asian Race	2.41 (0.11—3.76)	0.39	1.11 (0.24—5.17)	0.89

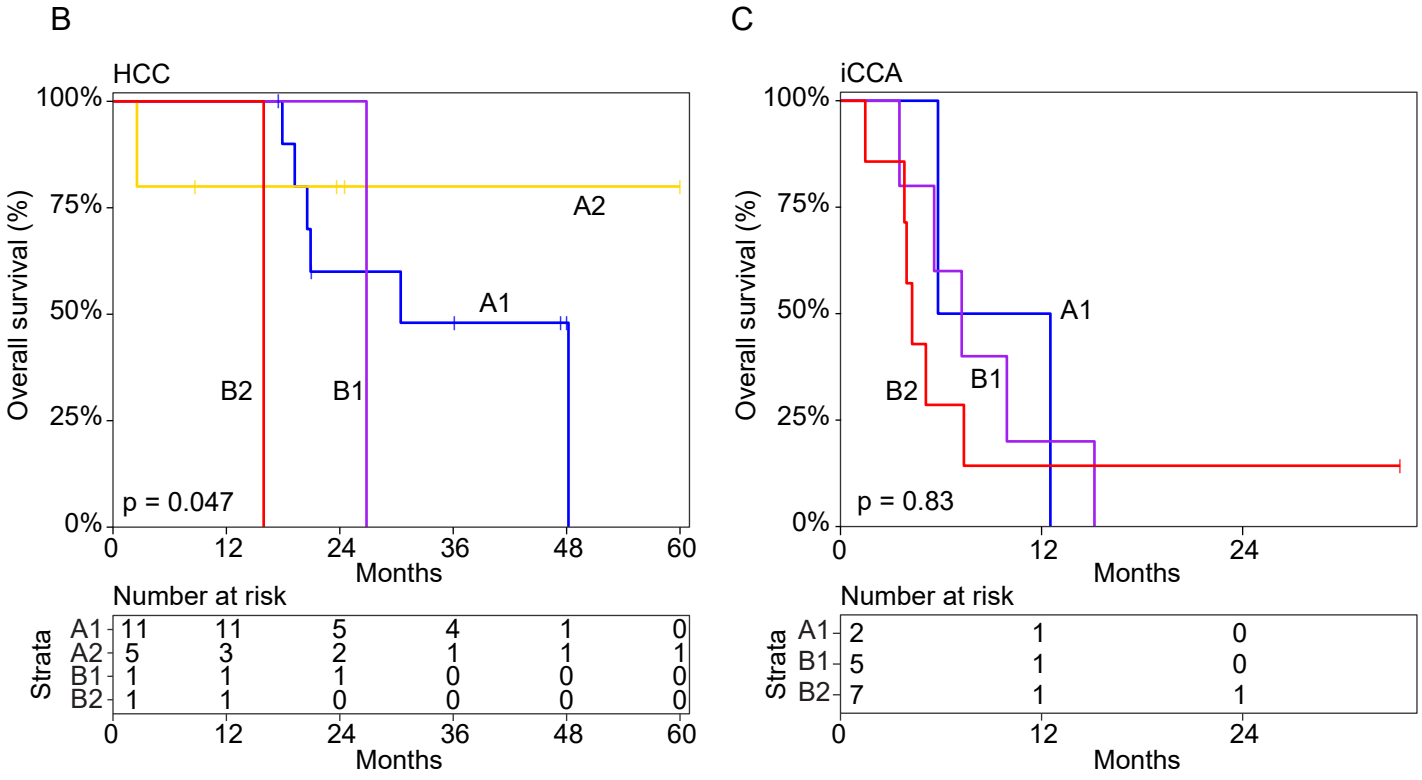

Figure S7. Application of CASCADE to the NCI-CLARITY cohort. Related to Figure 5.
(A) Hazard ratio with 95% confidence interval (CI) from multivariate and univariate analysis. Only quadrants A1 and B2 were used in the analysis since the two quadrants had the most number patients for modeling.
(B and C) Kaplan-Meier plots of baseline samples of HCC patients (B, n = 18) and iCCA patients (C, n=14) from the NCI-CLARITY cohort. Samples were divided into CASCADE quadrants using the global medians from the entire cohort in Figure 5C. The p value was calculated using log-rank test.

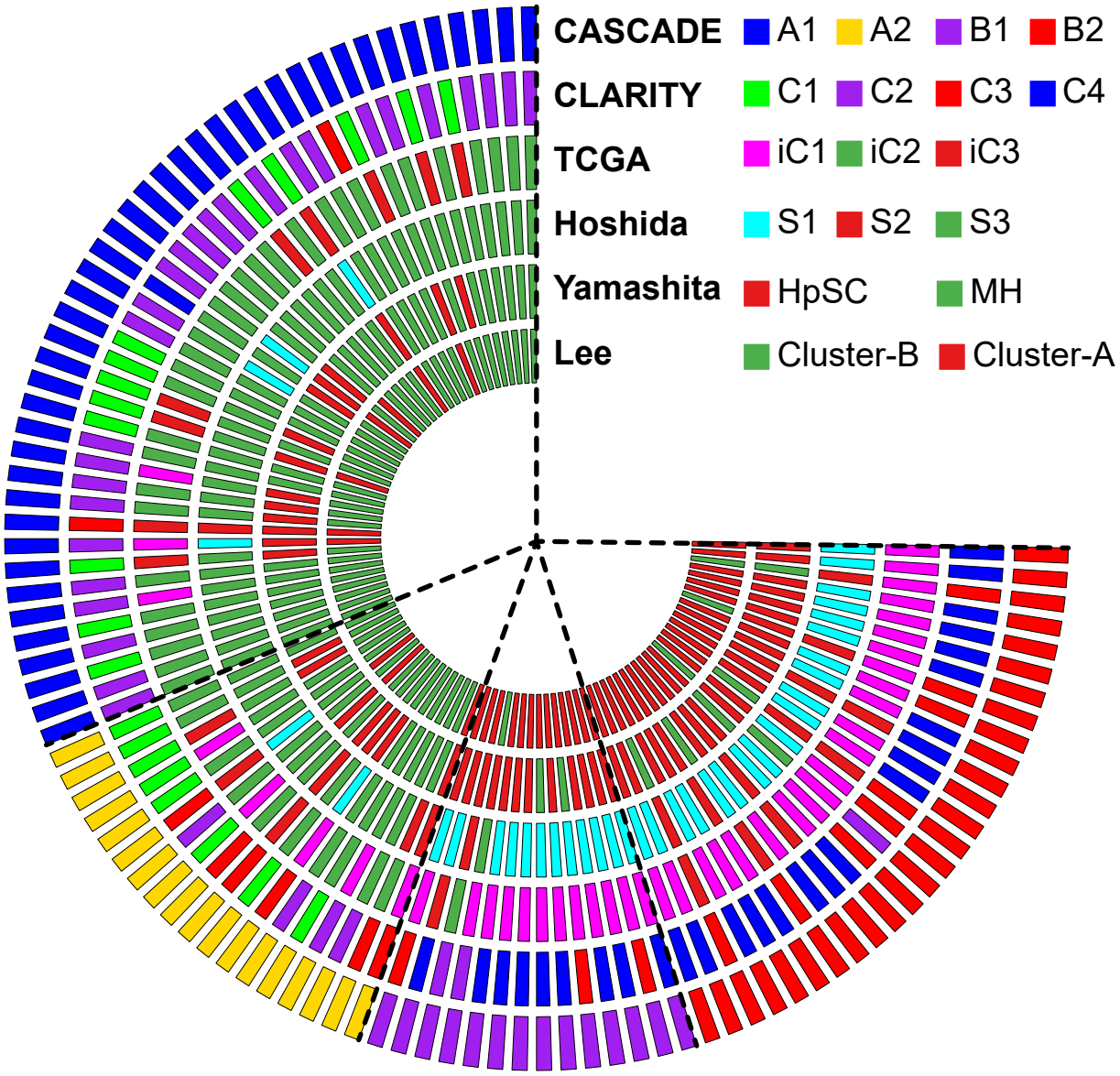

Figure S8. Comparison of HCC signatures in prospective cohort. Related to Figure 5.
Classification of NCI-CLARITY prospective cohort samples using CASCADE, CLARITY, TCGA, Hoshida, Yamashita, and Lee HCC signatures, respectively, from the outermost to innermost circles. Dashed lines segment the figure into samples of each CASCADE quadrant.