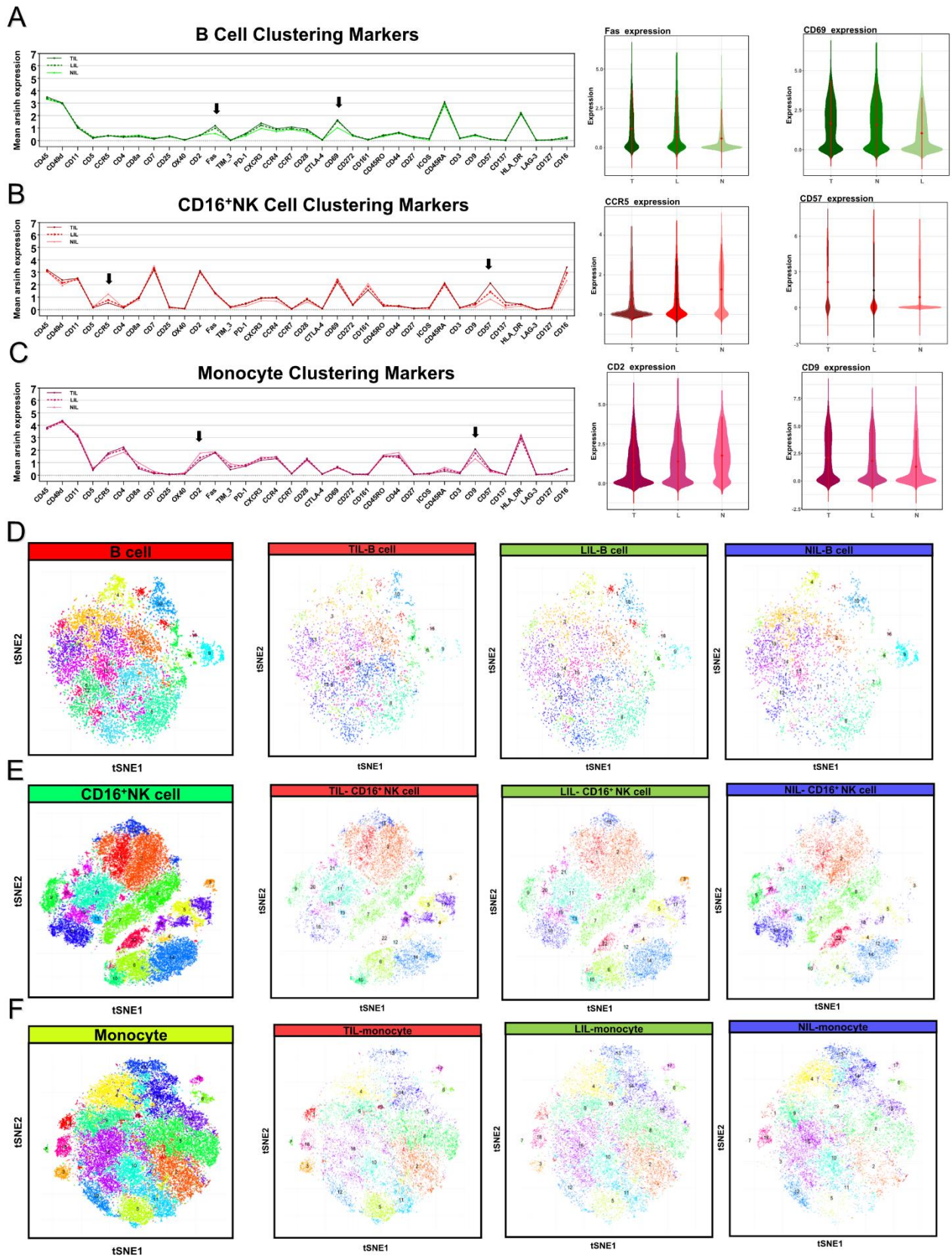
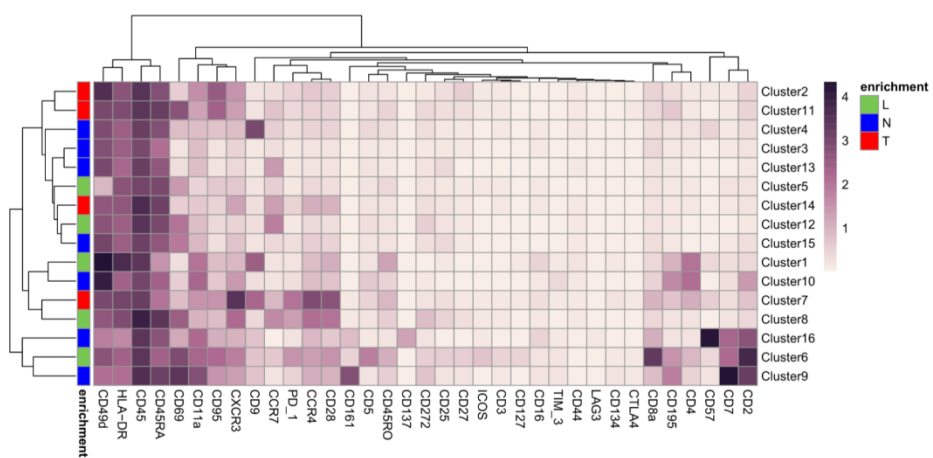


Supporting information:



G



H



I

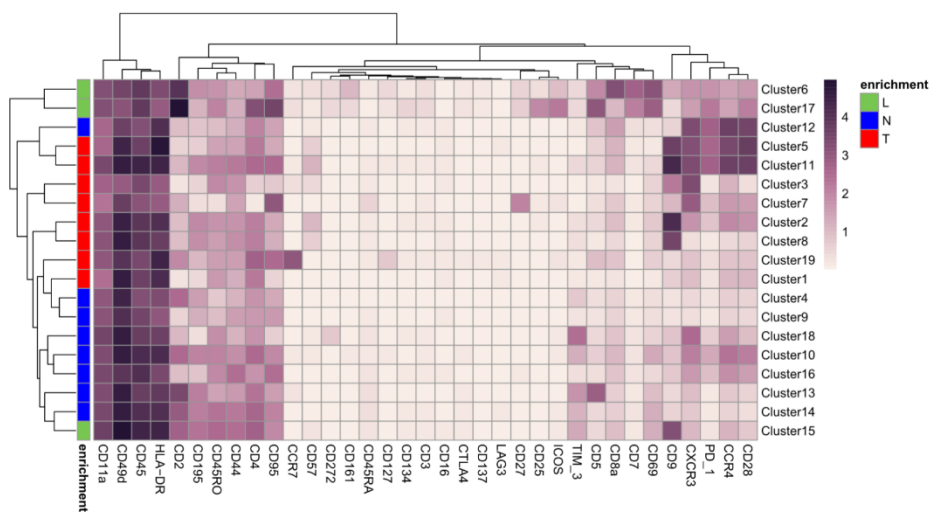


Figure S1 A. Average mean expression levels of each of the 3 different regions in HCC were shown for the 35 clustering proteins in B cells (left). Certain differentially expressed markers (Fas, CD69) were shown in violin plots (right). B. Average mean expression levels of each of the 3 different regions in HCC were shown for the 35 clustering proteins in NK cells (left). Certain differentially expressed markers (CCR5, CD57) were shown in violin plots (right). C. Average mean expression levels of each of the 3 different regions in HCC were shown for the 35 clustering proteins in Monocyte (left). Certain differentially expressed markers (CD2, CD9) were shown in violin plots (right). D. tSNE plots of B cells. B cells from three regions were shown in merge (left) and separately (right). E. tSNE plots of CD16+NK cell. CD16+NKs from three regions were shown in merge (left) and separately (right). F. tSNE plots of monocytes. Monocytes from three regions were shown in merge (left) and separately (right). G. Heatmap showed the mean expression level of all 35 makers in all B clusters. Cluster analysis was performed on both columns and rows. T/L/N enriched clusters were marked on the right. H. Heatmap showed the mean expression level of all 35 makers in all CD16+NK clusters. Cluster analysis was performed on both columns and rows. T/L/N enriched clusters were marked on the right. I. Heatmap showed the mean expression level of all 35 makers in all monocyte clusters. Cluster analysis was performed on both columns and rows. T/L/N enriched clusters were marked on the right.

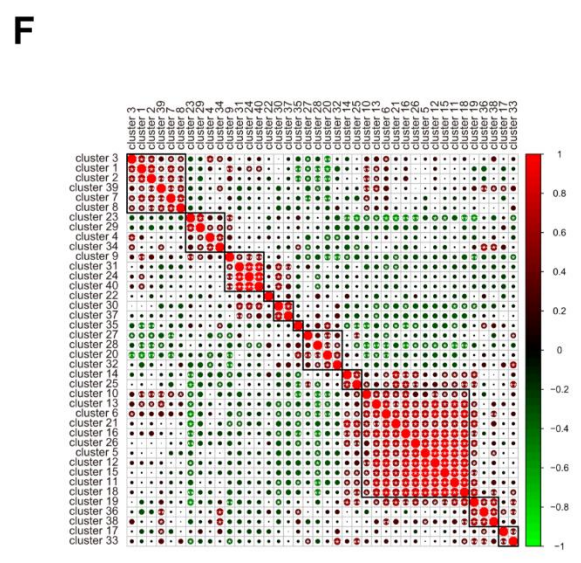
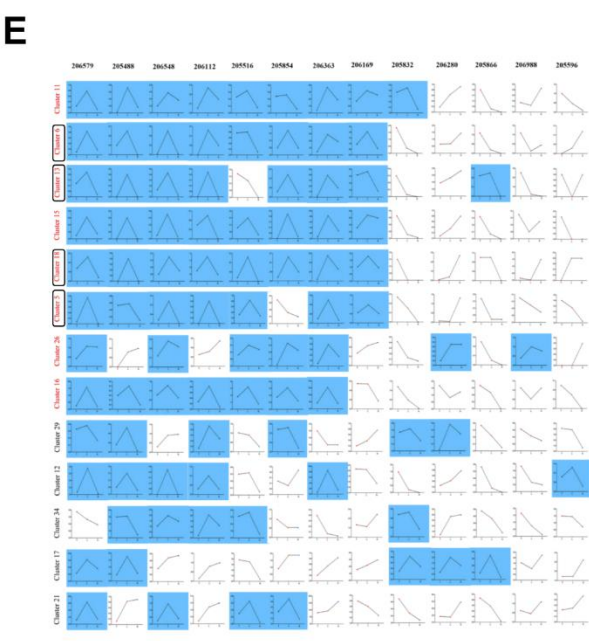
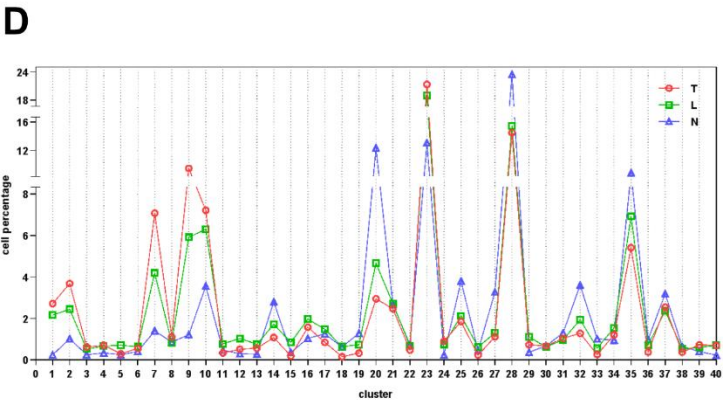
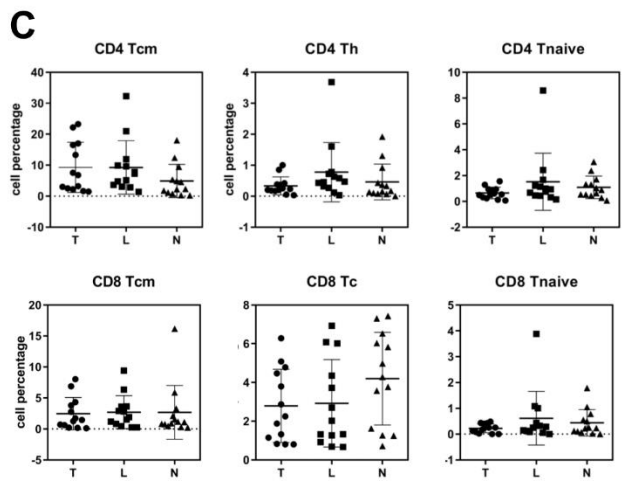
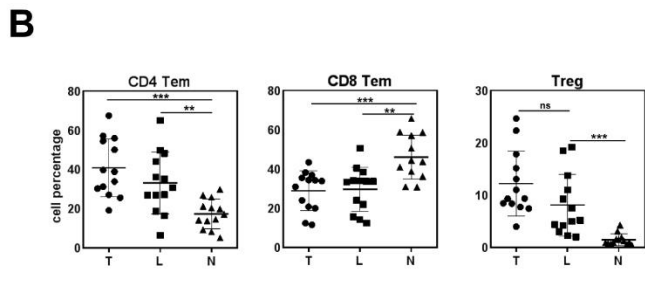
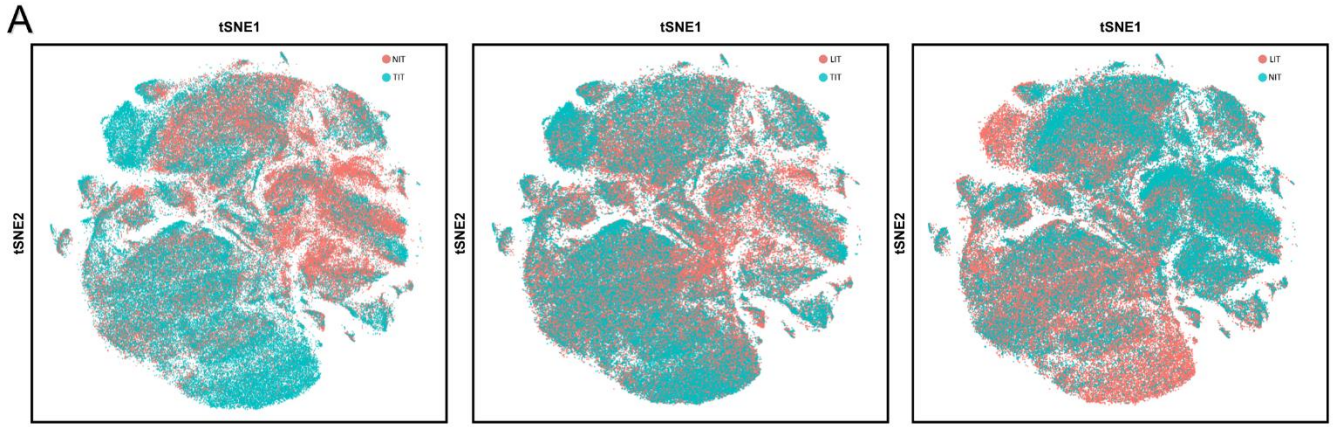
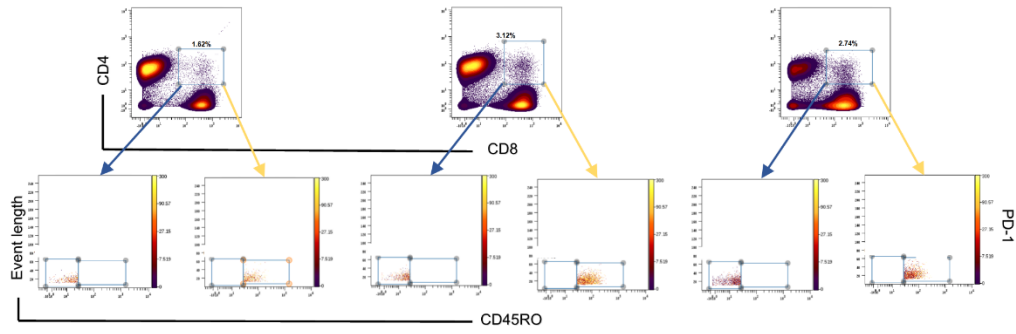
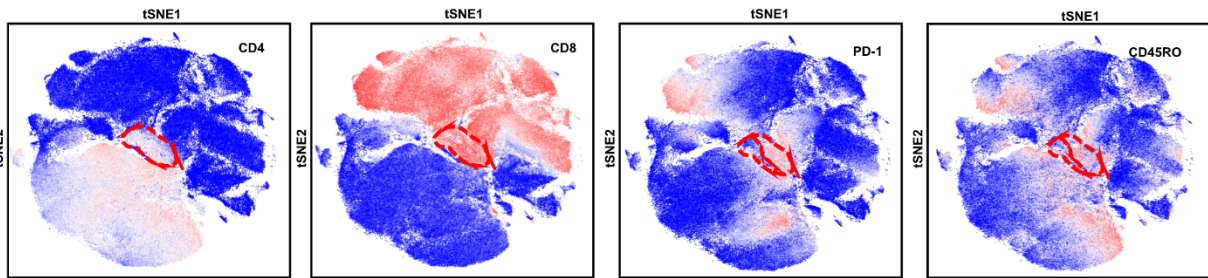


Figure S2 A. The similarity between tumor infiltrating T cell, L region infiltrating T cell and non-tumor region infiltrating T cell is compared by the overlap of tSNE plots. B. Cell percentage of each of the 40 clusters in T/L/N was plotted and compared. C. The cell percentage of CD4 Tem, CD8 Tem and Treg from different regions was compared. *P<0.05, **P<0.01, ***P<0.001. D. Comparison of cell percentage of certain T cell subtypes between different regions in HCC. Results are shown as mean $\pm$ SD, n=13. *P<0.05, **P<0.01, ***P<0.001, based on the student's t-test. E. Cell percentage of L-enriched T clusters in T/L/N of 13 enrolled patients. F. Heatmap showing the correlation among 40 T cell clusters. *P<0.05, **P<0.01, ***P<0.001.

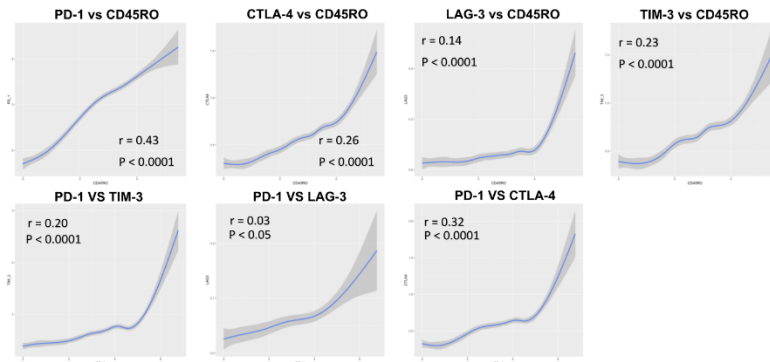
A



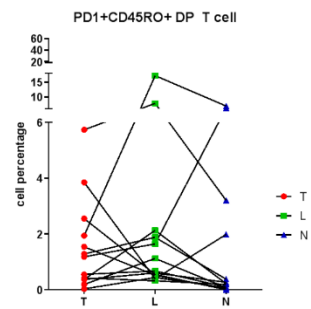
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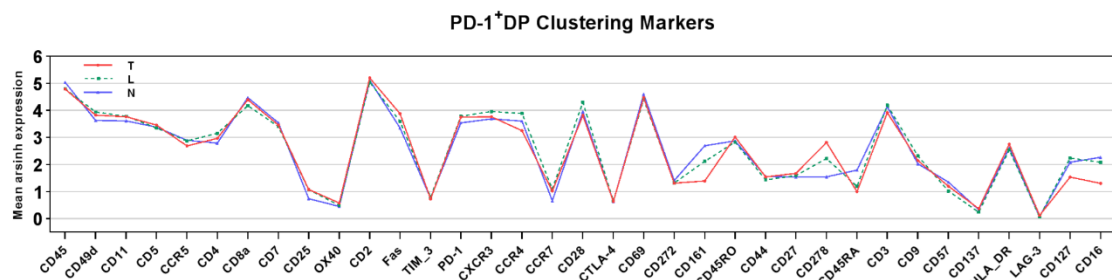
C



D



E



F

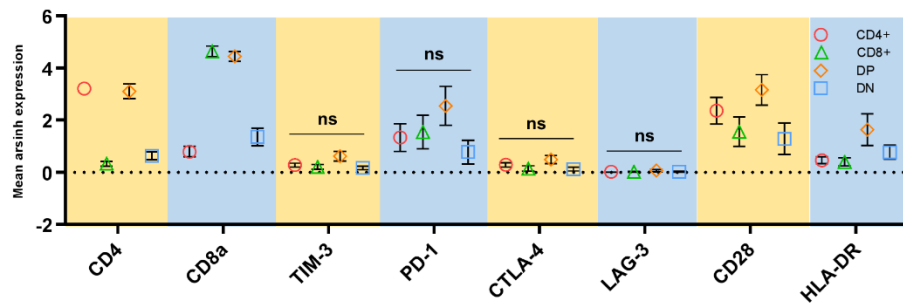
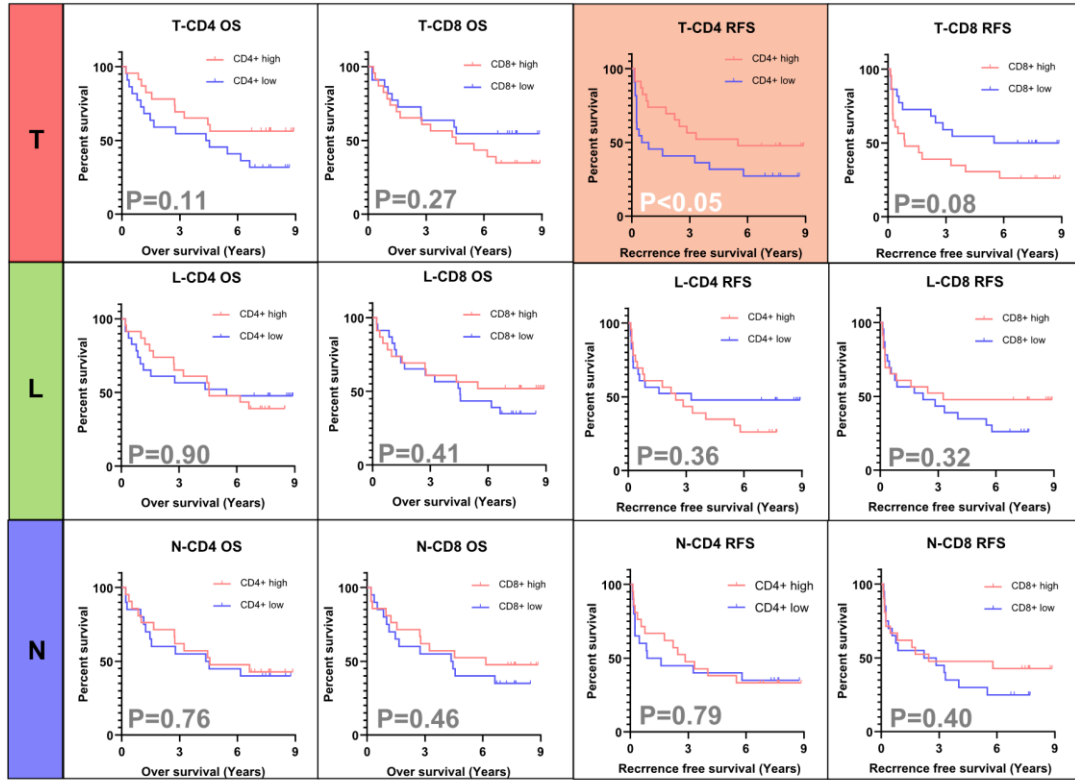


Figure S3 A. Flow plots show cells gated as DPT cells. DPT cells are then divided into 2 groups based on the expression level of CD45RO. Levels of CD45RO are shown for each of these populations. The bar chart depicts the expression intensity of PD-1. B. The expression level of CD4, CD8, PD-1 and CD45RO in 2D tSNE of total T cell pool. DPT cells are circled with a red line. DP CD45RO+/DP CD45RO- cells are separated with a red line. C. The correlations of the expression level of multiple immune checkpoint molecules were plotted. PD-1 vs CD45RO ($r=0.43$, $P<0.0001$). CTLA-4 vs CD45RO ($r=0.26$, $P<0.0001$). LAG-3 vs CD45RO ($r=0.14$, $P<0.0001$). TIM-3 vs CD45RO ($r=0.23$, $P<0.0001$). PD-1 vs TIM-3 ($r=0.20$, $P<0.0001$). PD-1 vs LAG-3 ($r=0.03$, $P<0.05$). PD-1 vs CTLA-4 ($r=0.32$, $P<0.0001$). D. Cell percentage of DP CD45RO+ PD-1+ T cells in total T cells from TIL/ LIL/ NIL of all 13 patients. E. Average mean expression levels of each of the 3 different regions in HCC were shown for the 35 clustering proteins in PD-1+DPT cell. F. In L region of HCC, the comparison of the expression level of several clustering markers and immune checkpoint molecules was made among single positive/ DP CD45RO+/DP CD45RO-/ double negative T cells. Results are shown as mean $\pm$ SD, $n=13$. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, based on the student's t-test.

A



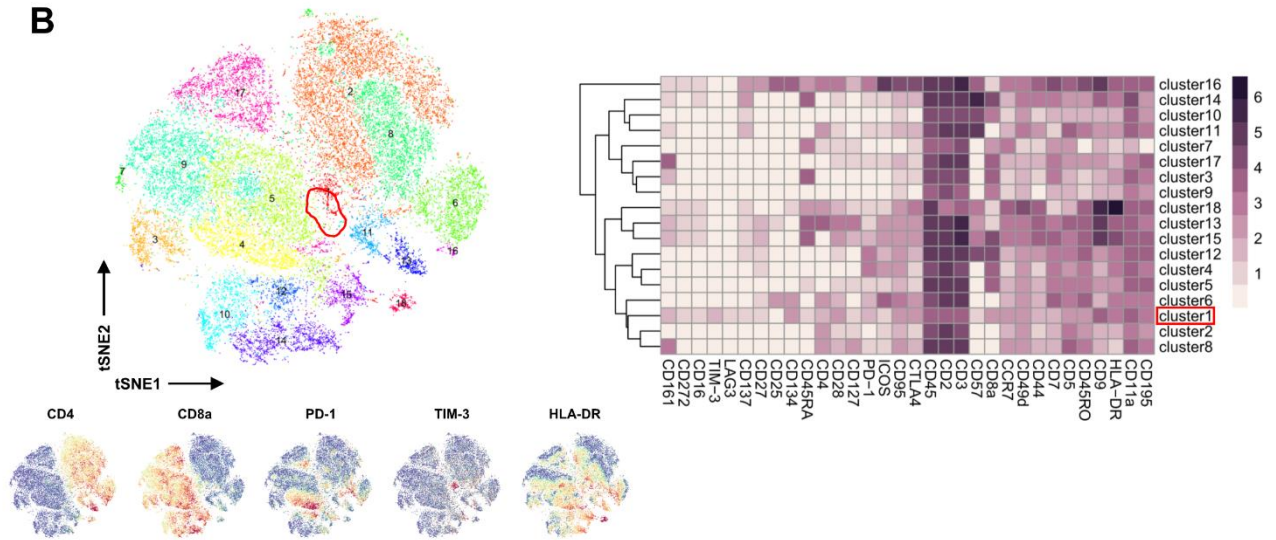
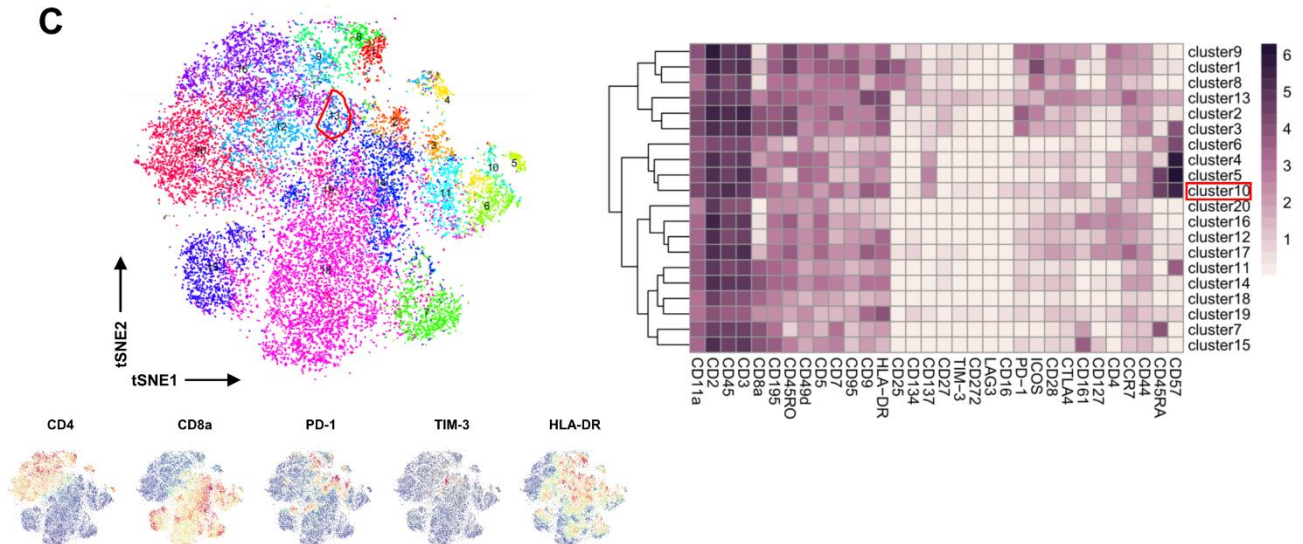
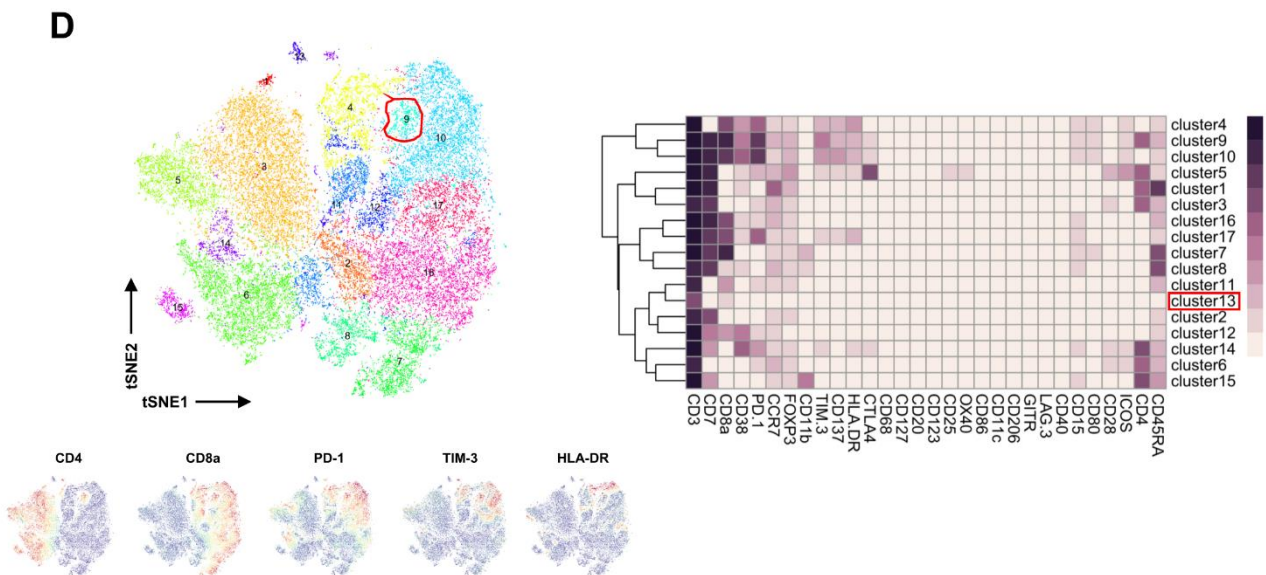
B**C****D**

Figure S4 A. Kaplan-Meier analysis of the correlation between CD4/CD8 single positive cell levels and overall survival (OS)/ recurrence-free survival (RFS). B. HCC cohort (30 cases): tSNE plots was drawn from a random sampling of 500 cells from each case. Phenograph clustering method was applied (left). Heatmap showed the expression feature of T cell clusters. C. ICC cohort (10 cases): tSNE plots was drawn from a random sampling of 500 cells from each case. Phenograph clustering method was applied (left). Heatmap showed the expression feature of T cell clusters. D. RCC cohort (70 cases): tSNE plots was drawn from a random sampling of 500 cells from each case. Phenograph clustering method was applied (left). Heatmap showed the expression feature of T cell clusters.

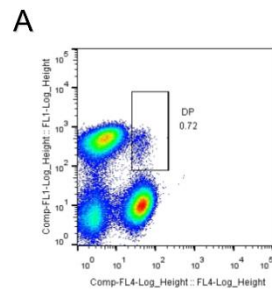


Figure S5 A. The gating information for DPT cells prepared for Single-cell sequencing.

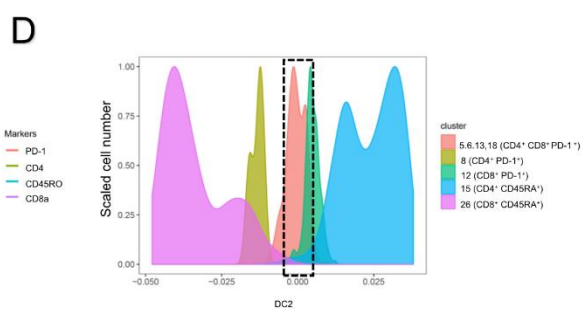
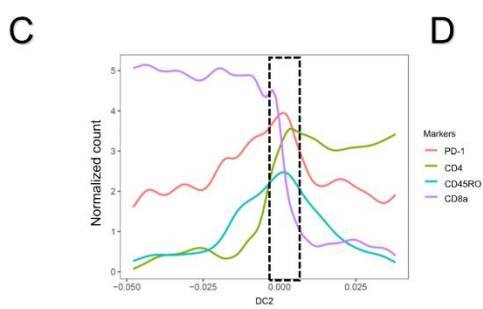
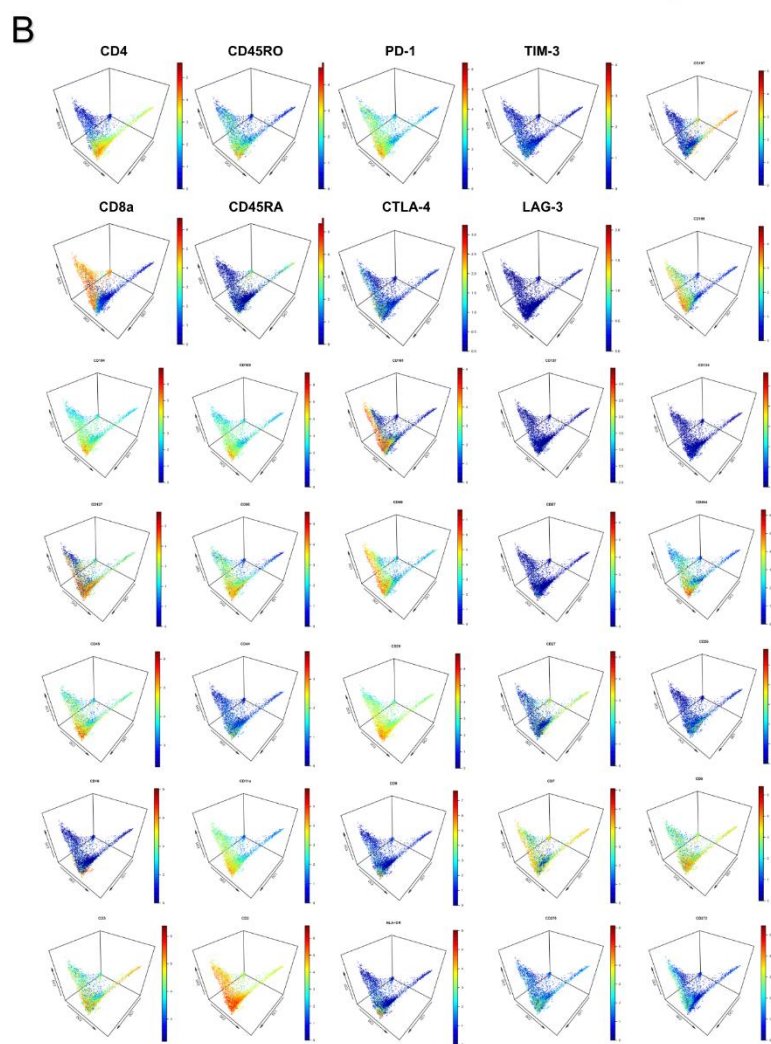
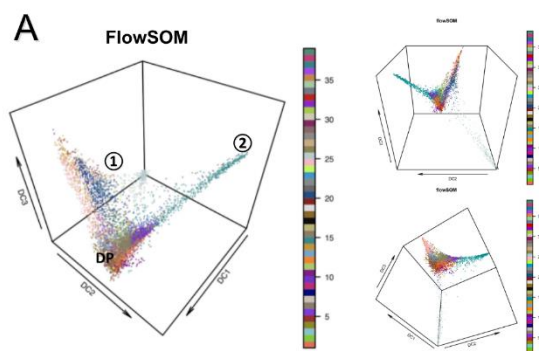


Figure S6 A. Visualization of T cell clusters using first, second and third components of a diffusion map. Cells are colored by FlowSOM clusters. The two main branches are indicated with number 1 and 2. B. The expression patterns of 35 clustering makers and immune checkpoint molecules were shown in the diffusion map plots. C. Conditional mean expression of the indicated markers along diffusion component two. D. Histogram demonstrating the distribution of certain T cell clusters (DP PD-1⁺, CD4⁺ PD-1⁺, CD8⁺ PD-1⁺, CD4⁺ CD45RA⁺, CD8⁺ CD45RA⁺) along diffusion component two.

Table S1. Chemical reagents used in CyTOF and analysis-related software/algorithm

Reagent name	Isotopes	Identifier	Source
Antibodies (Human)			
CD45	Y89	Cat# 3089003B	Fluidigm
CD45RA	169Tm	Cat# 3169008B	Fluidigm
CD45RO	165Ho	Cat# 3165011B	Fluidigm
CD49d	141Pr	Cat# 3141004B	Fluidigm
CD5	143Nd	Cat# 3143007B	Fluidigm
CD57	172Yb	Cat# 3172009B	Fluidigm
CD69	162Dy	Cat# 3162001B	Fluidigm
CD7	147Sm	Cat# 3147006B	Fluidigm
CD8a	146Nd	Cat# 3146001B	Fluidigm
CD9	171Yb	Cat# 3171009B	Fluidigm
CD95	152Sm	Cat# 3152017B	Fluidigm
HLA-DR	174Yb	Cat# 3174001B	Fluidigm
Tim-3	153Eu	Cat# 3153008B	Fluidigm
CD278	168Er	Cat# 3168024B	Fluidigm
CD11a	142Nd	Cat# 3142006B	Fluidigm
CD127	176Yb	Cat# 3176004B	Fluidigm
CD134	150Nd	Cat# 3150023B	Fluidigm
CD137	173Yb	Cat# 3173015B	Fluidigm
CD152	161Dy	Cat# 3161004B	Fluidigm
CD16	209Bi	Cat# 3209002B	Fluidigm
CD161	164Dy	Cat# 3164009B	Fluidigm
CD183	156Gd	Cat# 3156004B	Fluidigm
CD194	158Gd	Cat# 3158032A	Fluidigm
CD195	144Nd	Cat# 3144007A	Fluidigm
CD197	159Tb	Cat# 3159003A	Fluidigm
CD2	151Eu	Cat# 3151003B	Fluidigm
CD223	175Lu	Cat# 3175033B	Fluidigm
CD25	149Sm	Cat# 3149010B	Fluidigm
CD27	167Er	Cat# 3167002B	Fluidigm
CD272	163Dy	Cat# 3163009B	Fluidigm
CD279	155Gd	Cat# 3155009B	Fluidigm
CD28	160Gd	Cat# 3160003B	Fluidigm
CD3	170Er	Cat# 3170001B	Fluidigm
CD4	145Nd	Cat# 3140005B	Fluidigm
CD44	166Er	Cat# 3166001B	Fluidigm
Antibodies (Mouse)			
CD8a	168Er	Cat#3168003B	Fluidigm
CD44	150Nd	Cat#3150018B	Fluidigm
CD197	164Dy	Cat#3164013A	Fluidigm

CD25	151Eu	Cat#3151007B	Fluidigm
CD69	145Nd	Cat#3145005B	Fluidigm
TIM-3	162Dy	Cat#3162029B	Fluidigm
CD16	144Nd	Cat# 3144009B	Fluidigm
PD-1	159Tb	Cat# 3159006B	Fluidigm
CD4	172Yb	Cat# 3172003B	Fluidigm
Chemicals, Peptides, and Recombinant Proteins			
Cisplatin		Cat# 201192A	Fluidigm
Iridium		#201192A	Fluidigm
Human TruStain FcX™		#422302	Biolegend
Software and Algorithms			
Cytobank		https://support.cytobank.org/hc/en-us/articles/206336147-FCS-file-concatenation-tool	
Concatenation tool			Cytobank
Cytofkit (tSNE, FlowSOM)		https://bioconductor.org/packages/release/bioc/html/destiny.html	Chen et al. (1)

1. H. Chen, M. C. Lau, M. T. Wong, E. W. Newell, M. Poidinger, J. Chen, Cytofkit: A Bioconductor Package for an Integrated Mass Cytometry Data Analysis Pipeline. PLoS Comput Biol 12, e1005112 (2016).

Table S2. Clinical characteristics of the 46 HCC patients

patients ID	RFS day	Os day	pathology	differentiation	capsule	age	gender	AFP(500)	CEA(2.5)	CA19-9(37)	vascular invasion	cirrhosis	tumor thrombus	tumor size	tumor number	HBsAg	Total bilirubin (34)	Prothrombin time	Albumin	Ascites (ml)	Hepatic encephalopathy	Child-Pugh	BCLC	DPT cell percentage	DP PD-1+ cell percentage
76739	2971	2971	hcc	2	0	67	m	3.1	3.3	923.8	0	1	0	4.5	1	1	11.5	12.3	44.2	-	-	A	A	0.197393	0.063315
77013	2957	2957	hcc	3	0	71	m	4.7	3.8	90.6	0	0	0	7.5	1	0	12.6	12.2	41.8	-	-	A	A	0.039728	0.004957
77159	37	69	hcc	2	0	55	m			1000	0	1	1	5.5	2	1	37.6	11.5	38.9	<200	-	B	C	0.133634	0.018962
77180	190	285	hcc	2	1	52	m	546.1	2.4	90.6	0	1	1	4.5	2	1	6.3	13.6	42.7	-	-	A	C	0.192095	0.060279
77390	820	910	hcc	3	1	61	m	13.4	1.4	9.8	0	1	0	9	2	1	8.8	12.3	45.1	200	-	A	B	0.059866	0.04215
77490	78	328	hcc	3	0	63	m	358			0	1	0	5.5	1	1	13.6	13.3	46.7	-	-	A	A	0.121892	0.087275
78065	2925	2925	hcc	3	0	50	m	2.1	2.4	90.6	0	1	0	4	1	1	19.6	10.7	49.5	-	-	A	A	0.068282	0.022059
78315	54	70	hcc	2	0	35	m	11	1.2	14.2	0	0	0	9	2	1	9.7	12.3	38.2	-	-	A	B	0.080046	0.030162
78393	153	413	hcc	3	0	42	m	329.1	1.6	7.1	0	1	0	6	1	1	15.3	13.1	43.8	-	-	A	A	0.04119	0.018879
78553	170	261	hcc	3	0	47	m	141.3	4.3	9.3	0	0	0	9	2	1	9.6	12.6	38.9	<200	-	A	B	0.135734	0.041551

78587	42	122	hcc	2	0	61	f					1	1	10	1	0	12.6	12.3	27.5	<200	-	B	C	0.088131	0.045092
78666	59	2828	hcc	2	0	38	f	15.2	1.8	16.2	0	0	0	5	1	1	13.3	12.5	34.7	-	-	A	A	0.09383	0.01928
78823	1088	1452	hcc	2	0	77	m	1.3	2.6		0	1	0	6	2	0	9.4	11.5	41.4	-	-	A	B	0.188537	0.076345
78861	2899	2899	hcc	3	0	53	m	169.3	2.9	14.8	0	0	0	11	1	1	9.5	12	37.1	-	-	A	A	0.126741	0.025766
79032	1341	2210	hcc		0	73	m	3.7	3.7	12.1	0	0	0	7	1	0	20.9	11.1	42.7	-	-	A	A	0	0
79308	85	1824	hcc	2	0	45	m	95.8	1.8	36.8	0	0	0	5.5	2	1	15.3	12.4	38.5	-	-	A	B	0.147541	0.036988
79830	589	1077	hcc	3	0	52	m	5.2	4.7	272.4	1	1	0	6	1	1	13.3	11.3	40.5	-	-	A	C	0.057441	0.002611
79957	250	340	hcc	3	0	50	m	7	1	6.6	0	0	1	2	1	1	16	11	43.9	-	-	A	C	0.028369	0.007092
79974	947	1524	hcc	3	0	76	m	2.3	4.5	6.7	0	0	0	4	1	0	14.7	12.7	46.3	-	-	A	A	0.021955	0.001568
80095	2863	2863	hcc		0	64	m	6.8	2.4	7.7	0	0	0	8.5	1	0	12.5	10.1	41.5	-	-	A	A	0.261658	0.070596
81072	277	2834	hcc	3	0	45	m	87881	0.7		0	0	0	13.5	1	1	21.8	12	40.8	-	-	A	A	0.108747	0.053783
89093	80	177	hcc	3	0	54	m	7259	1.7	7.2	0	0	0	14	1	0	9.3	11	42.5	-	-	A	A	0.049254	0.049254
89284	2571	2571	hcc	3	0	68	m	4.2	8	0.6	0	0	1	5	2	0	240. 9	11.6	41.8	-	-	B	C	0.199472	0.152576

89352	2574	2574	hcc	3	0	71	m	0	0		0	0	0	5	1	0	15.7	11.6	42.3	-	-	A	A	0.173865	0.130676
89581	42	72	hcc	3	0	42	m	1210			1	1	1	8.5	1	1	41.2	11.1	38.5	<200	-	B	C	0.172693	0.046595
89626	2568	2568	hcc	3	0	50	m	1210	0.6	58.8	0	1	0	8	1	1	10.7	12.1	38.9	-	-	A	A	0.204744	0.156471
89802	2557	2557	hcc	3	0	51	m	176.7	0.8	21.8	0	1	0	11	1	1	9.3	11.7	45.6	-	-	A	A	0.280744	0.177747
89826	2488	2488	hcc	3	0	49	m	70	1.8	23.9	0	0	0	11	1	0	10	12.5	39.2	-	-	A	A	0.239617	0.159744
89863	123	551	hcc	3	0	43	m	1210			1	1	0	6	2	1	6.8	11.3	40.6	-	-	A	C	0.099034	0.068438
89990	58	89	hcc	3	0	29	m				0	0	0	3.5	1	1	15.5	13.8	39.9	-	-	A	A	0.155729	0.107898
90074	2554	2554	hcc	3	0	38	f	1210			0	0	0	8	1	0	14.9	10.8	43.5	-	-	A	A	0.159166	0.071899
90125	2555	2555	hcc	3	0	44	m	8.6	6.4	44.6	1	1	0	6.5	1	1	14.1	12.3	48.5	-	-	A	C	0.15873	0.126984
90265	1111	1484	hcc	2	0	66	m	3.5	5.8	46.3	0	0	0	6.5	1	0	7.8	10.1	45.9	-	-	A	A	0.110708	0.001815
90301	2548	2548	hcc	3	0	54	m	917.5		15.2	1	1	0	5.5	1	1	21.8	12.3	45.7	-	-	A	C	0.195074	0.160591
90336	84	1514	hcc	3	0	61	m	1210			0	1	0	5.5	1	0	11.7	11.9	42.5	-	-	A	A	0.110475	0.086743
90403	2541	2541	hcc	3	0	56	m	9.1	1.2	17.3	1	1	0	9	1	1	15.5	11.3	43.1	-	-	A	C	0.212722	0.097595

90494	291	382	hcc	3	0	57	m	1210			1	1	1	8	1	1	28.3	12.1	31.8	-	-	A	C	0.120869	0.112236
94815	2436	2436	hcc	3	0	43	m	0			0	1	0	8	1	1	14.6	12.5	45.2	-	-	A	A	0.172391	0.073316
95094	56	513	hcc	3	0	65	m	2.2	1.9	14.6	0	0	0	5.5	1	1	7.3	10.8	34	-	-	A	A	0.103837	0.081264
95612	1832	2409	hcc	3	0	59	m	6.2	3.5	40.9	0	0	0	3	1	0	31.9	12.1	44.1	-	-	A	A	0.121287	0.071782
99930	2298	2298	hcc	3	0	52	m	2.9			0	1	0	8	1	1	8	12.5	39.5	-	-	A	A	0.237223	0.230473
99998	530	926	hcc		0	51	m	1210	1.4	9.1	0	0	0	16	1	0	24.5	11.5	37.7	-	-	A	A	0.021186	0.006356
117829	741	917	hcc		0	81	m	28.8			0	0	0	5	1	0	9.9	11.2	38.8	-	-	A	A	0.016704	0.011136
118230	2239	2239	hcc	3	0	40	m	0	0	0	0	1	0	2.5	1	1	17.2	13	41.8	-	-	A	A	0.147092	0.04656
118292	80	488	hcc	3	0	66	m	18.9	3.6	19.9	0	0	0	12	1	1	14.9	11.3	45.2	-	-	A	A	0.03838	0.007463
118361	1932	2055	hcc		0	66	m	6			0	1	1	4	1	0	19.3	12.7	32	-	-	A	C	0.077299	0.052533

Table S3. Clinical characteristics of the 13 HCC patients

No	Patient ID	Viral status	Grades	Sex	Race	Age	Tumor size(cm)	Tumor multiplicity	AFP level(ng/ml)	Pre treatment
1	207587	Hep B	III	M	China	52	4.5	2	3.8	Nil
2	207560	Hep B	III	M	China	57	9	1	16.1	Nil
3	209020	Hep B	I	F	China	75	3.9	1	1281	Nil
4	209270	Hep B	III	M	China	46	4.6	1	4.8	Nil
5	209283	Hep B	III	M	China	54	6.4	1	69.18	Nil
6	205596	N	IV	M	China	55	9.9	2	11199	Nil
7	205488	Hep B	III	M	China	50	7.3	1	3.6	Nil
8	205516	N	III	M	China	69	3	1	169	Nil
9	205832	N	III	M	China	66	7.2	1	9.9	Nil
10	205854	Hep B	III	F	China	65	5.5	1	34.2	Nil
11	205866	Hep B	III	F	China	71	3.2	1	9.9	Nil
12	206112	Hep B	III	M	China	61	4	1	1210	Nil
13	206169	Hep B	III	M	China	53	6.6	2	2.7	Nil

Table S4. Primary antibodies used in multiple immunohistochemistry

Species	Antigen	Source	Clone	Supplier	Application	Dilution
Human	CD4	Rabbit	EPR6855	abcam	WB, IHC-P, Flow Cyt, ICC/IF	1:80
Human	CD8	Rabbit		abcam	ICC/IF, WB, IHC-P, IHC-Fr	1:50
Human	PD-1	Mouse	MX033	MXB	IHC	1:100

Table S5. Primers used for real-time PCR

Primer names (human)	Sequences	
	Forward (5'~3')	Reverse (5'~3')
<i>18S</i>	CGGCTACCACATCCAAGGAA	GCTGGAATTACCGCGGCT
<i>XCL1</i>	TGCTCTCTCACTGCATACATTG	TGGTGTAGGTCTTGATTCTGCT
<i>FOS</i>	CCGGGGATAGCCTCTCTTACT	CCAGGTCCGTGCAGAAGTC
<i>CRTAM</i>	GACGCTCACTCTAAAGTGTGTC	CTTGCAGGGTTACGTTAGGCA
<i>CCL4</i>	CTGTGCTGATCCCAGTGAATC	TCAGTTCAGTTCAGGTCATACA
<i>GZMB</i>	CCCTGGGAAAACACTCACACA	GCACAACCTCAATGGTACTGTCTG
<i>AHI1</i>	ATTGAGGAACACACAGTTAGCAA	GGCACCGTCTTTATCACCTTTT
<i>GZMH</i>	CTGGCTGGGGTTATGTCTCAA	GGCTACGTCCTTACACACGAG
<i>CHST12</i>	CTTCTACTTGCACACGTCCTT	CTCCGTCTCCTTTCTGGGAA
<i>TNFRSF9</i>	AGCTGTTACAACATAGTAGCCAC	GGACAGGGACTGCAAATCTGAT
<i>PDCD1</i>	CCAGGATGGTTCTTAGACTCCC	TTTAGCACGAAGCTCTCCGAT
<i>KLRD1</i>	CAGGACCCAACATAGAACTCCA	GGAAATGAAGTAACAGTTGCACC
<i>TUBA4A</i>	TGAGATCCGAAATGGCCATA	TAGTGACCACGGGCATAGTTG
<i>HLA-DRB1</i>	GAGCAGGTTAAACATGAGTGTCA	CTCTCCACAACCCCGTAGT
<i>CCL3</i>	AGTTCTCTGCATCACTTGCTG	CGGCTTCGCTTGGTTAGGAA
<i>HLA-DQA1</i>	TCGCTCTGACCACCGTGAT	AGGGACCGTAAACTGGTACAA
<i>CST7</i>	GTGTGAAGCCAGGATTTCTTAA	TGTCGTTTCGTGCAGTTGTTGA
<i>SLC7A5</i>	CCGTGAACTGCTACAGCGT	CTTCCCGATCTGGACGAAGC
<i>DUSP4</i>	GGCGGCTATGAGAGGTTTCC	TGGTCGTGTAGTGGGGTCC
<i>ITM2A</i>	ATCCTGCAAATTCCTTCGTG	CAGGTAAGCAGTCATTCCCTTT

Table S6. Fluorochrome-conjugated antibodies used in flow cytometry

Species	Source	Antigen	Fluorochrome	Clone	Supplier
Human	Mouse	CD45	APC-Cy™7	2D1	BD Bioscience
Human	Mouse	CD3	Alexafluor700	500A2	BD Bioscience
Human	Mouse	CD8	BB515	RPA-T8	BD Bioscience
Human	Mouse	CD45RO	APC	UCHL1	BD Bioscience
Human	Mouse	IFN- γ	PE	4S.B3	BD Bioscience
Human	Mouse	TNF α	BV650	MAb11	BD Bioscience
Human	Mouse	IL-2	BV421	5344.111	BD Bioscience
Human	Mouse	Fosp3	PE-CF594	259D/C7	BD Bioscience