Supplementary tables and figures

Table S1: Clinical and demographic information of patients involved in CyTOF analysis (Total n=23)

Characteristics	HBV (n=12)	NV (n=11)
Age [Median (range)]	64 (52, 79)	72 (48, 87)
Tumor size (cm) [Median (range)]	6.3 (1.7, 14.0)	6.3 (1.5, 20.0)
Pre surgery AFP (*ng/mL*) [Median (range)]	12.0 (5.8, 11737.0)	7.9 (1.8, 17349.0)
Ethnicity		
Chinese	11 (91.7%)	9 (81.8%)
Others	1 (8.3%%)	2 (18.2%)
Gender		
Male	10 (83.3%)	10 (90.9%)
Female	2 (16.7%)	1 (9.1%)
Edmondson Grading		
I, II or I-II	3 (25.0%)	7 (63.6%)
III or II-III	8 (66.7%)	4 (36.4%)
IV	1 (8.3%)	0 (0.0%)
TMN Staging		
1	5 (41.7%)	5 (45.4%)
2	2 (16.6%)	0 (0.0%)
3	5 (41.7%)	5 (45.4%)
4	0 (0.0%)	1 (9.2%)
Barcelona classification		
0/A	10 (83.3%)	7 (63.6%)
B	2 (16.7%)	4 (36.4%)
Fibrosis status (Stage)		
0	5 (41.7%)	11 (100%)
2-4	7 (58.3%)	0 (0%)
Liver enzyme level (U/L) [Median (range)]		
AST	35.5 (12.0, 141.0)	35.0 (21.0, 203.0)
ALT	34.5 (21.0, 87.0)	34.0 (16.0, 69.0)
HBV DNA (log IU/ml) * [Median (range)]	2.58 (1.30, 6.42)	NA

Footnote:
AFP= Alpha fetoprotein level
HBV= Hepatitis B viral; NV= Non viral
*= anti-viral treatment was given before surgery
NA= non-applicable
n=7 HBV vs n=7 NV (n=14) were compared in each figure however only 5 of them have enough NILs hence additional 9 cases were included for comparison of NILs

Table S2: Clinical and demographic information of TMA cohort involved in multiplex immunofluorescence FFPE tissues staining and survival analysis (n=102)

Characteristics	HBV (n=48)	Non-viral (n=54)
Age (Median (range))	64 (44, 83)	64 (15, 88)
Tumor size (cm) (Median (range))	3.8 (0.9, 30.2)	5.75 (1.1, 21.0)
Pre surgery AFP (*ng/mL*) (Median (range))	13.4 (1.8, 34272.0)	11 (1.0, 70700.0)
Ethnicity		
Chinese	43 (89.6%)	40 (74.1%)
Others	5 (10.4%)	14 (25.9%)
Gender		
Male	37 (77.1%)	44 (81.5%)
Female	11 (22.9%)	10 (18.5%)
Median Survival (yrs)	3.57 (0.03, 12.48)	3.85 (0.02, 13.34)
Edmondson Grading		
I-II	46 (95.8%)	40 (74.1%)
III-IV	2 (4.2%)	14 (25.9%)
TMN Staging		
1	32 (66.7%)	26 (48.1%)
2	12 (25.0%)	13 (24.1%)
3	2 (4.2%)	14 (25.9%)
4	2 (4.2%)	1 (1.9%)

Table S3: Clinical and demographic information of international cohort involved in survival analysis (Total n=116 from Singapore n=37, Hong Kong n=42 and Zurich n=37)

Characteristics	HBV (n=75)	Non-viral (n=41)
Age (Median (range))	56 (23, 78)	69 (20, 83)
Tumor size (cm) (Median (range))	4.3 (0.7, 27.0)	9.0 (1.2, 23.0)
Pre surgery AFP (*ng/mL*) (Median (range))	102.0 (2.0, 468600.0)	5.0 (1.0, 12630.5)
Ethnicity		
Chinese	65 (86.7%)	13 (31.7%)
Others	10 (13.3%%)	28 (68.3%)
Gender		
Male	63 (84.0%)	30 (73.2%)
Female	12 (16.0%)	11 (26.8%)
Median Survival (yrs) (range)	5.0 (0.05, 16.31)	3.9 (0.16, 15.91)
Edmondson Grading		
I-II	60 (80.0%)	29 (70.7%)
III-IV	15 (20.0%)	12 (29.3%)
TMN Staging		
1	21 (28.0%)	15 (36.6%)
2	21 (28.0%)	13 (31.7%)
3	27 (36.0%)	11 (26.8%)
4	6 (8.0%)	2 (4.9%)

Table S4: Antibodies used for CyTOF staining

Isotopes	Antibodies	Clone	Vendor
112/114	CD14	TüK4	Lifetechnologies
139	CD3	UCHT1	Biolegend
141	CD45 (Barcode 1)	HI30	Biolegend
142	CD45RO	UCHL1	Biolegend
143	HLA-DR	L243	Biolegend
144	CD8	SK1	Biolegend
145	T-bet	4B10	Biolegend
146	CD28	CD28.2	Biolegend
147	PD-1	EH12.2H7	Biolegend
148	CD4	SK3	Biolegend
149	CD154	24-31	Biolegend
150	CD103	B-Ly7	Ebioscience
151	CD134	Ber-ACT35	Biolegend
152	TNF-a	MAb11	Biolegend
153	CD45 (Barcode 2)	HI30	Biolegend
154	CD27	O323	Biolegend
155	CD152	BN13	BD bioscience
156	PD-L1	29E.2A3	Biolegend
157	CD244	CL7	Biolegend
158	IL-10	JES3-9D7	Biolegend
159	LAG-3	17B4	Abcam
160	TIM-3	F38-2E2	Biolegend
161	CCR7	G043H7	Biolegend
162	CD56	NCAM16.2	BD bioscience
163	CXCR3	G025H7	Biolegend
164	GITR	621	Biolegend
165	FoxP3	PCH101	Ebioscience
166	Ki67	20Raj1	Ebioscience
167	CD80	2D10	Biolegend
168	IFN-y	B27	Biolegend
169	IL-17A	BL168	Biolegend
170	CD45 (Barcode 3)	HI30	Biolegend
171	CD45RA	JS-83	Ebioscience
172	CD86	IT2.2	Biolegend
173	GranzymeB	CLB-GB11	Abcam
174	CD137	4B4-1	Biolegend
175	CCR5	T21/8	Biolegend
176	CD69	FN50	Biolegend
191/193	Ir Intercalator		Fluidigm

Table S5: Antibodies used for multiplexed immmunoflourecence tissue staining (Opal)

Antibody	Host	Clone	Company and location
Anti-Human CD4	Mouse	EPR6855	Abcam, Cambridge, UK
Anti-Human CD8	Mouse	C8/144B	DAKO
Anti-Human FOXP3	Mouse	236A/E7	Abcam, Cambridge, UK
Anti-Human Integrin alpha E (CD103)	Rabbit	EPR4166 (2)	Abcam, Cambridge, UK

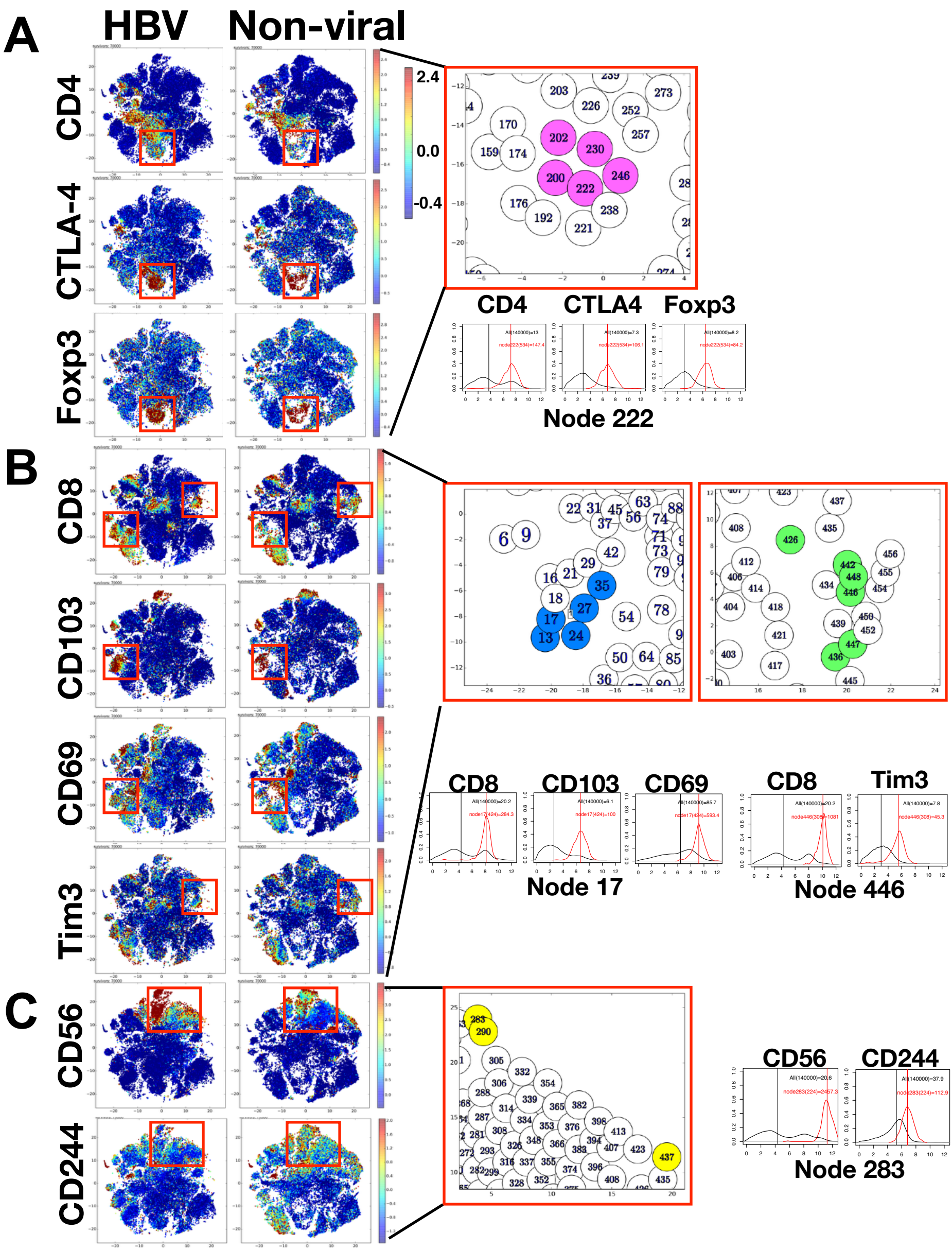


Figure S1: 2D cellular t-SNE plots of CyTOF data from HBV- or non-viral-related TILs.

A. t-SNE and density plots showing T_{REG} markers: CD4, CTLA4 and Foxp3 represented by Node no. 222.

B. t-SNE and density plots showing T_{RM} markers: CD8, CD103 and CD69 (represented by Node 17) and $CD8^{+}Tim3^{+}$ T cells (represented by Node 446).

C. t-SNE and density plots showing $CD244^{+}CD56^{+}$ NK cells represented by Node 283.

The density plots: red curve showing the expression profile of the indicated Node and black curve showed the marker expression profile of all nodes. Red and black lines showed the respective average marker expression levels. HBV: hepatitis B virus; TILs: tumor-infiltrating lymphocytes.

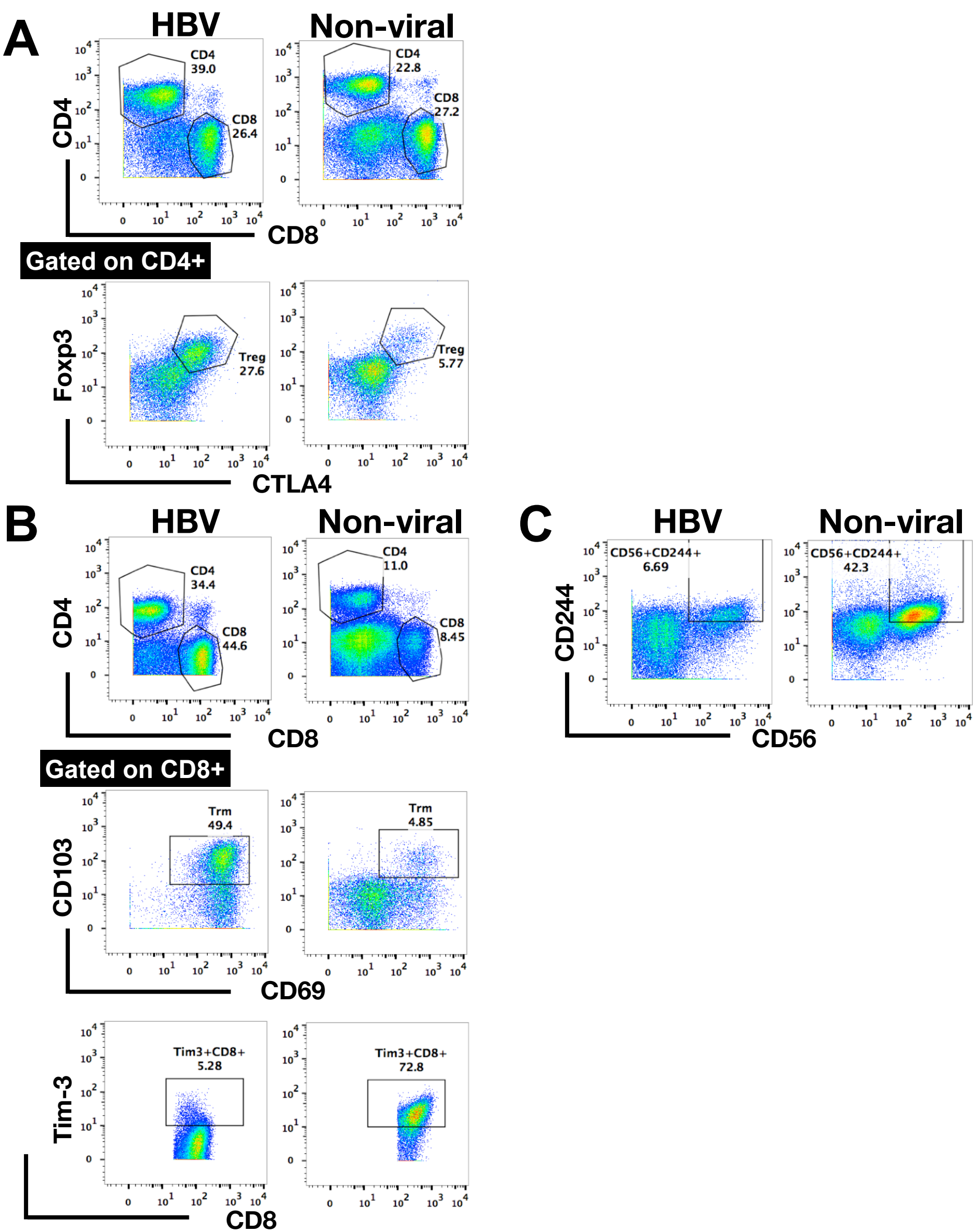


Figure S2: Representative manual gating plots for the major immune subsets enriched in either HBV- or non-viral-related HCC TILs.

A. Representative plots for T_{REG} manual gating on TILs from HEP2050 (HBV) and HEP178 (non-viral) HCC patients.
B. Representative plots for T_{RM} and $Tim3^{+}CD8^{+}$ T cells manual gating on TILs from HEP125 (HBV) and HEP152 (non-viral) HCC patients.
C. Representative plots for $CD244^{+}CD56^{+}$ NK cells manual gating on TILs from HEP0302 (HBV) and HEP0301 (non-viral) HCC patients.

HBV: hepatitis B virus; HCC: hepatocellular carcinoma; TILs: tumor-infiltrating lymphocytes.

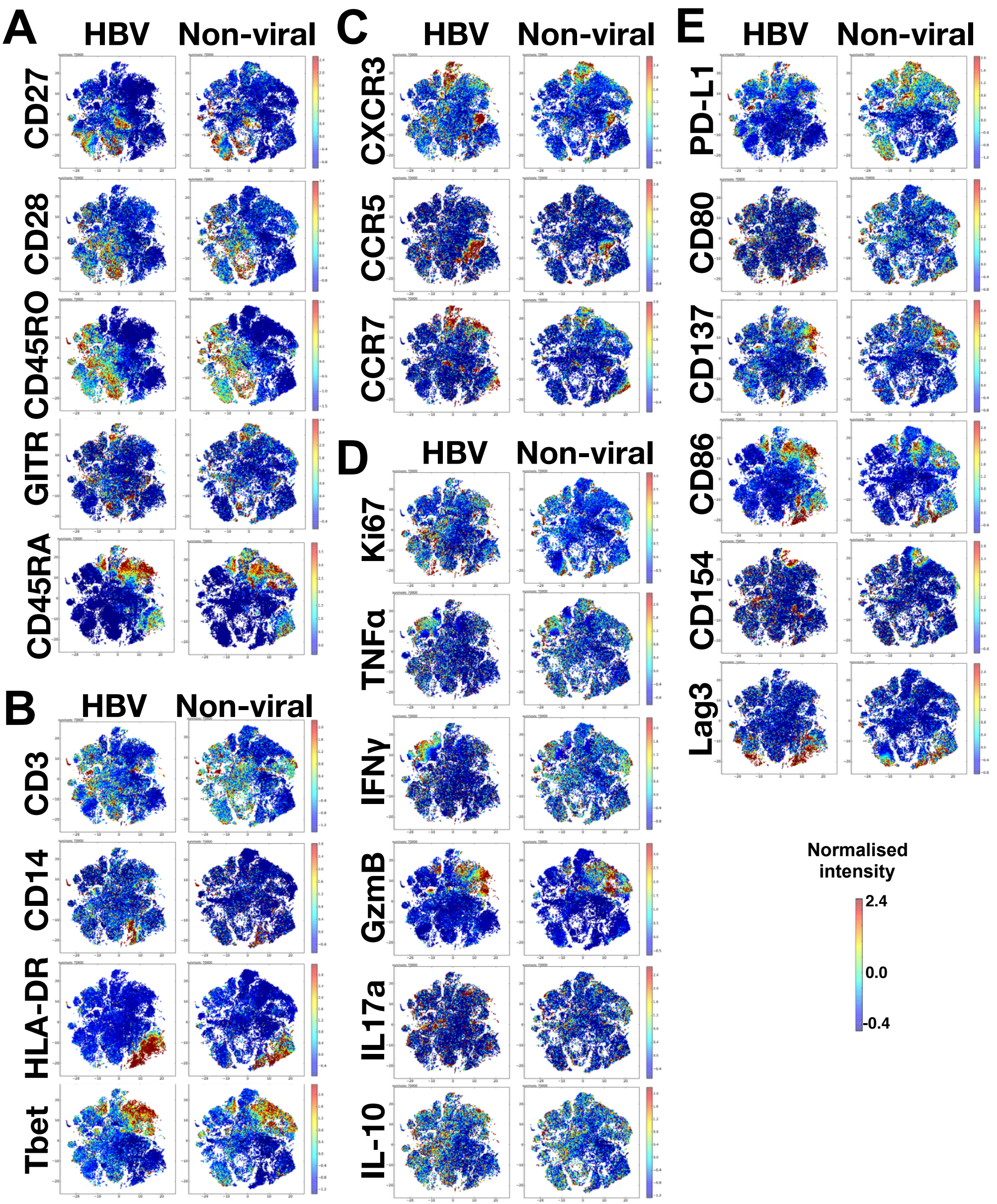


Figure S3: 2D cellular t-SNE plots of CyTOF data from HBV- or non-viral-related HCC TILs.

A. CD4-related markers.
B. Lineages-related markers
C. Chemokine receptors
D. Activation and cytokines markers
E. Other immune markers without distinct differences

HBV: hepatitis B virus; HCC: hepatocellular carcinoma; TILs: tumor-infiltrating lymphocytes.

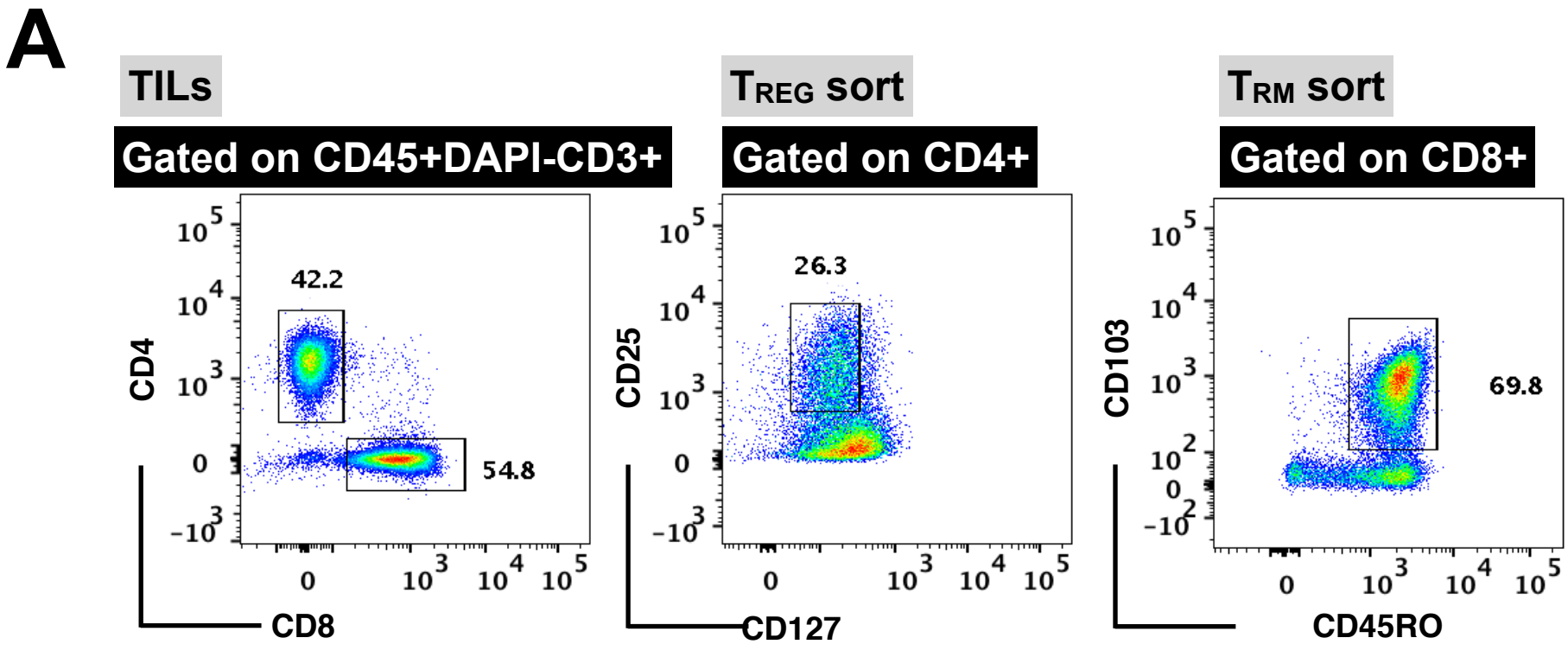


Figure S4: Plots showing the sorting strategies of TILs subsets from HBV- or non-viral-related HCCs

A. Representative pseudocolor plots illustrating sorting strategy for CD4CD25+CD127- (T_{REG}) and CD8+CD45RO+CD103+ (T_{RM}) populations from TILs.

HBV: hepatitis B virus; HCC: hepatocellular carcinoma; TILs: tumor-infiltrating lymphocytes.

Supplementary Table S6: Selective list of significantly enriched genes in TREG isolated from HBV-related HCC

CELL CYCLE

Ensembl ID	Gene Symbol	Annotation	logFC
ENSG00000145386	CCNA2	cyclin A2	1.8194
ENSG00000077380	DYNC1I2	dynein cytoplasmic 1 intermediate chain 2	1.0474
ENSG00000039068	CDH1	cadherin 1	1.5848
ENSG00000102384	CENPI	centromere protein I	2.1195
ENSG00000174442	ZWILCH	zwilch kinetochore protein	1.0560
ENSG00000101057	MYBL2	MYB proto-oncogene like 2	1.4493
ENSG00000167900	TK1	thymidine kinase 1	1.2061
ENSG00000137812	KNL1	kinetochore scaffold 1	1.0641
ENSG00000073111	MCM2	minichromosome maintenance complex component 2	1.0071

IL-10 SIGNALLING

Ensembl ID	Gene Symbol	Annotation	logFC
ENSG00000115594	IL1R1	interleukin 1 receptor type 1	1.1430
ENSG00000115590	IL1R2	interleukin 1 receptor type 2	1.3602
ENSG00000028137	TNFRSF1B	TNF receptor superfamily member 1B	1.0937

COMPLEMENT ACTIVATION

Ensembl ID	Gene Symbol	Annotation	logFC
ENSG00000211937	IGHV2-5	immunoglobulin heavy variable 2-5	9.2278
ENSG00000159403	C1R	complement C1r	1.9038
ENSG00000211956	IGHV4-34	immunoglobulin heavy variable 4-34	3.2238
ENSG00000211679	IGLC3	immunoglobulin lambda constant 3	8.0135

Supplementary Table S7: Selective list of significantly enriched genes in TRM isolated from HBV-related HCC

TCR SIGNALLING

Ensembl ID	Gene Symbol	Annotation	logFC
ENSG00000198146	ZAP70	zeta chain of T cell receptor associated protein kinase 70	2.1272
ENSG00000198821	CD247	CD247 molecule	2.3690
ENSG00000179344	HLA-DRB1	major histocompatibility complex, class II, DR beta 1	2.0547
ENSG00000196126	HLA-DQB1	major histocompatibility complex, class II, DQ beta 1	1.5021
ENSG00000277791	PSMB3	proteasome subunit beta 3	1.0930
ENSG00000092010	PSME1	proteasome activator subunit 1	1.3391
ENSG00000240065	PSMB9	proteasome subunit beta 9	1.2111

COSTIMULATION BY THE CD28 FAMILY

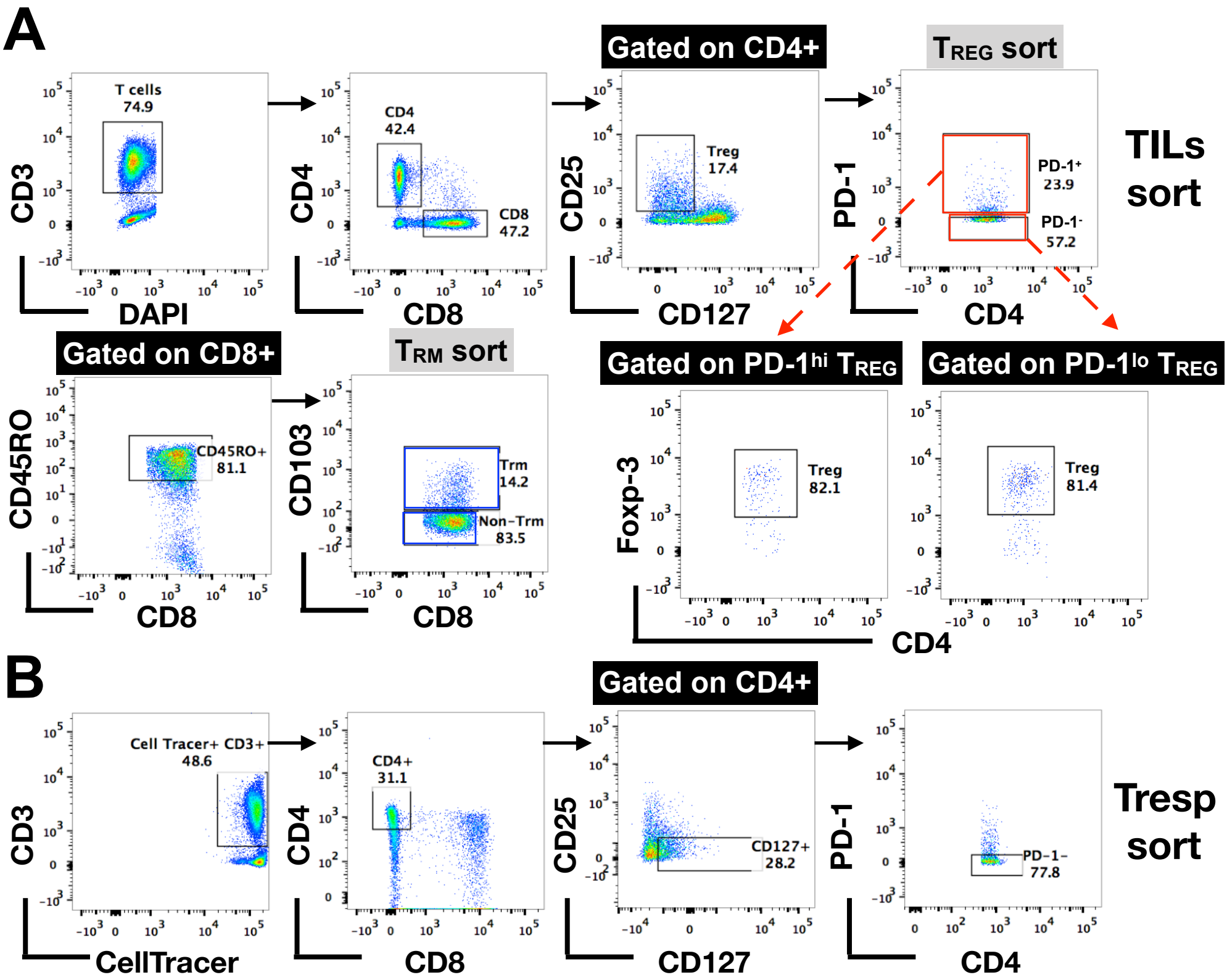
Ensembl ID	Gene Symbol	Annotation	logFC
ENSG00000121594	CD80	CD80 molecule	2.1157
ENSG00000142208	AKT1	AKT serine/threonine kinase 1	1.3451
ENSG00000163599	CTLA4	cytotoxic T-lymphocyte associated protein 4	2.2112
ENSG00000198821	CD247	CD247 molecule	2.3690
ENSG00000188389	PDCD1	programmed cell death 1	1.6145
ENSG00000179344	HLA-DRB1	major histocompatibility complex, class II, DR beta 1	2.0547
ENSG00000196126	HLA-DQB1	major histocompatibility complex, class II, DQ beta 1	1.5021

PD-1 SIGNALLING

Ensembl ID	Gene Symbol	Annotation	logFC
ENSG00000198821	CD247	CD247 molecule	2.3690
ENSG00000188389	PDCD1	programmed cell death 1	1.6145
ENSG00000179344	HLA-DRB1	major histocompatibility complex, class II, DR beta 1	2.0547
ENSG00000196126	HLA-DQB1	major histocompatibility complex, class II, DQ beta 1	1.5021

MHC CLASS II ANTIGEN PRESENTATION

Ensembl ID	Gene Symbol	Annotation	logFC
ENSG00000019582	CD74	CD74 molecule	1.3253
ENSG00000089692	LAG3	lymphocyte activating 3	3.1539
ENSG00000179344	HLA-DRB1	major histocompatibility complex, class II, DR beta 1	2.0547
ENSG00000196126	HLA-DQB1	major histocompatibility complex, class II, DQ beta 1	1.5021



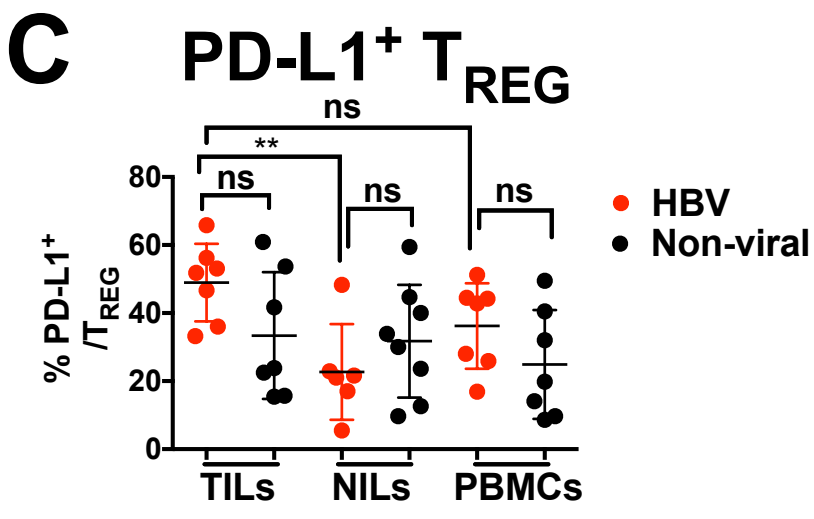


Figure S5: Sorting Strategy for TILs and Tresp cells for T_{REG} suppression assay

A. Representative plots for sorting of TILs from HEP287 (HBV-related HCC patient): PD-1$^+$ and PD-1$^-$ T_{REG} (Red boxes) and T_{RM} and Non-T_{RM} (blue boxes)
B. Representative plots for sorting Tresp cells from HEP287 PBMCs marked as CD3$^+$Cell Tracer$^+$ CD4$^+$CD127$^+$CD25$^-$ and PD-1$^-$ T cells.
C. Percentages of PD-L1$^+$$T_{REG}$ from TILs, NILs or PBMCs from either HBV-related HCCs or non-viral-related HCCs as obtained by manual gating using FlowJo software. Data represent the means ± standard deviations;**p <0.01; ns= statistically non-significant (unpaired Student's t-test, two-tailed).

TILs: tumor-infiltrating lymphocytes; Tresp: T-responders cells

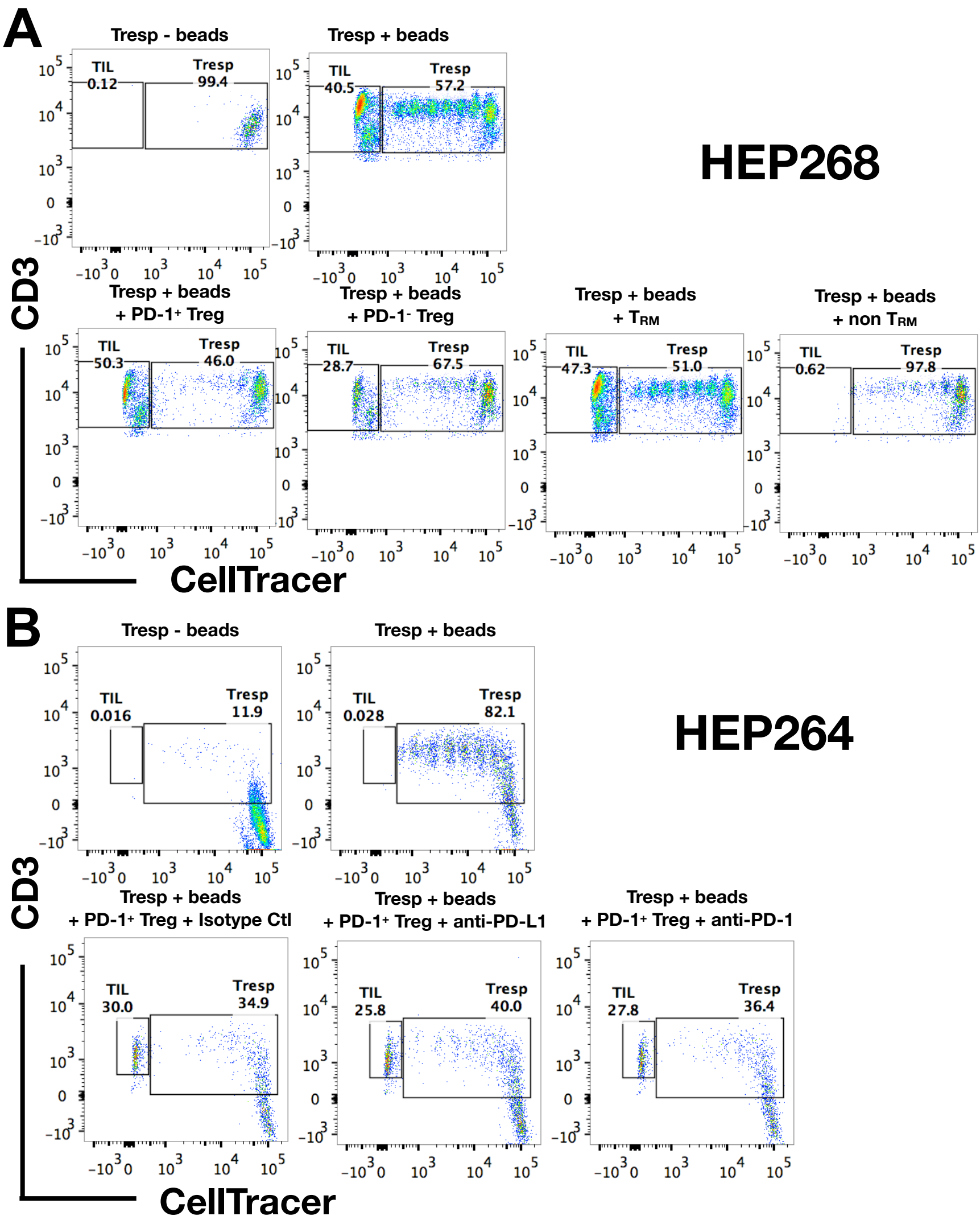


Figure S6 : Tresp cell proliferation profiles incubated with various TILs and check-point inhibitors

A. Proliferation of Tresp cells determined according to dilution cycles of CellTracer signal when co-cultured without or with T_{REG} proliferation beads (top panel) or with T_{REG} proliferation beads and PD-1$^+$ T_{REG}, PD-1$^-$ T_{REG}, T_{RM} or non T_{RM} from TILs (lower panel). Representative plots from HBV-related HCC patient HEP268.
B. Proliferation of Tresp cells when co-cultured without or with T_{REG} proliferation beads (top panel) or with T_{REG} proliferation beads, PD-1$^+$ T_{REG} and with either isotype control, anti-PD-L1 or anti-PD-1 antibody (lower panel). Representative plots from HBV-related HCC patient HEP264.

TILs: tumor-infiltrating lymphocytes; Tresp: T-responders cells