Supplementary Data

Supplementary Table 1. Comparison of clinical and demographic characteristics of HCC patients in Singapore, Hong Kong and Zurich cohorts

Variables		Sg cohort (n=40)	HK cohort (n=45)	Zurich cohort (n=27)	p-value	method
Sex, F/M	Number (percent)	6/34(15/85)	6/39 (13/87)	9/16(33/59)	=0.0491	*
*Age, years	Median (Range)	59 (31-84)	56 (28-74)	60(20-83)	ns	@
Race, Asian/European	Number (percent)	39/0(98/0)	45/0(100/0)	3/22(11/81)	<0.0001	*
Viral status, Non-infected/HepB ,C,D	Number (percent)	10/29(25/73)	3/41(7/91)	13/12(48/44)	=0.0001	*
Grade, 1+2/ 3+4	Number (percent)	22/16 (55/40)	44/0 (98/0)	16/5(59/19)	<0.0001	*
TMN Staging, I/ II+III+IV	Number (percent)	23/17 (58/42)	3/41 (7/91)	7/18(26/67)	<0.0001	*
α-fetoprotein, ng/ml	Median (Range)	30.3 (1.5-71,000)	229(2-468,600)	19(1-78171.3)	=0.03	@
Tumor size, cm	Median (Range)	5(0.7-23)	5 .5(2-27)	5.2(1.2/18)	ns	@
Survival, years	Median (25th/75th	4.5 (0.6/5.8)	6.3 (3.1/9.2)	2.9(1.1/4.7)	ns	#

%)

@ Kruskal-Wallis test
* Chi squared test
# Both Cox proportional hazards regression and Kaplan Meier with log-rank test show the differences are not significant.

Reagents and Antibodies

Supplementary Table 2: List of antibodies.

The list of antibodies used in immunohistochemistry (IHC) and flow cytometry (FC) was listed in Supplementary table 1 below. The working dilutions are 1:50 for all antibodies.

Antibody	Host	Clone	Company and location	Application
CD3	Mouse	MIH5	BD Biosciences, San Jose, CA	IHC
CD3	Rabbit	Polyclonal	Dako, Glostrup, Denmark	IHC
CD3	Goat	Polyclonal	Santa Cruz Biotechnology, Inc. Dallas, Texas.	IF
CD8	Mouse	1A5	Novocastra, Newcastle upon Tyne, UK	IHC
CD20	Rabbit	BV11	Novocastra, Newcastle upon Tyne, UK	IHC/IF
CD20	Goat	Polyclonal	Santa Cruz Biotechnology, Inc.	IF
CD27	Mouse	137B4	Dako	IHC/IF
CD40	Mouse	HB14	Abcam, Cambridge, MA	IHC/IF
CD38	Rabbit	Polyclonal	Abcam	IHC/IF
CD56	Mouse	123C3	Monosan	IHC
CD68	Mouse	PG-M1	Dako	IF
CD138	Mouse	B-A38	Abcam	IHC/IF
S100	Rabbit	Polyclonal	Dako	IF
Granyzme B	Rabbit	Polyclonal	Abcam	IHC
Activate Caspase 3	Rabbit	Polyclonal	Promega, Madison, WI	IHC
Ki67	Rabbit	SP6	AbCam	IHC
CD45	Mouse	H130	BioLegend, San Diego, CA	FC
CD19	Mouse	SJ25-C1	Life Technologies, Carlsbard, CA	FC

CD56	Mouse	NCAM 16.2	BD Biosciences.	FC
CD14	Mouse	61D3	eBioscience, Inc., San Diego, CA	FC
CD24	Mouse	ML5	BioLegend	FC
CD27	Mouse	M-T271	BioLegend	FC
CD38	Mouse	HIT2	BioLegend	FC
CD40	Mouse	HB14	BioLegend	FC
CD138	Mouse	DL-101	BioLegend	FC
HLA-DR	Mouse	L243	eBioscience, Inc.	FC
IFN-gamma	Mouse	4S.B3	BioLegend	FC
Granyzme B	Mouse	GB11	BioLegend	FC

A.

tumor

peritumor

Non-tumor

Infiltrating margin
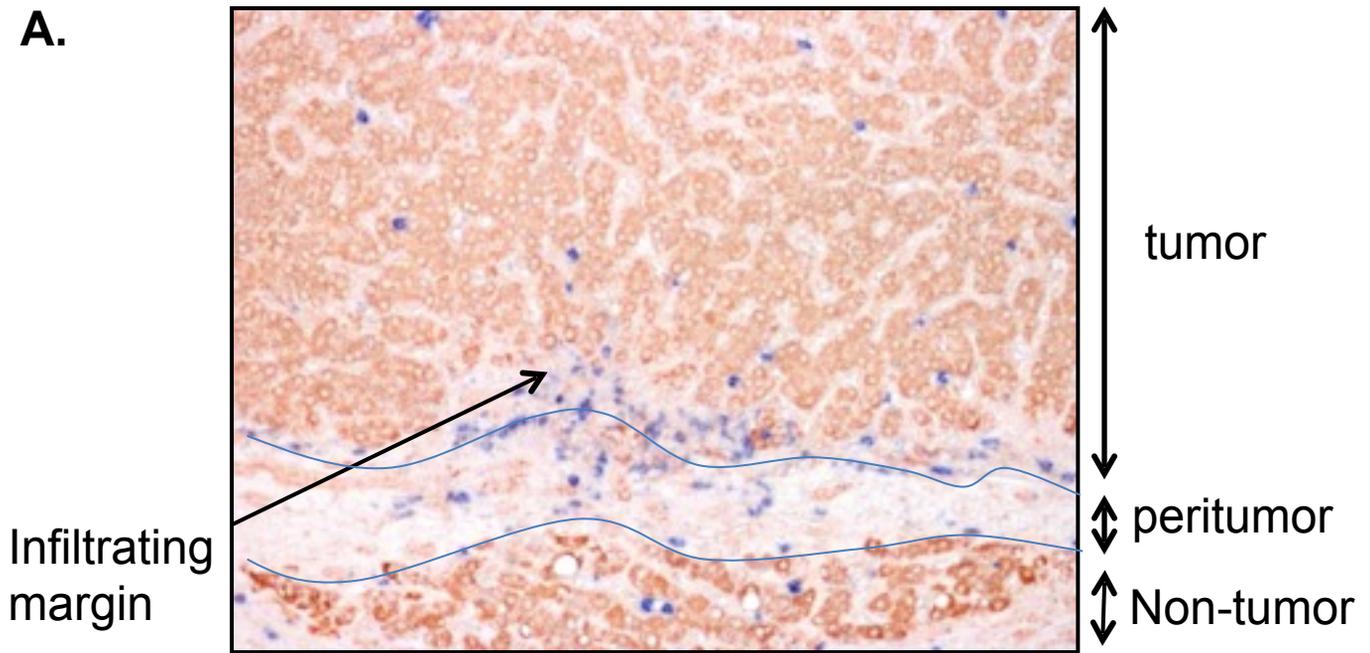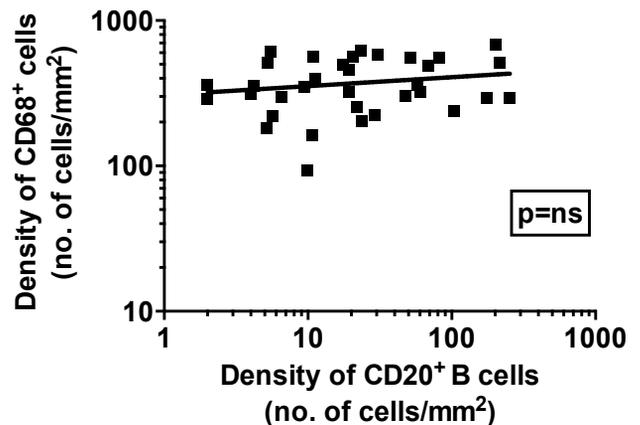
CD20 stained in blue.

Supplementary figure 1:
A. Margin definition of tumor region. Representative IHC image of HCC tumor sample at 400x magnification showing the tumor, peritumor and non-tumor regions which marks the basis of our quantification. In blue is CD20 staining on TIB. We considered the immune cells at the infiltrating margin as intratumoral.

A.

B.
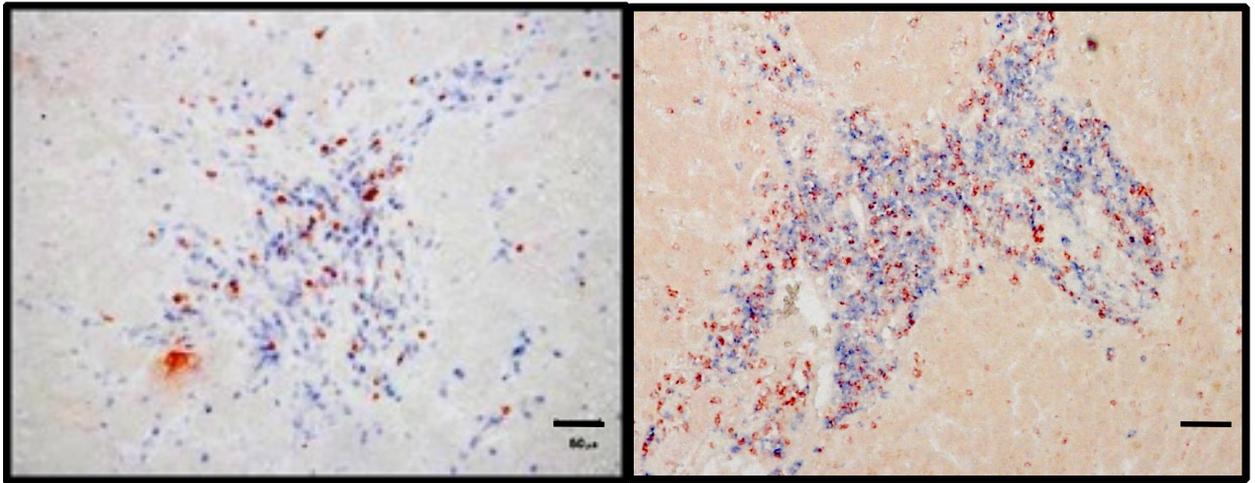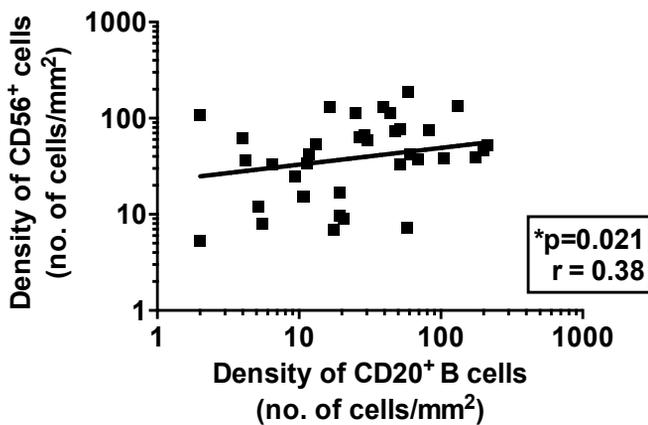

Supplementary figure 2: CD20 IHC staining and its correlation to other TIL in HCC samples.
A. Representative IHC images showing staining of CD20, CD3 and CD8 in HCC samples. On the left, CD20 (red) and CD3 (blue) and on the right, CD8 (red) and CD20 (blue). Bar= 50μm. 200x magnification.
B. Graphs showing correlation of densities of CD20$^+$ TIB with CD56$^+$ NK cells and lack of correlation with CD68$^+$ macrophages in HCC samples.

A.
CD56(R)+GZB(B)   CD8(R)+GZB(B)

B.
GZB⁺ TILs
IFNγ⁺ TILs

C.
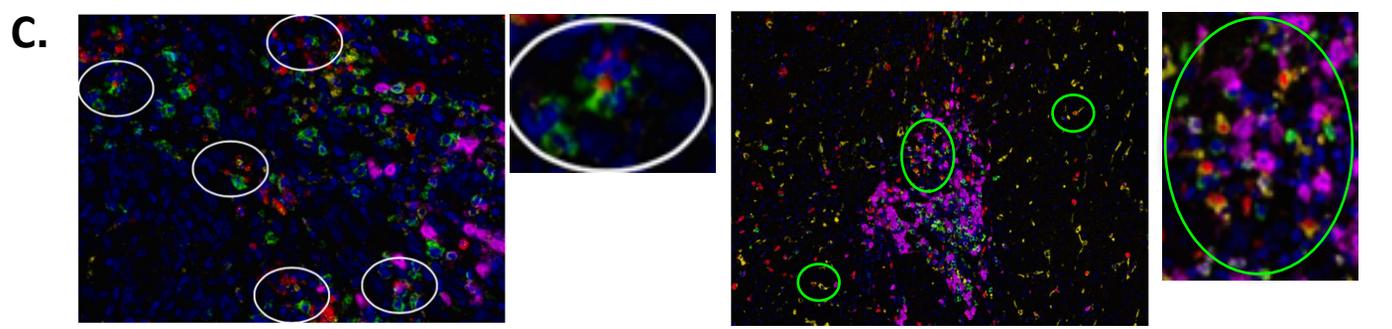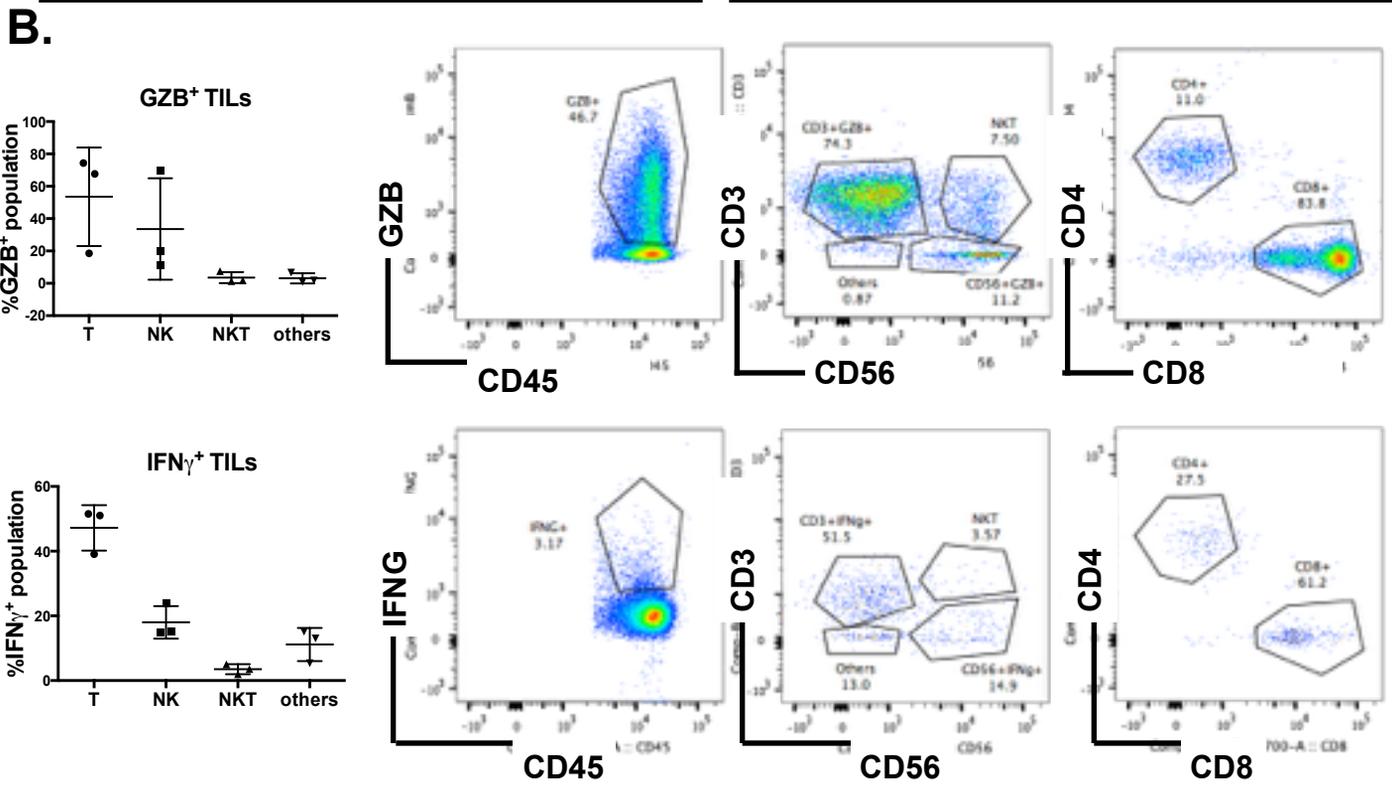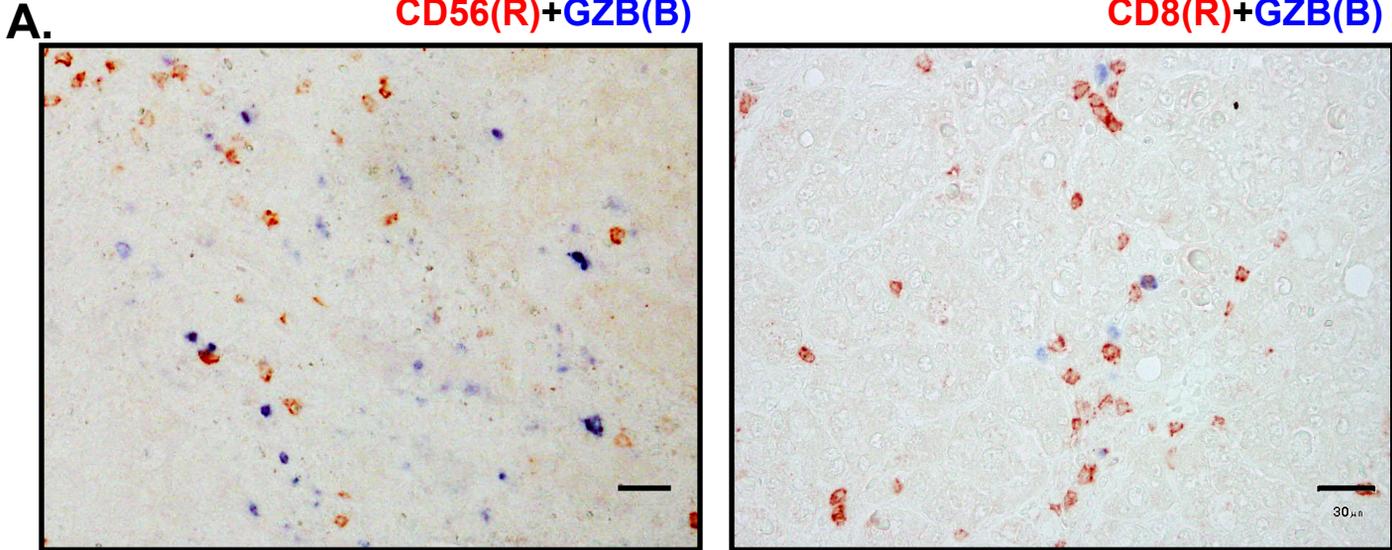
Supplementary figure 3

Supplementary figure 3: Tumor-infiltrating B cells correlates to T and NK cell activation marker Granzyme B and IFN-gamma.

A. Matched IHC images from serial sections of the same tissue sample from a single HCC patient. CD20(red) was observed to be at close proximity to Granzyme-B (GZB) (blue) in the tumor. CD8(red) and CD56(blue) are seen in the same region with high CD20 staining. Bar= 100μm. 100x magnification.

B. Freshly isolated TILs from three HCC patients samples were analyzed using flow cytometry. CD45$^+$GranzymeB$^+$ (above) or IFNγ^+ (below) TILs were majority T or NK cells. Graphs on the left, shown mean and SD of three tumor samples from HCC patients. Granzyme B (GZB) expressing TILs are mostly CD3$^+$ T (53.5±30.5%) and CD56$^+$ NK cells (33.6±31.3%). IFNγ expressing TILs are also mostly CD3$^+$ T (47.2±7.0%) and CD56$^+$ NK cells (18.0±5.0%).

C. Representative IF image from HCC tumor region stained using multiplex tissue staining technique with Opal. CD8=Green, CD20=Pink, CD68=Yellow, Granzyme B= Red, DAPI=Blue. Left, circles in white show co-localization of Granzyme (intracellular in Red) with CD8$^+$ (surface in green) T cells. Right, green circles show co-localization of Granzyme (intracellular in Red) with CD68$^+$ (surface in yellow) macrophages. Magnified images are presented at the side. Bar= 50μm. 200x magnification.
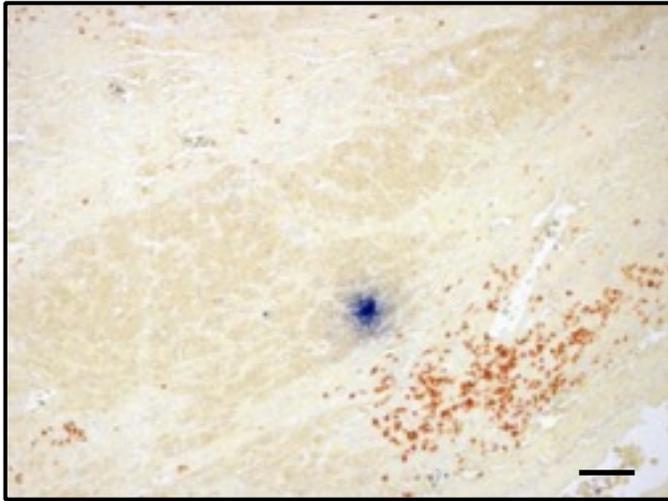
A. CD20(R)+Caspase 3(B)   **B.** CD20(R)+Ki67(B)
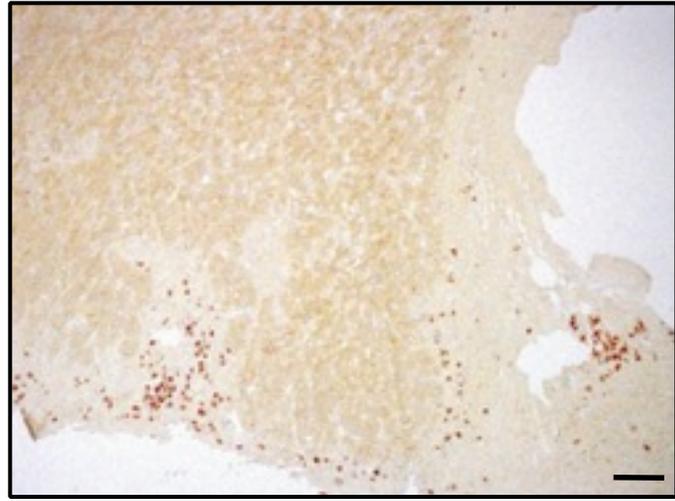
Supplementary figure 4: Tumor-infiltrating B cells correlates to decreased tumor cell viability.

A. Representative IHC images showing staining of CD20 (red) in close proximity with activated Caspase 3 (blue) and **B.** lack of staining of Ki-67 (blue) in HCC samples. Bar= 100µm. 100x magnification.

A.

IgGs(R)+CD20(B)

Tumor | Normal liver | 100x

400x

B.

IgGs(R)+CD138(B)
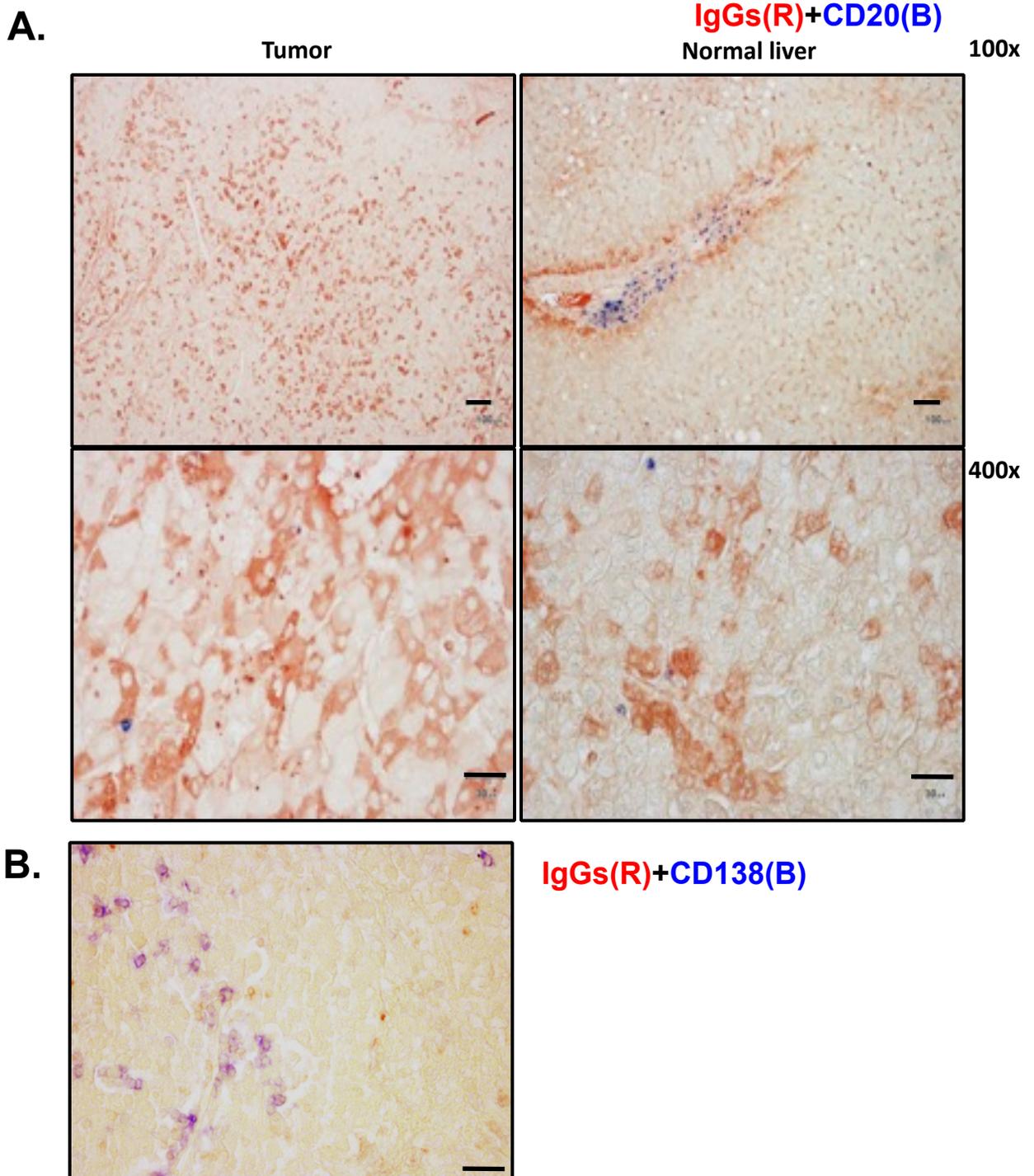
Supplementary figure 5: Expression of human IgGs in HCC samples.

A. Representative IHC images showing staining of IgGs (red) and CD20 (blue) for tumor (left) or normal liver (right) at 100x magnification (bar=100μm) or 400x magnification (bar= 30μm). **B.** Representative IHC images showing staining of IgGs (red) and CD138 (blue) in tumor. 400x magnification (bar=30μm)