***Supplementary Material***

## Supplementary Tables

**Supplementary Table 1** Clinicopathological characteristics of the 49 individual patients analysed for immunotherapy response.

| Patient ID | ICB Treatment (target) | Line of systemic treatment | Response status | Hepatitis status | AFP Level | Age (years) | Stage* | Grade# |
|---|---|---|---|---|---|---|---|---|
| HCC 001 | Combination PD-1/PD-L1 + CTLA-4 | 1st | Progressive Disease (PD) | None | 34206 | 70 | C | 3 |
| HCC 002 | PD-1/PD-L1 | 1st | Partial Response (PR) | HCV | 2.6 | 60 | C | 2 |
| HCC 003 | Combination PD-1/PD-L1 + others | 1st | Stable Disease (SD) | HBV | 4.2 | 78 | C | 3 |
| HCC 004 | Combination PD-1/PD-L1 + CTLA-4 | 1st | Progressive Disease (PD) | None | 1384 | 76 | C | 3 |
| HCC 005 | PD-1/PD-L1 | 1st | Progressive Disease (PD) | None | 60500 | 73 | C | 2 |
| HCC 006 | PD-1/PD-L1 | 1st | Progressive Disease (PD) | HBV | 6 | 52 | C | 2 |
| HCC 007 | Combination PD-1/PD-L1 + others | 2nd | Progressive Disease (PD) | HBV | 1138 | 62 | B | 3 |
| HCC 008 | PD-1/PD-L1 | 1st | Stable Disease (SD) | HBV | 4 | 65 | C | 3 |
| HCC 009 | PD-1/PD-L1 | 1st | Partial Response (PR) | HCV | 48392 | 65 | C | 3 |
| HCC 010 | Combination PD-1/PD-L1 + CTLA-4 | 1st | Partial Response (PR) | HBV | 832 | 79 | C | 3 |
| HCC 011 | PD-1/PD-L1 | 2nd | Progressive Disease (PD) | None | 20 | 67 | B | 2 |
| HCC 012 | PD-1/PD-L1 | 1st | Partial Response (PR) | None | 11.5 | 81 | C | 2 |
| HCC 013 | PD-1/PD-L1 | 1st | Progressive Disease (PD) | None | 16.8 | 71 | C | 2 |
| HCC 014 | PD-1/PD-L1 | 1st | Stable Disease (SD) | HBV | 87 | 79 | C | 3 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| HCC 015 | PD-1/PD-L1 | 1st | Progressive Disease (PD) | None | 2.3 | 66 | C | 1 |
| HCC 016 | PD-1/PD-L1 | 1st | Progressive Disease (PD) | HBV | 1359 | 72 | C | N.A. |
| HCC 017 | Combination PD-1/PD-L1 + CTLA-4 | 1st | Stable Disease (SD) | HBV | 2104 | 35 | C | 3 |
| HCC 018 | PD-1/PD-L1 | 2nd | Partial Response (PR) | None | 14450 | 68 | C | 1 |
| HCC 019 | PD-1/PD-L1 | 1st | Stable Disease (SD) | HBV | 5.8 | 72 | C | 2 |
| HCC 020 | PD-1/PD-L1 | 2nd | Progressive Disease (PD) | None | 4.1 | 76 | C | N.A. |
| HCC 021 | PD-1/PD-L1 | 1st | Progressive Disease (PD) | HBV | 11.2 | 63 | C | N.A. |
| HCC 022 | PD-1/PD-L1 | 1st | Stable Disease (SD) | HBV | 4373 | 78 | C | N.A. |
| HCC 023 | PD-1/PD-L1 | 2nd | Progressive Disease (PD) | None | 2309 | 56 | C | N.A. |
| HCC 024 | Combination PD-1/PD-L1 + CTLA-4 | 1st | Progressive Disease (PD) | HBV | 1.8 | 70 | C | N.A. |
| HCC 026 | PD-1/PD-L1 | 1st | Progressive Disease (PD) | None | 31 | 63 | C | 2 |
| HCC 027 | Combination PD-1/PD-L1 + others | 2nd | Stable Disease (SD) | None | 858 | 76 | B | 2 |
| HCC 028 | Combination PD-1/PD-L1 + CTLA-4 | 3rd | Progressive Disease (PD) | None | 764 | 55 | C | N.A. |
| HCC 029 | Combination PD-1/PD-L1 + CTLA-4 | 1st | Partial Response (PR) | HCV | 45.4 | 69 | C | 3 |
| HCC 030 | PD-1/PD-L1 | 2nd | Progressive Disease (PD) | None | 11 | 80 | C | 2 |
| HCC 032 | PD-1/PD-L1 | 2nd | Progressive Disease (PD) | HBV | 164 | 55 | C | 2 |
| HCC 033 | Combination PD-1/PD-L1 + CTLA-4 | 1st | Progressive Disease (PD) | HBV | 60500 | 68 | C | 3 |
| HCC 034 | Combination PD-1/PD-L1 + CTLA-4 | 2nd | Progressive Disease (PD) | HBV | 27.2 | 60 | C | 3 |
| HCC 035 | Combination PD-1/PD-L1 + CTLA-4 | 1st | Stable Disease (SD) | HBV | 1.7 | 45 | C | 2 |
| HCC 036 | PD-1/PD-L1 | 1st | Partial Response (PR) | None | 9.9 | 79 | C | 2 |
| HCC 037 | PD-1/PD-L1 | 1st | Progressive Disease (PD) | HBV | 121 | 58 | C | 2 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| HCC 038 | PD-1/PD-L1 | 1st | Partial Response (PR) | HBV | 70 | 54 | C | 3 |
| HCC 040 | Combination PD-1/PD-L1 + others | 1st | Partial Response (PR) | HBV | 69 | 72 | B | 2 |
| HCC 041 | Combination PD-1/PD-L1 + others | 1st | Stable Disease (SD) | HBV | 5.4 | 80 | C | 2 |
| HCC 042 | PD-1/PD-L1 | 2nd | Progressive Disease (PD) | HBV | 253 | 62 | B | |
| HCC 043 | Combination PD-1/PD-L1 + others | 2nd | progressive Disease (PD) | None | 4.7 | 64 | C | 2 |
| HCC 044 | Combination PD-1/PD-L1 + CTLA-4 | 2nd | Partial Response (PR) | HCV | 1.5 | 63 | C | N.A. |
| HCC 045 | Combination PD-1/PD-L1 + CTLA-4 | 2nd | Partial Response (PR) | HBV | 301 | 51 | C | N.A. |
| HCC 046 | PD-1/PD-L1 | 4th | Progressive Disease (PD) | HBV | 392 | 50 | C | N.A. |
| HCC 047 | PD-1/PD-L1 | 2nd | Stable Disease (SD) | None | 16.4 | 76 | C | N.A. |
| HCC 050 | Combination PD-1/PD-L1 + CTLA-4 | 1st | Progressive Disease (PD) | None | 4.1 | 78 | C | N.A. |
| HCC 053 | PD-1/PD-L1 | 1st | Progressive Disease (PD) | HBV | 60500 | 53 | C | 3 |
| HCC 055 | PD-1/PD-L1 | 1st | Progressive Disease (PD) | HCV | 60500 | 66 | C | 2 to 3 |
| HCC 057 | PD-1/PD-L1 | 1st | Progressive Disease (PD) | HBV | 3.3 | 77 | C | N.A. |
| HCC 060 | PD-1/PD-L1 | 1st | Progressive Disease (PD) | HBV | 73.8 | 56 | C | N.A. |

* Staged according to the BCLC staging system [1]. ICB, immune checkpoint blockade # Graded according to the 4-scale Edmondson and Steiner grading system [2]. HBV, hepatitis B virus; HCV, hepatitis C virus; AFP, alpha-fetoprotein; PD, progressive disease; PR, partial response; SD, stable disease; PD-1, programmed cell death protein-1; PD-L1, programmed death-ligand 1; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; TKI, Tyroxine Kinase Inhibitor; VEGF, Vascular endothelial growth factor; N.A, not applicable.

**Supplementary Table 2.** List of antibodies used for multiplex immunofluorescence and immunohistochemistry.

| **Antibody** | **Source** | **Labelling pattern** |
|---|---|---|
| DAPI | PerkinElmer, Inc. (FP1490) | Cell nucleus |
| CD38 | Novocastra (NCL-CD38-290) | Immune cells, cell membrane |
| CD68 | Dako (M0876) | Immune cells, cytoplasm |
| CD8 | Novocastra (NCL-CD8-4B11) | Immune cells, cell membrane |
| PD-L1 | Ventana Medical Systems (SP263) | Tumour and immune cells, cell membrane |

**Supplementary Table 3** List of antibodies used for flow cytometry.

| # | Antigen | Fluorochrome | Clone | Manufacturer | Catalogue number |
|---|---|---|---|---|---|
| 1 | CD16 | eFluor 450 | CB16 | Invitrogen; Thermo Fisher Scientific, Inc. | 48-0168-42 |
| 2 | CD14 | BV510 | M5E2 | BioLegend | 301842 |
| 3 | CD11c | BB515 | B-ly6 | BD Biosciences | 564490 |
| 4 | CD11b | PerCP-Cy5.5 | ICRF44 | BioLegend | 301328 |
| 5 | CD38 | PerCP-eFluor710 | HB7 | Invitrogen; Thermo Fisher Scientific, Inc. | 46-0388-42 |
| 6 | CD123 | Alexa 647 | 6H6 | BioLegend | 306024 |
| 7 | HLA-DR | APC-Fire 750 | L243 | BioLegend | 307658 |
| 8 | CD68 | PE-Cy7 | Y1/82A | Invitrogen; Thermo Fisher Scientific, Inc. | 25-0689-42 |

**Supplementary Table 4.** Ten significantly enriched biological pathways associated with differentially expressed genes between high- and low-CD38 hepatocellular carcinoma, as determined by ingenuity pathway analysis.

| **Pathway** | **Genes** | **-Log (P-value)** |
|---|---|---|
| Th1 and Th2 Activation Pathway | CCR1,CCR4,CCR8,CD247,CD274,CD28,CD3D,CD3E,CD3G,CD4,CD40LG,CD80,CD86,CD8A,CXCR3,CXCR4,CXCR6,HAVCR1,HAVCR2,HLA-DMA,HLA-DMB,HLA-DOA,HLA-DOB,HLA-DQA2,HLA-DRA,HLA-DRB1,HLA-DRB5,ICAM1,ICOS,IFNG,IKZF1,IL10,IL10RA,IL12A,IL12RB1,IL18,IL18R1,IL1RL1,IL24,IL2RA,IL2RB,IL2RG,IL6,ITGB2,JAK3,KLRC1,KLRD1,LTA,PIK3CD,PIK3CG,PIK3R5,PRKCQ,RUNX3,SOCS1,SPI1,STAT4,TIMD4,VAV1 | 2.64E+01 |
| Granulocyte Adhesion and Diapedesis | CCL11,CCL13,CCL17,CCL18,CCL19,CCL2,CCL20,CCL21,CCL22,CCL23,CCL24,CCL3,CCL3L1,CCL3L3,CCL4,CCL4L1/CCL4L2,CLDN11,CSF3R,CXCL1,CXCL10,CXCL11,CXCL12,CXCL14,CXCL3,CXCL5,CXCL6,CXCL8,CXCL9,CXCR4,FPR1,FPR3,HRH2,ICAM1,IL18,IL18RAP,IL1B,IL1RL1,ITGA4,ITGB2,MMP12,MMP16,MMP2,MMP23B,MMP25,MMP7,MMP9,SELE,SELL,SELP,SELPLG,TNF,TNFRSF11B,VCAM1,XCL1,XCL2 | 2.09E+01 |
| Th1 Pathway | CD247,CD274,CD28,CD3D,CD3E,CD3G,CD4,CD40LG,CD80,CD86,CD8A,CXCR3,HAVCR2,HLA-DMA,HLA-DMB,HLA-DOA,HLA-DOB,HLA-DQA2,HLA-DRA,HLA-DRB1,HLA-DRB5,ICAM1,ICOS,IFNG,IL10,IL10RA,IL12A,IL12RB1,IL18,IL18R1,IL6,ITGB2,JAK3,KLRC1,KLRD1,LTA,PIK3CD,PIK3CG,PIK3R5,PRKCQ,RUNX3,SOCS1,STAT4,VAV1 | 1.95E+01 |
| Th2 Pathway | CCR1,CCR4,CCR8,CD247,CD28,CD3D,CD3E,CD3G,CD4,CD80,CD86,CXCR4,CXCR6,HAVCR1,HLA-DMA,HLA-DMB,HLA-DOA,HLA-DOB,HLA-DQA2,HLA-DRA,HLA-DRB1,HLA-DRB5,ICAM1,ICOS,IFNG,IKZF1,IL10,IL12A,IL12RB1,IL1RL1,IL24,IL2RA,IL2RB,IL2RG,ITGB2,JAK3,PIK3CD,PIK3CG,PIK3R5,PRKCQ,RUNX3,SPI1,STAT4,TIMD4,VAV1 | 1.81E+01 |
| Agranulocyte Adhesion and Diapedesis | ACTC1,CCL11,CCL13,CCL17,CCL18,CCL19,CCL2,CCL20,CCL21,CCL22,CCL23,CCL24,CCL3,CCL3L1,CCL3L3,CCL4,CCL4L1/CCL4L2,CLDN11,CXCL1,CXCL10,CXCL11,CXCL12,CXCL14,CXCL3,CXCL5,CXCL6,CXCL8,CXCL9,CXCR4,ICAM1,IL18,IL1B,ITGA4,ITGB2,MMP12,MMP16,MMP2,MMP23B,MMP25,MMP7,MMP9,SELE,SELL,SELP,SELPLG,TNF,VCAM1,XCL1,XCL2 | 1.80E+01 |
| Communication between Innate and Adaptive Immune Cells | CCL3,CCL3L3,CCL4,CCR7,CD28,CD4,CD40LG,CD79A,CD80,CD86,CD8A,CXCL10,CXCL8,FCER1G,HLA-DRA,HLA-DRB1,HLA-DRB5,IFNG,IL10,IL12A,IL18,IL1B,IL6,TLR10,TLR2,TNF,TNFRSF13B,TNFRSF13C,TNFRSF17,TNFSF13B | 1.63E+01 |

| | | |
|---|---|---|
| Altered T Cell and B Cell Signaling in Rheumatoid Arthritis | CCL21,CD28,CD40LG,CD79A,CD80,CD86,FASLG,FCER1G,HLA-DMA,HLA-DMB,HLA-DOA,HLA-DOB,HLA-DRA,HLA-DRB1,HLA-DRB5,IFNG,IL10,IL12A,IL18,IL1B,IL6,LTA,LTB,SLAMF1,TLR10,TLR2,TNF,TNFRSF13B,TNFRSF13C,TNFRSF17,TNFSF13B | 1.61E+01 |
| T Helper Cell Differentiation | CD28,CD40LG,CD80,CD86,FCER1G,HLA-DMA,HLA-DMB,HLA-DOA,HLA-DOB,HLA-DRA,HLA-DRB1,HLA-DRB5,ICOS,IFNG,IL10,IL10RA,IL12A,IL12RB1,IL18,IL18R1,IL21R,IL2RA,IL2RG,IL6,STAT4,TNF,TNFRSF11B | 1.42E+01 |
| iCOS-iCOSL Signaling in T Helper Cells | CAMK4,CD247,CD28,CD3D,CD3E,CD3G,CD4,CD40LG,CD80,CD86,FCER1G,GRAP2,HLA-DMA,HLA-DMB,HLA-DOA,HLA-DOB,HLA-DRA,HLA-DRB1,HLA-DRB5,ICOS,IKBKE,IL2RA,IL2RB,IL2RG,INPP5D,ITK,LCK,LCP2,PIK3CD,PIK3CG,PIK3R5,PRKCQ,PTPRC,VAV1,ZAP70 | 1.42E+01 |
| Allograft Rejection Signaling | CD28,CD40LG,CD80,CD86,FASLG,FCER1G,GZMB,HLA-DMA,HLA-DMB,HLA-DOA,HLA-DOB,HLA-DQA2,HLA-DRA,HLA-DRB1,HLA-DRB5,IFNG,IL10,PRF1,TNF | 1.35E+01 |

**Supplementary Table 5**. Analysis of mPFS in patients with HCC treated with anti PD-1/PD-L1 single agent (n=30).

| Factor | mPFS (months) | OR (95% CI) | P-value |
|---|---|---|---|
| **Intratumoural total $CD38^+$ cell proportion** | | | |
| Low | 1.45 | Reference | |
| High | 11.56 | 0.397 (0.177, 0.892) | **0.0253*** |
| **Intratumoural $CD38^+CD68^+$ macrophage density** | | | |
| Low | 1.58 | | |
| High | 19.09 | 0.381 (0.157, 0.923) | **0.0325*** |
| **Intratumoural $CD38^-CD68^+$ macrophage density** | | | |
| Low | 1.58 | Reference | |
| High | 2.20 | 1.000 (0.467, 2.170) | 0.9998 |
| **Intratumoural $CD38^+CD68^-$ cells density** | | | |
| Low | 2.66 | Reference | |
| High | 1.61 | 1.025 (0.476, 2.206) | 0.9503 |
| **Intratumoural $CD8^+$ T cell density** | | | |
| Low | 2.68 | Reference | |
| High | 1.61 | 0.710 (0.295, 1.707) | 0.4435 |
| **PD-L1 tumour proportion score (TPS)** | | | |
| < 1 | 1.74 | Reference | |
| ≥ 1% | 1.68 | 0.952 (0.426, 2.129) | 0.9056 |

*P<0.05 indicated a statistically significant difference. #PD-L1, programmed death-ligand 1; mPFS, median progression free survival.

**Supplementary Table 6**. Analysis of mOS in patients with HCC treated with anti PD-1/PD-L1 single agent (n=30).

| Factor | mOS (months) | OR (95% CI) | P-value |
|---|---|---|---|
| **Intratumoural total $CD38^+$ cell proportion** | | | |
| Low | 6.87 | Reference | |
| High | 13.96 | 0.418 (0.175, 0.993) | **0.0483*** |
| **Intratumoural $CD38^+CD68^+$ macrophage density** | | | |
| Low | 6.87 | | |
| High | 34.43 | 0.374 (0.145, 0.966) | **0.0422*** |
| **Intratumoural $CD38^-CD68^+$ macrophage density** | | | |
| Low | 12.65 | Reference | |
| High | 8.41 | 1.336 (0.582, 3.066) | 0.4946 |
| **Intratumoural $CD38^+CD68^-$ cells density** | | | |
| Low | 6.87 | Reference | |
| High | 10.53 | 0.895 (0.391, 2.049) | 0.7926 |
| **Intratumoural $CD8^+$ T cell density** | | | |
| Low | 7.03 | Reference | |
| High | 12.65 | 0.769 (0.304, 1.946) | 0.5792 |
| **PD-L1 tumour proportion score (TPS)** | | | |
| < 1 | 13.96 | Reference | |
| ≥ 1% | 7.03 | 1.076 (0.454, 2.553) | 0.8672 |

*P<0.05 indicated a statistically significant difference. # PD-L1, programmed death-ligand 1; mOS, median overall survival.

**Supplementary Table 7**. Analysis of mPFS and mOS in patients with viral-related HCC (n=31) treated with anti PD-1/PD-L1 single agent.

| **Progression free Survival (PFS)** | | | |
|---|---|---|---|
| Factor | mPFS (months) | OR (95% CI) | P-value |
| Stage | | | |
| B (n=3) | 1.317 | Reference | |
| C (n=28) | 2.701 | 0.558 (0.163, 1.907) | 0.3518 |
| Age | | | |
| <65 (n= 16) | 1.318 | Reference | |
| ≥65 (n=15) | 3.887 | 0.776 (0.358, 1.678) | 0.5185 |
| AFP marker | | | |
| <400 (n=22) | 2.701 | Reference | |
| ≥400 (n=9) | 1.515 | 1.231 (0.530, 2.863) | 0.6289 |
| ECOG | | | |
| 0 (n=22) | 2.701 | Reference | |
| ≥ 1 (n=9) | 1.746 | 1.090 (0.454, 2.614) | 0.8475 |
| Child-Pugh score | | | |
| A5 (n = 17) | 5.271 | Reference | |
| A6 (n=12) | 1.515 | 1.233 (0.530, 2.870) | 0.6273 |
| A6, B7, and B8 (n=2) | 1.515 | 1.460 (0.658, 3.243) | 0.3521 |
| Macrovascular invasion | | | |
| Yes (n=9) | 2.701 | Reference | |
| No (n=22) | 1.746 | 1.689 (0.670, 4.256) | 0.2667 |
| Extra-hepatic spread | | | |
| Yes (n=23) | 5.271 | Reference | |
| No (n=7) | 1.285 | 4.019 (1.546, 10.444) | 0.0043 |
| **Intratumoural total $CD38^+$ cell proportion** | | | |
| Low (n=20) | 1.680 | Reference | |
| High (n=11) | 19.141 | 0.254 (0.097, 0.663) | **0.0051*** |
| **Intratumoural $CD38^+CD68^+$ macrophage density** | | | |
| Low (n=8) | 1.680 | Reference | |
| High (n=23) | 5.535 | 0.338 (0.137, 0.854) | **0.0217*** |
| **Overall Survival (OS)** | | | |

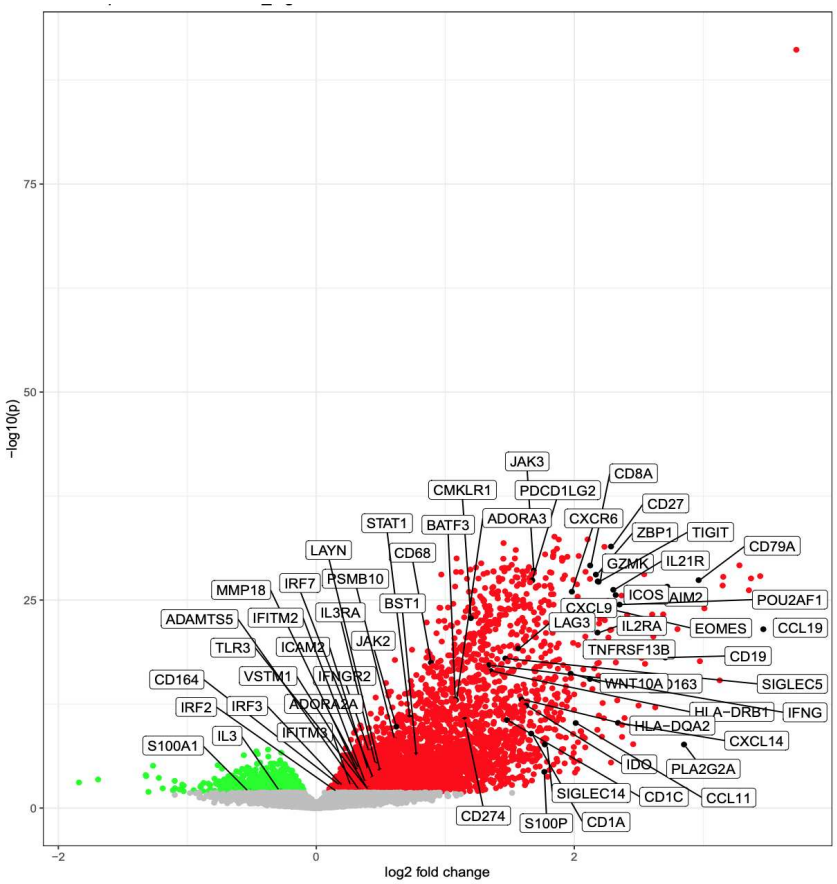
| Factor | mOS (months) | OR (95% CI) | P-value |
|---|---|---|---|
| Stage | | | |
| B (n=3) | 7.874 | Reference | |
| C (n=28) | 9.656 | 0.576 (0.167, 1.985) | 0.3823 |
| Age | | | |
| <65 (n= 16) | 7.050 | Reference | |
| ≥65 (n=15) | 14.232 | 0.949 (0.417, 2.162) | 0.9017 |
| AFP marker | | | |
| <400 (n=22) | 8.335 | Reference | |
| ≥400 (n=9) | 18.581 | 0.920 (0.375, 2.259) | 0.8563 |
| ECOG | | | |
| 0 (n=22) | 9.686 | Reference | |
| ≥ 1 (n=9) | 6.885 | 1.403 (0.567, 3.471) | 0.4643 |
| Child-Pugh score | | | |
| A5 (n = 17) | 15.879 | Reference | |
| A6 (n=12) | 5.106 | 1.448 (0.576, 3.639) | 0.4313 |
| A6, B7, and B8 (n=14) | 5.139 | 1.715 (0.724, 4.065) | 0.2204 |
| Macrovascular invasion | | | |
| Yes (n=9) | 9.686 | Reference | |
| No (n=22) | 8.434 | 1.147 (0.448, 2.935) | 0.7747 |
| Extra-hepatic spread | | | |
| Yes (n=23) | 15.879 | Reference | |
| No (n=7) | 5.139 | 3.799 (1.425, 10.126) | 0.0076 |
| **Intratumoural total $CD38^+$ cell proportion** | | | |
| Low (n=20) | 7.050 | Reference | |
| High (n=11) | 34.525 | 0.334 (0.121, 0.919) | **0.0337*** |
| **Intratumoural $CD38^+CD68^+$ macrophage density** | | | |
| Low (n=8) | 8.433 | Reference | |
| High (n=23) | 15.879 | 0.492 (0.198, 1.222) | 0.1264 |

**Supplementary Table 8**. Analysis of mPFS and mOS in patients with non-viral related HCC (n=18) treated with anti PD-1/PD-L1 single agent.

| **Progression free Survival (PFS)** | | | |
|---|---|---|---|
| Factor | mPFS (months) | OR (95% CI) | P-value |
| **Stage** | | | |
| B (n=2) | 1.581 | Reference | |
| C (n=16) | 1.680 | 1.082 (0.239, 4.893) | 0.9186 |
| **Age** | | | |
| <65 (n= 4) | 1.614 | Reference | |
| ≥65 (n=14) | 1.680 | 0.602 (0.180, 2.008) | 0.4086 |
| **AFP marker** | | | |
| <400 (n=11) | 1.614 | Reference | |
| ≥400 (n=7) | 3.624 | 0.579 (0.207, 1.616) | 0.2963 |
| **ECOG** | | | |
| 0 (n=13) | 1.680 | Reference | |
| **≥ 1** (n=5) | 1.581 | 1.282 (0.436, 3.774) | 0.6517 |
| **Child-Pugh score** | | | |
| A5 (n = 10) | 1.614 | Reference | |
| A6 (n=7) | 2.998 | 0.864 (0.306, 2.440) | 0.7821 |
| A6, B7, and B8 (n=8) | 2.998 | 0.974 (0.361, 2.627) | 0.9587 |
| **Macrovascular invasion** | | | |
| Yes (n=5) | 4.052 | Reference | |
| No (n=13) | 1.614 | 2.302 (0.728, 7.274) | 0.1556 |
| **Extra-hepatic spread** | | | |
| Yes (n=13) | 1.680 | Reference | |
| No (n=5) | 1.680 | 0.637 (0.200, 2.031) | 0.4457 |
| **Intratumoural total $CD38^+$ cell proportion** | | | |
| Low (n=8) | 2.998 | Reference | |
| High (n=10) | 1.680 | 0.727 (0.254, 2.080) | 0.5528 |
| **Intratumoural $CD38^+CD68^+$ macrophage density** | | | |
| Low (n=4) | 1.548 | Reference | |
| High (n=14) | 3.624 | 0.341 (0.099, 1.182) | 0.0898 |

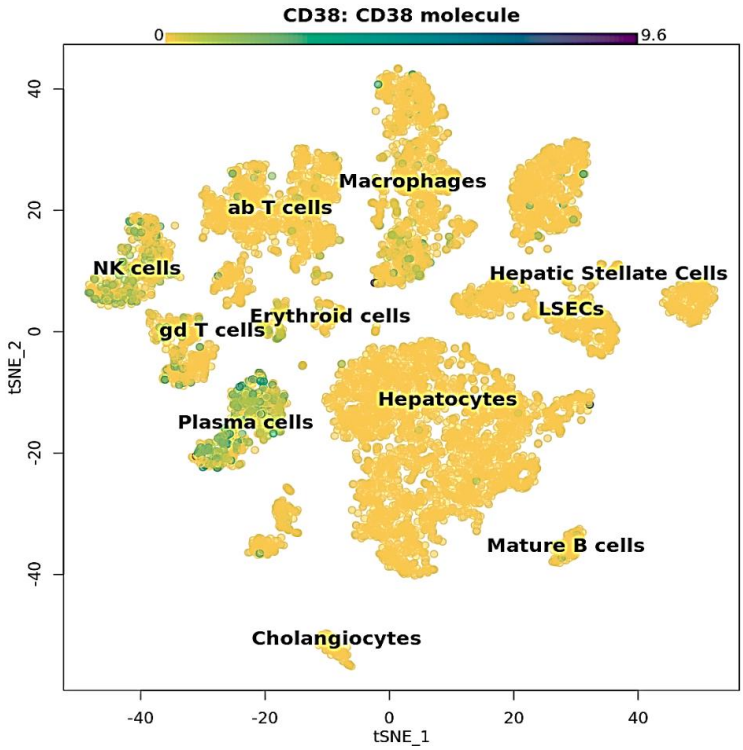
| **Overall Survival (OS)** | | | |
|---|---|---|---|
| Factor | mOS (months) | OR (95% CI) | **P-value** |
| **Stage** | | | |
| B (n=2) | 22.633 | Reference | |
| C (n=16) | 12.684 | 3.154 (0.397, 25.080) | 0.2776 |
| **Age** | | | |
| <65 (n= 4) | 9.620 | Reference | |
| ≥65 (n=14) | 14.001 | 0.621 (0.164, 2.362) | 0.4849 |
| **AFP marker** | | | |
| <400 (n=11) | 14.001 | Reference | |
| ≥400 (n=7) | 18.976 | 0.865 (0.268, 2.788) | 0.8077 |
| **ECOG** | | | |
| 0 (n=13) | 14.001 | Reference | |
| **≥ 1** (n=5) | Not reached | 0.563 (0.121, 2.606) | 0.4620 |
| **Child-Pugh score** | | | |
| A5 (n = 10) | 14.001 | Reference | |
| A6 (n=7) | 12.684 | 1.134 (0.329, 3.910) | 0.8425 |
| A6, B7, and B8 (n=8) | 12.684 | 1.366 (0.429, 4.353) | 0.5977 |
| **Macrovascular invasion** | | | |
| Yes (n=5) | 22.633 | Reference | |
| No (n=13) | 14.001 | 1.688 (0.443, 6.429) | 0.4432 |
| **Extra-hepatic spread** | | | |
| Yes (n=13) | 9.620 | Reference | |
| No (n=5) | 22.633 | 0.489 (0.127, 1.894) | 0.3010 |
| **Intratumoural total $CD38^+$ cell proportion** | | | |
| Low (n=8) | 14.528 | Reference | |
| High (n=10) | 12.684 | 0.676 (0.204, 2.246) | 0.5229 |
| **Intratumoural $CD38^+CD68^+$ macrophage density** | | | |
| Low (n=4) | 2.504 | Reference | |
| High (n=14) | 14.528 | 0.342 (0.085, 1.385) | 0.1327 |

**Supplementary Figure 1** High resolution volcano plot of the genes that are differentially expressed between high and low CD38 HCC (as seen in Fig. 1C)


−log10(p)
75
50
25
0
−2
0
2
log2 fold change
JAK3
CD8A
CMKLR1
PDCD1LG2
CD27
STAT1
BATF3
ADORA3
CXCR6
ZBP1
TIGIT
LAYN
CD68
GZMK
IL21R
CD79A
MMP18
IRF7
PSMB10
ICOS
AIM2
POU2AF1
BST1
CXCL9
ADAMTS5
IFITM2
IL3RA
LAG3
IL2RA
EOMES
CCL19
TLR3
ICAM2
JAK2
TNFRSF13B
CD19
CD164
VSTM1
IFNGR2
WNT10A
SIGLEC5
IRF2
IRF3
ADORA2A
HLA-DRB1
IFNG
IL3
IFITM3
HLA-DQA2
S100A1
CXCL14
IDO
PLA2G2A
SIGLEC14
CD1C
CCL11
CD274
S100P
CD1A

**Supplementary Figure 2** Single cell CD38 gene expression levels in the human liver.

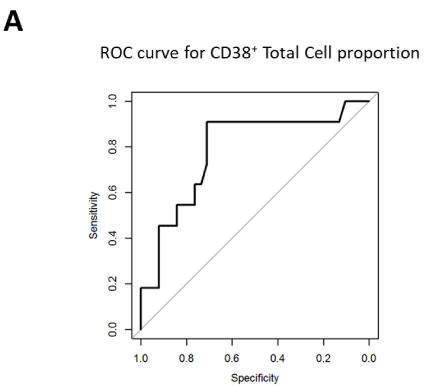
tSNE dimension reduction plot showing the expression of CD38 in the various identified cell populations. Low expression is shown in yellow, while high expression is shown in purple.



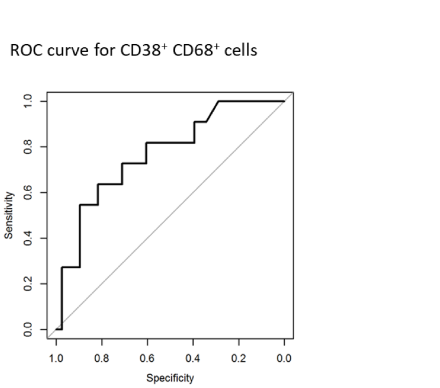
**Supplementary Figure 3** Receiver operating characteristic curves used to predict responders.

(A) Receiver operating characteristic curve for $CD38^+$ immune cells (AUC=0.785). (B) Receiver operating characteristic curve for $CD38^+$ $CD68^+$ macrophages (AUC=0.768).

*Sensitivity refers to the proportion of true positive subjects with the disease among subjects with disease. Specificity refers to the proportion of true negative subjects without the disease among subjects without disease. PPV refers to the proportion of patients with positive results among subjects with positive results. NPV refers to the proportion of subjects without disease with a negative result among subjects with negative results. Accuracy refers to the proportion of subjects correctly classified among all subjects. AUC, area under the curve; PPV, positive predictive value, NPV, negative predictive value. PFS, progression free survival.*




| Threshold | Specificity | Sensitivity | Accuracy | NPV | PPV |
|---|---|---|---|---|---|
| 5.0% | 71.1% | 90.9% | 75.5% | 96.4% | 47.6% |




| Threshold | Specificity | Sensitivity | Accuracy | NPV | PPV |
|---|---|---|---|---|---|
| 1 | 71.1% | 72.7% | 71.4% | 90.0% | 42.1% |

**Supplementary Figure 4** Response to anti-PD-1/PD-L1 single agent therapy in patients with hepatocellular carcinoma, in relation to intratumoural total $CD38^-$ $CD68^+$ macrophage density and $CD38^+$ $CD68^-$ cells density.
(A) The total $CD38^+$ cell proportion within the tumours of responders and non-responders treated with anti-PD-1/PD-L1 single agent. (B) Kaplan Meier curve showing the association between a high total $CD38^+$ cell proportion and improved PFS after treatment with anti-PD-1/PD-L1 single agent. (C) Kaplan Meier curve showing the association between a high total $CD38^+$ cell proportion and improved OS after treatment with anti-PD-1/PD-L1 single agent. (D) The $CD38^+$ $CD68^+$ macrophage density of responders and non-responders treated with anti-PD-1/PD-L1 single agent. (E)Kaplan Meier curve showing the association between high $CD38^-$ $CD68^+$ macrophage density and improved PFS after treatment with anti-PD-1/PD-L1 single agent. (F) Kaplan Meier curve showing the association between high $CD38^-$ $CD68^+$ macrophage density and improved OS after treatment with anti-PD-1/PD-L1 single agent. (G)Kaplan Meier curve showing the association between high $CD38^+$ $CD68^-$ cells density and improved PFS after treatment with anti-PD-1/PD-L1 single agent. (H) Kaplan Meier curve showing the association between high $CD38^+$ $CD68^-$ cells density and improved OS after treatment with anti-PD-1/PD-L1 single agent.

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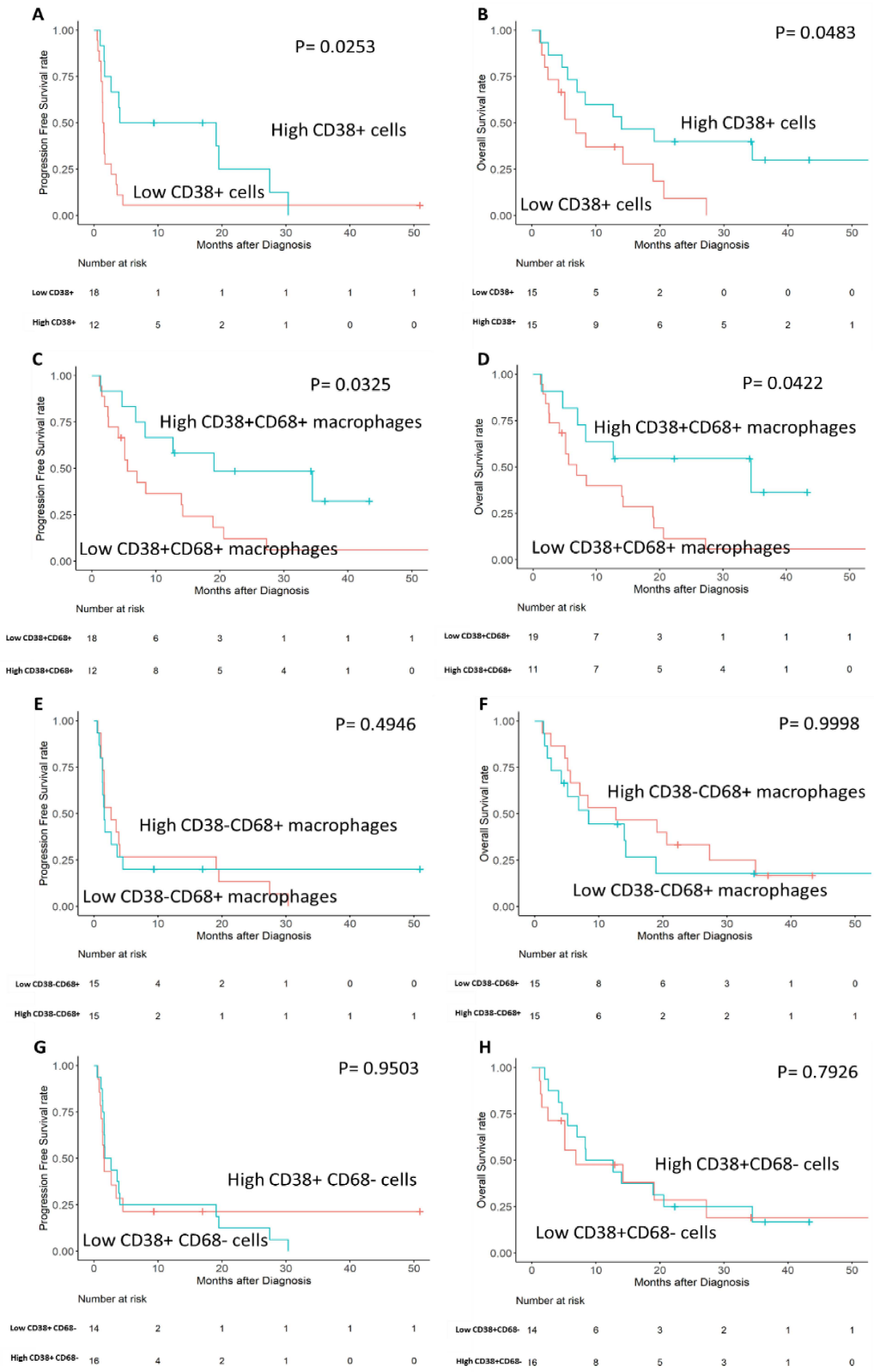
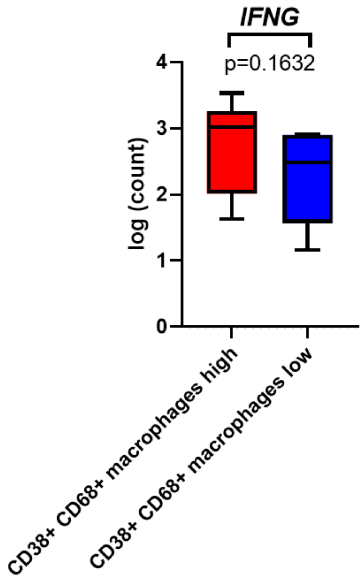
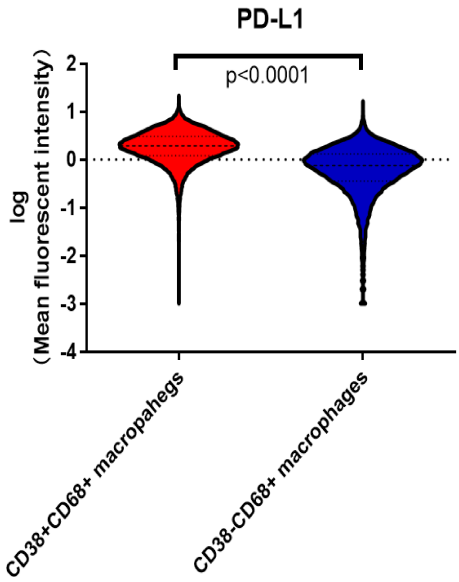

A
P= 0.0253
High CD38+ cells
Low CD38+ cells
Progression Free Survival rate
Months after Diagnosis
Number at risk
Low CD38+ 18 1 1 1 1 1
High CD38+ 12 5 2 1 0 0
B
P= 0.0483
High CD38+ cells
Low CD38+ cells
Overall Survival rate
Months after Diagnosis
Number at risk
Low CD38+ 15 5 2 0 0 0
High CD38+ 15 9 6 5 2 1
C
P= 0.0325
High CD38+CD68+ macrophages
Low CD38+CD68+ macrophages
Progression Free Survival rate
Months after Diagnosis
Number at risk
Low CD38+CD68+ 18 6 3 1 1 1
High CD38+CD68+ 12 8 5 4 1 0
D
P= 0.0422
High CD38+CD68+ macrophages
Low CD38+CD68+ macrophages
Overall Survival rate
Months after Diagnosis
Number at risk
Low CD38+CD68+ 19 7 3 1 1 1
High CD38+CD68+ 11 7 5 4 1 0
E
P= 0.4946
High CD38-CD68+ macrophages
Low CD38-CD68+ macrophages
Progression Free Survival rate
Months after Diagnosis
Number at risk
Low CD38-CD68+ 15 4 2 1 0 0
High CD38-CD68+ 15 2 1 1 1 1
F
P= 0.9998
High CD38-CD68+ macrophages
Low CD38-CD68+ macrophages
Overall Survival rate
Months after Diagnosis
Number at risk
Low CD38-CD68+ 15 8 6 3 1 0
High CD38-CD68+ 15 6 2 2 1 1
G
P= 0.9503
High CD38+ CD68- cells
Low CD38+ CD68- cells
Progression Free Survival rate
Months after Diagnosis
Number at risk
Low CD38+ CD68- 14 2 1 1 1 1
High CD38+ CD68- 16 4 2 1 0 0
H
P= 0.7926
High CD38+CD68- cells
Low CD38+CD68- cells
Overall Survival rate
Months after Diagnosis
Number at risk
Low CD38+CD68- 14 6 3 2 1 1
High CD38+CD68- 16 8 5 3 1 0

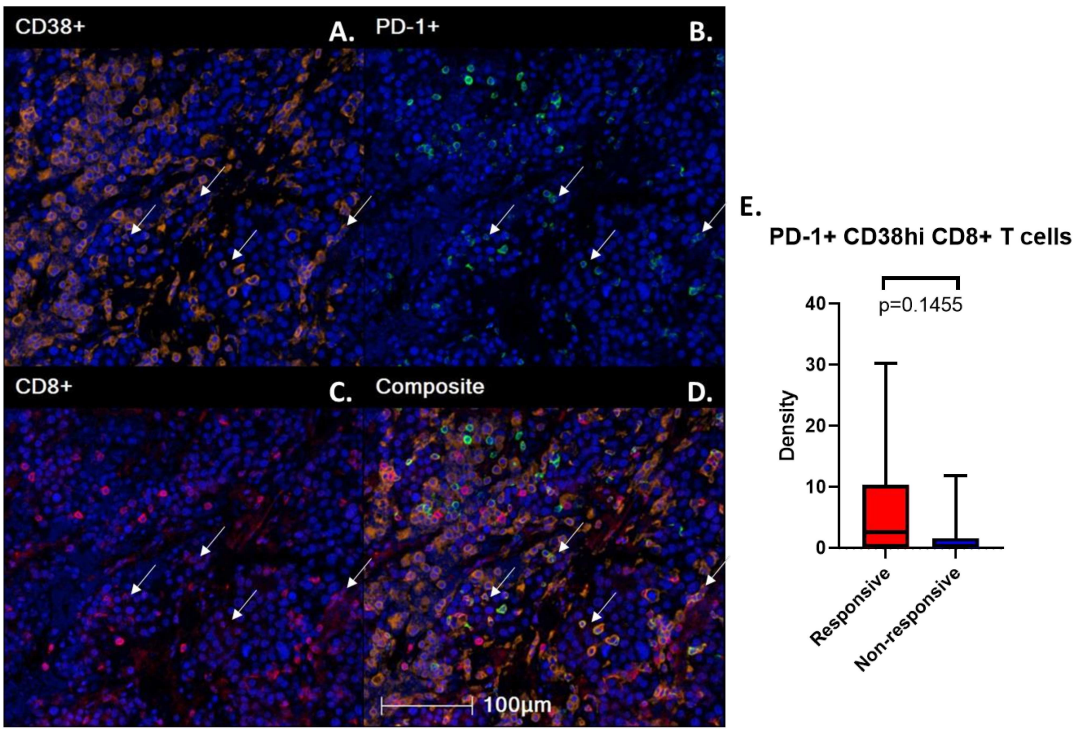
**Supplementary Figure 5.** IFNG gene level showed a trend higher in HCC patients that harboured higher $CD38^+$ $CD68^+$ macrophages



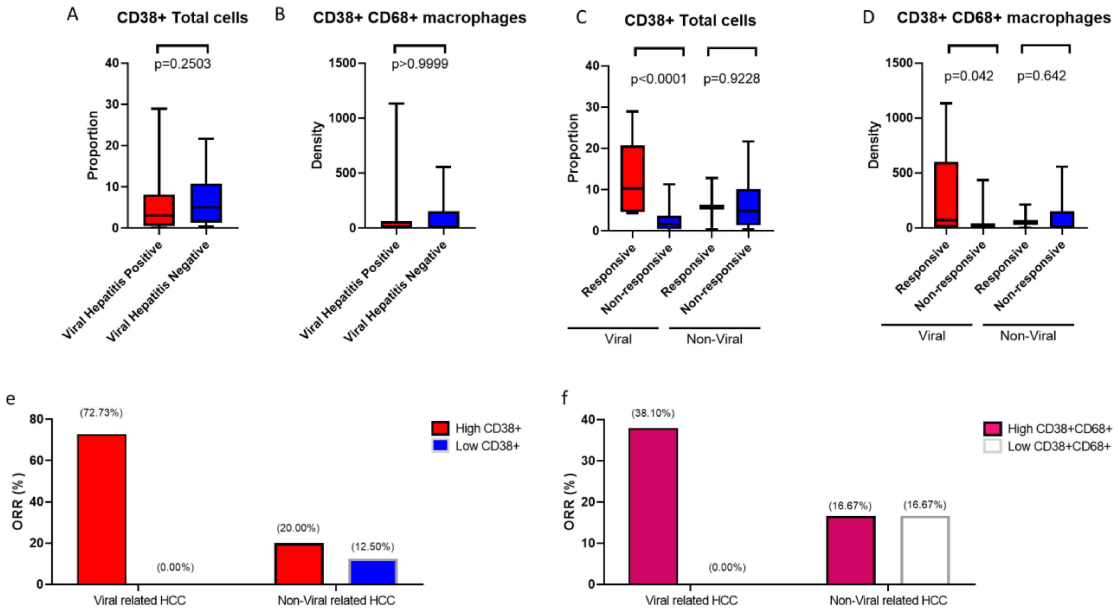
**Supplementary Figure 6.** The mean fluorescent intensity of PD-L1 on $CD38^{+}CD68^{+}$ macrophages are significantly higher than the $CD38^{-}CD68^{+}$ macrophages in HCC



**Supplementary Figure 7.** (A-D) mIHC/IF revealed that CD38 (orange), PD-1 (green) and the T- cell lymphocyte marker CD8 (red) were expressed in the HCC TME. CD8 (red) is co-localised with PD-1 (green) and CD38 (orange) in the HCC TME. Cell nuclei are counterstained with DAPI for mIHC/IF (blue). Images are shown at a magnification of 400X for A, B, C, and D. (E) There is no statistical significance (p=0.1455) of the densitiy of $CD38^{+}PD\text{-}1^{+}CD8^{+}$ cells between responsive and non-responsive patients.
*HCC, hepatocellular carcinoma; TME, tumour microenvironment, IHC, immunohistochemistry; mIHC/IF, multiplex immunohistochemistry/immunofluorescence.*



**Supplementary Figure 8.** Abundance of (A) $CD38^+$ cells and (B) $CD38^+CD68^+$ macrophages between patients with or without viral hepatitis in our cohort (31 vs 18). The proportion of (C) $CD38^+$ cells is associated with responsiveness to ICB in the viral-related HCC but not in the non-viral related HCC. (D) The density of $CD38^+CD68^+$ macrophages is associated with responsiveness to ICB in the viral-related HCC but not in the non-viral related HCC. (E-F) Overall response rates of each biomarker in the viral-related HCC and the non-viral related HCC, respectively.



**Supplementary Figure 9.** (A) Kaplan Meier curve showing the association between a high total $CD38^+$ cell proportion and improved PFS after treatment with ICB in viral-related HCC. (B) Kaplan Meier curve showing the association between a high total $CD38^+$ cell proportion and improved OS after treatment with ICB in viral-related HCC. (C) Kaplan Meier curve showing the association between high $CD38^+$ $CD68^+$ macrophage density and improved PFS after treatment with ICB in viral-related HCC. (D) Kaplan Meier curve showing the association between high $CD38^+$ $CD68^+$ macrophage density and improved OS after treatment with ICB in viral-related HCC. (E) Kaplan Meier curve showing the association between a high total $CD38^+$ cell proportion and improved PFS after treatment with ICB in non-viral-related HCC. (F) Kaplan Meier curve showing the association between a high total $CD38^+$ cell proportion and improved OS after treatment with ICB in non-viral-related HCC. (G) Kaplan Meier curve showing the association between high $CD38^+$ $CD68^+$ macrophage density and improved PFS after treatment with ICB in non-viral-related HCC. (H) Kaplan Meier curve showing the association between high $CD38^+$ $CD68^+$ macrophage density and improved OS after treatment with ICB in non-viral-related HCC. ICB, immune checkpoint blockade.

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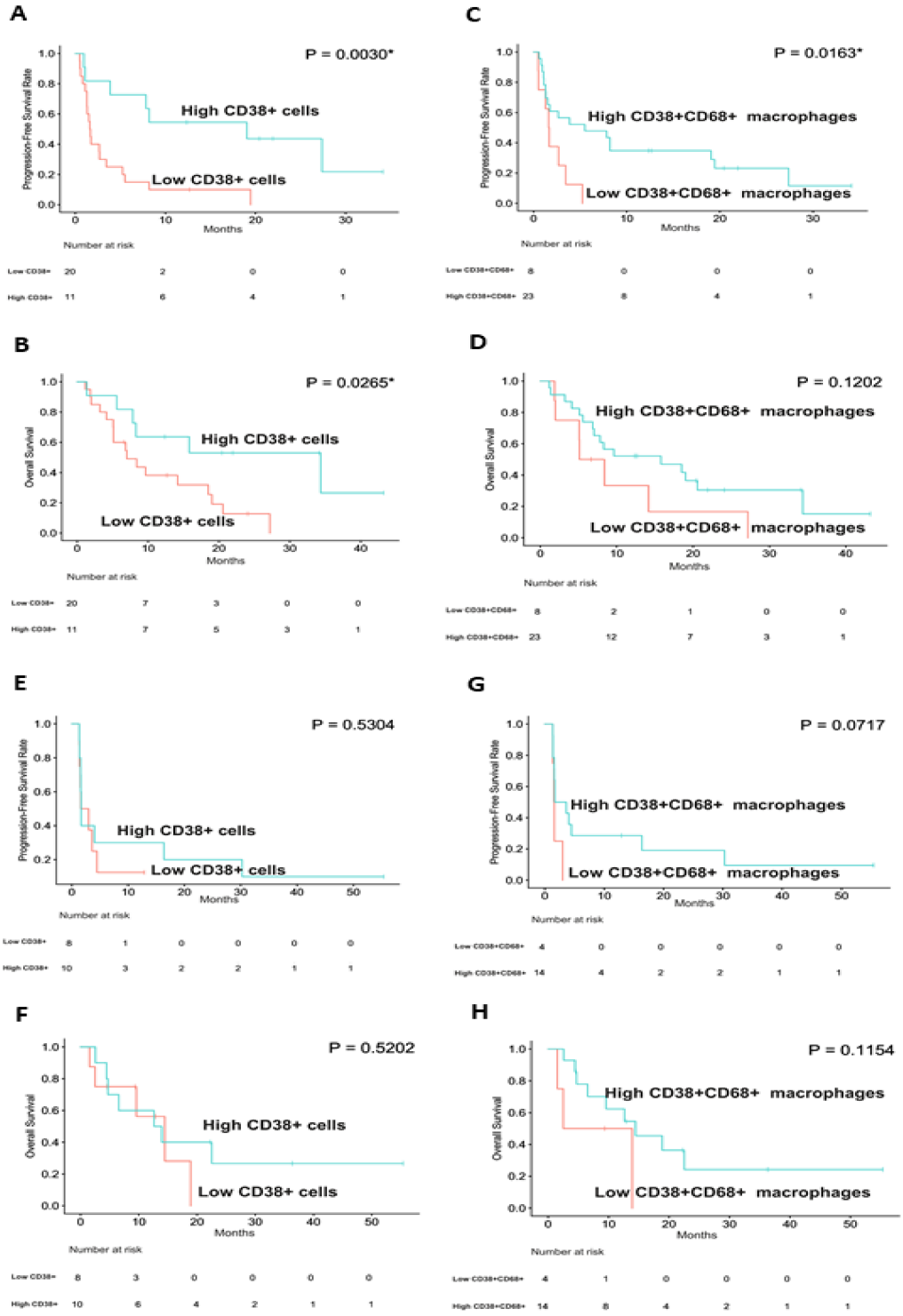
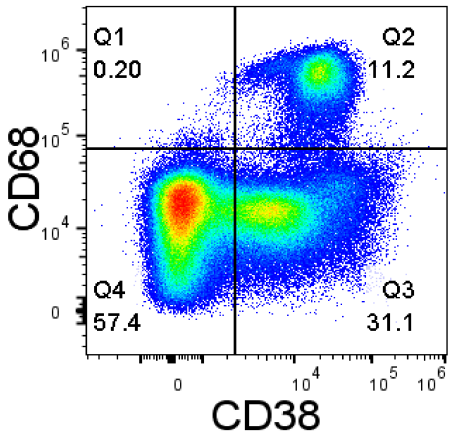
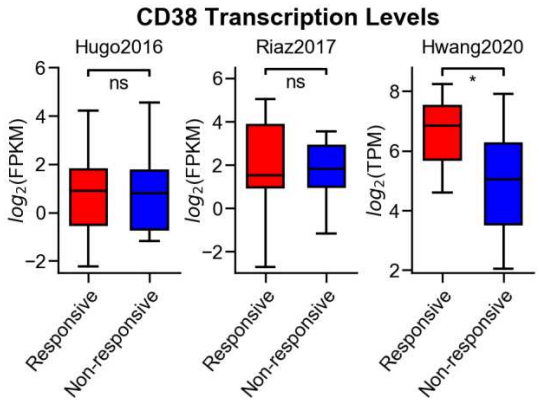

A
P = 0.0030*
High CD38+ cells
Low CD38+ cells
Progression-Free Survival Rate
Months
Number at risk
Low CD38+ 20 2 0 0
High CD38+ 11 6 4 1
C
P = 0.0163*
High CD38+CD68+ macrophages
Low CD38+CD68+ macrophages
Progression-Free Survival Rate
Months
Number at risk
Low CD38+CD68+ 8 0 0 0
High CD38+CD68+ 23 8 4 1
B
P = 0.0265*
High CD38+ cells
Low CD38+ cells
Overall Survival
Months
Number at risk
Low CD38+ 20 7 3 0 0
High CD38+ 11 7 5 3 1
D
P = 0.1202
High CD38+CD68+ macrophages
Low CD38+CD68+ macrophages
Overall Survival
Months
Number at risk
Low CD38+CD68+ 8 2 1 0 0
High CD38+CD68+ 23 12 7 3 1
E
P = 0.5304
High CD38+ cells
Low CD38+ cells
Progression-Free Survival Rate
Months
Number at risk
Low CD38+ 8 1 0 0 0 0
High CD38+ 10 3 2 2 1 1
G
P = 0.0717
High CD38+CD68+ macrophages
Low CD38+CD68+ macrophages
Progression-Free Survival Rate
Months
Number at risk
Low CD38+CD68+ 4 0 0 0 0 0
High CD38+CD68+ 14 4 2 2 1 1
F
P = 0.5202
High CD38+ cells
Low CD38+ cells
Overall Survival
Months
Number at risk
Low CD38+ 8 3 0 0 0 0
High CD38+ 10 6 4 2 1 1
H
P = 0.1154
High CD38+CD68+ macrophages
Low CD38+CD68+ macrophages
Overall Survival
Months
Number at risk
Low CD38+CD68+ 4 1 0 0 0 0
High CD38+CD68+ 14 8 4 2 1 1

**Supplementary Figure 10.** Co-expression of CD38 and CD68 observed in HCC PBMC. The representative pseudocolour plot was gated from the total single live cell population. HCC, hepatocellular carcinoma.



**Supplementary Figure 11.** CD38 transcription levels in pre-anti-PD-1 therapy melanoma samples as measured by RNA-sequencing. In two melanoma cohorts (Hugo2016 and Riaz2017[3,4]) and one NSCLS cohort (Hwang2020[5]), patients were classified as being responsive (R) or non-responsive (NR) to anti-PD-1 therapy (Hugo2016: R n=15, NR n=13, p-value=0.925; Riaz2017: R n=26, NR n=23, p-value=0.226; Hwang2020: R n=9, NR n=12, p-value=0.032). Transcription levels of CD38 were retrieved based on publicly available RNA-seq data from these studies. Two RNA-seq datasets from Hugo2016 were from different sites in the same patient. One RNA-seq dataset from Riaz2017 was dropped as the CD38 transcription level was not recorded. p-value was calculated by a two-tailed t-test.



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5. Hwang S, Kwon A-Y, Jeong J-Y, et al: Immune gene signatures for predicting durable clinical benefit of anti-PD-1 immunotherapy in patients with non-small cell lung cancer. Scientific Reports 10:643, 2020