Supplementary Materials and methods

Isolation of mononuclear cells from peripheral blood and tissues

Peripheral leukocytes were isolated by Ficoll density gradient centrifugation. Thereafter, the mononuclear cells were washed and resuspended in RPMI 1640 supplemented with 10% FBS. Monocytes/macrophages and T cells were purified from the leukocytes using a MACS column purification system (Miltenyi Biotec). Human $CD8^+$ T cells, PD-$L1^+$ and PD-$L1^-$ monocytes/macrophages, HLA-DR^{high} and HLA-DR^{low} monocytes/macrophages, as well as mouse F4/80^+ macrophages and $CD8^+$ T cells were further sorted by FACS (MoFlo, Beckman Coulter). These cells were used in subsequent experiments.

Animal experiments

All mice were maintained under specific pathogen-free conditions in the animal facilities of the First Affiliated Hospital of Nanjing Medical. All mice were randomly grouped. Animal experiments were performed with the approval of the Institutional Animal Care and Use Committee of the First Affiliated Hospital of Nanjing Medical. 1×10^6 mouse hepatoma Hepa1-6 cells in 25 μl of Matrigel (Corning) were injected under the hepatic capsule of 5–7-week-old female C57/BL6 mice. In some experiments, Hepa1-6 cells were pre-transfected with shNC or sh*Fn1* by lentiviral vectors delivery system. The detailed procedures of the animal experiments are shown in figure 1J, figure 4C, and figure 7L.

Immunohistochemistry and immunofluorescence

Paraffin-embedded and formalin-fixed samples were cut into 5-μm sections, followed by procedures for immunohistochemistry. After incubation with primary antibody against human CD68, PD-L1, HLA-DR, and PKM2, sections were stained with corresponding secondary antibodies and visualized in an Envision System. For immunofluorescence analysis of patient samples, frozen sections were initially incubated with mouse anti-human CD68 and rabbit anti-human PD-L1; or mouse anti-human CD68, rabbit anti-human PKM2 and rabbit anti-human PD-L1. Thereafter, sections were stained with Alexa Fluor 488-conjugated donkey anti-rabbit IgG and Alexa Fluor 555-conjugated donkey anti-mouse IgG (Invitrogen); or Alexa Fluor 488-conjugated donkey anti-mouse IgG, Alexa Fluor 555-conjugated donkey anti-rabbit IgG, and Cyanine 5-conjugated streptavidin detect biotinylated corresponding secondary antibodies anti-

rabbit IgG. Nuclei were counterstained with DAPI. Immunofluorescence staining images were visualized by confocal microscopy (LSM 880, AxioObserver; Carl Zeiss, Oberkochen, Germany).

Evaluation of immunohistochemical variables

Analysis was performed by two independent observers who were blinded to the clinical outcomes. At low-power field (× 100), the tissue sections were screened, and the 5 most representative fields were selected using a Leica DM IRB inverted research microscope (Leica, Wetzlar, Germany). For evaluating the density of tissue-infiltrating $CD68^{+}PKM2^{+}$ cells, the respective areas of HCC tissues were then scanned at × 400 magnification (0.146 mm^2 per field). The number of nucleated $PKM2^{high}$ cells in each area was then counted manually and expressed as cells per field. The $PKM2^{high}$ cells that were negative for CD68 were excluded from counting. Positively stained cells that are smaller than the size of circulating monocytes/macrophages (15 µm) were excluded from counting. There was a significant linear correlation between the counting data of two independent observers ($P= 2.77 \times 10^{-24}$), and the average of counts by 2 investigators was applied in the following analysis to minimize interobserver variability.

Flow cytometry (FACS)

Monocytes/macrophages and T cells from peripheral blood, tissues, or ex vivo culture were stained with fluorochrome-conjugated antibodies and then analyzed by FACS. In some cases, T cells from ex vivo culture system were stimulated with Leukocyte Activation Cocktail (BD Bioscience) at 37°C for 5 hours. Thereafter, the cells were stained with surface markers, fixed and permeabilized with IntraPrep reagent (Beckman Coulter), and finally stained with intracellular markers. Data were acquired using a Gallios flow cytometer (Beckman Coulter).

Real-time polymerase chain reaction (PCR)

Trizol reagent (Invitrogen) was used to isolate total RNA of cells or tissue. Aliquots (2 µg) of the RNA were reverse-transcribed using MMLV reverse transcriptase. The PCR was performed in triplicate using Hieff qPCR SYBR Green Master Mix in a Roche LightCycler 480 System.

Immunoblotting

Monocytes/macrophages from in vitro culture system were washed three times with PBS and

the pellets were resuspended in lysis buffer for 20 minutes on ice. After centrifugation at 10,000 g for 10 minutes, the supernatants were dissolved in Laemmli sample buffer and heated at 95 °C for 5 minutes. Equal amount of cellular proteins was separated on 10% SDS–polyacrylamide gel electrophoresis and electrotransfered to nitrocellulose membranes. The membranes were blocked with 3% bovine serum albumin, and the presence of indicated protein on the blots was detected with specific antibodies and commercial ECL kit.

Glucose uptake assay

Purified monocytes/macrophages were starved of glucose by incubation for 1 hour in PBS and then stained with 2-NBDG for 30 minutes at 37 °C and subjected to FACS analysis.

Extracellular acidification (ECAR) analyses

Measurement of the ECAR of monocytes/macrophages was done using an XF-24 Extracellular Flux Analyzer (Seahorse Bioscience). HLA-DR^{high} and HLA-DR^{low} monocytes/macrophages isolated from tissue or macrophages cultured in vitro were suspended in XF Base Medium Minimal DMEM (pH 7.4) with L-Glutamine (2 mM) and then placed on a cell culture microplate (2×10^5 cells/well; XF-24, Seahorse Bioscience). Glucose (10 mM), oligomycin A (1 μM), and 2-DG (50 mM) were added to the cells during performing real-time measurement of the ECAR.

Lactate assay

The supernatants of monocytes/macrophages were collected from ex vivo or in vitro culture systems. Concentrations of the lactate in the supernatants were detected using L-lactate assay kit according to the manufacturers' instructions (Eton bioscience).

In vitro and ex vivo culture of monocytes/macrophages

Monocytes/macrophages, PD-L1$^+$ or HLA-DR^{high} monocytes/macrophages isolated from HCC tissues were left untreated or treated with 2-DG (25 mM), oligomycin (Oym, 0.5 μM), etomoxir (ETO, 50 μM), or with neutralizing antibodies against human TNF-α (10 μg/mL), IL-1β (10 μg/mL), IL-6 (40 μg/mL), IL-12 (10 μg/mL). In some experiments, purified monocytes from human blood were untreated or treated with human recombinant TNF-α (20 ng/ml), IL-1β (10 ng/ml), IL-6 (40 ng/ml), IL-12 (10 ng/ml), or with 30% primary liver-SN or primary HCC-SN,

or FN1 (20 μg/ml) in the presence or absence of 2-DG, 3-PO (30 nM), 3-BP (20 nM), ML-265 (50 μM), α-KG (20 μg/mL), WP1066 (1 ug/ml), or antibody against TLR-4 (20 μg/ml). In other experiments, monocytes were pre-transfected with 300 nM negative control siRNA, or PKM2-specific siRNA (sense: 5'-CCU GUA UGU CAA UAA ACA ACA-3'; antisense: 5'-UUG UUU AUU GAC AUA CAG GUA-3') using P3 primary cell 4D-Nucleofector X kit (V4XP-3024, Lonza) before exposure to HCC-SN. All siRNA duplexes were purchased from GenePharma.

Ex vivo T cell culture system

$CD8^+$ T cells, PD-$L1^-$ and PD-$L1^+$ monocytes/macrophages were isolated from human HCC tumors by FACS-sorting. PD-$L1^+$ monocytes/macrophages were pretreated with glycolysis inhibitor 2-DG for 24 hours. Thereafter, 1×10^5 T cells were left untreated or were cultured with autologous PD-$L1^-$ or PD-$L1^+$ monocytes/macrophages (5:1) in the presence or absence of a specific blocking antibody against PD-L1 (20 μg/mL) for 20 hours. Meanwhile, 25 μg/ml of tumor mass lysates were added to trigger tumor specific T cell response. In some cases, T cells and PD-$L1^+$ monocytes/macrophages were cocultured with specific blocking antibodies against IL-12 (10 μg/mL) or HLA-DR (20 μg/mL) in the presence of PD-L1 blocking antibody.

Enzyme-linked immunospot assay (ELISpot)

ELISpot assays were performed using commercial kits (BD Bioscience) according to the manufacturer's instructions. In brief, 96-well nitrocellulose plates (Millipore) were coated with 5 μg/ml anti–human IFN-γ capture antibody at 4°C overnight. The wells were then washed and blocked for 2 hours at room temperature with 10% FBS-RPMI 1640 medium. 1×10^5 purified $CD8^+$ T cells from human HCC tissues were left untreated or were cultured with autologous monocytes/macrophages for 20 hours in the plate. After wash, the plates were incubated with 2 μg/ml of biotinylated anti–human IFN-γ detection antibody and developed with streptavidin–horseradish peroxidase, followed by the addition of 3-amino-9-ethylcarbazole substrate reagent. In some experiments, Mock or PD-L1 HEK293T transfectants were primary pretreated with 10 μg/mL mitomycin C for 20 minutes to abolish the growth or cytokine production activity of cells. Thereafter, purified $CD8^+$ T cells were left untreated or cultured for 24 hours with indicated mitomycin C–treated cells in the presence or absence of 10 ng/mL human recombinant IL-12 antibody (eBioscience). The production of IFN-γ by tumor $CD8^+$ T cells

was determined by ELISpot Assay. The images were scanned with an ELISpot reader (CTL), and spot numbers were counted manually.

Ex vivo tumor-specific T cell cytotoxicity assay

$CD8^+$ T cells and macrophages were isolated from mice Hepa1-6 tumors. Macrophages were pretreated with glycolysis inhibitor 2-DG for 24 hours. 1×10^5 $CD8^+$ T cells were left untreated or were cultured with autologous macrophages and CFSE-labelled Hepa1-6 cells (10:2:1) in the presence or absence of a specific blocking antibody against PD-L1 (20 μg/mL) for 12 hours. Meanwhile, 25 μg/ml of tumor mass lysates were added to trigger tumor specific T cell response. Propidum iodide$^+$ apoptotic tumor cells were analyzed by flow cytometry.

Enzyme-linked immunosorbent assay (ELISA)

Concentrations of the cytokines TNF-α, IL-1β, IL-6, and IL-12p70 in the supernatants from in vitro or ex vivo culture systems were detected using ELISA kits according to the manufacturers' instructions (eBioscience).

Preparation of culture supernatant from primary tumors

Culture supernatants were acquired by culture of completely digested HCC tumor. All specimens were from individuals without concurrent autoimmune disease, HBV, HCV, HIV, or syphilis. The digested tumor or liver cells were washed in medium containing polymyxin B (20 μg/mL; Sigma-Aldrich) to exclude endotoxin contamination. Thereafter, 1×10^7 digested cells were resuspended in 10 mL of complete medium and cultured in 100-mm dishes. In some experiments, Huh7 cells were pre-transfected with shNC or shFN1 by lentiviral vectors delivery system. Tumor culture supernatants were prepared by plating 5×10^6 tumor cells in 10 mL complete medium in 100-mm dishes. After 2 days, the supernatants were harvested, centrifuged, and stored at −80°C.

Construction of PD-L1 stable cell lines

The PD-L1 gene was amplified by PCR from human cDNA and confirmed by DNA sequencing. The gene fragment was inserted into retrovirus vector pBABE-puro and cotransfected into the package cell HEK293T with helper virus vector pBABE-ampho in the context of Lipofectamine (Invitrogen). The supernatant of these HEK293T cells was used to infect original HEK293T

cells. The cell lines stably expressing PD-L1 and the mock transfectant were selected with puromycin (1 μg/mL, Sigma-Aldrich).

Supplementary Tables

Table S1. Clinical characteristics of HCC patients

Patients characteristics	**Cohort 1**	**Cohort 2**
No. of patients	91	93
Age, years (median, range)	51, 19–81	50.7, 27–78
Gender (male: female)	76:15	71:22
HBsAg (negative: positive)	14:77	23:70
Cirrhosis (absent: present)	24:67	28:65
ALT, U/L (median, range)	41.4, 12.3–533.7	39.4, 9.9–453.7
AFP, ng/ml (≤ 25: > 25)	43:48	47:46
Tumor size, cm (≤ 5: > 5)	37:54	38:55
Tumor multiplicity (solitary: multiple)	71:20	71:22
Vascular invasion (absent: present)	63:28	64:29
TNM stage (I+II: III+IV)	43:48	63:30
Tumor differentiation (I+II: III+IV)	62:29	58:35
Fibrous capsule (absent: present)	35:56	39:54
Tumor $PKM2^{+}CD68^{+}$ cells density (median, range)	90, 26–211	N/A

Abbreviations: HBsAg, hepatitis B surface antigen; AFP, alpha fetoprotein; ALT, alanine aminotransferase; TNM, tumor-node-metastasis; N/A, not applicable.

Note: Patients in cohort 1 contributed to the paraffin embedded samples for immunohistochemical staining that were used in analyses of cell distribution, correlation, and patient prognosis; patients in cohort 2 contributed fresh samples.

Table S2. Fluorochrome-conjugated antibodies used in flow cytometry

Antibody	**Supplier**	**Catalogue**	**RRID**
Anti-Human CD14 Antibody, AF 700, Clone M5E2	BD Biosciences	557923	AB_396944
Anti-Human CD23 Antibody, APC Conjugated, Clone M-L233	BD Biosciences	558690	AB_1645456
Anti-Human CD3 Antibody, AF 700, Clone OKT3	eBioscience	56-0037-42	AB_10714978
Anti-Human CD15 Antibody, eFluor 450, Clone MMA	eBioscience	48-0158-42	AB_1907348
Anti-Human CD19 Antibody, PE-CY7, Clone J4.119	Beckman Coulter	PM IM 3628U	NA
Anti-Human CD56 Antibody, PE-CY7, Clone N901	Beckman Coulter	A21692	AB_2892144
Anti-Human CD45 Antibody, KO, Clone J.33	Beckman Coulter	PN A96416	NA
Anti-Human CD86 Antibody, PE, Clone HA5.2B7	Beckman Coulter	IM2729U	NA
Anti-Human CD163 Antibody, PE, Clone GHI/61	eBioscience	12-1639-42	AB_1963570
Anti-Human CD206 Antibody, PE/Cy7, Clone 15-2	Biolegend	321124	AB_10933248
Anti-Human CD274 (PD-L1) Antibody, PE, Clone MIH1	eBioscience	12-5983-42	AB_11042286
Anti-Human CD274 (PD-L1) Antibody, PE-Cy7, Clone MIH1	BD Biosciences	558017	AB_396986
Anti-Human Glut1 Antibody, APC, Clone 202915	BD Biosciences	FAB1418A	AB_1207948
Anti-Human HLA-DR Antibody, PE-CF594, Clone G46-6	BD Biosciences	562304	AB_11154415
Anti-Mouse CD3 Antibody, AF 700, Clone 17A2	eBioscience	56-0032-82	AB_529507
Anti-Mouse CD8a Antibody, FITC, Clone 53-6.7	Invitrogen	11-0081-82	AB_464915
Anti-Mouse CD45 Antibody, BV 570, Clone 30-F11	Biolegend	103136	AB_2562612

Antibody	Supplier	Catalogue	RRID
Anti-Mouse CD86 Antibody, FITC, Clone GL-1	Biolegend	105005	AB_313148
Anti-Mouse CD274 Antibody, PE/Cyanine7, Clone 10F-9G2	Biolegend	124314	AB_10643573
Anti-Mouse F4/80 Antibody, APC, Clone BM8	Biolegend	123116	AB_893481
Anti-Mouse I-A/I-E Antibody, PE/Cyanine7, Clone M5/114.115.2	Biolegend	107629	AB_2290801
Anti-Mouse IFN-γ Antibody, ef450, Clone XMG1.2	eBioscience	48-7311-82	AB_1834366

Table S3. Antibodies for immunoblotting, IHC, and IF

Antibody	Supplier	Identifier
Antibodies for IHC/IF		
Anti-Human CD68 Antibody (PG-M1)	ZSBio	Cat# ZM-0464
Anti-Human CD8 Antibody	ZSBio	Cat# ZA-0508
Anti-Human HLA-DR Antibody (L243)	Biolegend	Cat# 307602; RRID:AB_314680
Anti-Human PD-L1 Antibody (E1L3N)	Cell Signaling Technology	Cat# 13684; RRID:AB_2687655
Anti-Human PKM2 Antibody (D78A4)	Cell Signaling Technology	Cat# 4053; RRID:AB_1904096
Cy 5-streptavidin	Invitrogen	Cat# 43-4316
Antibodies for immunoblotting		
Mouse Anti-Human HIF-1α	BD Biosciences	Cat# 610958; RRID:AB_398271
Rabbit Anti-Human HK2 (C64G5)	Cell Signaling Technology	Cat# 2867; RRID:AB_2232946
Rabbit Anti-Human LDHA Antibody	Proteintech	Cat# 19987-1-AP; RRID:AB_10646429
Rabbit Anti-Human PFKFB3	Proteintech	Cat# 13763-1-AP; RRID:AB_2162854
Rabbit Anti-Human PKM2	Cell Signaling Technology	Cat# 4053; RRID:AB_1904096
Rabbit Anti-Human Stat3 (Tyr705) (D3A7)	Cell Signaling Technology	Cat# 9145; RRID:AB_2491009
Mouse Anti-Stat3 Antibody (124H6)	Cell Signaling Technology	Cat# 9139; RRID:AB_331757
β-Actin (ACTB) Antibody (8H10)	Origene	Cat# TA310155; RRID:AB_10691552

Table S4. Primers for real-time PCR

Gene	Forward	Reverse
Primers for human gene		
IL6	TCAGCCCTGAGAAAGGAGACA	GATTTTCACCAGGCAAGTCTCC
TNF	AAGCCTGTAGCCCATGTTG	TGGTAGGAGACGGCGATG
IL1B	CGAATCTCCGACCACCACTAC	GATGAAGGGAAAGAAGGTGCTC
IL12A	GCTTCTTCATCAGGGACATCATC	GTCAGGGAGAAGTAGGAATGTGG
HK1	CGAGAGTGACCGATTAGCACT	AGACAGGAGGAAGGACACGTT
HK2	GATTGTCCGTAACATTCTCATCG	CAGGCAGTCACTCTCAATCTGAG
PFKFB3	CTCGCATCAACAGCTTTGAGG	TCAGTGTTTCCTGGAGGAGTC
ALDOA	AGATGAGTCCACTGGGAGCAT	AGATGAGTCCACTGGGAGCAT
GAPDH	GGAGTCAACGGATTTGGTCGT	TCTCGCTCCTGGAAGATGGT
PGK1	GGGTCGTTATGAGAGTCGACT	AGGTGGCTCATAAGGACTACC
ENO2	AACAGTGAAGCCTTGGAGCTG	TCCTCAATGGAGACCACAGGA
PKM	TCTGTACCATTGGCCCAGCTT	TGGCTGTGCGCACATTCTTGA
LDHA	GATTCCAGTGTGCCTGTATGG	CTACAGAGAGTCCAATAGCCC
CS	GGGCTGCAAGAACAAGACA	CTCCCTTTCTTACCTCCCCA
ACO2	AATGGATGTACTCGTTGGGC	ACAGCCTACTGGTGACTCGG
IDH2	AACCGTGACCAGACTGATGAC	ATGGTGGCACACTTGACAGC
OGDH	TTGGCTGGAAAACCCCAAAAG	TGTGCTTCTACCAGGGACTGT
SUCLA2	TTGCTTCAGGAGACTCAGCA	GTGAGCGAAAATATCCCAGG
SHDA	CGAACGTCTTCAGGTGCTTT	AAGAACATCGGAACTGCGAC
SHDB	CACAGATGCCTTCTCTGCAT	AAGGCTGGAGACAAACCTCA
FH	CCTCATCTGCTGCCTTCATT	GGAGGTGTGACAGAACGCAT
MDH2	TCGGCCCAGAACAATGCTAAA	GCGGCTTTGGTCTCGATGT
ACTB	GGATGCAGAAGGAGATCACT	CGATCCACACGGAGTACTTG
FN1	ACCTCGGTGTTGTAAGGTGG	CCATAAAGGGCAACCAAGAG
Primers for Mouse gene		
Il1b	GAAATGCCACCTTTTGACAGTG	TGGATGCTCTCATCAGGACAG
Il6	TGGAGTCACAGAAGGAGTGGCT	GCATAACGCACTAGGTTTGCCG
Il12a	TTCCTGCACTGCTGAAGACATC	CAAGGCACAGGGTCATCATCAA
Tnf	CCTGTAGCCCACGTCGTAG	GGGAGTAGACAAGGTACAACCC
Hk2	ATGATCGCCTGCTTATTCACG	CGCCTAGAAATCTCCAGAAGGG
Pfkfb3	ACGAAGATGCCGTTGGAACT	TCGACGGGCACCCAGAT
Pkm	TTGACTCTGCCCCCATCAC	GCAGGCCCAATGGTACAAAT
Actb	ACACCCGCCACCAGTTCGC	ATGGGGTACTTCAGGGTCAGGATA
Primers for si and sh		
si*PKM2*	CCUGUAUGUCAAUAAACAACA	UUGUUUAUUGACAUACAGGUA
shFN1	GGTTGTTATGACAATGGAA	TTCCATTGTCATAACAACC
sh*Fn1*	CTGGACCTGTCCAAGTAATTA	TAATTACTTGGACAGGTCCAG

Table S5. Recombinant proteins, peptides, chemicals, and critical commercial assays

Name	Supplier	Identifier
Recombinant proteins, peptides		
Human IL-1 beta/IL-1F2 antibody	R&D Systems	Cat# MAB601; RRID:AB_358545
Human IL-6 antibody, Clone 6708	R&D Systems	Cat# MAB206; RRID:AB_2127617
Human IL-12p70 antibody (24910)	R&D Systems	Cat# MAB219; RRID:AB_2123616
Human TNF-alpha antibody, Clone 28401	R&D Systems	Cat# MAB610; RRID:AB_2203945
Human TLR4 Affinity Purified antibody	R&D Systems	Cat# AF1478; RRID:AB_354816
Human CD274 (PD-L1) Antibody (MIH1)	eBioscience	Cat# 14-5983-82; RRID:AB_467784
InVivoMAb anti-mouse PD-L1 (B7-H1)	Bio X Cell	Cat# BE0101; RRID:AB_10949073
nVivoMab anti-mouse/rat IL-1β antibody	Bio X Cell	Cat# BE0246; RRID:AB_2687727
InVivoMab anti-mouse/rat/rabbit TNF-α antibody	Bio X Cell	Cat# BE0244; RRID:AB_2687725
Recombinant Human IL-1 beta/IL-1F2 Protein	R&D Systems	Cat# 201-LB-010
Recombinant Human IL-6 Protein	R&D Systems	Cat# 206-IL-010
Recombinant Human IL-12 Protein	R&D Systems	Cat# 219-IL-025
Recombinant Human TNF-alpha Protein	R&D Systems	Cat# 210-TA-020
Human Fibronectin Protein	R&D Systems	Cat# 1918-FN
Chemicals		
2-DG	Sigma-Aldrich	Cat# D8375 CAS: 154-17-6
2-NBDG	Sigma-Aldrich	Cat# 72987 CAS: 186689-07-6
3-BP	Sigma-Aldrich	Cat# 376817 CAS: 1113-59-3
3-PO	Merck Millipore	Cat# 525330 CAS: 18550-98-6
Etomoxir	Sigma-Aldrich	Cat# 236020 CAS: 828934-41-4
α-KG	Sigma-Aldrich	Cat# K2010 CAS: 22202-68-2

Chemicals		
ML-265	Cayman Chemical	Cat# 13942 CAS: 1221186-53-3
Oligomycin	Sigma-Aldrich	Cat# 495455 CAS: 1404-19-9
Propidium iodide	Sigma-Aldrich	Cat# P4170 CAS: 25535-16-4
WP1066	Sigma-Aldrich	Cat# 573097 CAS: 857064-38-1
Critical commercial assays		
IL-1β Human Uncoated ELISA kit	eBioscience	Cat# 88-7349
IL-6 Human Uncoated ELISA Kit	eBioscience	Cat# 88-7066
IL-12 p70 Human Uncoated ELISA kit	eBioscience	Cat# 88-7126
TNF-α Human Uncoated ELISA Kit	eBioscience	Cat# 88-7347
Human Fibronectin Platinum ELISA 10 x 96 tests Kit	Invitrogen	Cat# BMS2028TEN
Human IFN-γ ELISPOT Pair	BD Biosciences	Cat# 551873
IntraPrep reagent	Beckman Coulter	Cat# A07803
Hieff qPCR SYBR Green Master Mix	Yeasen	Cat# 11201ES03
Leukocyte Activation Cocktail	BD Biosciences	Cat# 550583
L-Lactate Assay Kit I -200 Assays	Eton bioscience	Cat# 1200012002
Trizol Reagent	Life Techonology	Cat# AM9738
XF Glycolysis Stress Test kit	Seahorse Bioscience	Cat# 102194-100
5X All-In-One RT MasterMix	abm	Cat# G492

Table S6. Association of tumor PKM2^{+} CD68^{+} cells with clinicopathological characteristics

Variables		Tumor PKM2^{+} CD68^{+} cells		
		High	Low	*p* value
Age, years	≤ 51	28	28	0.895
	> 51	17	18	
Gender	Male	38	38	0.814
	Female	7	8	
HBsAg	Negative	8	6	0.531
	Positive	37	40	
Cirrhosis	Absent	7	17	**0.021**
	Present	38	29	
ALT, U/L	≤ 40	23	21	0.602
	> 40	22	25	
AFP, ng/ml	≤ 25	24	19	0.250
	> 25	21	27	
Tumor size, cm	≤ 5	14	23	**0.045**
	> 5	32	22	
Tumor multiplicity	Solitary	35	36	0.956
	Multiple	10	10	
Vascular invasion	Absent	35	28	0.081
	Present	10	18	
TNM stage	I+II	15	28	**0.009**
	III+IV	30	18	
Tumor differentiation	I+II	31	31	0.878
	III+IV	14	15	
Fibrous capsule	Absent	15	20	0.320
	Present	30	26	

Abbreviations: HbsAg, hepatitis B surface antigen; ALT, alanine aminotransferase; AFP, α-fetoprotein; TNM, tumor-node-metastasis.

Table S7. Univariate and multivariate analysis of factors associated with disease-free survival of HCC patients

Variables				DFS			
				Univariate	**Multivariate**		
				***p* value**	**HR**	**95%CI of HR**	***p* value**
Age, years	> 51	vs.	≤ 51	0.118			
Gender	female	vs.	male	0.246			
HBsAg	positive	vs.	negative	0.171			
Cirrhosis	present	vs.	absent	0.227			
ALT, U/L	> 40	vs.	≤ 40	0.242			
AFP, ng/ml	> 25	vs.	≤ 25	0.051			
Tumor size, cm	> 5	vs.	≤ 5	0.071			
Tumor multiplicity	multiple	vs.	solitary	0.076			
Vascular invasion	present	vs.	absent	**0.004**	2.014	1.073–3.781	**0.029**
TNM stage	III+IV	vs.	I+II	**0.001**	5.395	2.583–11.270	**0.001**
Tumor differentiation	III+IV	vs.	I+II	**0.004**	0.349	0.194–0.628	**0.032**
Fibrous capsule	present	vs.	absent	0.535			
Tumor PKM2^{+}CD68^{+} cells	high	vs.	low	**0.019**	1.823	1.005–3.308	**0.048**

Abbreviations: HBsAg, hepatitis B surface antigen; ALT, alanine aminotransferase; AFP, α-fetoprotein; TNM, tumor-node-metastasis; N/A, not applicable.

Figure S1

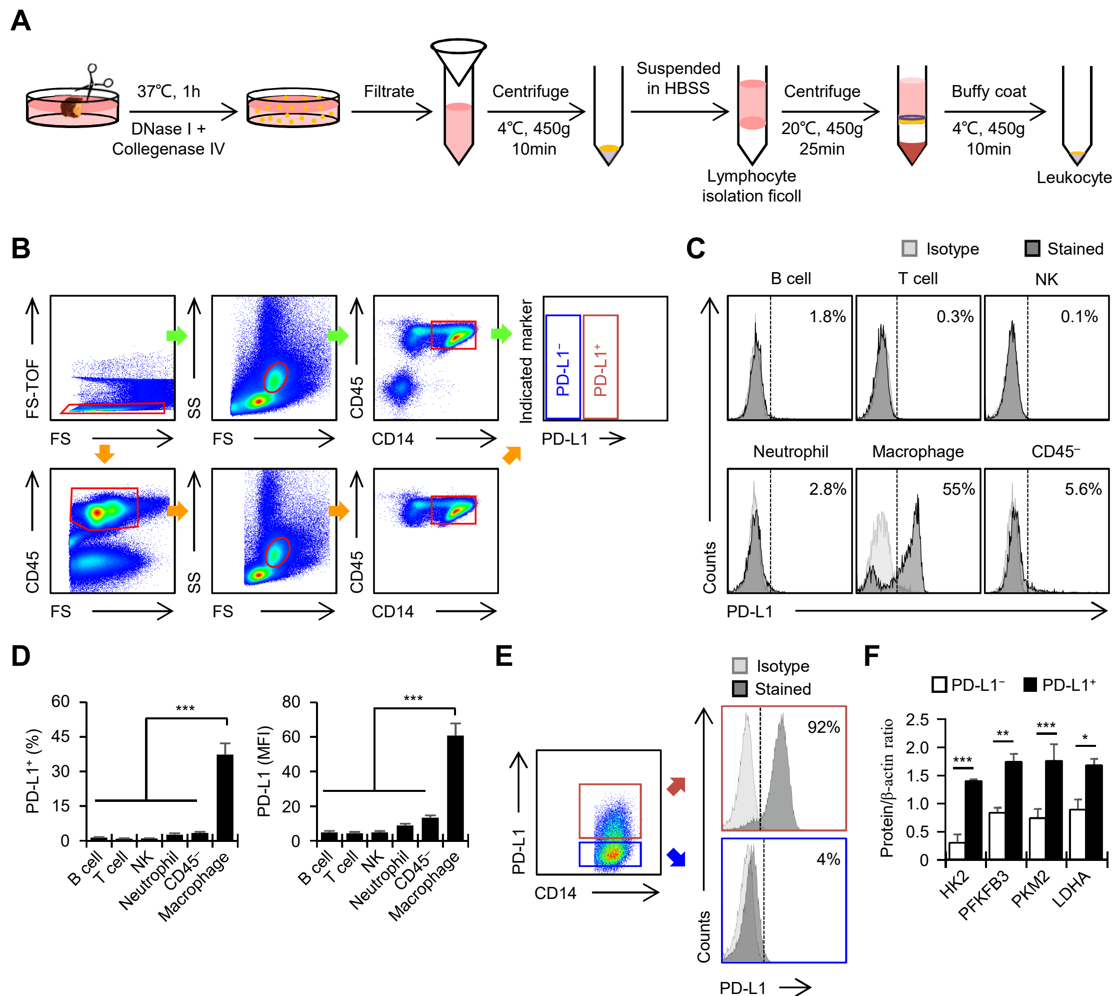

Figure S1. The expression of PD-L1 in human HCC tumors are mainly in macrophages. (A) Procedures for preparing single-cell leukocyte suspensions from human HCC tissues. **(B)** Representative plots of cells isolated from blood and tumor tissues showing that mononuclear cells from samples were first gated for singlets (FS-TOF vs. FS) and myeloid cells (SS vs. FS; green arrows), or alternatively, they were first gated for singlets (FS-TOF vs. FS) and leukocytes (CD45 vs. FS) then myeloid cells (SS vs. FS; orange arrows). Thereafter, the $CD45^{+}CD14^{+}$ macrophages were further divided into $PD\text{-}L1^{-}$ and $PD\text{-}1^{+}$ subsets. **(C and D)** FACS analysis of PD-L1 expression on B cells ($CD19^{+}$), T cells ($CD3^{+}CD56^{-}$), NK cells ($CD56^{+}CD3^{-}$), neutrophils ($CD15^{+}$), monocytes/macrophages ($CD14^{+}$), and $CD45^{-}$ non-hematopoietic cells isolated from HCC tumors (n=6). **(E)** Gating strategy for FACS sorting. $PD\text{-}L1^{-}$ and $PD\text{-}L1^{+}$ subsets were sorted according to patterns of PD-L1 expression. **(F)** Expression of key glycolytic enzymes gene in $PD\text{-}L1^{+}$ and $PD\text{-}L1^{-}$ macrophages purified from human HCC tumors (n=3).

Figure S2

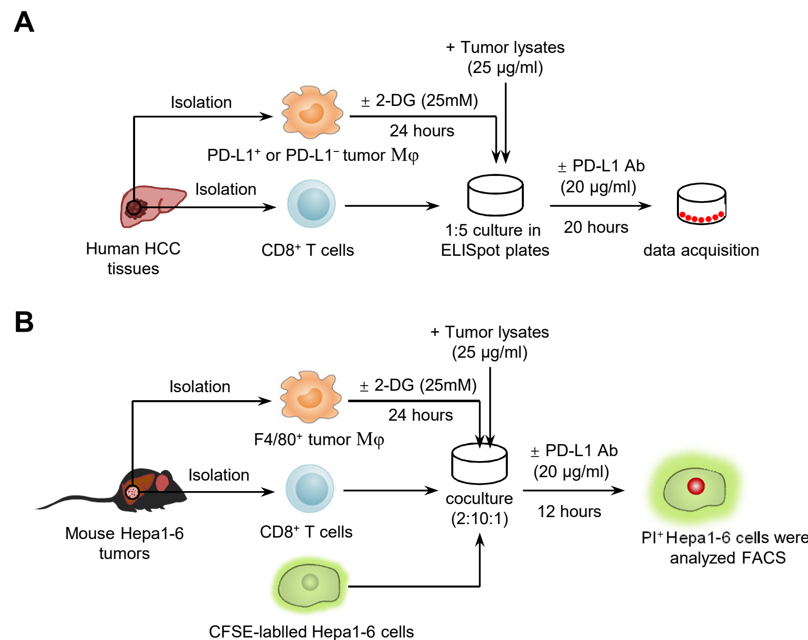

Figure S2. The effect of PD-L1 blockade and glycolysis inhibition on tumor macrophages -elicited cytotoxic T cell function. (**A**) Schematic procedures for Figure 2A. IFN-γ detection by ELISpot in HCC tumor-derived T cells cultured alone or with PD-L1^{-} or PD-L1^{+} macrophages (Mφ), or with those cells pre-treated with 2-DG, anti-PD-L1 antibody, or IgG1 (n=5). (**B**) Schematic procedures for Figure 2B. Cytotoxic effects of tumor T cells on CFSE-labelled autologous mouse Hepa1-6 hepatoma cells in the presence or absence of tumor Mφ that were left untreated or pre-treated with 2-DG, anti-PD-L1 antibody, or IgG1 (n=6). Propidium iodide^{+} apoptotic tumor cells were measured by FACS.

Figure S3

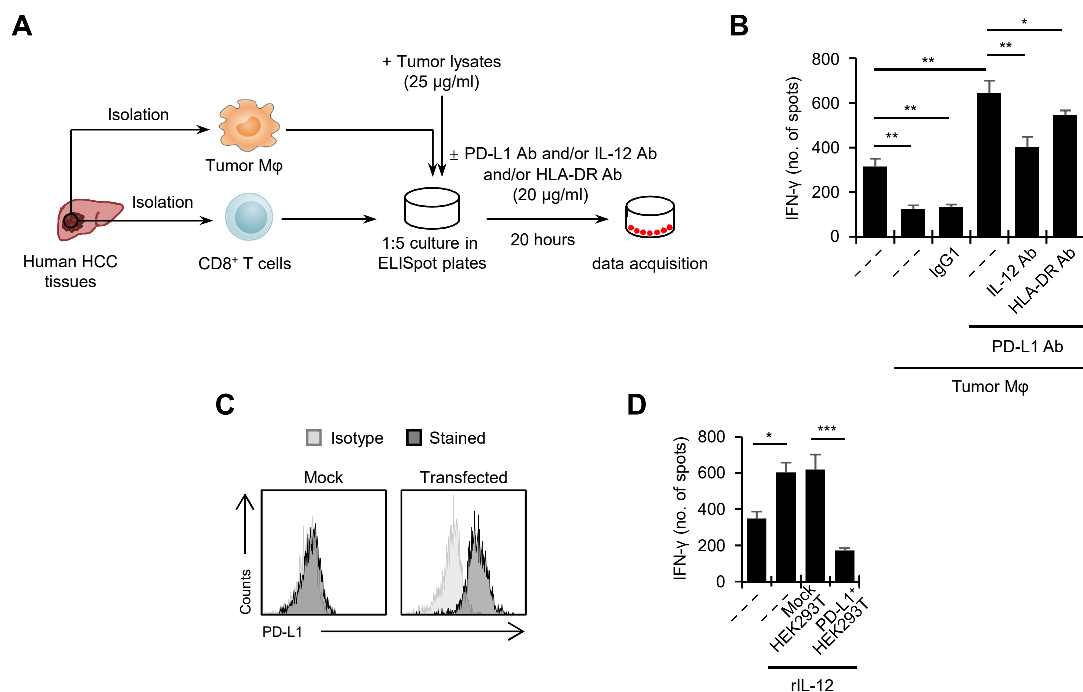

Figure S3. The effect of IL-12 or HLA-DR on tumor macrophages-elicited cytotoxic T cell function upon PD-L1 blockade. (A and B) Macrophages (Mφ) from human HCC tumors were cultured with autologous tumor-derived $CD8^+$ T cells for 20 hours in the presence or absence of an anti-PD-L1 antibody, and/or anti-IL-12 antibody, and/or anti-HLA-DR antibody. Meanwhile, 25 µg/ml of tumor mass lysates were added to trigger tumor specific T cell response **(A)**. IFN-γ production in $CD8^+$ T cells was analyzed by ELISpot **(B)**. **(C)** Efficiency of PD-L1 over-expression in Huh7 cells (n=3). **(D)** IFN-γ detection by ELISpot in HCC tumor-derived T cells cultured alone or with Mock or PD-L1 HEK293T transfectants in the presence or absence human recombinant IL-12 antibody (n=5). Results represent mean ± SEM of four independent experiments (n=5). *P<0.05, **P<0.01, ***P<0.001, one-way ANOVA followed by Bonferroni's correction.

Figure S4

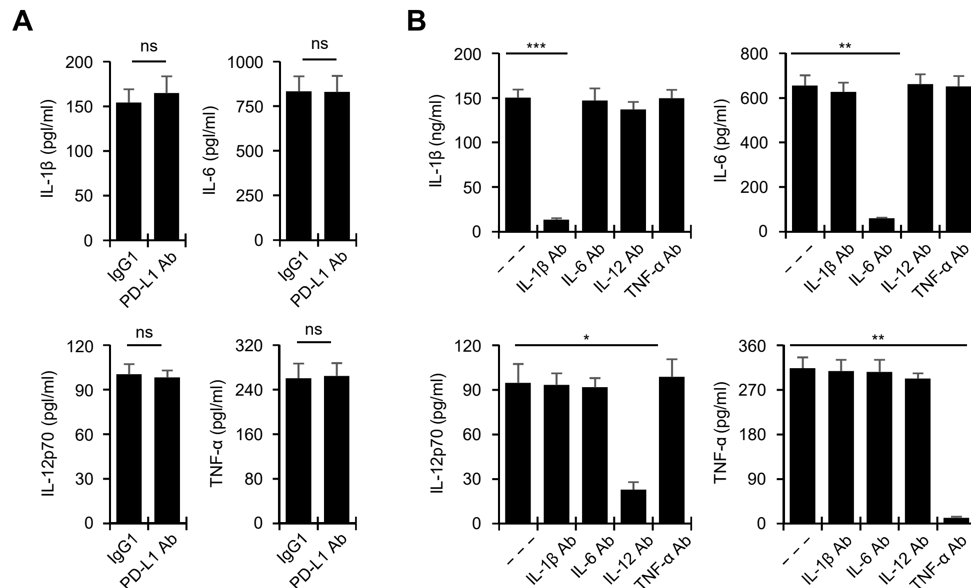

Figure S4. Blocking the PD-L1 in PD-L1⁺ macrophages did not affect the production of proinflammatory cytokines. (A) Effects of PD-L1 blocking antibody on proinflammatory cytokines expression in macrophages isolated from human HCC tumors (n=4). **(B)** Macrophages from human HCC tumors were treated with indicated neutralizing antibodies or isotype IgG for 24 hours. IL-1β, IL-6, IL-12, and TNF-α production were determined by ELISA (n=5). Results represent mean ± SEM of four independent experiments. *P<0.05, **P<0.01, ***P<0.001, one-way ANOVA followed by Bonferroni's correction.

Figure S5

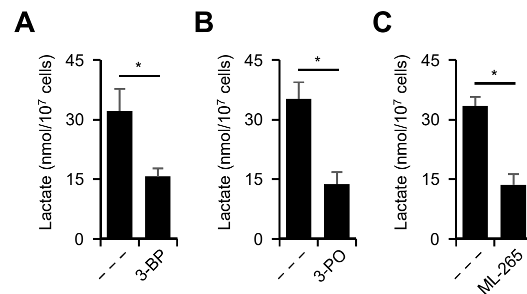

Figure S5. Effects of key glycolytic inhibitors on lactate production. (A-C) CD14^{+} cells purified from peripheral blood of HCC patients were treated with supernatant from primary HCC cells (HCC-SN) in the absence or presence of 3-BP **(A)**, 3-PO **(B)**, or ML-265 **(C)** for 24 hours (n=7). Lactate production was measured with a lactate assay kit. Results represent mean ± SEM of four independent experiments. *P<0.05, Student's t-test.

Figure S6

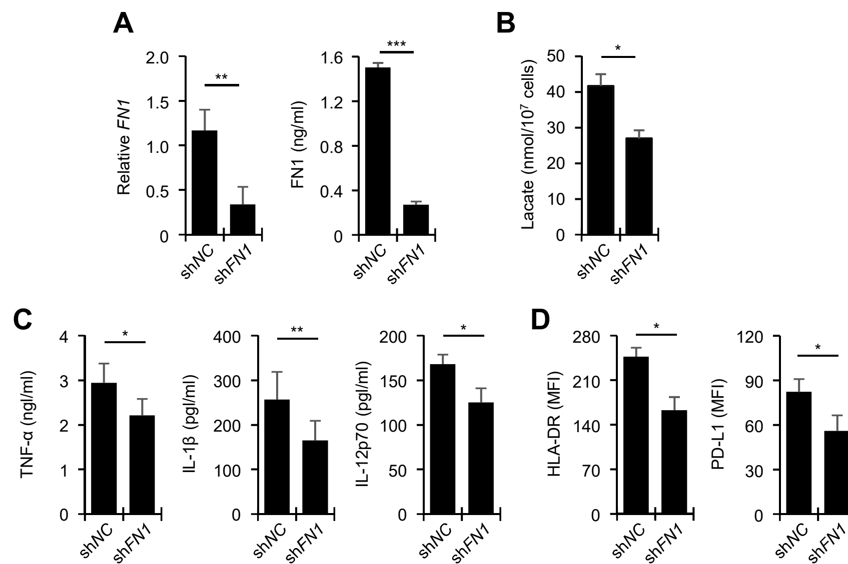

Figure S6. FN1-knockdown cells showed impaired ability to induce PD-L1 expression in monocytes. (A-D) Knock-down of *FN1* **(A)** in Huh7 cells suppressed lactate production **(B)**, inflammatory cytokine expression **(C)**, as well as HLA-DR and PD-L1 expression **(D)**, in Huh7 culture supernatants treated monocytes (each n=4). Results are expressed as the mean ± SEM. *P<0.05, **P<0.01, ***P<0.001.