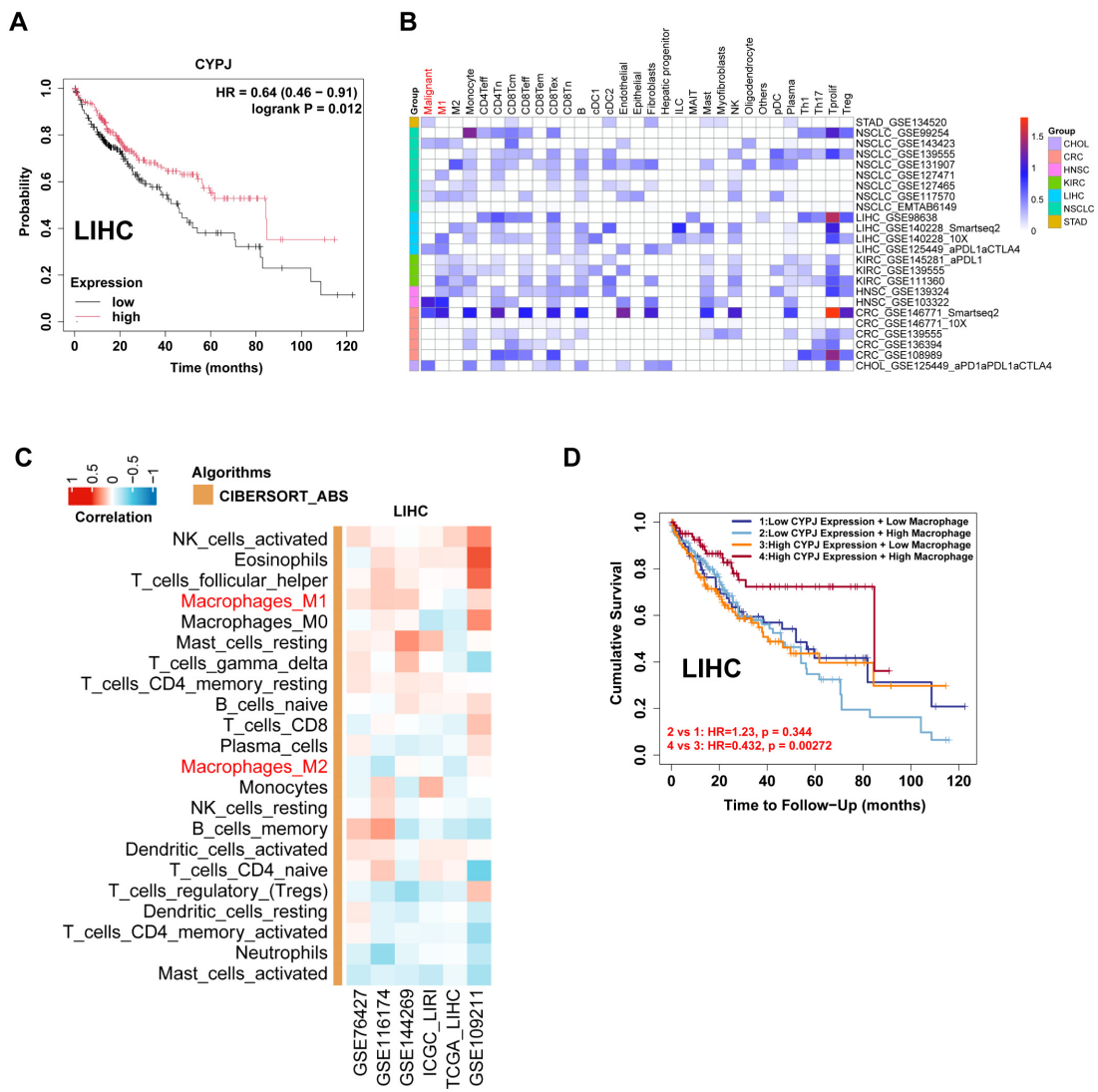


Supplementary Figures

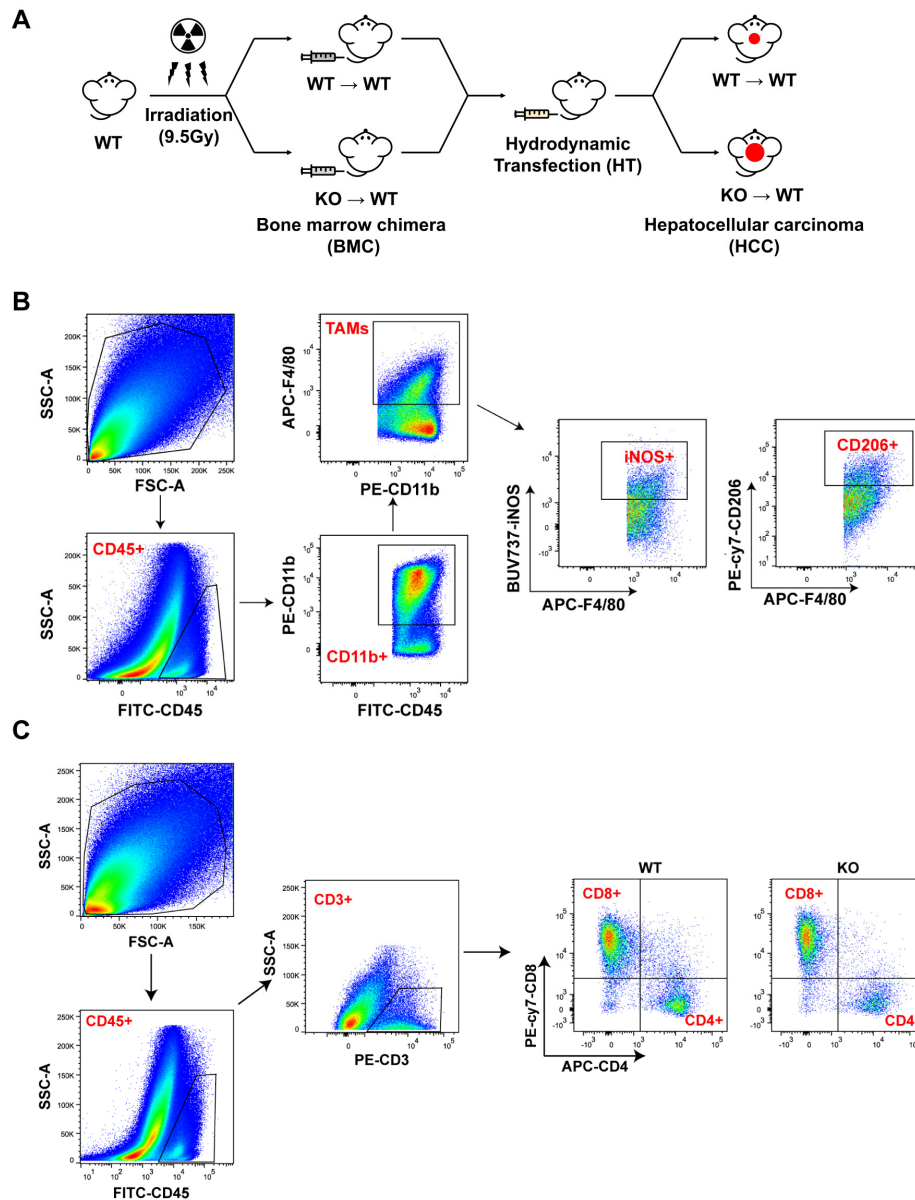
Supplementary Figure 1



Supplementary Figure 1. CYPJ is Associated with M1 TAMs in LIHC.

(A) The survival analysis of CYPJ in LIHC was done on the Kaplan-Meier Plotter website. (B) Heatmap of CYPJ expression in the single cell expression of tumors with high CYPJ expression using the TISCH1 website. (C) Analysis of the relationship between immune cell infiltration and CYPJ using the CIBERSORT-ABS algorithm in the BEST website. (D) The survival analysis of CYPJ and macrophage in LIHC was done on the TIMER2.0 website.

Supplementary Figure 2



Supplementary Figure 2. The Strategy of Bone Marrow Chimera and Flow Cytometry Analysis of Immune Cell Infiltration in Tumors.

(A) Schematic diagram of bone marrow chimerism experiment. After feeding the recipient male mice with neomycin water, the recipient mice were lethally irradiated with 9.5 Gy. Bone marrow cells (5×10^6) harvested from the donor male mice were injected intravenously into irradiated recipients. (B) Gating strategy for TAMs in

tumors. Tumors were directly ground into single cells and resuspended in 3% BSA for staining. Immune cells were labeled with CD45 in tumors, and TAMs were defined by CD11b and F4/80. Then, iNOS⁺ were identified as M1 TAMs, while CD206⁺ were identified as M2 TAMs. (C) Gating strategy for T cells in tumors. Single cells are resuspended in 3% BSA, CD45 labels are used for immune cells, and CD3 labels total T cells. Further, T cells were defined as CD4⁺ T cells and CD8⁺ T cells.

A

Time / h

WT-BMDM

KO-BMDM

Hepa1-6 CM

Cypj

Gapdh

15 KD

35 KD

B

Relative mRNA levels

Il1β

Il10

Cd80

Arg1

iNos

Cd206

MOCK

Hepa1-6-2h

Hepa1-6-4h

Hepa1-6-6h

C

SSC-A

FSC-A

CD11b+

PE-CD11b

PE-sy7-CD206

PE-CD11b

Hepa1-6 CM

Hepa1-6 CM

WT-BMDM

KO-BMDM

Relative mRNA levels

hCYP4

iNos

MOCK

AAV-GFP

AAV-CYP4

AAV-miCYP4

D

Mouse_Cypj

Human_CYPJ

E

SSC-A

FSC-A

CD11b+

APC-CD11b

WT-BMDM

KO-BMDM

Relative mRNA levels

iNos

MOCK

AAV-GFP

AAV-CYP4

AAV-miCYP4

F

SSC-A

FSC-A

CD11b+

PE-CD11b

PE-sy7-CD206

PE-CD11b

Hepa1-6 CM

Hepa1-6 CM

WT-BMDM

KO-BMDM

Relative mRNA levels

hCYP4

iNos

MOCK

AAV-GFP

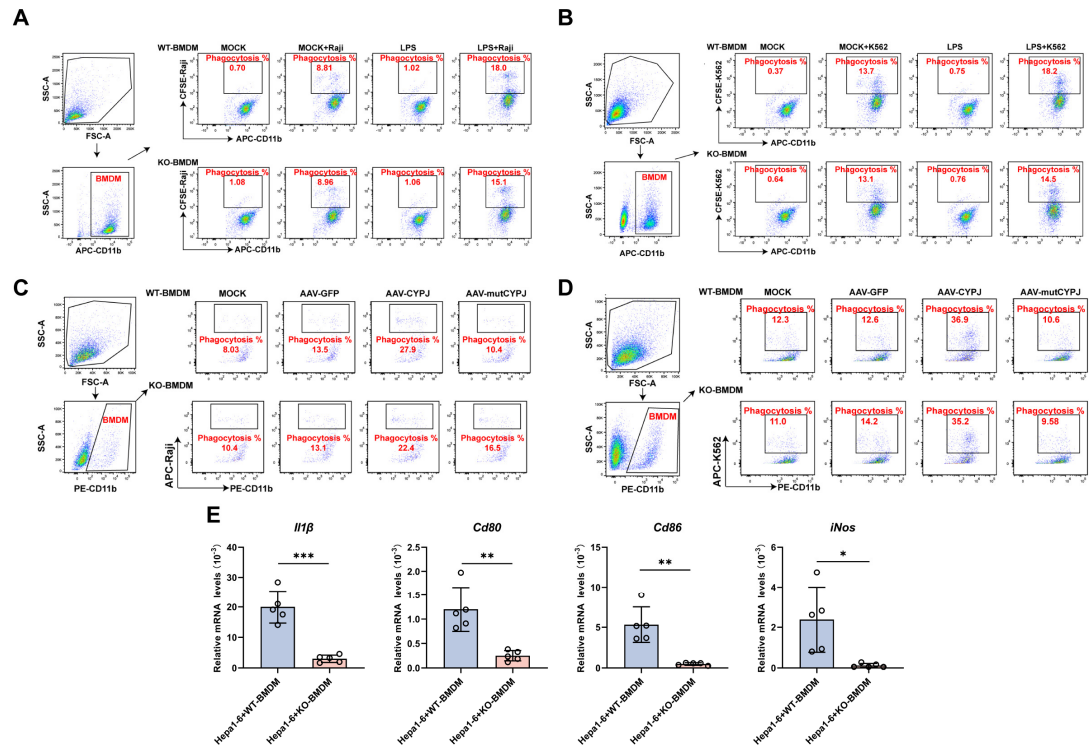
AAV-CYP4

AAV-miCYP4

(A) Expression of CYPJ in WT-BMDM and KO-BMDM under the stimulation of tumor cell conditioned-medium (Hepa1-6 CM). **(B)** qRT-PCR detects M1(*Il1 β* , *Cd80* and *iNos*) or M2 (*Il10*, *Arg1* and *Cd206*) markers under Hepa1-6 CM stimulation. **(C)** Flow cytometry analysis of the proportion of M1 (iNOS⁺) and M2 (CD206⁺) macrophages with the stimulation of Hepa1-6 CM. **(D)** Comparative conservation analysis of the

amino acid sequence of CYPJ between humans and mice. (E) Gating strategy of macrophage polarization after AAV infection. The CD11b indicates BMDMs, and then the iNOS⁺ cells were deemed to be M1 macrophages. (F) qRT-PCR analysis to measure the expression of hCYPA and M1 markers (*Il1 β* and *iNos*) in WT-BMDM after AAV infection. MOCK cells served as the negative control. (*p <0.05, ** p <0.01, *** p <0.001, **** p <0.0001, ns=non-significant).

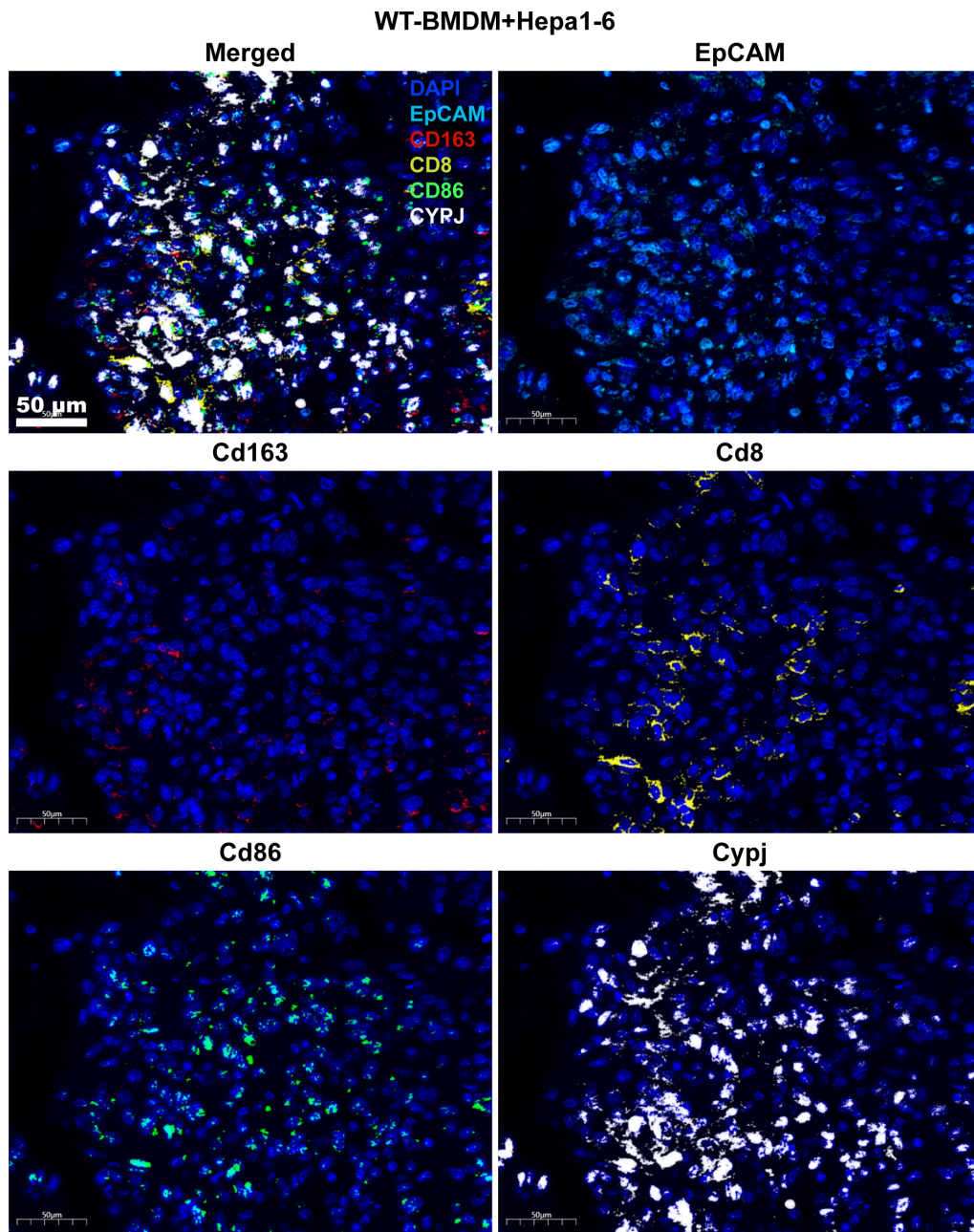
Supplementary Figure 4



Supplementary Figure 4. Gating Strategy of Phagocytosis Experiments.

(A, B) Gating strategy of macrophage phagocytosis to Raji (A) and K562 (B) in WT/KO-BMDM under LPS stimulation. Raji and K562 are labeled with CFSE, and BMDMs are labeled with APC-CD11b. (C, D) Gating strategy of macrophages phagocytosis to Raji (C) and K562 (D) cells after AAV infection. Raji and K562 are labeled with CellTrace™ Far Red (APC channel), and BMDMs are labeled with PE-CD11b. (E) qRT-PCR detects M1 markers (*Il1β*, *Cd80*, *Cd86*, and *iNos*) in tumors of Hepa1-6 mixed with WT-BMDM or KO-BMDM. (*p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001, ns=non-significant).

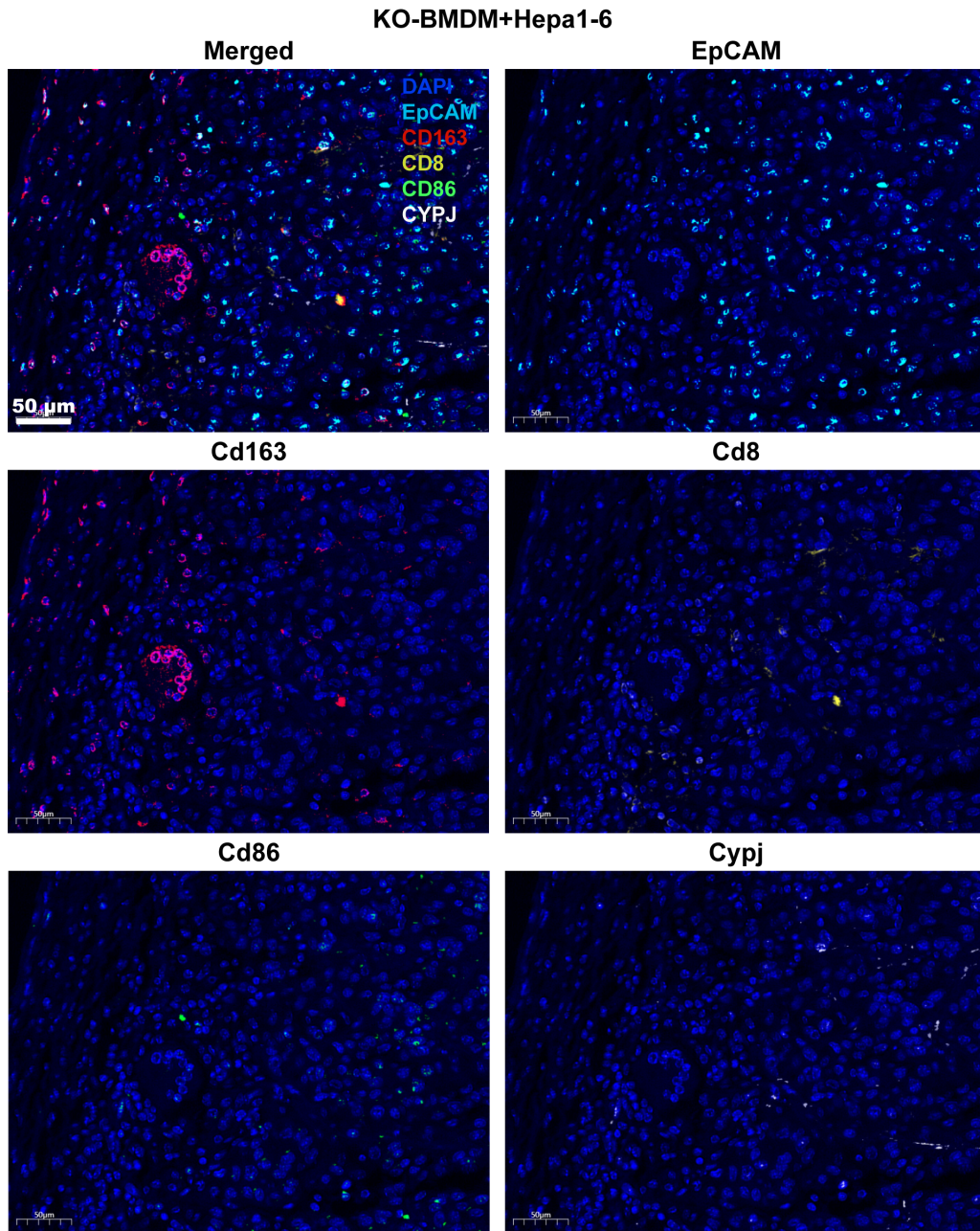
Supplementary Figure 5



Supplementary Figure 5. Analysis of mIHC in Hepa1-6 Tumors Mixed with WT-BMDM.

mIHC of Hepa1-6 tumors mixed with WT-BMDM labeled with DAPI (blue), CYPJ (white), CD86 (green), CD163 (red), CD8 (yellow), and EpCAM (cyan). Scale bar, 50 µm.

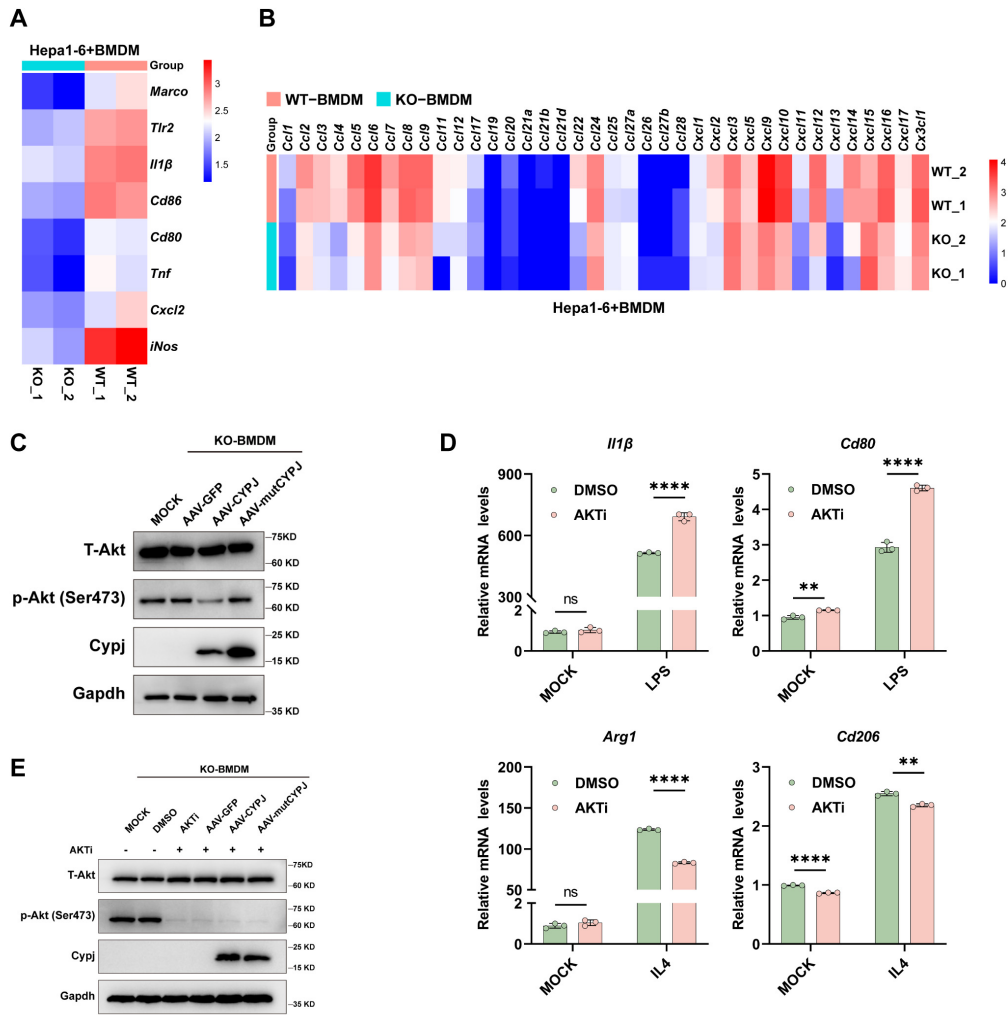
Supplementary Figure 6



Supplementary Figure 6. Analysis of mIHC in Hepa1-6 Tumors Mixed with KO-BMDM.

mIHC of Hepa1-6 tumors mixed with KO-BMDM labeled with DAPI (blue), CYPJ (white), CD86 (green), CD163 (red), CD8 (yellow), and EpCAM (cyan). Scale bar, 50 µm.

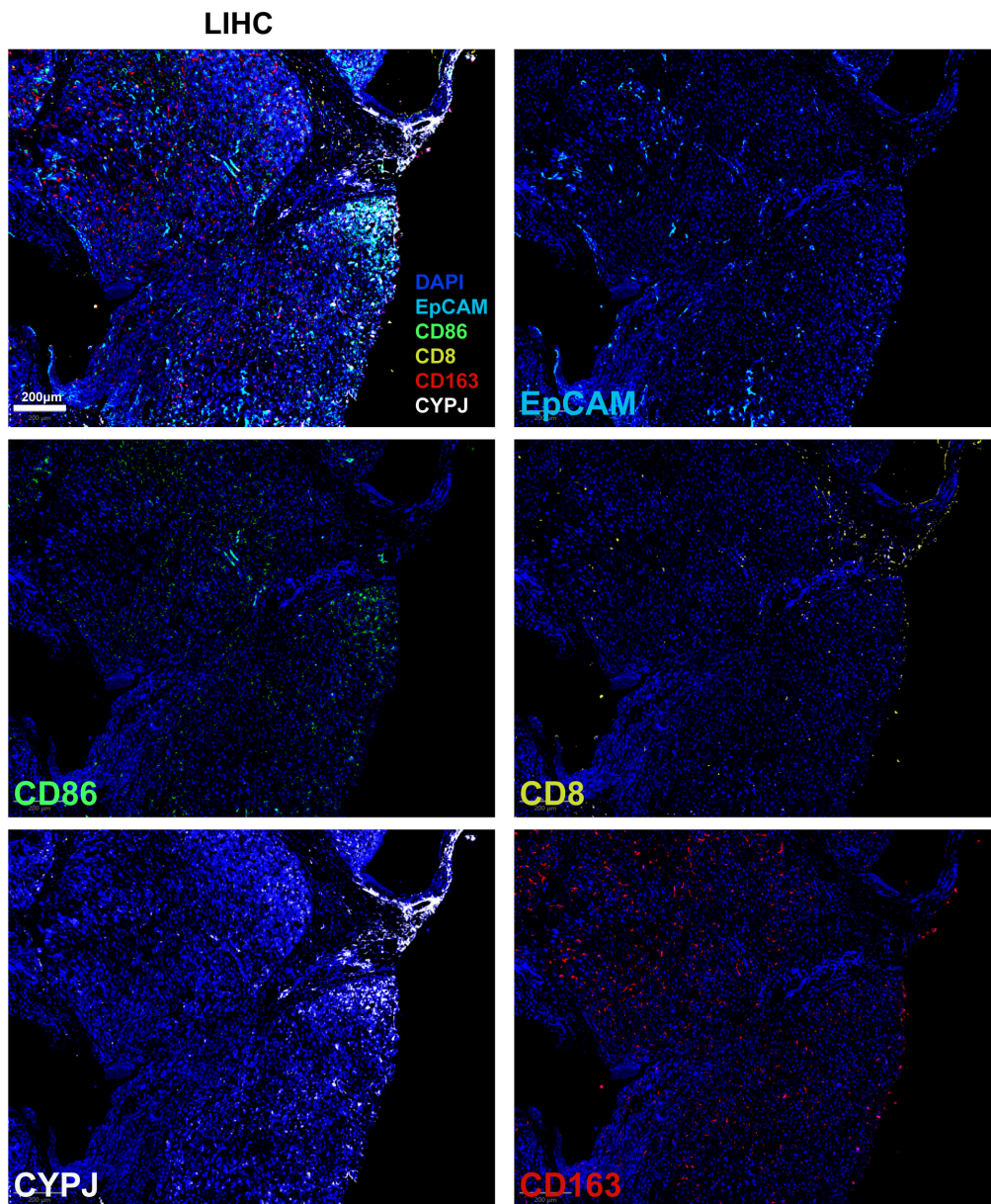
Supplementary Figure 7



Supplementary Figure 7. Mechanisms of CYPJ Regulates Macrophages.

(A, B) Heatmap of markers associated with M1 polarization (A) and chemokines (B) in tumors of Hepa1-6 cells mixed with WT-BMDM (WT_1 and WT_2) or KO-BMDM (KO_1 and KO_2), with expression levels normalized to $\log_{10}(\text{count}+1)$. (C) WB analysis of AKT in KO-BMDM after AAV infection. (D) qRT-PCR detects M1 markers (*Il1β* and *Cd80*) and M2 markers (*Arg1* and *Cd206*) in KO-BMDM after treatment with AKT inhibitor (AKTi) and rAAV virus. (E) WB analysis of AKT treated with AKT inhibitor (AKTi). (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns=non-significant).

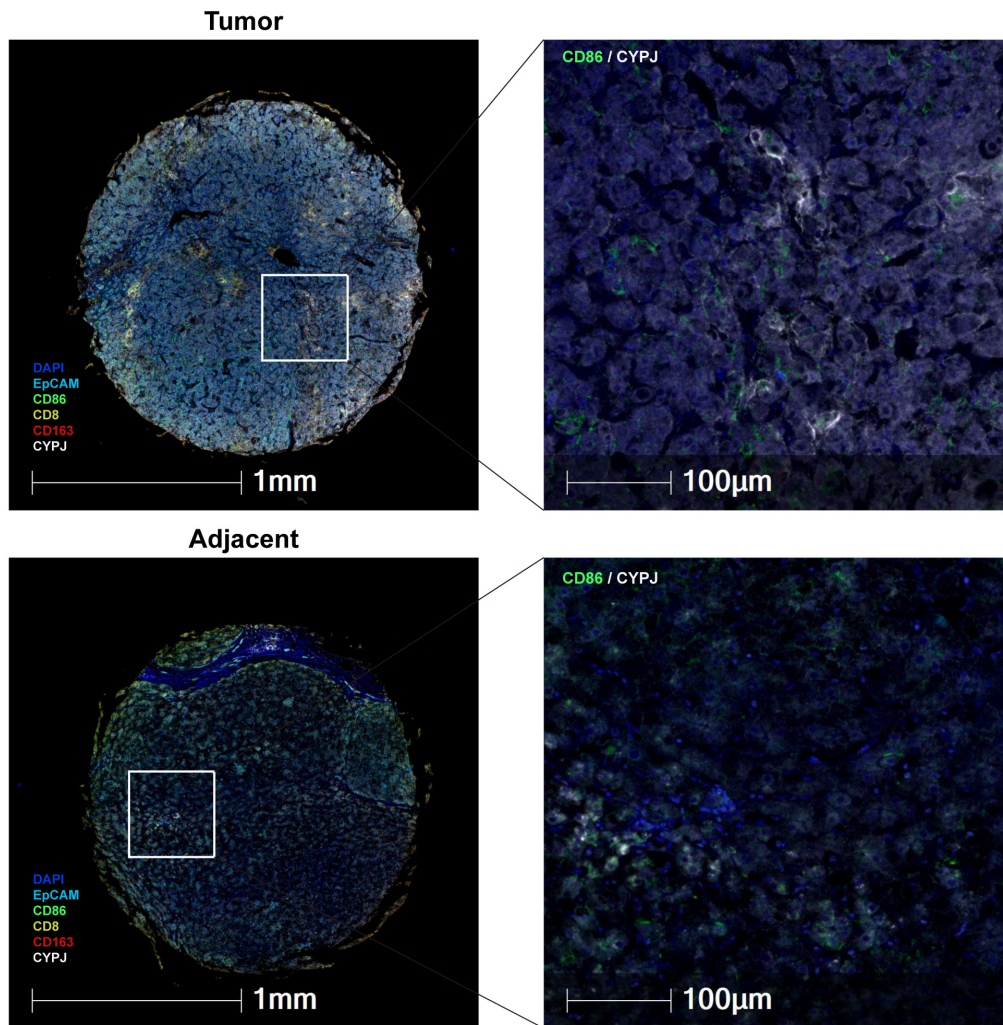
Supplementary Figure 8



Supplementary Figure 8. Analysis of mIHC in LIHC Patients.

mIHC of liver cancer tissue, labeled with DAPI (blue), CYPJ (white), CD86 (green), CD163 (red), CD8 (yellow), and EpCAM (cyan), then scanned using the Vectra Polaris Pathology Imaging System. Scale bar, 200 μ m.

Supplementary Figure 9



Supplementary Figure 9. Analysis of mIHC in Liver Cancer and Adjacent Normal Tissues.

The mIHC detects the expression of CYPJ, CD86 (M1 TAMs), CD163 (M2 TAMs), CD8 (CD8⁺ T cell), and EpCAM (tumor cell) in liver cancer and adjacent normal tissues.

Scale bar, 1 mm and 100 µm.

Supplementary Tables

Supplementary Table 1. Primer sequences of qRT-PCR

Genes	Forward (5'-3')	Reverse (5'-3')
Mouse		
<i>IL1β</i>	TGGACCTTCCAGGATGAGGACA	G TTCATCTCGGAGCCTGTAGTG
<i>Cd80</i>	CCTCAAGTTTCCATGTCCAAGGC	GAGGAGAGTTGTAACGGCAAGG
<i>Cd86</i>	ACGTATTGGAAGGAGATTACAGCT	TCTGTCAGCGTTACTATCCCGC
<i>iNos</i>	CTATGGCCGCTTTGATGTGC	TTGGGATGCTCCATGGTCAC
<i>Il10</i>	CGGGAAGACAATAACTGCACCC	CGGTTAGCAGTATGTTGTCCAGC
<i>Arg1</i>	GTAGACCCTGGGGAACACTAT	ATCACCTTGCCAATCCCCAG
<i>Cd206</i>	TCATTCCCTCAGCAAGCGAT	GTGGATACTTGCCAGGTCCC
<i>Cypj</i>	CTGTGAGAGAACACCCAAAACA	TTTTTGGCCCAGATGCTGCTA
<i>Gapdh</i>	CATCACTGCCACCCAGAAGACTG	ATGCCAGTGAGCTTCCCGTTCAG
Human		
hCYPJ	TGTAGGTGATATTAAAATTGAAGTCTTC	CTTCTTGCCCCAAATACTGTTG

Supplementary Table 2. The information on antibodies

Antibodies	Source	Identifier	Dilution Ratio
Antibodies for IHC, mIHC, WB and IP			
Rabbit polyclonal anti-CD86	Proteintech	Cat#26903-1-AP	IHC (1:100) mIHC (1:100)
Mouse monoclonal anti-CD163	OriGene	Cat#TA506381	IHC (1:100) mIHC (1:100)
Mouse monoclonal anti-CD8	Servicebio	Cat#GB12068	mIHC (1:100)
Rabbit polyclonal anti-CYPJ	Abcam	Cat#ab251870	IHC (1:100) mIHC (1:100) WB (1:1000)
Rabbit polyclonal anti-EpCAM	Proteintech	Cat#21050-1-AP	mIHC (1:200)
Rabbit polyclonal anti-GAPDH	HUABIO	Cat#R1210-1	WB (1:5000)
Rabbit polyclonal anti- β Tubulin	Fdbio	Cat#FD0064-100	WB (1:1000)
Rabbit polyclonal anti-mTOR	Cell Signaling Technology	Cat#2983	WB (1:1000)
Rabbit polyclonal anti- PI3K (P85)	Signalway Antibody	Cat#48184	WB (1:1000)
Mouse polyclonal anti-AKT (pan)	Cell Signaling Technology	Cat#2920	WB (1:1000)
Rabbit polyclonal anti- Phospho- AKT (Ser473)	Cell Signaling Technology	Cat#4060	WB (1:1000)

Mouse anti-Myc	Proteintech	Cat#16286-1-AP	WB (1:1000) IP (1 µg /mL)
Mouse anti-Flag	Sigma-Aldrich	Cat#F1804	WB (1:1000) IP (1 µg/mL)
Mouse anti-GFP	Proteintech	Cat#66002-1-AP	WB (1:1000) IP (1 µg/mL)
Mouse IgG	Santa Cruz	Cat#sc-2025	IP (1 µg/mL)
Antibodies for Flow Cytometry			
FITC anti-mouse CD45	Biolegend	Cat#103108	1:200
PE anti-mouse CD11b	Biolegend	Cat#101207	1:200
APC anti-mouse CD11b	sungene	Cat#M10117-11A	1:200
APC anti-mouse F4/80	Biolegend	Cat#123116	1:200
BUV737 anti-mouse iNOS	Invitrogen	Cat#367-5920-82	1:100
PE-cy7 anti-mouse CD206	Biolegend	Cat#141719	1:100
PE anti-mouse CD3	Biolegend	Cat#100206	1:200
APC anti-mouse CD4	Elabscience	Cat#E-AB-1097E	1:200
PE-cy7 anti-mouse CD8	Biolegend	Cat#100722	1:200

Supplementary Table 3. Expression of markers associated with M1 polarization

Original count value				
Group	Hepa1-6+KO-BMDM	Hepa1-6+KO-BMDM	Hepa1-6+WT-BMDM	Hepa1-6+WT-BMDM
Gene	KO_1	KO_2	WT_1	WT_2
<i>Marco</i>	27	14	153	284
<i>Tlr2</i>	87	111	505	579
<i>Il1β</i>	149	125	674	805
<i>Cd86</i>	84	73	768	577
<i>Cd80</i>	36	24	173	161
<i>Tnf</i>	34	14	223	146
<i>Cxcl2</i>	74	59	147	333
<i>Nos2</i>	128	71	1636	2749
Normalized to log10(count+1)				
Group	Hepa1-6+KO-BMDM	Hepa1-6+KO-BMDM	Hepa1-6+WT-BMDM	Hepa1-6+WT-BMDM
Gene	KO_1	KO_2	WT_1	WT_2
<i>Marco</i>	1.447158031	1.176091259	2.187520721	2.45484486
<i>Tlr2</i>	1.944482672	2.049218023	2.704150517	2.763427994
<i>Il1β</i>	2.176091259	2.100370545	2.829303773	2.906335042
<i>Cd86</i>	1.929418926	1.86923172	2.88592634	2.761927838
<i>Cd80</i>	1.568201724	1.397940009	2.240549248	2.209515015
<i>Tnf</i>	1.544068044	1.176091259	2.350248018	2.167317335
<i>Cxcl2</i>	1.875061263	1.77815125	2.170261715	2.523746467
<i>Nos2</i>	2.11058971	1.857332496	3.214048679	3.439332694

Supplementary Table 4. Expression of chemokines

Original count value				
Group	Hepa1-6+KO-BMDM	Hepa1-6+KO-BMDM	Hepa1-6+WT-BMDM	Hepa1-6+WT-BMDM
Gene	KO_1	KO_2	WT_1	WT_2
<i>Ccl1</i>	2	5	9	38
<i>Ccl2</i>	191	171	398	838
<i>Ccl3</i>	70	57	370	345
<i>Ccl4</i>	36	20	241	210
<i>Ccl5</i>	89	145	888	1528
<i>Ccl6</i>	814	822	3938	4388
<i>Ccl7</i>	90	153	348	689
<i>Ccl8</i>	255	517	1883	1818
<i>Ccl9</i>	343	417	1515	1707
<i>Ccl11</i>	0	52	148	153
<i>Ccl12</i>	90	53	100	142
<i>Ccl17</i>	10	20	14	50
<i>Ccl19</i>	0	0	0	0
<i>Ccl20</i>	1	3	3	7
<i>Ccl21a</i>	0	0	0	0
<i>Ccl21b</i>	0	0	0	1
<i>Ccl21d</i>	0	0	0	0
<i>Ccl22</i>	13	25	108	243
<i>Ccl24</i>	828	486	1222	1039
<i>Ccl25</i>	76	79	55	68
<i>Ccl27a</i>	93	108	50	82
<i>Ccl26</i>	1	0	0	0
<i>Ccl27b</i>	1	0	0	0
<i>Ccl28</i>	1	3	3	0
<i>Cxcl1</i>	62	85	74	79
<i>Cxcl2</i>	74	59	147	333
<i>Cxcl3</i>	1312	1431	897	1310
<i>Cxcl5</i>	343	344	251	290
<i>Cxcl9</i>	998	1179	11987	10800
<i>Cxcl10</i>	438	487	4023	3232
<i>Cxcl11</i>	7	12	34	74
<i>Cxcl12</i>	57	323	1327	1666
<i>Cxcl13</i>	1	4	20	71
<i>Cxcl14</i>	13	128	694	970
<i>Cxcl15</i>	2322	1293	697	479
<i>Cxcl16</i>	350	420	2340	1833
<i>Cxcl17</i>	146	106	132	172
<i>Cx3cl1</i>	740	1021	2403	1563

Normalized to log10(count+1)				
Group	Hepa1-6+KO-BMDM	Hepa1-6+KO-BMDM	Hepa1-6+WT-BMDM	Hepa1-6+WT-BMDM
Gene	KO_1	KO_2	WT_1	WT_2
<i>Ccl1</i>	0.477121255	0.77815125	1	1.591064607
<i>Ccl2</i>	2.283301229	2.235528447	2.600972896	2.923761961
<i>Ccl3</i>	1.851258349	1.763427994	2.56937391	2.539076099
<i>Ccl4</i>	1.568201724	1.322219295	2.383815366	2.324282455
<i>Ccl5</i>	1.954242509	2.164352856	2.948901761	3.184407485
<i>Ccl6</i>	2.911157609	2.915399835	3.595385981	3.642365581
<i>Ccl7</i>	1.959041392	2.187520721	2.542825427	2.838849091
<i>Ccl8</i>	2.408239965	2.71432976	3.275080898	3.259832699
<i>Ccl9</i>	2.536558443	2.621176282	3.180699201	3.232487866
<i>Ccl11</i>	0	1.72427587	2.173186268	2.187520721
<i>Ccl12</i>	1.959041392	1.73239376	2.004321374	2.155336037
<i>Ccl17</i>	1.041392685	1.322219295	1.176091259	1.707570176
<i>Ccl19</i>	0	0	0	0
<i>Ccl20</i>	0.301029996	0.602059991	0.602059991	0.903089987
<i>Ccl21a</i>	0	0	0	0
<i>Ccl21b</i>	0	0	0	0.301029996
<i>Ccl21d</i>	0	0	0	0
<i>Ccl22</i>	1.146128036	1.414973348	2.037426498	2.387389826
<i>Ccl24</i>	2.918554531	2.687528961	3.087426457	3.017033339
<i>Ccl25</i>	1.886490725	1.903089987	1.748188027	1.838849091
<i>Ccl27a</i>	1.973127854	2.037426498	1.707570176	1.919078092
<i>Ccl26</i>	0.301029996	0	0	0
<i>Ccl27b</i>	0.301029996	0	0	0
<i>Ccl28</i>	0.301029996	0.602059991	0.602059991	0
<i>Cxcl1</i>	1.799340549	1.934498451	1.875061263	1.903089987
<i>Cxcl2</i>	1.875061263	1.77815125	2.170261715	2.523746467
<i>Cxcl3</i>	3.118264726	3.155943018	2.953276337	3.117602692
<i>Cxcl5</i>	2.536558443	2.537819095	2.401400541	2.463892989
<i>Cxcl9</i>	2.999565488	3.071882007	4.078746734	4.033463966
<i>Cxcl10</i>	2.64246452	2.688419822	3.604657972	3.509605705
<i>Cxcl11</i>	0.903089987	1.113943352	1.544068044	1.875061263
<i>Cxcl12</i>	1.763427994	2.51054501	3.123198075	3.2219356
<i>Cxcl13</i>	0.301029996	0.698970004	1.322219295	1.857332496
<i>Cxcl14</i>	1.146128036	2.11058971	2.841984805	2.98721923
<i>Cxcl15</i>	3.36604921	3.111934276	2.843855423	2.681241237
<i>Cxcl16</i>	2.545307116	2.624282096	3.369401414	3.263399331
<i>Cxcl17</i>	2.167317335	2.029383778	2.123851641	2.238046103
<i>Cx3cl1</i>	2.869818208	3.009450896	3.380934463	3.194236749