Materials and methods

Cell lines and reagents

Human HCC cell lines (Huh7, HCCLM3, MHCC97H, PLC/PRF/5), THP1 cells and 293T cells were obtained from the American Type Culture Collection (Manassas, VA, USA). Cells were cultured using Dulbecco's modified Eagle's medium (DMEM) or RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), penicillin (100 μg/ml), and streptomycin (100 μg/ml), and were incubated in a humidified environment at 37°C with 5% CO2. No mycoplasma contamination was detected in all cells and the authenticity of all cell lines was verified by genomic short tandem repeat profiling. The compound PMA(P1585) was purchased from Sigma-Aldrich (StLouis, MO, USA). Oleic acid(A600868) was obtained from Sangon Biotech (Shang Hai). DMEM, RPMI 1640, FBS, and trypsin were purchased from gibico (USA).

ELISA

We measured WWOX levels in serum from HCC patients using the Human WWOX ELISA Kit (Biorbyt, orb1146837). Briefly，standards or samples are added to the appropriate microtiter plate wells then with a biotin-conjugated antibody specific to WWOX. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain WWOX, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm ± 10nm.

Flow cytometry analysis

Firstly, human HCC cells were co-cultured with THP-1 cells, and the co-cultured THP-1 cells were taken and resuspended with 100 μl PBS to make a single cell suspension. Single cell suspensions were counted and 1×106 were aspirated into a Test Tube. Add an appropriate amount of Human TruStain FcXTM blocking antibody,

mix well and leave on ice for 10min. Antibodies against CD68 and CD206 were continued to be added, mixed and incubated for 30 min at 4°C, protected from light. After centrifugation at 3000 rpm for 5 min, the cells were resuspended in 400 μl PBS and detected by flow cytometry (FACS Aria II, BD, USA).

Immunofluorescence staining and confocal microscopy

The appropriate number of cells were inoculated in a glass bottom cell culture dish (Biosharp, BS-20-GJM) and left to stand for 24h. Cells were fixed with 4% paraformaldehyde for 20 min and membranes were broken with 0.3% Triton-100. After being blocked with 5% goat serum for 1h, primary antibodies (WWOX, 1:100, M01223, Boster; NME2, 1:100, AF0222, Affinity) were added according to the instructions and incubated at 4°C overnight. The next day, cells were incubated with Alexa Fluor 488 (ab150113, Abcam, USA) coupled goat anti-mouse IgG or Alexa Fluor 594 (ab150080, Abcam, USA) coupled goat anti-rabbit IgG for 1 hour. Finally, nuclei were stained with DAPI (C1006, Beyotime) and images were captured under a confocal fluorescence microscope (Leica, Germany).

Dual-luciferase reporter assay

The SCD5 promoter region was cloned into a luciferase reporter vector. 293T cells were inoculated into 96-well plates and left to stand for 24h. The pGL3.0 vector, SCD5 promoter reporter plasmid and transcription factor NME2 plasmid were then co-transfected into the cells. 48 h later, the cells were collected, and luciferase activity was assayed using a luciferase reporter kit (Promega, E2920) according to the instructions.

More details on the materials and methods used are described in the Supplementary data.

Plasmids construction and transfection

For plasmids construction, PCR-amplified cDNA sequence of human WWOX, NME2 and SCD5 with an N-terminal tag were subcloned into pcDNA3.1(+) vectors based on

the manufacture's protocol. The lysine mutations at 31 of NME2 was created using a MultiS Fast Mutagenesis kit V2 (Vazyme, China). The lentivirus vector for WWOX knockdown and overexpression were purchased from Zorin Co., Shanghai, China. Stable cell lines were selected using 2μg/ml puromycin after transfection with 5μg/ml polybrene for 48h.

Immunohistochemistry (IHC) and Multiplexed Immunofluorescence staining

Immunohistochemical staining was performed using our tissue microarray containing 176 patients with HCC. Briefly, the microarray was dewaxed, hydrated, and antigenically repaired using EDTA buffer. Blocked for 30 min using goat serum, and subsequent incubation with primary antibody and HRP-conjugated secondary antibody. Analysis of staining results was carried out independently by 2 experienced pathologists. IHC results were interpreted as previously described(1).

mIF staining was performed with the HCC tissue specimens. We used anti-WWOX, anti-CD68 and anti-CD8 monoclonal antibodies as the primary antibodies, where were incubated with the sections at 4 °C overnight. Next, the specimens were incubated with CY3 (B0059), CY5 (B0060) and FITC (B0061) for 10 min. Finally, the specimens were incubated with DAPI (B0025) for 10 min, and then, the specimens were treated with anti-fluorescence quenching agent. The cells were observed with a fluorescence microscope (Olympus Japan). The number of tumor-infiltrating immune cells per high-power field (HPF, 10×40) of three replicates were counted independently HPF.

RNA extraction and quantitative real-time PCR (qRT-PCR)

Total RNA was extracted using TRIzol and reverse transcribed to cDNA using the Hifair III 1st Strand cDAN Synthesis kit (Yeasen, 11141ES60) according to the instructions. Real-time PCR assay was performed using qPCR SYBR Green Master Mix (Yeasen, 11202ES03). actin was used as an internal reference. The primers specific to each gene are shown in Supplementary Table 5.

Western blot

Cells were lysed in RIPA (Beyotime, P0013B) lysis buffer, and the protein concentration of cell lysate was determined by BCA protein assay (Thermo Scientific, 23227). Equal amounts of quantified proteins were loaded onto 8-15% SDS-PAGE gels and transferred to PVDF membranes (Millippore, IPVH00010). 5% nonfat milk (Sangon, A600669) was blocked for 1 hour at room temperature. Western blotting was performed using primary and secondary antibodies conjugated to HRP. Antibodies used for immunoblotting were as in supplementary Table 6.

Cytometry by time-of-flight (CyTOF)

Fresh HCC tissues were treated into single-cell suspension using the Tumor Dissociation Kit (Cat.130-096-730; Miltenyi Biotec). Immediately following this, filtration was performed using a 70 μm filter, followed by centrifugation and lysis of the erythrocytes. Incubating for 30 min with a panel of metal-conjugated antibodies and permeabilized for intracellular marker staining. Subsequently, signal detection was performed in the Helios3 CyTOF system by PLTTech (Hangzhou, China). The antibodies used are listed in supplementary Table 7, and the cluster annotation are listed in supplementary Table 8.

For data analysis, the results were normalized as before(2). Briefly, the CyTOF data was then normalized and analyzed on the Cytobank platform. Unsupervised clustering and t-SNE dimensionality reduction were performed based on the expression profiles of these markers using the 'cytofkit' package in R software to identify cell types.

DNA pull-down assay

The DNA pull-down kit (F188705, FITGENE) was used for this experiment. Biotin-labeled and unlabeled SCD5 promoter probes were first prepared. 2×10^7 human HCC cells were taken, 400 μl of lysis buffer was added and placed on ice for 30 min. centrifugation was performed at 12000 g for 15 min at 4°C, and the supernatant was collected into a new EP tube. Prepare magnetic beads according to the instructions, add 3μg of DNA probe into the prepared magnetic beads, incubate at

room temperature for 30min on a mixer, remove the supernatant, resuspend the magnetic beads and add an equal amount of total protein, incubate at 4℃ for 2h on the mixer, remove the supernatant and rinse twice with rinsing solution. After 15 min of elution, the supernatant was collected and used for subsequent experiments.

Co-culture and Transwell assay

Appropriate amount of THP-1 cells were inoculated at the bottom of 6-well plate and continued to be cultured with RPMI 1640 containing PMA (100ng/ml) for 48h. Take the appropriate Transwell chamber (37006, SPL life science) and inoculate the appropriate amount of human HCC cells in the chamber. Place the chamber on top of the 6-well plate inoculated with THP-1 cells, and continue to incubate with RPMI 1640 medium for 12-24h, and take the co-cultured THP-1 cells for subsequent experiments.

Appropriate amount of THP-1 cells was inoculated at the bottom of 6-well plate and incubation was continued with RPMI 1640 containing PMA (100ng/ml) for 48 h. After trypsin digestion, THP-1 cells were inoculated into Transwell chambers (36224, SPL life science). Inoculate appropriate amount of human hepatocellular carcinoma cells at the bottom of the 24-well plate, place the inoculated Transwell chambers in the 24-well plate, and continue to culture for 48-72h. The cells were fixed with 4% paraformaldehyde for 20 min, continued to be stained with 1% crystal violet for 20 min, rinsed with water and wiped off the surface of the filter membrane with a cotton swab. After sufficient drying, images were captured using an inverted microscope (IX71, Olympus, JAPAN).

Nuclear/cytoplasmic protein extraction assay

Separation of cytoplasm/nuclei was performed using the NE-PER^{TM} Nuclear and Cytoplasmic Extraction Reagents (78833, Thermo). Briefly, human HCC cells were inoculated into 10-cm dishes, and when the cells were about 90% full, PBS was rinsed, cytoplasmic extraction buffer was added, and the cells were allowed to stand on ice for 10 min, and then centrifuged at 12,000 rpm for 10 min at 4°C. Transfer the

supernatant (cytoplasmic fraction) to a new EP tube. Resuspend the cellular precipitates by vortexing using cell nucleus extraction buffer and then leave on ice for 10 min ×4 times. Centrifuge at 12000 rpm for 10 min at 4°C. Transfer the supernatant (nucleus fraction) to a new EP tube. The cytoplasmic and nuclear fractions can be placed at -80°C for further use.

Animal experiments

Six-week-old male humanized huPBMC-NOG-MHC I/II-2 KO mice were purchased from Charles River Laboratories, China and were housed in the SPF Laboratory Animal Center, Zhongshan Hospital, Fudan University, China. Prior to the experiment, all animals had free access to sterile food and water and were habituated for 7 days. Regarding humanized mice, briefly, $5\text{-}6\times10^6$ huPBMC were injected into NOG-MHCI/II-2 KO mice via tail vein. Subsequent experiments can be performed after 2-4 weeks when reconstitution of the human immune system is complete. Mice were injected subcutaneously with Huh7-shWWOX or control cells (5×10^6 cells in 100 μl PBS) near the axilla. After four weeks, the mice were euthanized and the removed subcutaneous tumors were used to construct a humanized orthotopic HCC mouse model. For antibody intervention, the PD-1 antibody treatment regimen was that mice were injected intraperitoneally with 200 μg of anti-human PD-1 antibody (Cat. BE0188; Bio X Cell) or IgG control twice a week for 2 weeks. For the removal of macrophages, Clophosome (FormuMax, 200μl/mouse) were injected intraperitoneally every five days for three times.

For further drug sensitivity experiments, Huh7 cell-derived humanized orthotopic HCC mouse models were first constructed. One week later, the mice were divided into 4 groups according to the experimental purpose. Among them, the PD-1 antibody treatment regimen was that mice were injected intraperitoneally with 200 μg of anti-human PD-1 antibody (Cat. BE0188; Bio X Cell) or IgG control twice a week for 2 weeks. For SCD5 blockage, mice were injected intraperitoneally with 5 mg/kg SCD5 inhibitor SCD1/5-IN-1 (Cat.HY-155403; MedChemExpress) or vehicle control daily for 10 days. Similarly, four weeks after the humanized orthotopic HCC mouse

model construction, the mice were euthanized, and the tumors and lung tissues were removed for subsequent experiments. All procedures were performed in strict accordance with the recommendations and guidelines of the Animal Care and Ethics Committee of Zhongshan Hospital, Fudan University.

Statistical analysis

Quantitative variables were presented as the mean ± SD and categorical variables as n(%). Group comparisons of quantitative data were tested by the Student's t test or one way ANOVA with a post hoc LSD test. Kaplan-Meier method and log-rank test were used for survival analysis. IBM SPSS Statistics 22.0 software was used with two-tailed P values < 0.05 as statistically significant.

REFERENCE

1. Zhou C, Liu C, Liu W, et al. SLFN11 inhibits hepatocellular carcinoma tumorigenesis and metastasis by targeting RPS4X via mTOR pathway. Theranostics 2020;10:4627-4643.
2. Finck R, Simonds EF, Jager A, et al. Normalization of mass cytometry data with bead standards. Cytometry A 2013;83:483-494.

Variables	Non-responder	Responder
Total	4	4
Age(years)		
≤55/>55	1/3	2/2
CA199		
≤34/>34U/L	3/1	3/1

Supplemental Tables

Supplemental Table 1. Clinicopathological characterization of HCC samples undergoing next-generation sequencing

DBIL		
≤6.8/>6.8ng/mL	3/1	4/0
TBIL		
≤20.4/>20.4ng/mL	4/0	3/1
AFP		
≤20/>20ng/mL	1/3	2/2
GGT		
≤60/>60U/L	0/4	1/3
ALT		
≤50/>50U/L	1/3	2/2
AST		
≤40/>40U/L	2/2	4/0
Tumor size		
≤5/>5cm	1/3	1/3
Cirrhosis		
No/yes	1/3	1/3
HBsAg		
Negative/Positive	2/2	1/3

Supplemental Table 2. Relationships between ICIs response and clinicopathological characteristics in the Zhongshan cohort

Variables	Responder	Non-responder	P value
Total	28	29	
Age(years)			0.707
≤55/>55	11/17	10/19	
CA199			0.410
≤34/>34U/L	22/6	20/9	
DBIL			0.516
≤6.8/>6.8ng/mL	15/13	18/11	
TBIL			0.470
≤20.4/>20.4ng/mL	19/9	17/12	
AFP			0.705
≤20/>20ng/mL	12/16	11/18	
Albumin			0.284
≤45/>45g/L	21/7	25/4	
GGT			0.707
≤60/>60U/L	9/19	8/21	
ALT			0.896
≤50/>50U/L	14/14	15/14	
AST			0.881
≤40/>40U/L	16/12	16/13	
Tumor size			**0.022**
≤5/>5cm	15/13	7/22	
Cirrhosis			0.707
No/yes	17/11	19/10	
BCLC			0.703
A/B/C	6/6/16	5/9/15	
PVTT			0.681
No/yes	16/12	15/14	
HBsAg			0.227
Negative/Positive	12/16	8/21	
WWOX expression			**<0.01**
Low/high	6/23	22/6	

Supplemental Table 3 Relationships between WWOX and clinicopathological characteristics in the Zhongshan cohort

Variables	Total	WWOX low	WWOX high	χ2	*P*
Age				0.269	0.604
≤50	76(43.2)	41(53.9)	35(46.1)		
>50	100(56.8)	50(50.0)	50(50.0)		
AFP				9.018	0.003
≤20	65(36.9)	24(36.9)	41(63.1)		
>20	111(63.1)	67(60.4)	44(39.6)		
CEA				0.018	0.894
≤5	162(92.0)	84(51.9)	78(48.1)		
>5	14(8.0)	7(50.0)	7(50.0)		
CA199				2.298	0.130
≤36	134(76.1)	65(48.5)	69(51.5)		
>36	42(23.9)	26(61.9)	16(38.1)		
Cirrhosis				4.124	0.042
No	29(16.5)	10(34.5)	19(65.5)		
Yes	147(83.5)	81(55.1)	66(44.9)		
Tumor size				1.421	0.233
≤5	85(48.3)	40(47.1)	45(52.9)		
>5	91(51.7)	51(56.0)	40(44.0)		
Differentiation				10.550	0.001
I/II	111(63.1)	47(42.3)	64(57.7)		
III/IV	65(36.9)	44(67.7)	21(32.3)		
Child grade				1.421	0.233
A	166(94.3)	84(50.6)	82(49.4)		
B/C	10(5.7)	7(70.0)	3(30.0)		
MVI				10.627	0.001
Negative	100(56.8)	41(41.0)	59(59.0)		
Positive	76(43.2)	50(54.8)	26(34.2)		
BCLC stage				5.801	0.016
0/A	87(49.4)	37(42.5)	50(57.5)		
B/C	89(50.6)	54(60.7)	35(39.3)		
ALT				0.051	0.821
≤40	102(58.0)	52(51.0)	50(49.0)		
>40	74(42.0)	39(52.7)	35(47.3)		
AST				1.850	0.174
≤37	124(70.5)	60(48.4)	64(51.6)		
>37	52(29.5)	31(59.6)	21(40.4)		
HBsAg				0.000	0.998
Negative	29(16.5)	15(51.7)	14(48.3)		
Positive	147(83.5)	76(51.7)	71(48.3)		

Continued

Supplemental Table 3 Relationships between WWOX and clinicopathological characteristics in the Zhongshan cohort

Variables	Total	WWOX low	WWOX high	χ2	*P*
CD68					<0.001
low	73(41.5)	26(35.6)	47(64.4)		
high	103(58.5)	65(63.1)	38(36.9)		
CD206					0.021
low	96(54.5)	42(43.8)	54(56.2)		
high	80(45.5)	49(61.3)	31(38.7)		

Supplemental Table 4 Univariate and multivariate analysis of clinicopathological characteristics with overall survival in the Zhongshan cohort

Variables	Univariate *P* value	Multivariate HR(95%CI)	Multivariate *P* value
Age ≤50/>50	0.643		
AFP ≤20/>20	**0.005**	1.362(0.825-2.249)	0.228
CEA ≤5/>5	0.559		
CA199 ≤36/>36	**0.044**	1.443(0.901-2.310)	0.127
Cirrhosis No/Yes	**<0.001**	0.683(0.364-1.283)	0.236
Tumor size ≤5/>5	**<0.001**	2.133(1.356-3.356)	**0.01**
Differentiation I/II/III/IV	**0.035**	1.420(0.898-2.245)	0.134
Child grade A/B/C	0.051		
MVI Negative/Positive	**0.024**	1.057(0.668-1.672)	0.812
BCLC stage 0/A/B/C	0.139		
ALT ≤40/>40	0.322		
AST ≤37/>37	**0.003**	1.419(0.900-2.239)	0.132
HBsAg Negative/Positive	0.203		
WWOX+CD68 $WWOX^{high}CD68^{low}$/ $WWOX^{low}CD68^{high}$	**<0.001**	1.594(0.694-3.664)	0.270
WWOX+CD206 $WWOX^{high}CD206^{low}$/ $WWOX^{low}CD206^{high}$	**<0.001**	2.345(1.124-4.891)	**0.023**

Supplemental Table 5. Sequences of primers (5'-3') used for qRT-PCR.

Genes		Sequences (5'-3')
Human ACTB	FORWARD	GGACCTGACTGACTACCTCAT
Human ACTB	REVERSE	CGTAGCACAGCTTCTCCTTAAT
Human WWOX	FORWARD	ATGTACTCCAACATTCATCGCAG
Human WWOX	REVERSE	GTCTCTTCGCTCTGAGCTTCT
Human SCD1	FORWARD	GGTGATGTTCCAGAGGAGGTACT
Human SCD1	REVERSE	TCGCAAGAAAGTGGCAACGA
Human SCD5	FORWARD	CTTGGCCTCTATTCTCCGCTA
Human SCD5	REVERSE	ATTATGGAAGCCTTCACCAATGGC
Human NME2	FORWARD	CCACCTCTTATTCATAGACCCA
Human NME2	REVERSE	AGATTCAAAGCCAGGCACCAT
Human CD206	FORWARD	ATTCAGATATGCCAGGGCGA
Human CD206	REVERSE	ATTTGGGTTCGGGAGTCGTC
Human ARG1	FORWARD	GTGGAAACTTGCATGGACAAC
Human ARG1	REVERSE	AATCCTGGCACATCGGGAATC
Human CD163	FORWARD	TTTGTCAACTTGAGTCCCTTCAC
Human CD163	REVERSE	TCCCGCTACACTTGTTTTCAC
Human IL10	FORWARD	TCAAGGCGCATGTGAACTCC
Human IL10	REVERSE	GATGTCAAACTCACTCATGGCT
Human TGF-β	FORWARD	GGCCAGATCCTGTCCAAGC
Human TGF-β	REVERSE	GTGGGTTTCCACCATTAGCAC

Supplemental Table 6. Primary antibodies used in the study

Antibody	Concentration	Application	Identifier	Company
Anti-human WWOX	1:1000/1:200	Western blot/CoIP	Ab238144	Abcam
Anti-human SCD5	1:1000	Western blot	PA5-89006	Invitrogen
Anti-α-Tubulin	1:1000	Western blot	AC007	ABclonal
Anti-human NME2	1:500/1:100	Western blot/CoIP	Sc-166937	Santa cruz
APC anti-human CD68	5μl/million cells	Flow cytometry	333810	Biolegend
PE/Cyanine7 anti-human CD206 (MMR)	5μl/million cells	Flow cytometry	321124	Biolegend
Anti-human SCD1	1:1000	Western blot	Ab236868	Abcam
Anti-human KAT1	1:1000/1:200	Western blot/CoIP	Sc-390562	Santa cruz
Anti-human HA	1:1000/1:200	Western blot/CoIP	30702ES60	Yeasen
Anti-human GFP	1:1000/1:200	Western blot/CoIP	31001ES50	Yeasen
Anti-human Lamin B1	1:1000	Western blot	12987-1-AP	Proteintech
Human TruStain FcX™	1:1000	Flow cytometry	422301	Biolegend
Human WWOX elisa kit		ELISA	Orb1146837	Biorbyt

Supplemental Table 7. 42 antibodies used in the CyTOF assay

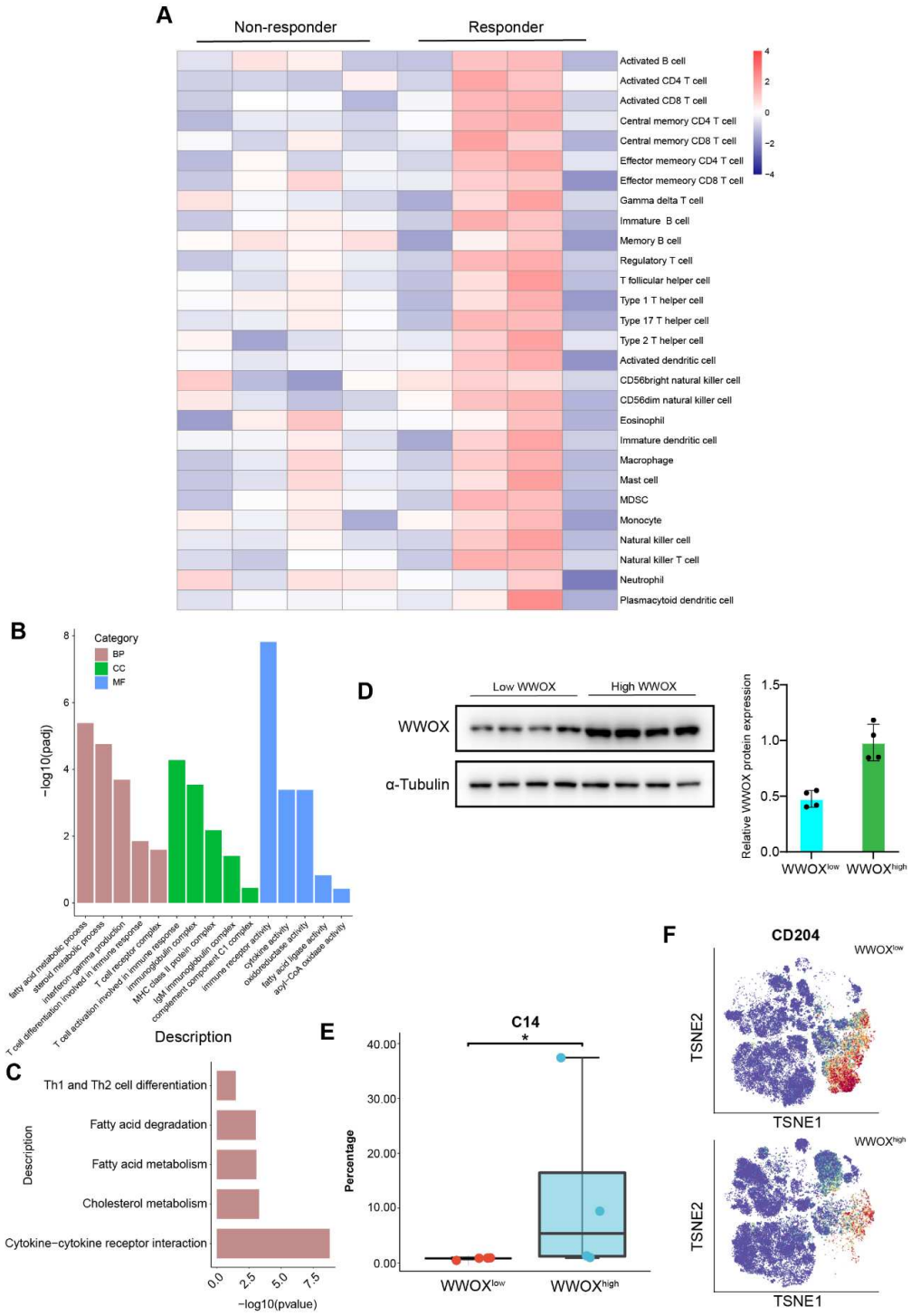
Label	Marker	Clone	Manufacturer
89Y	CD45	HI30	BioLegend
115In	CD3	UCHT1	Bio Cell
139La	Ki-67	SolA15	eBioscience
141Pr	CD56	NCAM16.2	BD
142Nd	TCR γ/δ	5A6.E9	ThermoFisher
143Nd	CD274(B7-H1,PD-L1)	29E.2A3	BioLegend
144Nd	CD38	HIT2	BioLegend
145Nd	CD103	B-Ly7	eBioscience
146Nd	CD197(CCR7)	G043H7	BioLegend
147Sm	CD366(Tim-3)	F38-2E2	BioLegend
148Nd	TIGIT(VSTM3)	A15153G	BioLegend
149Sm	CD19	HIB19	BioLegend
150Nd	CD192(CCR2)	K036C2	BioLegend
151Eu	CD107a(LAMP-1)	H4A3	BioLegend
152Sm	CD27	O323	BioLegend
153Eu	CD204(SR-AI)	351615	R&D
154Sm	CD152(CTLA-4)	14D3	eBioscience
155Gd	CD279(PD-1)	EH12.2H7	BioLegend
156Gd	CD88(C5aR)	S5/1	BioLegend
157Gd	CD206(MMR)	15-2	BioLegend
158Gd	CD80(B7-1)	2D10.4	eBioscience
159Tb	CD11c	BU15	BioLegend
160Gd	CD25(IL-2Rα)	24212	R&D
161Dy	CD163	GHI/61	BioLegend
162Dy	CD169(Siglec-1)	7-239	BioLegend

163Dy	Galectin-9	9M1-3	BioLegend
164Dy	CD68	Y1/82A	BioLegend
165Ho	CD66b	G10F5	BioLegend
166Er	PPT1(C-term)	1117CT11.2.1.4	Abcepta
167Er	Granzyme B Recombinant	QA16A02	BioLegend
168Er	CD45RO	UCHL1	BioLegend
169Tm	CD47	CC2C6	BioLegend
170Er	CD172a(SIRPα)	15-414	BioLegend
171Yb	CD86	Fun-1	BD
172Yb	FOXP3	PCH101	eBioscience
173Yb	TREM-1	193015	R&D
174Yb	CD14	M5E2	BioLegend
175Lu	CD16	3G8	BioLegend
176Yb	HLA-DR	L243	BioLegend
197Au	CD4	RPA-T4	BioLegend
198Pt	CD8a	RPA-T8	BioLegend
209Bi	CD11b	M1/70	BioLegend

Supplemental Table 8. Cluster annotation in the CyTOF assay

Cluster	Annotation	Cluster	Annotation
C01	NK cell	C17	Macrophage
C02	$CD4^+$T cell	C18	Macrophage
C03	$CD4^+$T cell	C19	Macrophage
C04	$CD4^+$T cell	C20	Macrophage
C05	$CD4^+$T cell	C21	Macrophage
C06	γ/δ T cell	C22	Neutrophil
C07	$CD8^+$T cell	C23	Eosinophil
C08	$CD4^+$T cell	C24	Eosinophil

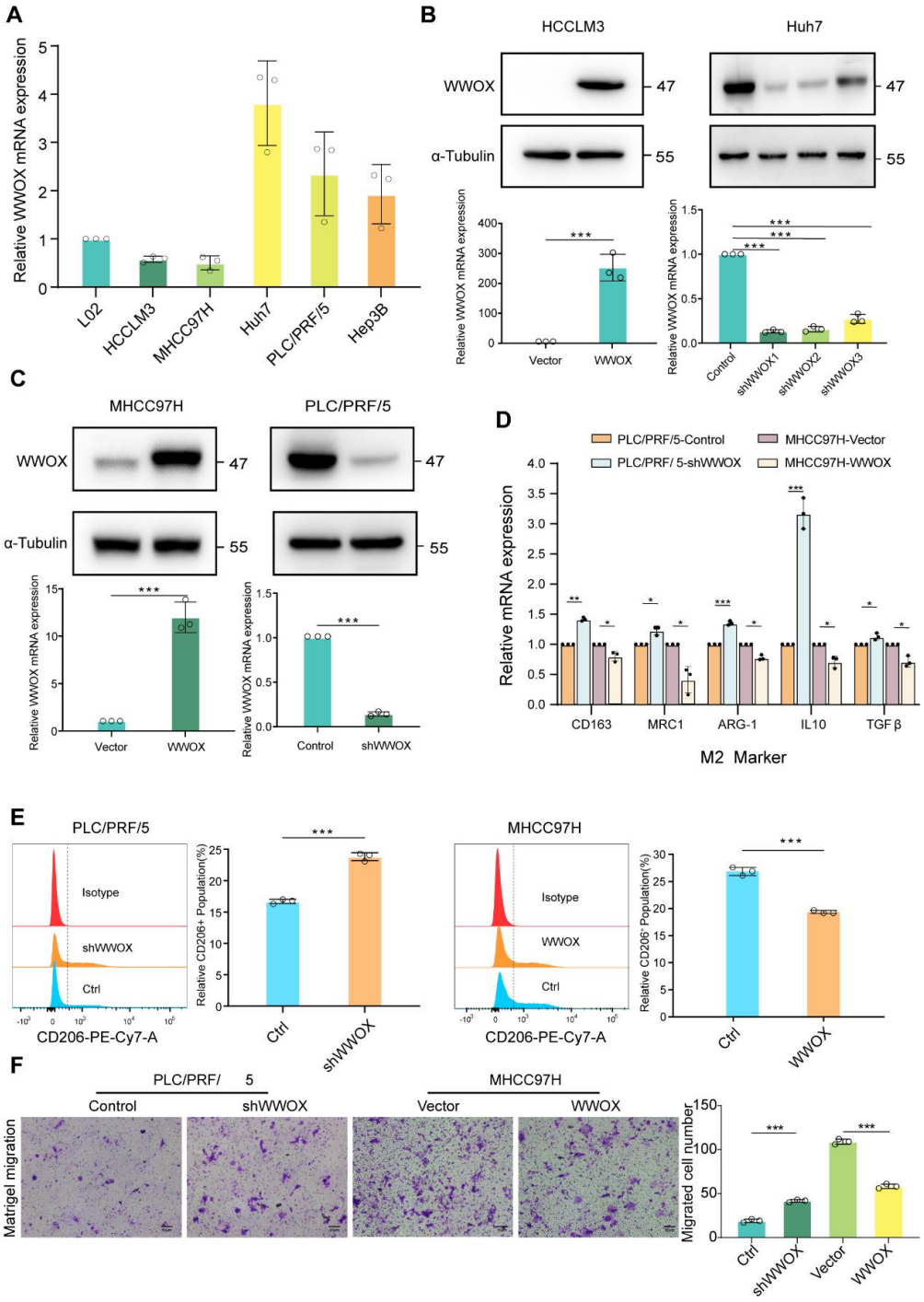
C09	$CD8^+$T cell	C25	Neutrophil
C10	$CD8^+$T cell	C26	Plasma cell
C11	DNT	C27	NK cell
C12	$CD8^+$T cell	C28	NK cell
C13	$CD8^+$T cell	C29	NK cell
C14	$CD8^+$T cell	C30	B cell
C15	B cell	C31	Other
C16	B cell	C32	Macrophage



Supplementary Figure 1 WWOX affects the response of HCC to ICIs and induces suppressive TIME after deficiency

(A) Heatmap of tumor immunoreactivity score calculated by ssGSEA for 8 cases. (B, C) GO and KEGG analysis of differentially expressed genes in two groups of tumor samples. (D) Western blot analysis of WWOX protein levels in tumor tissues of 8

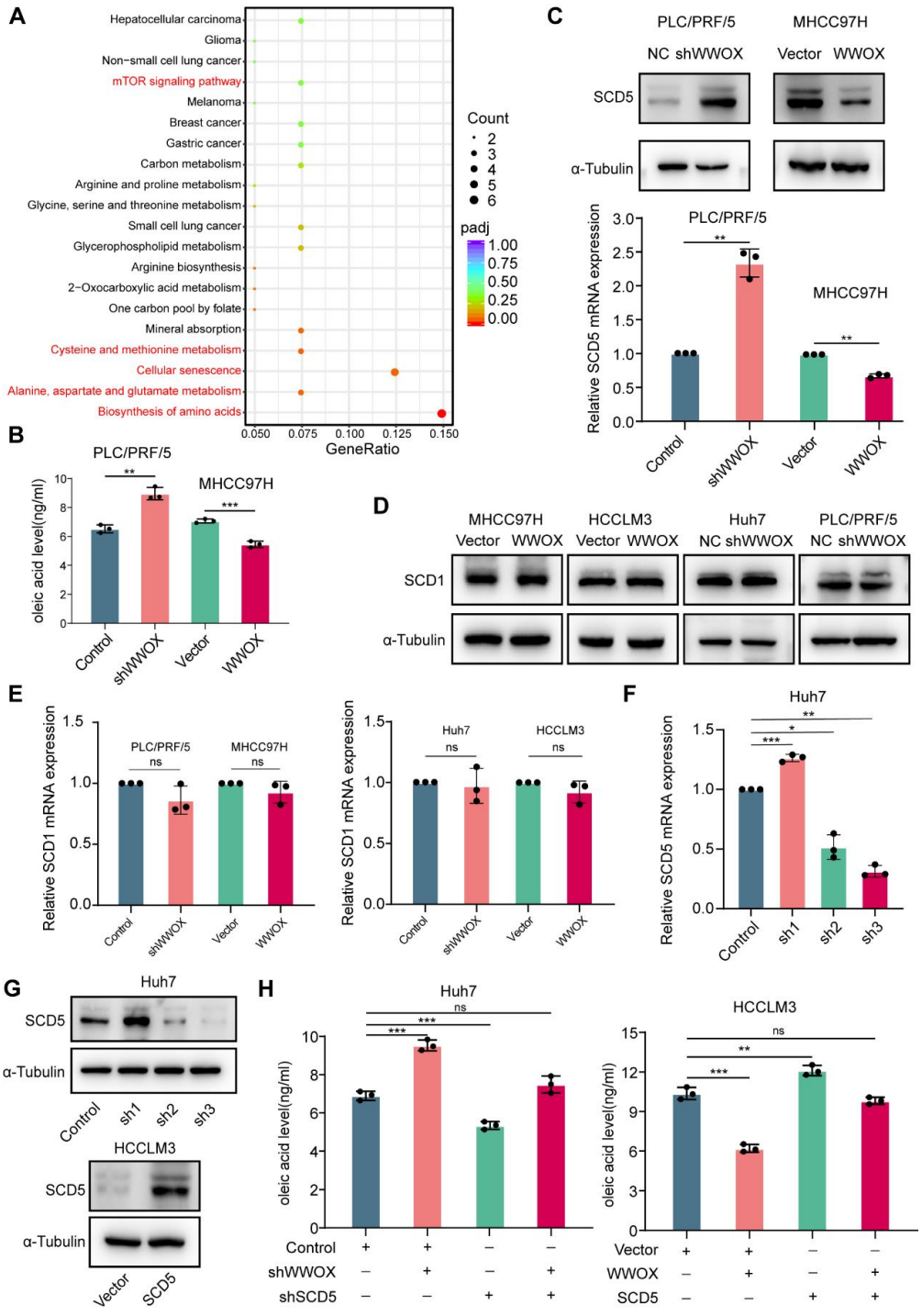
cases. (E) Distribution of $CD8^+$ T cell subset C14 infiltrated in two groups of tissues. (F) t-SNE plots of CD204 expression levels in the indicated groups. BP: Biological Process; CC: Cellular Component; MF: Molecular Function. WWOX, WW domain-containing oxidoreductase; TIME, tumor immune microenvironment; ssGSEA, single-sample gene set enrichment analysis; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.



Supplementary Figure 2 WWOX deficiency induces macrophage recruitment and immunosuppresive polarization

(A) qPCR analysis of mRNA levels of WWOX in HCC cell lines. (B) Western blot and qPCR analysis of protein and mRNA levels of WWOX after lentiviral WWOX transfection of HCCLM3 cells and lentiviral shWWOX transfection of Huh7 cells. (C) Western blot and qPCR analysis of protein and mRNA levels of WWOX after

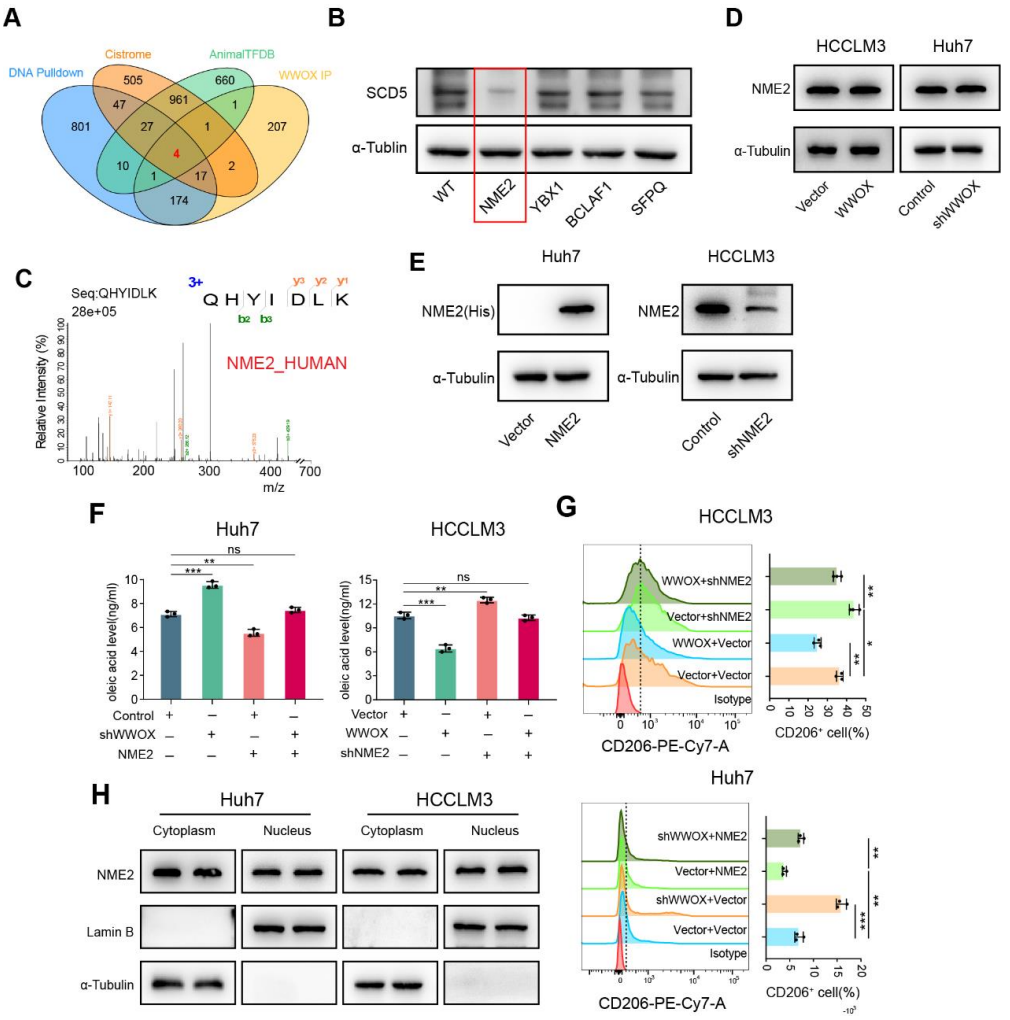
lentiviral WWOX transfection of MHCC97H cells and lentiviral shWWOX transfection of PLC/PRF/5 cells. (D) qPCR analysis of mRNA levels of CD163, MRC1, ARG1, IL-10 and TGF-β in macrophages (PMA treated) after co-culture with the indicated HCC. (E) Flow cytometry analysis of CD206 levels on the surface of macrophages (PMA treated) after co-culture with the indicated HCC. (F) Chemotaxis assay to analyze the effect of PLC/PRF/5-shWWOX and MHCC97H-WWOX cell supernatants on macrophage (PMA treated) migration. Scale bar, 50 μm. **P*<0.05, ***P*<0.01, and ****P*<0.001, Student's *t* test. WWOX, WW domain-containing oxidoreductase; qPCR, quantitative real-time PCR; HCC, hepatocellular carcinoma; PMA, phorbol-12-myristate-13-acetate.



Supplementary Figure 3 Oleic acid from HCC cells mediated by SCD5 promotes immunosuppresive macrophage polarization

(A) KEGG analysis of differential genes in OA-treated and untreated macrophages. (B) ELISA to detect OA content in the supernatants of the indicated HCC cells. (C)

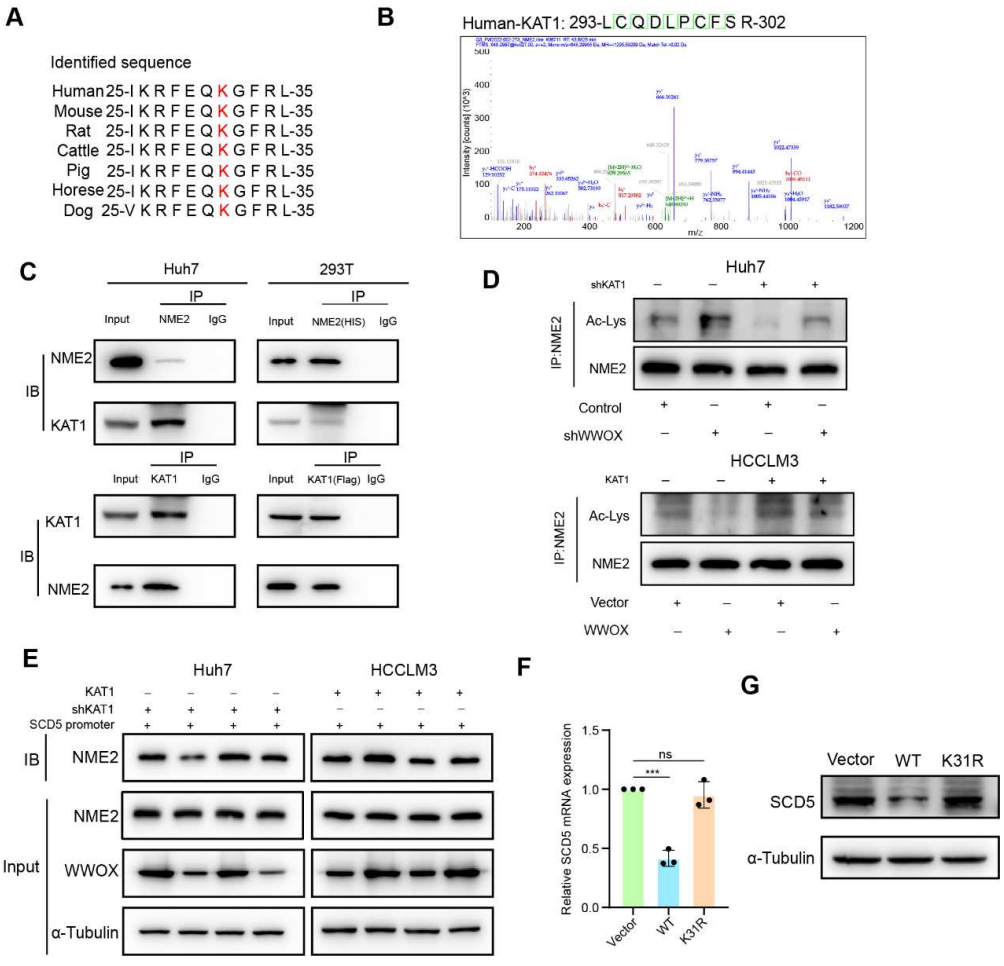
Western blot and qPCR analysis of protein and mRNA levels of SCD5 in the indicated HCC cells. (D-E) Western blot and qPCR analysis of protein and mRNA levels of SCD1 in the indicated HCC cells. (F) qPCR analysis of mRNA levels of SCD5 after lentiviral shSCD5 transfection of Huh7 cells. (G) Western blot analysis of protein levels of SCD5 after lentiviral SCD5 transfection of HCCLM3 cells and lentiviral shSCD5 transfection of Huh7 cells. (H) ELISA analysis of OA in supernatants of lentiviral shSCD5-transfected Huh7-shWWOX cells (Left) and supernatants of lentiviral SCD5-transfected HCCLM3-WWOX cells. **P*<0.05, ***P*<0.01, and ****P*<0.001, Student's *t* test or one-way ANOVA with a post hoc LSD test. HCC, hepatocellular carcinoma; SCD5, stearoyl-Coa desaturase 5; KEGG, Kyoto Encyclopedia of Genes and Genomes; OA, oleic acid; qPCR, quantitative real-time PCR; SCD1, stearoyl-Coa desaturase 1; WWOX, WW domain-containing oxidoreductase.



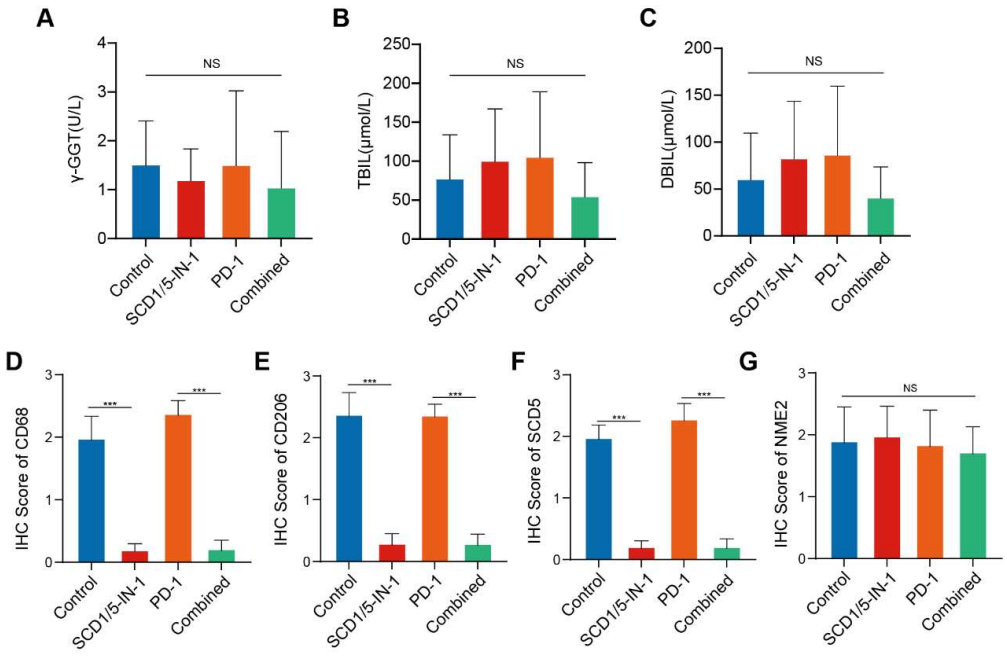
Supplementary Figure 4 WWOX directly binds NME2 and inhibits transcription

of SCD5

(A) Venn diagram of SCD5 transcription factor prediction and mass spectrometry analysis. (B) Western blot analysis of SCD5 protein levels after overexpression of four transcription factors in huh7 cells. (C) Secondary mapping of mass spectrometry peptides of NME2. (D) Western blot analysis of NME2 protein levels in the indicated HCC cells. (E) Western blot analysis of NME2 protein levels after lentiviral NME2 and lentiviral shNME2 transfection of the indicated HCC cells. (F) ELISA analysis of OA content in supernatants of lentiviral NME2-transfected Huh7-shWWOX cells (Left) and supernatants of lentiviral shNME2-transfected HCCLM3-WWOX cells. (G) (Left): Flow cytometry analysis of CD206 levels in macrophages co-cultured with MHCCLM3-WWOX cells transfected with lentivirus-shNME2; (Right): Flow cytometry analysis of CD206 levels in macrophages co-cultured with Huh7-shWOX cells transfected with lentivirus-NME2. (H) Western blot analysis of the effect of WWOX on NME2 nuclear translocation in the indicated HCC cells. **P*<0.05, ***P*<0.01, and ****P*<0.001, One-way ANOVA with a post hoc LSD test. WWOX, WW domain-containing oxidoreductase; NME2, nucleoside diphosphate kinase 2; SCD5, stearoyl-Coa desaturase 5; HCC, hepatocellular carcinoma; OA, oleic acid; KAT1, histone acetyltransferase 1; IP, immunoprecipitation.



Supplementary Figure 5 WWOX binds NME2 in a competitive manner with KAT1 to inhibit the acetylation of NME2 at site 31 mediated by KAT1 (A) Comparison of NME2 acetylated residues characterized by mass spectrometry between different species. (B) Secondary mapping of mass spectrometry peptides of KAT1. (C) Co-IP assay to detect endogenous and exogenous interactions of NME2 with KAT1. (D) Co-IP analysis of NME2 acetylation levels after transfection of KAT1 and shKAT1 plasmids into Huh7-shWWOX and HCCLM3-WWOX cells. (E) A DNA pulldown assay was used to analyze the binding of NME2 to the SCD5 promoter after lentiviral transfection of the indicated HCC cells with KAT1 and shKAT1. (F, G) Vector, His-NME2 WT, and K31R were transfected into Huh7 cells, and SCD5 mRNA and protein levels were analyzed using qPCR and Western blotting.



Supplementary Figure 6 Pharmacological blockade the SCD5 signaling enhances the anti-PD-1 therapeutic effect of humanized WWOX^{low} tumor-bearing mice. (A-C) Effects of blockade of SCD5 combined with anti-PD-1 treatment on liver function in orthotopic HCC tumor-bearing humanized mice. (D-G) IHC score of CD68, CD206, SCD5 and NME2 in orthotopic HCC tumor tissues from the indicated groups. **P*<0.05, ***P*<0.01, and ****P*<0.001, one-way ANOVA with a post hoc LSD test. SCD5, stearoyl-Coa desaturase 5; WWOX, WW domain-containing oxidoreductase; γ-GGT, γ-glutamyl transpeptidase; TBIL, total bilirubin; DBIL, direct bilirubin.