Supplementary information

Isolation of peritoneal macrophages

BALB/c mice were euthanized by cervical dislocation. 4 mL sterile PBS was injected into the peritoneal cavity, gently massaged, and withdrawn. Cells were collected, centrifuged at 1,400 rpm for 5 min, counted, and seeded at 5×10^5 cells per well in 12-well plates, yielding peritoneal macrophages.

Differentiation of macrophages from peripheral blood

Mouse peripheral blood was collected into EDTA-containing tubes and subjected to red blood cell lysis. Remaining cells were counted and seeded at 1.4×10^6 cells per well in 12-well plates. Cells were cultured in medium containing macrophage colony-stimulating factor (M-CSF, 20 ng/mL) for 5 days to generate M0 macrophages, followed by stimulation with 100 ng/mL LPS to induce M1 polarization.

Isolation of tumor-infiltrating and tissue-resident immune cells

Tumor tissues or adjacent normal intestinal tissues were excised, minced into small fragments using sterile scissors, and digested in 2 mL RPMI-1640 containing 0.1% collagenase (STEMCELL, 07247) and 0.01% DNase (Roche, 10104159001) at 37°C for 1 h with gentle agitation. Digestion was terminated by adding 4 mL RPMI-1640 supplemented with 1% heat-inactivated serum. Cell suspensions were filtered through a 70-μm cell strainer, centrifuged, and resuspended in PBS. After a second filtration through a 70-μm strainer, cells were counted. For flow cytometric staining, 3×10^6 cells per sample were used.

Isolation of $CD8^+$ T cells and MDSCs

C57BL/6 or BALB/c mice were euthanized, and spleens were harvested under sterile conditions. Splenocytes were mechanically dissociated using the rubber end of a 5 mL syringe and passed through a 70 μm cell strainer. Cells were centrifuged at 2,000 rpm for 5 min, and red blood cells were lysed using 2 mL red blood cell lysis buffer for 5 min at room temperature. Lysis was neutralized with an equal volume of PBS, followed by centrifugation at 2,000 rpm for 5 min. Cells were counted, and $CD8^+$ T cells were isolated using a $CD8^+$ T Cell Isolation Kit (STEMCELL Technologies, #19853) according to the manufacturer's instructions. Briefly, cells were resuspended in MACS buffer at a concentration of 1×10^8 cells/mL and incubated with Fc Blocker for 5 min at room temperature. The isolation cocktail was added at 50 μL/mL

and incubated for 10 min. Magnetic beads were vortexed for 30 s prior to use and added at 125 μL/mL, followed by incubation for 5 min at room temperature. Samples were transferred to flow cytometry tubes, adjusted to a final volume of 2.5 mL, and placed on a magnetic rack for 3 min. Magnetic separation was repeated three times. The supernatant containing untouched $CD8^+$ T cells was collected, centrifuged at 2,000 rpm for 5 min, and cells were counted and seeded at 2×10^5 cells per well in 96-well plates for downstream experiments.

The MDSC isolation procedure followed the same magnetic negative-selection strategy as described above for $CD8^+$ T cells. The primary difference lies in the antibody cocktail composition, which is specifically designed to deplete non-MDSC populations while enriching $CD11b^+Gr\text{-}1^+$cells.

Preparation of tumor cell-conditioned medium

Human or murine tumor cell lines were cultured in the appropriate growth medium. Cells at the logarithmic growth phase were detached and washed once with serum-free DMEM. Cells were seeded into culture dishes at approximately 60% confluence in DMEM supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% penicillin-streptomycin. After 48 h of culture, conditioned media were collected, filtered through a 0.22 μm filter, aliquoted, and stored at -80°C. Repeated freeze-thaw cycles were avoided.

SIRT1 enzymatic activity assay

SIRT1 enzymatic activity was measured using a commercial SIRT1 activity assay kit (Elabscience, E-BC-F056) according to the manufacturer's instructions.

Immunohistochemistry

Tumor tissues were fixed with 4% paraformaldehyde immediately after mice were sacrificed. Subsequent immunohistochemistry analysis was performed by Shanghai Zuocheng Biological Technology. Images of sections were captured with NanoZoomer S210 (Hamamatsu, Japan) and further analyzed using NDP. View 2 software.

Enzyme-linked immunosorbent assay (ELISA)

The quantitative detection kit for mouse IL-1α/IL-6 was purchased from ABclonal (RK00103/RK00008). The operation process was carried out according to the product instructions provided by the company. The concentrations of BDNF in tumor interstitial fluid or indicated cell samples were measured using ELISA kits (Multi Science, EK2127) according

to the manufacturer's instructions.

Reverse transcription and real-time qPCR (RT-qPCR)

Total RNA was extracted from cells (5×10^5) or tissues (0.2 mg of Tumor) using either the SteadyPure Quick RNA Extraction Kit (AG, AG21023) or TRIzol reagent. For cells, total RNA was extracted using the SteadyPure Quick RNA Extraction Kit according to the manufacturer's instructions. RNA was then used for reverse transcription with the All-in-One cDNA Synthesis SuperMix. Gene expression levels were quantified using the Bio-Rad CFX Connect system with a fast two-step amplification protocol and SYBR Green Premix Pro Tag HS qPCR Tracking Ki (Accurate Biology, AG11735). Expression values were normalized to β-actin. Primer sequences used for qPCR are listed as follows:

mCD38 F	TCCCTCCGTGAGCCATTTTAC
mCD38 R	CGATGTCGTGCATCACCCA
mCD73 F	AACCCCTTTCCTCTCAAATCCA
mCD73 R	CAGGGCGATGATCTTATTCACAT
mIDO1 F	GCTTTGCTCTACCACATCCAC
mIDO1 R	CAGGCGCTGTAACCTGTGT
mTDO2 F	ATGAGTGGGTGCCCGTTTG
mTDO2 R	GGCTCTGTTTACACCAGTTTGAG
mIL4I1 F	AACACTTGTTGGTGGAAACGA
mIL4I1 R	TCCTTGCGATTAGGAGTGGTC
mKMO F	ATGGCATCGTCTGATACTCAGG
mKMO R	CCCTAGCTTCGTACACATCAACT
mACMSD F	CTACCAAAGGAATGGCCCGAT
mACMSD R	GTGGAAAGAGCTTGGACTGTC
mKYNU F	GTCAAGCCTGCGTTAGTGG
mKYNU R	GGAGGGTTTGAAATTCGGAATCC
mQPRT F	CATCCTTGGTTACCGGGTCG
mQPRT R	GCCAGGGTGTTAAGAGCCA
mNADS1 F	AATATGCGGCTATGGATGTTGG
mNADS1 R	GGAGCGAATGCAGGAGAGT
mNAPRT F	AGGACTGTATGCGCTTTCTTC
mNAPRT R	CCAGAGCAATCAAGGGCTCG
mNMNAT1 F	TGGCTCTTTTAACCCCATCAC
mNMNAT1 R	TCTTCTTGTACGCATCACCGA
mNAMPT F	GCAGAAGCCGAGTTCAACATC
mNAMPT R	TTTTCACGGCATTCAAAGTAGGA
mNMRK1 F	GATGTCAGCAGTTTCCTGTTGG
mNMRK1 R	GCCATCGAAGTACCCTGGAG

mACTIN F	GGCTGTATTCCCCTCCATCG
mACTIN R	CCAGTTGGTAACAATGCCATGT
mIL-1α F	CGAAGACTACAGTTCTGCCATT
mIL-1α R	GACGTTTCAGAGGTTCTCAGAG
mIL-1β F	GAAATGCCACCTTTTGACAGTG
mIL-1β R	TGGATGCTCTCATCAGGACAG
mMMP12 F	CTGCTCCCATGAATGACAGTG
mMMP12 R	AGTTGCTTCTAGCCCAAAGAAC
mIL-6 F	ACCAGAGGAAATTTTCAATAGGC
mIL-6 R	TGATGCACTTGCAGAAAACA
mMMP13 F	CTTCTTCTTGTTGAGCTGGACTC
mMMP13 R	CTGTGGAGGTCACTGTAGACT
mP21 F	GTGGGTGTCAAAGCACTTAG
mP21 R	ACAGTCCAGACCAGGATGTTA
hCD73 F	GCCTGGGAGCTTACGATTTTG
hCD73 R	TAGTGCCCTGGTACTGGTCG
hACTIN F	CATGTACGTTGCTATCCAGGC
hACTIN R	CTCCTTAATGTCACGCACGAT

siRNA or shRNA knockdown

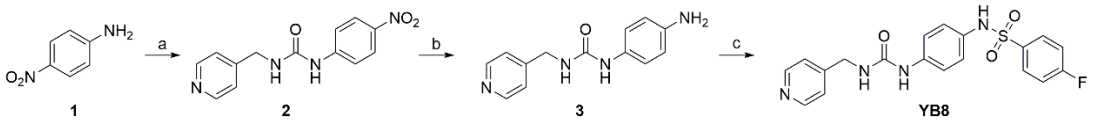
Mouse bone marrow-derived cells or human cell lines were seeded at 1 × 106 cells per well in 12-well plates and differentiated into M0 macrophages with M-CSF (20 ng/mL), with medium replacement every other day. On day 4, culture medium was removed and replaced with 300 μL Opti-MEM. For siRNA transfection, siRNA (40 nM) was diluted in 50 μL Opti-MEM, and RNAiMAX (2 μL) was diluted separately in 50 μL Opti-MEM. After 5 min incubation at room temperature, the two solutions were combined and incubated for an additional 15 min. The transfection mixture was added dropwise to the cells. After 4-6 h, medium was replaced with complete culture medium. Cells were harvested 48 h or 72 h post-transfection for analysis of mRNA and protein expression. siRNA/shRNA sequences are listed as follows:

siRNA: CD73 #1 forward	CCUCCUCAGAAACGUUAAATT
reverse	UUUAACGUUUCUGAGGAGGTT
CD73 #2 forward	GCCUCUAGCACAUCAGAUATT
reverse	UAUCUGAUGUGCUAGAGGCTT
mNAMPT #1 forward	CCACCUUAUCU UAGAGUCAUU
reverse	AAUGACUCUAAGAUAAGGUGG
mNAMPT #2 forward	GCCACCUUAUC UUAGAGUCAU

reverse	AUGACUCUAAGAUAAGGUGGC
hNAMPT #1 forward	GGCCAAAUAUU UGUUAGAATT
reverse	UUCUAACAAAUAUUUGGCCTT
hNAMPT #2 forward	GCAUCUUCCAA UAGAAAUATT
reverse	UAUUUCUAUUGGAAGAUGCTT
hNAMPT #3 forward	GCAGAACACAG UACCAUAATT
reverse	UUAUGGUACUGUGUUCUGCTT
shRNA: mBDNF #1 forward	GGUGAUGCUCAGCAGUCAATT
reverse	UUGACUGCUGAGCAUCACCTT
mBDNF #2 forward	GGCGAUUCAUAAGGAUAGATT
reverse	UCUAUCCUUAUGAAUCGCCT

Chemistry

Scheme: Synthesis of compound YB8



YB8 was synthesized through a three-step reaction. Reagents and conditions: (a) i) Triphosgene, Et3N, DCM, 0°C to room temperature, 1.5 h; ii) pyridin-4-ylmethanamine, room temperature, overnight. (b) H2, 10% Pd/C, MeOH, room temperature, overnight; (c) 4-fluorobenzenesufonyl chloride, DCM, Et3N, 0°C to room temperature, 5 h.

Synthesis of 1-(4-Nitrophenyl)-3-(pyridin-4-ylmethyl) urea (2)

Triphosgene (150 mg, 0.50 mmol) was dissolved in dry dichloromethane (DCM, 10 mL) and cooled to 0°C. A solution of 4-nitroaniline (207 mg, 1.5 mmol) and DIPEA (388 mg, 3.0 mmol) in DCM was added dropwise under stirring. The reaction mixture was stirred at 0°C for 30 min, then allowed to warm to room temperature and stirred for an additional 2 h. Reaction progress was monitored by TLC until completion. The solvent was removed under reduced pressure, and the residue was redissolved in dry DCM (10 mL). 4-(Aminomethyl) pyridine (162 mg, 1.5 mmol) was added dropwise, and the reaction was stirred at room temperature overnight. The mixture was concentrated in vacuo and purified by flash chromatography (DCM/MeOH, gradient elution from 50:1 to 33:1) to yield compound **2** as a white solid (170 mg, 42%). ^{1}H

NMR (600 MHz, DMSO-d_6) δ: 9.59 (s, 1H), 8.53 (d, J = 6.0 Hz, 2H), 8.15 (d, J = 9.2 Hz, 2H), 7.65 (d, J = 9.2 Hz, 2H), 7.35 (d, J = 6.2 Hz, 2H), 7.11 (t, J = 6.1 Hz, 1H), 4.38 (d, J = 6.1 Hz, 2H).

Synthesis of 1-(4-Aminophenyl)-3-(pyridin-4-ylmethyl) urea (3)

Compound 2 (0.54 g, 2.0 mmol) was dissolved in dry DCM (10 mL), and 10% Pd/C (50 mg) was added. The mixture was stirred under an atmosphere of H_2 (1 atm) at room temperature overnight, and reaction completion was confirmed by thin layer chromatography. The catalyst was removed by filtration through a pad of Celite and rinsed with MeOH. The combined filtrates were concentrated under reduced pressure, and the crude residue was purified by flash chromatography (DCM/MeOH, gradient elution from 33:1 to 25:1) to afford compound **3** as a pale yellow solid (456 mg, 94%). ^{1}H NMR (600 MHz, DMSO-d_6) δ: 8.49 (d, J = 6.1 Hz, 2H), 8.09 (s, 1H), 7.26 (d, J = 6.2 Hz, 2H), 7.01 (d, J = 8.6 Hz, 2H), 6.49 – 6.45 (m, 3H), 4.69 (s, 2H), 4.28 (d, J = 6.1 Hz, 2H).

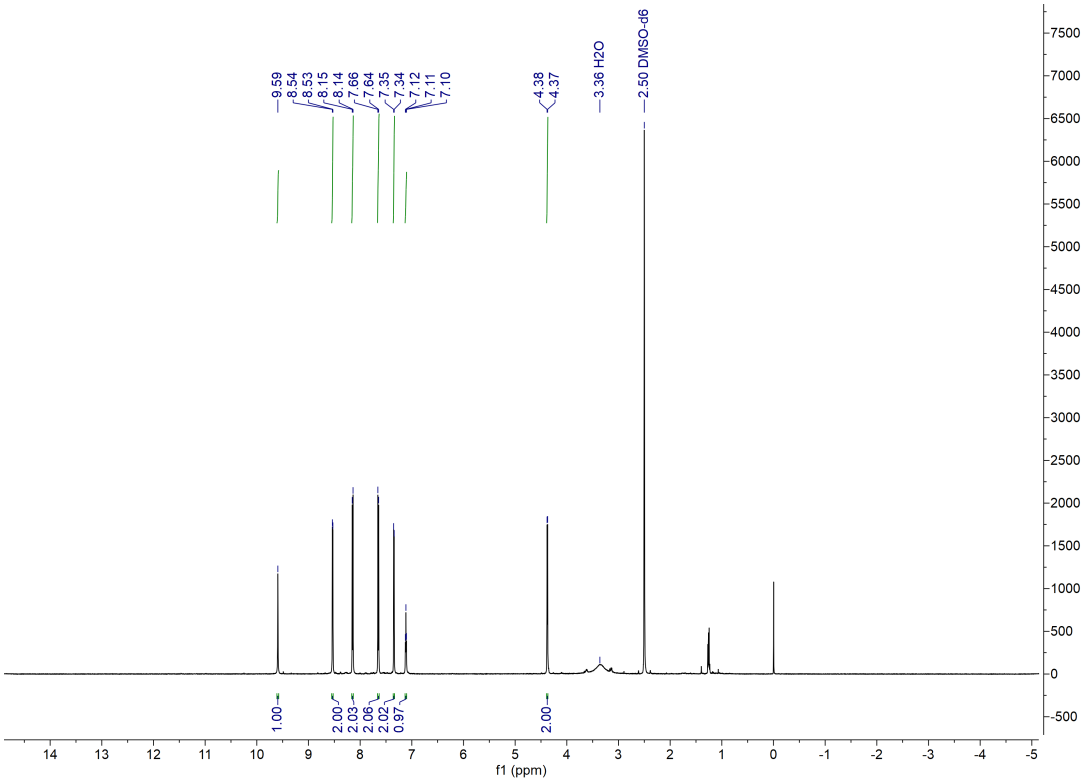
Synthesis of 4-Fluoro-N-(4-(3-(pyridin-4-ylmethyl) ureido) phenyl) benzenesulfonamide (YB8)

A solution of compound **3** (242 mg, 1.0 mmol) and triethylamine (303 mg, 3.0 mmol) in dry dichloromethane (10 mL) was cooled to 0°C, followed by dropwise addition of 4-fluorobenzenesulfonyl chloride (194 mg, 1.0 mmol). The reaction mixture was stirred at 0 °C for 30 min, then at room temperature for another 3 h. After completion, the reaction mixture was concentrated under reduced pressure, and the crude product was purified by flash chromatography (DCM/MeOH, gradient eluent from 33:1 to 25:1) to afford YB8 as an off-white solid (342 mg, 86%). ^{1}H NMR (600 MHz, DMSO-d_6) δ: 9.93 (s, 1H), 8.63 (s, 1H), 8.48 (d, J = 6.1 Hz, 2H), 7.73 (dd, J = 8.9, 5.2 Hz, 2H), 7.37 (t, J = 8.7 Hz, 2H), 7.27 – 7.25 (m, 4H), 6.92 (d, J = 8.9 Hz, 2H), 6.68 (t, J = 6.1 Hz, 1H), 4.29 (d, J = 6.1 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 165.53, 163.87, 155.72, 150.08, 149.93, 138.06, 136.34, 131.04, 130.25, 130.19, 123.04, 122.43, 118.96, 116.89, 116.74, 41.81. HRMS (ESI, positive) m/z calcd for $C_{19}H_{17}FN_4O_3S$ $[M + H]^+$: 401.1078; found 401.1086. HPLC purity: 96%.

NMR Spectra

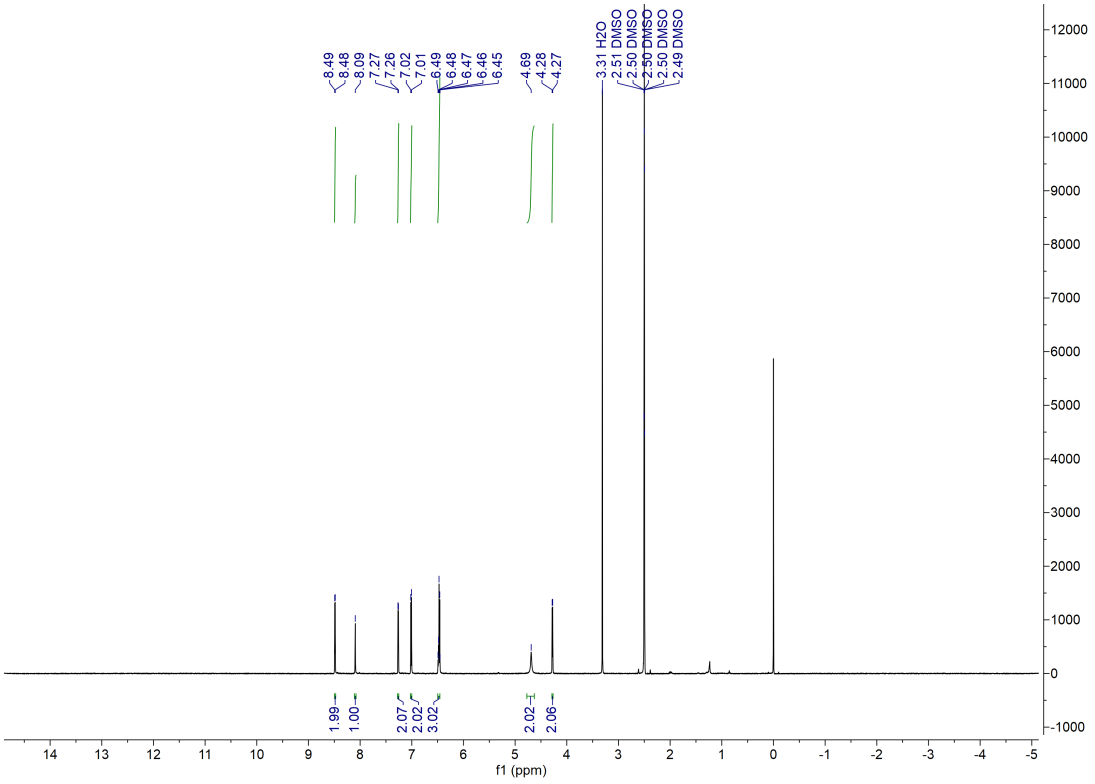
1-(4-Nitrophenyl)-3-(pyridin-4-ylmethyl) urea (2)

^{1}H NMR



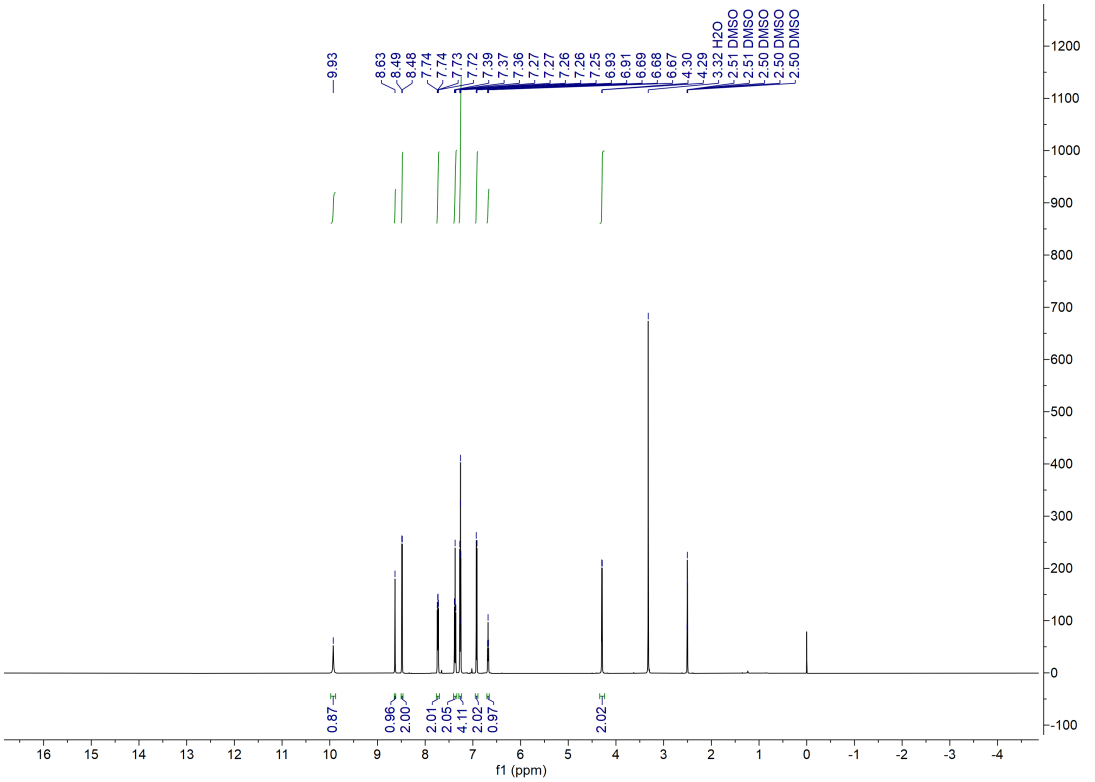
1-(4-Aminophenyl)-3-(pyridin-4-ylmethyl) urea (3)

^{1}H NMR



4-Fluoro-N-(4-(3-(pyridin-4-ylmethyl) ureido) phenyl) benzenesulfonamide (YB8)

^{1}H



^{13}C