Supplementary Methods:

Cell lines

CT26 (CRL-2638), DLD1 (CCL-221), HCT116 (CCL-247), SW480 (CCL-228), and Caco2 (HTB-37) were acquired from American Type Culture Collection (ATCC, Manassas, Virginia, USA), authenticated, according to ATCC instructions. MC38 (ENH204-FP) was acquired from Kerafast and NCM-460 (CVCL_0460) from INCELL. All cell lines were cultured in an incubator set at 37 °C in 5% CO2 and 95% atmospheric air, maintained with DMEM (Gibco; cat. no. 11965118) supplemented with 10% (v/v) Fetal Bovine Serum (Gibco; cat. no. 16140071) and Antibiotic-Antimycotic (Gibco; cat. no. 15240112). All cell lines were assessed regularly to ensure they were free of mycoplasma contamination.

CRISPR/Cas9 knockout

To knockout YTHDF1, we designed independent single guide RNAs (sgRNAs) using the CRISPR tool (http://crispr.mit.edu) and had them constructed into vector pSpCas9(BB)-2A-Puro (PX459) V2.0 (Addgene; #62988), following the instructions on Addgene. The following sgRNA sequences against murine *YTHDF1* were used: sgRNA1 (KO1): ATCCCGTATCTCACTACCTA; sgRNA2 (KO2): ATTTCCTTACTCCCTCAGCG. A nontargeting sequence GTAACCTCTCGAGCGATAGA was used in the control vector (NC). To generate the knockout clones, MC38 or CT26 cells were transfected with the constructed vector using Lipofectamine LTX (Thermo Fisher Scientific; cat. no. 15338030), according to the manufacturer's instructions. One day after the transfection, the cells were selected with puromycin at 5 or 8 μg/ml for MC38 or CT26, respectively. The selected cells were then trypsinized, and 2000 cells were plated in one well of a 6-well dish in one ml of growth medium. Single cells were collected using a pipette on a microscope and seeded to a well of a 96-well dish containing growth medium. Medium was refreshed every 5-7 days, and after a total of 10-16 days' culture single cells that grew into colonies were expended to dishes with a bigger format. Clones validated to show loss of YTHDF1 were used in the subsequent experiments.

Stable cell line with YTHDF1 overexpression

Human wide type YTHDF1 or YTHDF1 mutant (K395A, Y397A) was cloned into pLenti CMV Blast empty (Addgene; #17486). The vectors were transfected to HEK293T for virus generation as described above, and DLD1 cells were infected by the virus at the presence of 4 μg/ml of polybrene. The infected cells were then selected with blasticidin S (Thermo Fisher Scientific; cat. no. A1113903) at 5 μg/ml, and the cells were subjected to further analyses after the selection.

Syngeneic mice models

All experimental protocols were conducted after approval by Department of Health, The Government of the Hong Kong Special Administrative Region, and by University Animal Experimentation Ethics Committee, The Chinese University of Hong Kong. MC38 and CT26 cells resuspended in PBS were 1:1 (v/v) mixed with matrigel (BD Biosciences; cat. no. 354248) and subcutaneously injected to 6–8-week-old male C57BL/6 and BALB/c, respectively. The cells were injected at one million cells in a total volume of 50 μl per injection. Mice were acquired from The Chinese University of Hong Kong and a minimum of five mice were used per group. Assessment of tumor size began 6–8 days after cell inoculation and was performed once every 2–4 days using calipers. Tumor volume was calculated with the formula: $V = (d^2 \times D)/2$, where d stood for minor tumour axis and D for major tumour axis; and the data was presented as mean ± s.e.m. in mm^3. All mice were maintained at 21 °C ± 1, 55 to 70% humidity, and with a 12 h light/ dark cycle, from 7 am to 7 pm.

Reverse transcription-quantitative PCR (RT-qPCR)

RNAs were isolated with Trizol reagent (Thermo Fisher Scientific) and converted into cDNA using the PrimeScript RT Reagent Kit (Takara; cat. no. RR037B) and a 96-Well thermal cycler (Applied Biosystems). Quantitative PCR (qPCR) was performed with One-Step TB Green PrimeScript RT-PCR Kit II (Takara; cat. no. RR086B), according to the instructions from the manufacturer. The assays were conducted in technical triplicates in the ViiA7 Real-Time PCR System (Thermo Fisher Scientific) in a 96- or 386-well plate format. *ACTB* was used as an internal control. Relative expression levels were examined using the $2^{-\Delta\Delta C}$T method. Gene-specific primers used in this study are shown in **Table S3**.

Immunohistochemistry

After embedded in paraffin, tumor samples were sectioned, deparaffinized, and rehydrated. Antigens were then retrieved with sodium citrate buffer (10 mM sodium citrate, 0.05% tween 20, pH 6.0), and the sections were treated with 3% H_2O_2 and blocked with 5% goat serum. The sections were then incubated with anti-p65 (Cell Signaling Technology; cat. no. 8242) overnight at 4 ℃, followed by incubation with Goat Anti-Rabbit IgG (H + L)-HRP Conjugate (1706515, Bio-Rad). The sections were further counterstained with hematoxylin. Images were acquired using a microscope and three random images for each tumor were used for quantification. The quantification was conducted with the brown layer deconvoluted from the image, using (Fiji Is Just) ImageJ downloadable online (https://imagej.net/software/fiji/downloads).

Immunofluorescence

Tumor samples were frozen with liquid nitrogen in TissueTek OCT Compound (Sakura Finetek) and stored at -80 ℃. The frozen tissue blocks were sectioned into 5 μm thickness and the sections were fixed with pre-cooled acetone or 4% paraformaldehyde for 10 mins, for MDSC or T cell detection, respectively. The sections were further permeabilized with 0.25% Triton X-100 for 15 mins, blocked with 5% goat serum, and incubated with specific antibodies with 1% goat serum overnight at 4 ℃. The sections were then washed with PBS twice, stained with Hoechst 33342 (Thermo Fisher Scientific; cat. no. H3570), and further washed with PBS for three times, followed by mounting with ProLong™ Glass Antifade Mountant (Thermo Fisher Scientific; cat. no. P36980). The fluorescence images were acquired with the Leica TCS SP8 multiphoton system. The following antibodies from Biolegend were used: Alexa Fluor® 594 anti-mouse Gr-1 (clone RB6-8C5), Alexa Fluor® 594 anti-mouse CD8a (clone 53-6.7), Alexa Fluor® 594 anti-mouse CD4 (clone GK1.5), Alexa Fluor® 488 anti-mouse CD3 (clone 17A2), and Alexa Fluor® 488 anti-mouse/human CD11b (clone M1/70).

Western blot

Cells were lysed using 4% (w/v) sodium dodecyl sulfate solution, and the protein concentration was assessed using the Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific; cat. no. 23227) following the manufacturer's instructions. Same amount of protein from each sample was loaded and separated using SDS-PAGE and transferred to PVDF membrane (Merck; cat. no. IPVH00010). The membrane was then blocked

using 5% (w/v) BSA or milk, incubated with specific primary antibody, and blotted with horseradish peroxidase (HRP)-linked secondary antibody, according to the manufacturer's instructions. Visualization was performed using chemiluminescent substrate (Thermo Fisher Scientific; cat. no. 34096) on the ChemiDocTM XRS + Imaging System (Bio-Rad). Primary antibodies were diluted at 1:1000, while secondary antibodies at 1:3000. The following antibodies anti-IκBα (cat. no. 4814), anti-p-IκBα (cat. no. 2859), anti-IKKα (cat. no. 11930), anti-p-p65 (cat. no. 3033), anti-p65 (cat. no. 8242), anti-histone H3 (cat. no. 9715), anti-p50 (cat. no. 3035), anti-β-actin (cat. no. 4970), HRP-linked anti-mouse IgG (cat. no. 7076), HRP-linked anti-rabbit IgG (cat. no. 7074) were acquired from Cell Signaling Technology, while anti-YTHDF1 antibody (cat. no. ab220162) was acquired from Abcam.

Enzyme-linked immunoassay (ELISA)

ELISA against murine (ab216951) and human CXCL1 (ab190805) were both acquired from Abcam and used according to the user manuals. Supernatant samples collected from in vitro cell culture were used without dilution, while tumor lysates and sera were used after dilution of five times prior to assessment.

RNA interference

To generate stable cell lines expressing shRNAs, the shRNAs were cloned into pLKO.1-puro vector (Addgene; cat. no. 8453) following the protocol provided on the Addgene website (http://www.addgene.org/tools/protocols/plko/). A pair of shRNAs that did not target any know human genome were used as negative control and was designated as shNC. shRNAs that targeted YTHDF1 were designated shYTHDF1. To generate viruses containing the shRNAs, HEK293T was transfected, using Lipofectamine 2000 (Invitrogen; cat. no. 11668019), with the shRNA-pLKO.1-puro vector and the viral packaging vectors pMDLg/pRRE (Addgene; cat. no. 12251), pRSV-REV (Addgene; cat. no. 12253), and pMD2.G (Addgene; cat. no. 12259). Media containing the viruses were collected 48–72 h post-transfection and filtered with 0.22 μm filter to remove cell debris. Target cells were infected with the viruses in addition with 4 μg/ml of polybrene (Sigma-Aldrich; cat. no. H9268). One day after the transduction, the cells were selected with appropriate concentration of puromycin for further experiments. The targeted sequences of the shRNAs were listed in **Table S4**.

Antibodies treatments

The anti-mouse PD1 (clone RMP1-14), anti-mouse CD8α (clone 2.43), and their isotype controls were all acquired from BioXcell. Randomization was performed when therapeutic effects had to be assessed in the experiments with anti-PD1. Anti-PD1 was administrated intraperitoneally at 100 μg per mouse for MC38 xenografts and 250 μg for CT26 xenografts, at days 7, 10, 13, 16, and 19. To deplete $CD8^+$ T cells in the immunocompetent mice, anti-mouse CD8α was injected intraperitoneally 4, 7, and 9 days after cancer cell inoculation, with a loading dose of 400 μg per mouse and 100 μg per mouse subsequently. The efficacy of $CD8^+$ T cell depletion was determined by measuring the fraction of $CD8^+$ T cells in the bloodstream of mice with flow cytometry analysis, one day after the last dose of anti-mouse CD8α treatment.

Vesicle-like PLGA-based nanoparticle (VNP) formulation

Vesicle-like PLGA-based nanoparticle were assembled by Guangzhou Kelan Biotechnology Co., Ltd (Guangzhou, China). 2'-O-Methyl (2'-OMe) modified siRNA with 2'-O-Methyl (2'-OMe) modification were purchased from GenePharma Co. Ltd (Shanghai, China). The sequencing of human YTHDF1 siRNA: sense (CCACUCAAACUCUUUCGGGTT), antisense (CCCGAAAGAGUUUGAGUGGAA); Mouse YTHDF1 siRNA: sense (GCACUGACUGGUGUCCUUUTT), antisense (AAAGGACACCAGUCAGUGCTT). For syngeneic tumor models, MC38 murine CRC cells and HCT116 human CRC cells were respectively injected into C57BL/6 mice (1×10^6 cells/mouse) and $CD34^+$ humanized mice (5×10^6 cells/mouse), VNP-siNC/siYTHDF1 were treated via intertumoral injection (40ug/mouse every 2 days) when tumors reached at 50~100 mm^3. Tumor weight and tumor volume were monitored and measured, the composition of immune cells in tumors were analysed by flow cytometry.

Flow cytometry analysis

Xenografts were cut into small pieces with a surgical blade, and digested with collagenase D (Roche; cat. no. COLLD-RO) at 0.5 mg/ml and DNase I (Roche; cat. no. 10104159001) at 0.25 mg/ml, at 37 ℃ for one hour with agitation. For measuring $CD8^+$ T cells in the bloodstream of mice after anti-CD8α treatment, 100 μl of blood was

collected retro-orbitally into a tube containing 50 µl 0.5 M EDTA solution (pH 8.0) The suspensions were then filtered with 70 µm cell strainers, blocked with anti-mouse CD16/32 (Biolegend; cat. no. 156604), and stained with Zombie Green™ Fixable Viability Kit (Biolegend; cat. no. 423112) and specific antibodies in PBS containing 1% (w/v) BSA. Flow cytometry was conducted with FACS Celesta instrument and data was analysed with FlowJo V10. The following antibodies were acquired from Biolegend and used in the flow cytometry analysis: BV421 anti-mouse CD19 (clone 6D5), BV605 anti-mouse CD45 (clone 30-F11), PE anti-mouse CD3 (clone 17A2), PE/Cy7 anti-mouse CD4 (clone RM4-5), BV711 anti-mouse CD8α (clone 53-6.7), BV421 anti-mouse Ly-6C (clone HK1.4), PE anti-mouse Gr-1 (clone RB6-8C5), PE/Cy5 anti-mouse CD11b (clone M1/70), PE/Cy7 anti-mouse Ly-6G (clone 1A8), BV711 anti-mouse IFN-γ (clone XMG1.2), BV421 anti-mouse CD8α (clone 53-6.7), PE anti-mouse granzyme B (clone QA16A02), PE/Cy5 anti-mouse CD3 (clone 17A2). Compensation was performed using single-color controls which were acquired by incubating the AbC™ Total Antibody Compensation Bead Kit (Thermo Fisher Scientific; cat. no. A10513) with the fluorochrome-conjugated antibodies.

RNA-sequencing

Total RNAs were extracted and purified with RNeasy Mini Kit (Cat. #74106; QIAGEN). The libraries were sequenced using the illumina HiSeq 4000 (PE150) according to the protocol from company (Novogene, China). FPKM (Fragments per Kilobase of transcript per Million mapped reads) using DESeq2 were used for calculating gene expression levels. WebGestalt (http://www.webgestalt.org) was utilized for pathway analysis of RNA-seq data. Pathway analysis was performed with Over-Representation Analysis (ORA) on KEGG database or WikiPathways database.

T cell suppression assay

The leukocytes were collected from allograft tumors with or without YTHDF1 knockout digesting by collagenase IV (Gibco, 0.5 mg/ml) and DNase I (Worthington, 0.25 mg/ml). MDSCs were isolated from leukocyte using EasySep™ Mouse MDSC ($CD11b^+Gr1^+$) Isolation Kit (STEMCELL). Autologous spleen was used for T cells isolation by EasySep™ Mouse T Cell Isolation Kit (STEMCELL), and then isolated T cells were labeled with carboxyfluorescein succinimidyl ester (CFSE) (Invitrogen).

CFSE-labeled T cells were co-cultured with MDSC cells at 96-well plates (T:MDSC=1:0 and 1:1) for 72 h, Dynabeads ™ Mouse TActivator CD3/CD28 (ThermoFisher) and IL-2 (BioLegend) was added into medium for culture. CFSE intensity were quantified by flow cytometry.

MDSC isolation and migration assay

EasySep™ Mouse MDSC ($CD11b^+Gr1^+$) Isolation Kit (STEMCELL) were used for MDSCs isolation from the spleens of CT26 tumor-bearing BALB/c or MC38 tumor-bearing C57BL/6 mice. MDSCs (1 x 10^5 cells/well) were seeded in the top chamber of the transwell (pore size: 0.8 μm), and the bottom chamber were filled with conditioned medium (CM, without FBS) which cultured CT26 or MC38 cells with or without YTHDF1. MDSCs migrated to the bottom chamber after 4 h incubation, and cells number were counted.

Cytokine multiplex immunoassay

The cytokine multiplex immunoassay was performed with the Mouse Cytokine 23-plex Assay (Bio-Rad; cat. no. M60009RDPD) on the Bio-Plex 200 System (Bio-Rad) according to the user guide. Three types of materials derived from MC38 or CT26 clones bearing YTHDF1 knockout were subjected to the assay. These include supernatants collected from cells cultured in vitro, tumor lysates derived from the subcutaneous xenografts, and sera from the mice bearing the subcutaneous xenografts. Supernatants were performed without dilution while tumor lysates and sera were diluted five times prior to assessment. Data was shown as value normalized to control, the NC clone. For data from tumor lysates or sera, the mean derived from four mice each group was shown.

Ribosome-sequencing

Cells with or without YTHDF1 knockout were treated with 100 μg/ml cycloheximide (CHX) for 5 min at 37°C. The unprotected mRNA regions in the cells were excluded by treating with RNAse I. According to the ribosome-sequencing (Ribo-seq) protocol from company (Novogene, China), The intact mRNA-ribosome complexes were sequenced by using illumina HiSeq 4000 (SE50). Reads mapping on human rRNAs, snoRNAs, snRNAs and tRNAs from GENCODE project (v30) were excluded. The

residual reads were mapped on the human genome via bowtie2 (version 2.3.4.3) with option -L 10. The featureCounts (version 1.6.4) with the parameters (M –fracOverlap 0.4 –largestOverlap) was utilized for calculating the expression of protein-coding genes. Pathway analysis of Ribo-seq was applied via *Gene Set Enrichment Analysis* (*GSEA*) methods.

MeRIP-sequencing and MeRIP-qPCR

Total RNAs were extracted with Trizol reagent (Thermo Fisher Scientific) and DNA contamination was removed using the DNase and DNase Buffer in the PrimeScript RT Reagent Kit (Takara; cat. no. RR037B). The RNAs were then fragmented with RNA Fragmentation Buffer (10 mM Tris-HCl, 10 mM $ZnCl_2$) for 5 min at 70°C. The fragmented RNAs were then incubated with the Protein A/G Magnetic Beads (HY-K0202, MedChemExpress) bound with the anti-m6A antibody (ab208577, Abcam) at 4°C for 4 hrs, at the presence of RNase inhibitor (10777019, Thermo Fisher Scientific). The beads were then washed twice in IP buffer (150 mM NaCl, 10 mM Tris-HCl, pH 7.5, 0.1% IGEPAL CA-630), twice in low-salt IP buffer (50 mM NaCl, 10 mM Tris-HCl, pH 7.5, 0.1% IGEPAL CA-630), and twice in high-salt IP buffer (500 mM NaCl, 10 mM Tris-HCl, pH 7.5, 0.1% IGEPAL CA-630). The RNAs were then eluted from the beads with the RLT buffer supplied in the RNeasy Mini Kit (74106, QIAGEN) and purified with the DireCTzol RNA Miniprep Kit (R2050, Zymo Research). The isolated RNAs were then subjected to RT-qPCR using the RT-qPCR procedures described above or RNA-sequencing. IGEPAL CA-630 (I8896) and $ZnCl_2$ solution (39059) were acquired from Sigma. The list of qPCR primers used in MeRIP-qPCR was in **Table S5**.

RIP-sequencing or RIP-qPCR

RIP was performed using anti-YTHDF1 antibody (Abcam, ab220162) and the EZ-Magna RIP™ RNA-Binding Protein Immunoprecipitation Kit (17-701, Sigma) according to the manufacturer's instructions. Briefly, cell pellets were lysed with the RIP Lysis Buffer. The cell lysates were then incubated with magnetic beads bound with the anti-YTHDF1 antibody overnight at 4°C. The beads were then washed with the RIP Wash Buffer for a total of 6 times and the RNAs were released by digesting the antibody with proteinase K in 1% (w/v) SDS at 55°C for 30 min. The RNAs then were isolated with phenol:chloroform:isoamyl alcohol (BP1754I-100, Fisher) and RNA precipitation

by ethanol. The resuspended RNAs were then subjected to RT-qPCR using the RT-qPCR procedures described above or RNA-sequencing.

Measurement of liver or kidney function indicators

Creatinine, blood urea nitrogen, alanine aminotransferase, and aspartate transaminase were measured in serum from the VNP-treated animals, using the Catalyst One Chemistry Analyzer (IDEXX) following the instructions from the user manual. Thirty microliters of each serum sample were diluted with PBS to a total volume of 120 μl, and then loaded to specific catalyst slides. The slides were then read with the analyzer automatically.

Statistical analysis

All measurements were acquired using distinct samples rather than collected with repeated measurements. GraphPad Prism version 8 (GraphPad Software; San Diego, CA) was used for data analysis, and the data were shown as means ± s.d., unless stated otherwise. Two-tailed student's t-test was used to conduct statistical analysis, unless stated otherwise. A *P* value lower than 0.05 was regarded as statistically significant, unless stated otherwise.