Anti-tumour immunity controlled through mRNA m\(^6\)A methylation and YTHDF1 in dendritic cells

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There is growing evidence that tumour neoantigens have important roles in generating spontaneous antitumour immune responses and predicting clinical responses to immunotherapies\(^{1,2}\). Despite the presence of numerous neoantigens in patients, complete tumour elimination is rare, owing to failures in mounting a sufficient and lasting antitumour immune response\(^3\). Here we show that durable neoantigen-specific immunity is regulated by mRNA N\(^\text{6}\)methyladenosine (m\(^6\)A) methylation through the m\(^6\)A-binding protein YTHDF1\(^5\). In contrast to wild-type mice, Ythdf1\(^−/−\) mice show an elevated antigen-specific CD8\(^+\) T cell antitumour response. Loss of YTHDF1 in classical dendritic cells enhanced the cross-presentation of tumour antigens and the cross-priming of CD8\(^+\) T cells in vivo. Mechanistically, transcripts encoding lysosomal proteases are marked by m\(^6\)A and recognized by YTHDF1. Binding of YTHDF1 to these transcripts increases the translation of lysosomal cathepsins in dendritic cells, and inhibition of cathepsins markedly enhances cross-presentation of wild-type dendritic cells. Furthermore, the therapeutic efficacy of PD-L1 checkpoint blockade is enhanced in Ythdf1\(^−/−\) mice, implicating YTHDF1 as a potential therapeutic target in anticancer immunotherapy.

Spontaneous priming of T cells against tumour neoantigens is crucial for the clinical efficacy of immunotherapies. However, in many patients, neoantigen recognition is insufficient to induce the lasting T cell response that is required for complete tumour rejection. The identification of molecular pathways that influence immunoreactivity to tumour neoantigens could provide new targets for improving the response to immunotherapy.

m\(^6\)A, the most abundant internal mRNA modification, is responsible for the post-transcriptional regulation of mRNA in diverse cell types\(^6\)–\(^10\). m\(^6\)A can affect the efficiency of mRNA translation via the m\(^6\)A-binding protein YTHDF1\(^5\). Dysregulation of m\(^6\)A pathway components could affect oncogene expression, thereby linking m\(^6\)A and tumorigenesis\(^11\)–\(^14\). As most studies focus on tumour-intrinsic oncogenic pathways, potential roles of the mRNA m\(^6\)A modification in host antitumour immune responses are unknown. Furthermore, the roles of various m\(^6\)A reader proteins in cancer have been largely unexplored.

We inoculated ovalbumin (OVA)-expressing B16 melanoma cells subcutaneously into wild-type and Ythdf1\(^−/−\) mice\(^9\) (Extended Data Fig. 1). Compared to wild-type mice, Ythdf1\(^−/−\) mice showed slower growth of B16-ova tumours and prolonged survival (Fig. 1a, Extended Data Fig. 2a, b). We also tested the MC38 colon carcinoma model, which has been reported to have a broader neoantigen pool\(^16\). Consistently, we observed a similar level of tumour inhibition in Ythdf1\(^−/−\) and wild-type mice (Fig. 1b, Extended Data Fig. 2c).

Immune infiltrates contained higher levels of CD8\(^+\) cytotoxic T cells and natural killer (NK) cells in tumours from Ythdf1\(^−/−\) mice than from wild-type mice, suggesting that immunosurveillance is enhanced in the absence of YTHDF1 (Fig. 1c). Accordingly, we observed reduced infiltration of myeloid-derived suppressor cells (MDSCs) in tumours from Ythdf1\(^−/−\) mice (Extended Data Fig. 2d, e), whereas there was no significant difference in the number of T regulatory (Treg) cells (Extended Data Fig. 2f, g). Both CD8\(^+\) T cells and NK cells are critical for controlling tumour growth\(^17\), so we dissected their contributions to the anti-tumour response in Ythdf1\(^−/−\) mice. NK cells from wild-type and Ythdf1\(^−/−\) mice showed similar degranulation responses (Extended Data Fig. 2h), and antibody-mediated depletion of NK cells had no effect on tumour growth in Ythdf1\(^−/−\) mice (Fig. 1d, Extended Data Fig. 2i). By contrast, the anti-tumour response in Ythdf1\(^−/−\) mice was completely abrogated in the absence of CD8\(^+\) T cells (Fig. 1d, Extended Data Fig. 2i), indicating that CD8\(^+\) T cells are essential for tumour control in the Ythdf1-deficient host.

To determine whether neoantigen-specific CD8\(^+\) T cell responses are generated in B16-OVA tumours, we analysed the frequency of tumour-infiltrating CD8\(^+\) T cells expressing the SIINFEKL MHC-I tetramer in wild-type and Ythdf1\(^−/−\) mice. Whereas wild-type mice did not accumulate antigen-specific CD8\(^+\) T cells within the tumour, Ythdf1\(^−/−\) mice showed a substantial increase in CD8\(^+\) T cells against tumour neoantigen in vivo (Fig. 2a, b). To investigate whether the infiltration of neoantigen-specific CD8\(^+\) T cells in Ythdf1\(^−/−\) mice was due to enhanced spontaneous CD8\(^+\) T cell priming at an early stage, we stimulated lymphocytes from tumour-draining lymph nodes (DLNs) in vitro with or without OVA-derived SIINFEKL peptide or tumour cells, and measured endogenous CD8\(^+\) T cell responses using enzyme-linked immune absorbent spot (ELISPOT) testing for interferon-γ (IFN-γ). There were substantially more IFN-γ-spot-forming cells in Ythdf1\(^−/−\) mice than in wild-type mice in both B16-OVA and MC38 tumour models (Fig. 2c, d), indicating that YTHDF1 depletion in host cells potentiates the early steps of T cell priming against tumour neoantigens.

Next, we showed that loss of Ythdf1 in T cells makes a minor contribution to the observed antitumour immunity (Extended Data Fig. 3a). As dendritic cells (DCs) are the main antigen-presenting cells (APCs) that cross-prime CD8\(^+\) T cells, we hypothesized that the increased T cell priming in Ythdf1\(^−/−\) mice could be attributed to improved recognition of tumour cells through an increased cross-priming ability of DCs\(^18,19\). To test this hypothesis, we used classical DCs cultured in medium supplemented with FLT3L (FLT3L-DCs) to model how cross-presentation occurs\(^20\)–\(^22\). We pulsed FLT3L-DCs with necrotic B16-OVA in vitro

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https://doi.org/10.1038/s41586-019-0916-x

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and evaluated their ability to cross-prime T cells expressing transgenic ovalbumin-specific (OT-1) T cell receptors. Ythdf1−/− FLt3L-DCs were able to cross-prime OT-1 T cells to a greater extent than wild-type DCs (Fig. 2e). To determine the cross-presentation capacity of classical DCs (cDCs) in vivo, we collected CD8α+ DCS and CD11b+ DCs from DLTs of B16-OVA- or MC38-OVA-tumour-bearing mice and cocultured them with OT-1 T cells. Whereas wild-type mice showed weak cross-presentation of CD8+ T cells, we observed substantially augmented T cell cross-presentation induced by both CD8α+ DCS and CD11b+ DCs in Ythdf1−/− mice (Fig. 2f, Extended Data Fig. 3b). In addition, Ythdf1−/− DCs showed enhanced cross-presentation for the less sensitive model antigen SIY (Extended Data Fig. 3c). To test whether cross-presentation in DCs depends on RNA m6A methylation in general, we compared the cross-presentation capacity of Mettl14-deficient DCs from C57Bl/6-CreMettL14/Cre conditional knockout mice and wild-type DCs. DCs deficient in Mettl14 showed enhanced cross-presentation ability (Extended Data Fig. 3d), confirming the critical role of the m6A-YTHDF1 axis in restricting the cross-presentation capacity of cDCs.

To investigate whether the increased cross-presentation of YTHDF1-deficient DCs for priming could be attributed to differential expression of co-stimulatory molecules, we evaluated the expression of CD80 and CD86 on DCs. Wild-type and Ythdf1−/− DCs expressed comparable levels of CD80 and CD86, and also exhibited a similar ability to directly prime OT-1 T cells with peptide stimulation (Extended Data Fig. 3e, f). In addition, loss of YTHDF1 did not affect the composition of DC subpopulations in naive mice (Extended Data Fig. 4), nor did it affect LPS-mediated activation of DCs (Extended Data Fig. 5a). These findings suggest that loss of YTHDF1 increases the cross-presentation capacity of DCs, rather than affecting the development or activation of DCs.

To determine whether YTHDF1 deficiency enhances the crosspresentation of tumour antigens on DCs, leading to better cross-presentation of CD8+ T cells,24,25 we assessed the abundance of H-2Kb-SIINFEKL complexes on DCs from wild-type and Ythdf1−/− mice bearing B16-OVA tumours. Although phagocytosis of tumour cells was similar in wild-type and Ythdf1−/− mice (Extended Data Fig. 5b, c), the level of H-2Kb-SIINFEKL complexes was markedly higher in tumour-infiltrating Ythdf1−/− DCs than in wild-type DCs (Fig. 2g, h). Furthermore, compared with splenic wild-type DCs, DCs from Ythdf1−/− mice exhibited a higher potential for cross-presentation of soluble OVA in vitro (Extended Data Fig. 5d). These data suggest that DCs from Ythdf1−/− mice possess improved antigen-presentation relative to DCs from wild-type mice.

To investigate whether the antitumour immunity relies on loss of Ythdf1 specifically in DCs, we generated chimeric, DC-specific Ythdf1 knockout mice. Specifically, we reconstituted irradiated mice with a 1:1 mixture of Ythdf1−/− bone marrow cells (BMCs) and wild-type BMCS with a Zbtb46-DTR transgene, which drives expression of the diphtheria toxin receptor in classical DCs. Upon administration of diphtheria toxin, mice show effective tumour control that depends on CD8+ T cells. A, Wild-type or Ythdf1−/− mice were injected subcutaneously with 106 B16-OVA cells. Tumour growth was monitored. One of three representative experiments is shown. B, Wild-type or Ythdf1−/− mice were injected subcutaneously with 106 MC38 cells. Tumour growth was monitored. One of three representative experiments is shown. C, Percentage of tumour-infiltrating T cells and NK cells on day 12 post tumour inoculation. D, Wild-type and Ythdf1−/− mice were injected subcutaneously with 106 B16-OVA cells and treated with 200 μg of CD8- or NK-depleting antibody twice a week starting on day 3. Tumour size was monitored over time. n, number of mice. Mean ± s.e.m., two-sided unpaired Student’s t-test.
transcripts were enriched for pathways associated with phagosomes and lysosomes (Fig. 3d). Limiting lysosomal proteolysis in DCs can enhance cross-presentation by minimizing destruction of internalized antigens. We noticed that translation of a group of transcripts that encode lysosomal cathepsins, which are responsible for antigen degradation in DC lysosomes, was repressed in Ythdf1−/− DCs compared with wild-type DCs (Fig. 3e). By contrast, the translational efficacy of co-stimulatory or inhibitory molecules (signal 2) and cytokines (signal 3) was not substantially altered in Ythdf1-deficient FLT3L-DCs (Extended Data Fig. 6e). In line with the observation in FLT3L-DCs, loss of Ythdf1 resulted in decreased translational efficacy of cathepsins in granulocyte–macrophage colony-stimulating factor (GM-CSF)-induced bone marrow DCs (GMDCs; Extended Data Fig. 7a–f).

Consistently, we found that multiple cathepsin transcripts are bound by YTHDF1, and that they subsequently affect the cross-priming capacity of DCs.

To identify the functional pathways that are associated with YTHDF1-targeted mRNAs, we analysed m6A-marked mRNAs that are both targets of YTHDF1 and translationally regulated by YTHDF1. We performed Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis, which showed that YTHDF1-targeted transcripts were enriched for pathways associated with phagosomes and lysosomes (Fig. 3d).

Consequently, we performed RNA immunoprecipitation and sequencing ( RIP–seq ) to map target transcripts bound by YTHDF1 in FLT3L-DCs. YTHDF1-binding sites were highly reproducible between two biological replicates (Extended Data Fig. 6a, b), and were predominantly distributed in the coding region and 3′ untranslated region (UTR; Extended Data Fig. 6c, d). Given that YTHDF1 is known to affect mRNA translation, we assessed the translational efficiency of wild-type and Ythdf1−/− DCs by ribosome profiling. We also performed antibody-based m6A profiling and RNA sequencing (RNA-seq) in the same cells. We categorized transcripts into three groups: non-m6A marked transcripts, m6A-containing transcripts, and m6A-marked transcripts bound by YTHDF1. As expected, we found a notable decrease in translation efficiency, particularly for YTHDF1-targeted and m6A-marked transcripts, in Ythdf1−/− DCs compared with wild-type DCs (Fig. 3a, b), whereas Ythdf1 deficiency did not substantially alter the distribution of m6A in mRNAs from DCs (Fig. 3c).

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cysteine protease inhibitor E64 or more selective inhibitors (CA-074 for cathepsin B and cathepsin L inhibitor II) notably enhanced the efficiency of cross-presentation in wild-type DCs (Extended Data Fig. 8d–f).

Moreover, the in vivo antitumour response was markedly improved by cathepsin blockade in wild-type mice (Fig. 4b, Extended Data Fig. 8g), suggesting that cathepsins are critical factors for determining the antitumour response in this model. Collectively, these data show that loss of YTHDF1 in DCs attenuates antigen degradation by restricting the expression of lysosomal proteases, leading to improved cross-presentation and better cross-presentation of CD8+ T cells.

Finally, we investigated whether loss of YTHDF1 with increased neoantigen-specific CD8+ T cells could enhance the antitumour response to immune checkpoint blockade, which targets the T cell inhibitor receptor PD1. As Ythdf1−/− mice showed a marked increase in IFNγ in CD8+ T cells, and IFNγ signalling upregulates the expression of PD-L1, we evaluated the level of PD-L1. PD-L1 expression was increased in tumour cells from Ythdf1−/− tumour-bearing mice compared with wild-type mice (Fig. 4c), whereas neutralizing IFNγ diminished the expression of PD-L1 (Extended Data Fig. 9). We then tested whether PD-L1 blockade could potentiate the antitumour response in Ythdf1−/− mice. We treated wild-type and Ythdf1−/− tumour-bearing mice with an anti-PD-L1 antibody (clone 10F.9G2). Although tumour regression occurred in 40% of untreated Ythdf1−/− mice and anti-PD-L1-treated wild-type mice, 100% of anti-PD-L1-treated Ythdf1−/− mice showed complete tumour regression (Fig. 4d). These data suggest that combining a checkpoint blockade with YTHDF1 depletion could be a potential new therapeutic strategy to improve outcomes in patients with low response to checkpoint blockade.

In line with the observations in mouse models, we found that patients with colon cancer who had low expression of YTHDF1 in the tumour stroma tended to have a higher number of CD8+ cells in tumour biopsies than patients with high expression of YTHDF1, who lacked CD8+ cell infiltrates (Fig. 4e, f), further supporting the notion that the reduced YTHDF1 expression may correlate with the T cell infiltrated tumour microenvironment.

Tumours can evade immune recognition despite expressing neoantigens. Our current results reveal that the m6A-marked mRNAs that encode lysosomal proteases are recognized by YTHDF1 in DCs. Binding of YTHDF1 promotes translation of lysosomal proteases, suppressing the cross-presentation of engulfed tumour neoantigens, which represents a previously unrecognized (to our knowledge) mechanism of immune evasion. Our data do not exclude potential contributions from other targets of YTHDF1; further investigation of complex regulatory pathways mediated by the m6A axis is necessary to expand our understanding and uncover additional features of antitumour immunity. Finally, this work suggests that YTHDF1 could be a therapeutic target for immunotherapy in combination with emerging checkpoint inhibitors or DC vaccines.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41586-019-0916-x.

Received: 14 November 2017; Accepted: 10 January 2019; Published online 6 February 2019.

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Acknowledgements This study was supported by the National Key Research and Development Program of China, Stem Cell and Translational Research (2018YFA0109700 to D.H.), Strategic Priority Research Program of the Chinese Academy of Science (XDA16010404 to D.H.), National Institute of Health (HG008935 and GM113194 to C.H.), Ludwig Center at the University of Chicago (to C.H. and R.R.W.), CAS Hundred Talent Program (to D.H.), National Natural Science Foundation of China (31870890 to M.M.X., 31741074 to D.H.), National Science Fund for Excellent Young Scholars (81622039 to B.S.), Science Foundation for Distinguished Young Scholars of Jiangsu Province (BK20160045 to B.S.) and Open Project of Key Laboratory of Genomic and Precision Medicine of the CAS. The Mass Spectrometry Facility of the University of Chicago is funded by National Science Foundation (CHE-1048528). C.H. is an investigator of the Howard Hughes Medical Institute. We thank J. Tauler for editing.

Reviewer information Nature thanks J. Hanna, J. Neefjes and the other anonymous reviewer(s) for their contribution to the peer review of this work.

Author contributions D.H. and M.M.X. conceived the project. D.H., M.M.X., J.L., L.D., X.H., Y.L. and R.C. performed experimental work. D.H. and C.C. performed bioinformatics analysis. Y.L., J.W. and B.S. generated Ythdf1 knockout mice. M.B.B. and U.D. provided human colon biopsy samples. D.H., M.M.X. and C.H. designed the study. D.H., M.M.X., C.H. and R.R.W. wrote the manuscript with input from all authors.

Competing interests C.H. is a scientific founder and a member of the scientific advisory board of Accent Therapeutics, Inc. A patent application on YTHDF1 has been filed by the University of Chicago.

Additional information Extended data is available for this paper at https://doi.org/10.1038/s41586-019-0916-x.
Supplementary information is available for this paper at https://doi.org/10.1038/s41586-019-0916-x.
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METHODS

Mice. *Ythdf1−/−* mice were generated as previously described. Founder mice with mutant alleles were backcrossed to C57BL/6 mice for two generations. Mice used for experiments were further backcrossed to C57BL/6 mice for seven generations (total nine generations). To ensure comparability in genetic background, mice were maintained by crossing heterozygous and heterozygous mice. *Ythdf1−/−* mice or their littermate control wild-type mice were used in all experiments. Littermates were co-housed during pregnancy with 2% FCS and 1 mM micromob environment and primers. Used for genotyping of *Ythdf1−/−* mice: CACCTGAGTTGACATCCTAAC and GCTCGAAGTGTCCATCC. Female *Rag2−/−* mice and 2C CD8+ TCR transgenic, Cd11c-Cre and Zbtb66-DTR mice were purchased from Jackson Laboratory. Female CD11c-Cre,Mettl14f/f conditional knockout mice were generated in-house. All mice were used at 6–12 weeks of age. All mice were maintained under specific pathogen-free conditions and used in accordance with the animal experimental guidelines set by the Institute of Animal Care and Use Committee. This study has been approved by the Institutional Animal Care and Use Committee of The University of Chicago.

Cell lines. MC38 is a mouse colon adenocarcinoma cell line that was provided by D. Bartlett (University of Pittsburgh, Pittsburgh). B16-OVA, an OVA-transfected clone derived from the mouse melanoma cell line B16, was provided by Y.-X. Fu (UT Southwestern). The B16F10 cell line was purchased from ATCC. MC38-zsGreen-OT-Ip (MC38-OZ) and B16F10-zsGreen-OT-Ip (B16-OZ) were selected for a single clone after being transfected by lentivirus expressing zsGreen-OT-Ip (SIINFEKEL). MC38-SIY is an EGRF-SIY-transfected clone derived from the mouse melanoma cell line MC38. Cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM; Invitrogen) supplemented with 10% FBS, 1% penicillin-streptomycin, 2 mM l-glutamine, 1 mM sodium pyruvate and 0.1 mM non-essential amino acids at 37 °C in 5% CO2.

Primary cell cultures. Single-cell suspensions of BMCs were cultured in RPMI-1640 medium containing 10% fetal bovine serum, supplemented with 20 ng/ml GM-CSF (Biolegend). Fresh medium with GM-CSF was added into culture on days 3 and 5. On day 6, CD11c+ cells were purified using the EasySep Mouse CD11c Positive Selection Kit II (STEMCELL Technologies). To culture FLT3L-DCs, single-cell suspensions of BMCs were cultured in Iscove’s modified Dulbecco’s medium (IMDM) containing 10% fetal bovine serum at a concentration of 1 × 106 per ml. Cells were supplemented with 100 ng/ml ETL3L (PEPROTECH) 3–9 days to obtain FLT3L-DCs.

Tumour growth and treatments. B16-OVA or MC38 tumour cells (1 × 106) were injected subcutaneously (s.c.) into the flank of mice. Tumour volumes were measured by length (L) and width (W) and calculated as tumour volume = L × W/2. Mice with tumour volumes less than 200 mm3 are considered to be surviving. For the in vivo depletion study, 200 μg of anti-CD8 antibody (clone YTS169.4) or anti-NK.1.1 (clone PK136) was injected intraperitoneally (i.p.) three days after tumour inoculation. For adoptive transfer of T cells, Rag mice were inoculated with 5 × 106 B16-OVA on day 0. On day 11, mice with established tumours were treated with E64 intratumorally. For anti-PD-L1 treatment, 1 × 106 B16-OVA tumour cells were s.c. injected into the flank of mice. Tumours were allowed to grow for seven days and treated i.p. with anti-PD-L1 (clone 10E9F2) or rat immunoglobulin. Tumour-free mice after treatment were monitored over time and the percentage of tumour regression was calculated. To block IFN-γ, tumour-bearing mice were treated with 50 μg anti-IFN-γ monoclonal antibody (clone XMG1.2) intratumorally and PD-L1 expression on tumour cells was evaluated by flow cytometry. All antibodies were InVivoMab from BioXCell. For adoptive transfer of T cells, Rag mice were inoculated with 5 × 106 B16-OVA on day 0. On the same day, T cells were purified from wild-type or *Ythdf1−/−* mice using a T cell negative isolation kit (STEMCELL Technologies). T cells (5 × 106) were injected intravenously (i.v.) into Rag2−/− mice. Tumour-bearing mice were killed before the tumour diameter reached 2 cm; this tumour size limit was approved by the Institutional Animal Care and Use Committee of The University of Chicago.

Generation of bone marrow chimera. To generate bone marrow chimeric mice, C57BL/6 mice were exposed to 8 Gy of X-ray radiation. After 24 h, 5 × 106 BMCs, consisting of 2.5 × 106 wild-type or *Ythdf1−/−* BMCs and 2.5 × 106 Zbtb66-DTR BMCs, were injected i.v. into irradiated mice. Six weeks after reconstitution, *Zbtb66-DTR:Ythdf1−/−* and *Zbtb66-DTR:Ythdf1−/−* mixed bone marrow chimera mice were inoculated with 106 B16-OVA cells and treated with 400 ng diethylthiocarbamic acid (DCT) over 3–12 days to obtain FLT3L-DCs.

A 1.0:10000 dilution was added and incubated for 2 h at room temperature or overnight at 4°C. Avidin–horseradish peroxidase (BD Bioscience) with a 1:10000 dilution was then added and the plate was incubated for 1 h at room temperature. IFN-γ spots were developed according to the manufacturer’s instructions (BD Bioscience).

Antigen-presentation assay. For cross-presentation of tumour neoantigens, CD11c+ or CD8+ DCs were purified from DLNs of wild-type or *Ythdf1−/−* mice 6 days after inoculation with B16-OVA, MC38-OT-Ip or MC38-EGRF-SIY. OT-I or 2C naive CD8+ T cells were isolated from lymph nodes and spleen of 6–12-week-old naive mice and cultured with tumour-infiltrating DCs (CD45+ CD11b+ CD11c+ MHCII+ CD8+ TC) transgenic mice, and adoptively transferred into naive OT-I mice at a ratio of 1:10 for three days with or without 1 μg/ml SIINFEKEL peptide. For cross-presentation of soluble OVA, splenic DCs were sorted and stimulated with 100 ng/ml LPS overnight. DCs were then pulsed with different concentrations of OVA (endotoxin free, Sigma) for 5 h. Cells were washed and co-cultured with OT-I naive CD8+ T cells for three days. For in vivo cross-presentation of tumour neoantigens, FLTL3-DCs were cultured on days 9–10 and co-cultured with necrotic B16-OVA tumour cells overnight. B127 CD11c+ cells were subsequently purified. GMDs from *Mettl14f/f* or CD11c-Cre,Mettl14f/f mice were collected on day 6 and co-cultured with necrotic B16-OVA tumour cells for 16 h. To inhibit cathepsins, GMDs were pre-treated with E64 (sigma) for 2 h followed by co-culturing with tumour cells. CD11c+ cells were then purified and incubated with naive CD8+ T cells from OT-I mice for three days. IFN-γ production was detected using an IFN-γ Flex Set CBA assay (BD Bioscience). To inhibit cathepsins in ex vivo cDCs, wild-type or *Ythdf1−/−* mice were inoculated with 5 × 106 MC38 cells. Thirty-six hours after tumour inoculation, spleens were removed and digested, and CD11c+ DCs were purified using the EasySep Mouse (Stemcell Technologies). CD11c+ DCs were then treated with 0.04 μM E64 (Sigma) overnight followed by co-culturing with OVA protein for 4 h. Any free OVA protein was then removed from the culture medium, and CD11c+ DCs were incubated with cell-trace violet (CTV)-labelled OT-1 cells for three days. The cross-presentation capacity of DCs was analysed by the dilution of CTV in CD8+ T cells. For the in vitro cathepsin inhibition assay, FLTL3-DCs were treated with 5 μg/ml CA-074 methyl ester (Selleck), 5 μg/ml cathepsin L inhibitor III (Sigma) or a combination (5 μg/ml CA-074 methyl ester and 5 μg/ml cathepsin L inhibitor III) for 2 h followed by co-culturing with necrotic B16-OVA cells for 16 h, and then FLTL3-DCs were purified using an EasySep Mouse CD11c Positive Selection Kit II (STEMCELL Technologies). The purified cells were incubated with OT-I cells at a ratio of 1:20 for three days. The cross-presentation capacity of DCs was then measured by IFN-γ production. To detect MHC-H2Kb−SIINFEKEL, mice were inoculated with B16-OVA. After 12 days, tumours were removed and tumour-infiltrating DCs (CD45+ CD11b+ Ly6C− MHCII− CD24+ CD11c+) were stained with monoclonal antibody 25D1.

Cell trace violet labelling. Ten million splenocytes from naive OT-I mice were re-suspended in 1 ml PBS followed by incubating with 5 μM CTV (ThermoFisher) at 37°C for 20 min. RPMI-1640 medium (5 ml) was added to the cells and incubated for 5 min to remove the free dye in the solution. These cells were then centrifuged and incubated with pre-warmed RPMI-1640 for at least 10 min at room temperature for subsequent analysis.

RIP−seq. Twenty million GMDs were harvested and co-cultured with or without necrotic B16-OVA overnight. The procedure was adapted from a previous report19. Five million FLTL3-DCs were harvested. DCs were then purified and pelleted by centrifugation for 5 min. Cells were washed twice with cold PBS and the cell pellet was re-suspended with two volumes of lysis buffer (150 mM KCl, 10 mM HEPES, pH 7.6, 2 mM EDTA, 0.05% Nonidet P-40, 1 mM protease inhibitor cocktail, 400 μM/mL Na4SO4 inhibitor). The lysate was incubated on ice for 5 min and centrifuged for 15 min to clear the lysate. One tenth-volume of cell lysate was saved as input and total RNA was extracted using Trizol. The rest of the
cell lysis was incubated with 5 μg anti-YTHDF1 (Proteintech) at 4°C overnight with gentle rotation followed by incubation with 40 μl protein G beads for 1 h at 4°C. The beads were then washed five times with 1 ml ice-cold washing buffer (200 mM NaCl, 50 mM HEPES pH 7.6, 2 mM EDTA, 0.05% NP-40, 0.5 mM DTT, 200 U/ml RNAse inhibitor). The immunoprecipitation complex was resuspended in 400 μl 1 × Proteinase K and digested with 2 mg Proteinase K at 55°C for 1 h. RNA was then extracted using an RNA isolation kit (Zymo). Input and immunoprecipitated RNA of each sample were used to generate the library using a TrueSeq stranded mRNA sample preparation kit (Illumina).

**m^A^-seq.** Total RNA was isolated from DCs. Polyadenylated RNA was further enriched from total RNA using the Dynabeads mRNA Purification Kit (Invitrogen). RNA samples were fragmented into ~100-nucleotide-long fragments by sonication. Fragmented RNA (100 ng mRNA or 5 μg total RNA) was used for m^A^-immunoprecipitation (m^A^-IP) with the EpiMark N^6^-methyladenosine enrichment kit (NEB E1610S) according to the manufacturer’s protocol. RNA was enriched through RNA Clean & Concentrate-5 (Zymo Research) and used for library generation with SMARTer Stranded Total RNA-Seq Kit (Takara). Sequencing was performed at the University of Chicago Genomics Facility on an Illumina HiSeq4000 machine in single-read mode with 50 bp per read. Sequencing reads were aligned to the mouse genome mm9 by STAR (version 2.6.0c)²⁰. The m^A^-enriched regions (peaks) in each m^A^-IP sample were detected by MACS2 (version 2.1.2.10160309)²¹ with q < 0.01 and the corresponding m^A^-input sample was used as the control. Peaks that were detected by both replicates were considered as high-confidence peaks. The peak annotation and binding motif were analysed by HOMER (version 4.9)²².

**Ribosome profiling.** DCs (5 × 10⁴) were treated with 100 μg/ml cycloheximide (CHX) for 7 min. The cells were then washed before using a cell liver. The cell suspension was spun at 400g for 5 min and the cell pellet was washed twice with 5 ml cold PBS with CHX (100 μg/ml). Lysis buffer (200 μl; 10 mM Tris, pH 7.4, 150 mM KCl, 5 mM MgCl₂, 100 μg/ml CHX, 0.5% Triton-X-100, freshly added 1:100 protease inhibitor, 40 U/ml SUPERaspin) was added to the cell pellet and the pellet was lysed on ice for 15 min with rotation. Ten per cent of the clarified lysate was saved as input and the rest of the lysate was separated through a 5 ml 10–50% sucrose gradient and centrifuged at 4°C for 2 h at 28,000 r.p.m. Fragments were collected separately and analysed using a Qubit RNA HS Assay Kit (Invitrogen). The fractions corresponding to monosome or polysome, respectively, were combined and concentrated on Amicon Ultra-100 K columns (Millipore). Two A260 units of ribosome fractions were digested with 60 U RNase I (Ambion) at room temperature for 30 min. RNA was extracted using RNA Clean & Concentrate (Zymo) and ribosomal RNA was deleted before size selection. RNA fragments (26–32 nt) were isolated using 15% denaturing Urea-PAGE (Calbiochem). The cell lysis solution was centrifuged at 16,100 g for 15 min. Cytokine production was measured using the Mouse Inflammation Kit (BD Biosciences).

**Maturation of DCs.** GMDcs were collected and co-cultured with 100 ng/ml LPS overnight. Cytokine production was measured using the Mouse Inflammation Kit (BD Biosciences).

**Identification of off-target site and T7E1 assay.** Two identified off-target loci for each sgRNA site with the highest scores were selectively amplified using the following primers: YTHDF1_For: TACATTGGTGTCACGATTTGACT; YTHDF1_Rev: TGGCGTCCATACAACTTGCTGG; MED20_Rev: AGGCACCACACAAACCAGGCA. A HiPure DNA Sample Mini Kit (Magen, D3121-03) was used to extract genomic DNA from the tails of wild-type and Yhdf1−/− mice. PCR reactions to amplify 350-bp fragments (for Yhdf1−/− mice) and 510-bp fragmentd (for wild-type mice) were carried out in 30 μl reactions, 15 μl of 2× EasyTaq PCR SuperMix (AS111 TransGen Biotech), 0.75 μl of each of forward and reverse primers and 1 μl genomic DNA. The reaction products were subjected to 1.5% agarose gel electrophoresis. For the T7EI cleavage assay, equal volumes of PCR products from Yhdf1−/− and wild-type mice were mixed and then denatured and annealed in NEBuffer 2 (NEB) using a thermal cycler. Hybridized PCR products were digested with T7 endonuclease I (NEB, M0302L) or ddH2O (as control) for 20 min at 37°C and subjected to 1% agarose gel electrophoresis.

**Statistical analysis and reproducibility.** No statistical method was used to determine sample size. Mice were assigned at random to treatment groups for all mouse studies and, where possible, mixed among cages. No mice were excluded from experiments. Blinded staining and blinded analysis were performed for IHC experiments. Experiments were independently repeated two to three times. Data were analysed using Prism 5.0 software (GraphPad) and presented as mean values ± s.e.m. P values were calculated using one-sided or two-sided unpaired Student’s t-tests. For survival curves, the log-rank (Mantel–Cox) test was used. For translational efficiency, P values were calculated using the likelihood ratio test and adjusted by the Benjamini–Hochberg method. For cumulative distribution, the two-sided Kolmogorov–Smirnov test was used to calculate P values. For multiple comparisons, the two-sided Fisher’s exact or Student’s t-test was used to calculate P values.

**Data processing and analysis.** Illumina reads were post-processed and aligned to the mouse genome (mm10) by Bowtie2 (version 2.1.0) with default parameters. To visualize sequencing signals in the genome browser, we generated RIP-seq and m^A^-seq bigwig files with bamCoverage function from deepTools (version 3.0.1)²³ with `b-w = 1–normalizeUsing BPM`. For RIP-seq, Piranha software (version 1.2.1)²⁴.
was to detect the binding sites of YTHDF1 with \`-b 100 -i 100\'. Metagene plots were created using the Bioconductor GUITAR\textsuperscript{39} package (version 1.16.0). Peaks that were detected by both replicates were considered as high-confidence peaks. GO term analyses were performed using metascape\textsuperscript{40}.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

The data that support the findings of this study are available from the corresponding author upon reasonable request. RIP–seq, Ribo-seq and m\textsuperscript{6}A-seq datasets have been deposited in the Gene Expression Omnibus (GEO) under the accession number GSE115106. A summary of sequencing experiments is provided in Supplementary Table 3. The differential translational efficiency results provided in Supplementary Table 4. Source Data for bar graphs and box-plots in the Figures and Extended Data Figures are provided in separate Excel files.

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Extended Data Fig. 1 | Deletion efficacy of Ythdf1−/− mice. a, b, Off-target analysis of the CRISPR–Cas9 system in Ythdf1−/− mice. a, Ythdf1 single guide RNA (sgRNA) targeting sites and four putative off-target sites were amplified. b, PCR products from Ythdf1−/− mice and wild-type mice were mixed and digested by T7EI. The PCR product from wild-type mice was used as negative control. c, Immunoblot assays are shown to validate changes in YTH protein expression in Ythdf1−/− DCs. Data are representative of one experiment (a, b) and two independent biological replications (c).
Extended Data Fig. 2 | Characterization of immune phenotypes of Ythdf1−/− mice. a, Data points for Fig. 1a. b, Wild-type or Ythdf1−/− mice were injected s.c. with 10⁶ B16-OVA cells. Survival was monitored. Mice with tumour volumes less than 200 mm³ are considered to be surviving. One of three representative experiments is shown. c, Data points for Fig. 1b. d–h, Wild-type or Ythdf1−/− mice were injected s.c. with 10⁶ B16-OVA cells. d, e, Frequency of tumour infiltrating MDSCs (Ly6C⁺CD11b⁺) was assessed 12 days after tumour inoculation. f, g, The percentages of Treg cells in spleen, DLN and tumour are shown. h, Degranulation of tumour NK cells in response to in vitro re-stimulation with PMA/ionomycin. i, Data points for Fig. 1d. Data are representative of two independent experiments (a, c, e, g–i); two-sided unpaired Student’s t-test (a, c, e, g–i); two-sided log-rank (Mantel–Cox) test (b).
Extended Data Fig. 3 | Cross-priming of tumour neoantigens is increased in Ythdf1\(^{-/-}\) mice. a, Rag2\(^{-/-}\) mice were inoculated with T cells isolated from wild-type or Ythdf1\(^{-/-}\) mice on day 0. On the same day, mice were injected s.c. with 5 \(\times\) 10\(^5\) B16-OVA cells. Tumour growth was measured over time. b, Wild-type or Ythdf1\(^{-/-}\) mice were injected s.c. with 10\(^6\) MC38-OTIp cells. Six days after tumour inoculation, CD8\(^+\) or CD11b\(^+\) DCs were sorted from DLNs. DCs were co-cultured with CD8\(^+\) T cells isolated from naive OT-I mice. Cross-priming capacity was determined by the production of IFN\(\gamma\). c, Wild-type or Ythdf1\(^{-/-}\) mice were injected s.c. with 10\(^6\) MC38-SIY cells. Six days after tumour inoculation, DCs were sorted from DLNs and co-cultured with CD8\(^+\) T cells isolated from naive 2C mice. Cross-priming capacity was determined by the production of IFN\(\gamma\). d, Wild-type or Mett14-deficient GMDCs were co-cultured with B16-OVA cells. Cross-priming capacity was determined by the production of IFN\(\gamma\). e, Wild-type and Ythdf1\(^{-/-}\) mice were injected s.c. with 10\(^6\) B16-OVA cells. Data are shown as the expression of CD80 and CD86 on tumour-infiltrating DCs. f, Wild-type and Ythdf1\(^{-/-}\) mice were injected s.c. with 10\(^6\) B16-OVA cells. Six days after tumour inoculation, DCs were sorted from DLNs. DCs were pulsed with 1 \(\mu\)g/ml exogenous OT-I peptide and co-cultured with isolated CD8\(^+\) T cells from naive OT-I mice for 3 days, and then analysed by IFN\(\gamma\)-CBA. Data are representative of two independent experiments with similar results (e). n, number of mice. Mean ± s.e.m., two-sided unpaired Student’s t-test (a–c, f) or one-sided unpaired Student’s t-test (d).
Extended Data Fig. 4 | Development of DCs and T cells is similar in Ythdf1+/+ and Ythdf1−/− mice. a, b, Percentages of CD11b+ and CD8α+ DCs in lymph node (LN) and spleen. c, d, Percentages of CD4+ and CD8+ T cells in lymph node and spleen. No significant difference was detected between wild-type and Ythdf1−/− mice. n, number of mice. Mean ± s.e.m., two-sided unpaired Student's t-test.
Extended Data Fig. 5 | In vitro functional analysis of GMDCs generated from Ythdf1−/− mice. 

a, Production of IL-6, CCL2 and TNFα upon stimulation of Ythdf1−/− GMDCs with LPS. 

b, c, Wild-type and Ythdf1−/− mice were injected s.c. with 10⁶ B16-OTI-zsGreen cells. The percentage of tumour-infiltrating zsGreen+ DCs six days after tumour inoculation is shown. Data are representative of two independent experiments (b).

d, Splenic DCs from wild-type and Ythdf1−/− mice were stimulated with LPS overnight. The cross-presentation capacity of DCs in response to soluble OVA was assessed. n = 3 independent experiments (a); n = 6 independent experiments (d). n, number of mice. Mean ± s.e.m., two-sided unpaired Student’s t-test.
Extended Data Fig. 6 | Transcriptome-wide analysis of YTHDF1-binding sites in FLT3L-DCs. 

**a**, High reproducibility of YTHDF1 RIP-seq data. For each potential YTHDF1 binding peak, the fold-enrichment of the RIP/input signal was determined for both replicate 1 (Rep1) and replicate 2. Peaks identified in both replicates were considered as high-confidence peaks and are indicated in red. 

**b**, Overlap of YTHDF1-binding transcripts revealed from RIP-seq of two biological replicates.

**c**, Meta-gene analysis to show the distribution of YTHDF1-binding sites along a normalized transcript.

**d**, Distribution of YTHDF1-binding sites in transcripts. TTS, transcription termination site.

**e**, Heatmap showing the translational efficiency of co-simulatory/inhibitory proteins (signal 2) and cytokines (signal 3) in wild-type and Ythdf1−/− FLT3L-DCs.
Extended Data Fig. 7 | THDF1-deficient GMDCs exhibit lower translational rates. a, High reproducibility of YTHDF1 RIP-seq data in GMDCs. For each potential YTHDF1 binding peak, the fold-enrichment of the RIP/input signal was determined for both Replicate 1 and Replicate 2. Peaks identified in both replicates were considered high-confidence peaks and are indicated in red. b, Volcano plots of genes with differential translational efficiencies in wild-type and Ythdf1−/− GMDCs. YTHDF1 targets are marked with yellow circles. *P* values calculated using two-sided likelihood ratio test with Benjamini–Hochberg adjustment; *n* = 4 (2 conditions × 2 biological replicates). c, Cumulative distribution of the fold change in translational efficiency between wild-type and Ythdf1−/− GMDCs. *P* values calculated using two-sided Kolmogorov–Smirnov test; *n* = 2 independent biological replicates. Box-plot elements: centre line, median; box limits, upper and lower quartiles; whiskers, 1–99%. d, Distribution of YTHDF1-binding sites in transcripts. e, Metagene plot depicting nearly unchanged distribution of m6A peaks and similar consensus motifs in wild-type and Ythdf1−/− GMDCs. *P* values of consensus motifs generated by HOMER29 using one-sided binomial test. f, KEGG and GO enrichment analysis of YTHDF1 target genes revealed enrichment of biological functions related to the innate immune system, lysosomes and phagosomes (n = 79). One-sided hypergeometric test was used to determine the statistical significance of enrichment. g, Heatmap showing translational efficiency of cathepsin genes in GMDCs and FLT3L-DCs. *n*, number of genes or m6A peaks.
Extended Data Fig. 8 | Antigen degradation is reduced in Ythdf1<sup>−/−</sup> mice and inhibition of protease cathepsins enhances cross-priming of wild-type DCs. a, GMDCs were co-cultured with necrotic B16-OVA cells overnight. Immunoblot analysis of cathepsins B, D and L (CTSB, CTSD and CTSL) in GMDCs. b, Wild-type and Ythdf1<sup>−/−</sup> DCs were treated with actinomycin D and RNAs were collected at different time points after treatment. mRNA levels were measured using RT–qPCR and represented as mRNA remaining after transcription inhibition (TI). c, GMDCs were co-cultured with necrotic B16-OVA cells overnight and OVA degradation in BMDCs was measured by immunoblot. d, Ex vivo purified wild-type cDCs were pre-treated with 0.04 μM E64 and pulsed with OVA protein for 4 h. The cross-priming capacity of DCs was compared by co-culturing DCs with CTV-labelled OT-I T cells. Proliferation was measured by the dilution of CTV. e, GMDCs were pre-treated with 0.2–2 μM E64 and co-cultured with B16-OVA cells. The cross-priming capacity of DCs was compared by co-culturing DCs with isolated CD8<sup>+</sup> T cells from naive OT-I mice and analysed by IFNγ CBA. f, FLT3L-DCs were pre-treated with cathepsin inhibitor CA-074 or/and cathepsin L inhibitor III (CASIII), followed by co-culturing with necrotic B16-OVA cells. Synergistic inhibition was observed. The cross-priming capacity of DCs was determined. g, Data points for Fig. 4b. n = 3 independent experiments (a, c); n = 2 independent experiments (b). n, sample size. Mean ± s.e.m., two-sided unpaired Student’s t-test (e) or one-sided unpaired Student’s t-test (f).
Extended Data Fig. 9 | IFNγ in tumour tissues is responsible for the upregulation of PD-L1 in Ythdf1−/− mice. Tumour-bearing mice were treated with 50 µg anti-IFNγ monoclonal antibody intratumorally and PD-L1 expression on tumour cells is shown. n, number of mice. Mean ± s.e.m., two-sided unpaired Student’s t-test.
Reporting Summary

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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

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Data collection

Cell images were collected with with a Nikon Eclipse Ti2 with NIS Elements Imaging Software (Version 5.02).

Data analysis

Images analyzed with ImageJ (Version 1.52e). Experimental data were analyzed using Prism 5.0 software (GraphPad). For sequencing data, reads were post-processed and aligned to the mouse mm9 assembly using STAR program (version 2.6.0c). To visualize sequencing signals in the genome browser, we generated bigwig files with bamCoverage function from deepTools (version 3.0.1). For RIP-Seq, Piranha (version 1.2.1) software was used to detect the binding sites of YTHDF1. For m6A-Seq, the m6A-enriched regions (peaks) in each m6AIP samples were detected by MACS2 (version 2.1.2.20160309). Metagene plots were performed by the Bioconductor GUITAR package (version 1.16.0). Peaks that were detected by both replicates were considered as high confident peaks. GO term analyses were performed by metascape. For Ribo-seq, adapter sequences were firstly trimmed from sequencing reads by using fastx_trimmer (version 0.0.14) and cutadapt (version 1.15). Trimmed reads were filtered for mitochondrial DNA and ribosomal RNA by Bowtie2 (version 2.3.4). Uniquely mapped reads were selected by using SAMtools (version 1.7). The raw counts of coding regions were calculated by HOMER (version 4.9). The differentially translational efficiency genes were detected by Bioconductor DESeq2 package (Version 1.18.1). The flow cytometry data were analyzed by Flowjo 7.0 (Treestar).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
Data

Policy information about availability of data
All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Ribo-seq, RIP-seq and m6A-seq data generated by this study have been deposited in the GEO database under the accession number GSE115106. A summary of the sequencing experiment can be found in Supplementary Table 3. The differential translational efficiency results provided in Supplementary Table 4. All other data supporting the findings of this study are available from the corresponding author on reasonable request.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences  ☐ Behavioural & social sciences  ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size  In general, no statistical methods were used to predetermine sample size. For the animal experiments, sample size was determined based on our prior experience of performing similar sets of experiments and literature reports. For human samples, samples were collected till we have sufficient number to obtain statistically reliable estimates. For cell and biochemical data, we aimed to collect data from three or more biological replicates when possible.

Data exclusions  No data were excluded from the analysis

Replication  Experiments were independently repeated two to three times. All attempt to reproduce the results were successful.

Randomization  Mice were assigned at random to treatment groups for all mouse studies and, where possible, mixed among cages.

Blinding  Reported in the 'Statistical analysis and reproducibility' section of the Methods. For IHC experiment, blinded staining and blinded analysis were performed. For other experiments, the investigators were not blinded to the group allocation, as group allocation was clearly visible in the samples due to phenotypic changes. Variations were controlled through replicates.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☒ | Antibodies |
| ☒ | Eukaryotic cell lines |
| ☒ | Palaeontology |
| ☒ | Animals and other organisms |
| ☒ | Human research participants |
| ☒ | Clinical data |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☒ | ChiP-seq |
| ☒ | Flow cytometry |
| ☒ | MRI-based neuroimaging |

Antibodies

Antibodies used

- Goat HRP anti-GAPDH WB 1:10000 ProteinTech HRP-60004
- Rabbit anti-YTHDF1 IP&WB 1:100 for IP, 1:1000 for WB ProteinTech 17479-1-AP
- Rabbit anti-YTHDF2 WB 1:1000 ProteinTech 24744-1-AP
- Anti-rabbit IgG-HRP WB 1:5000 cell signaling technology 70745
- Mouse anti-YTHDF3 WB 1:500 Santa-Cruz sc-377119 F-2
- Anti-mouse IgG-HRP WB 1:5000 cell signaling technology 70765
- Rabbit anti-YTHDC1 WB 1:1000 Abcam ab122340
Goat anti-YTHDC2 WB 1:500 Santa-Cruz sc-249370 G-19
Anti-goat IgG-HRP WB 1:5000 Abcam ab6741
Anti-Chicken Egg Albumin antibody produced in rabbit WB 1:5000 Sigma C6534-2.4G2
CD11c Monoclonal Antibody (N418), PE-Cyanine7 Flow cytometry 1:500 eBioscience 25-0114-82 N418
PerCP/Cy5.5 Anti-Mouse/Human CD11b Flow cytometry 1:500 Biologend 102212 M1/70
FITC Anti-Mouse Ly-6C Flow cytometry 1:500 Biologend 128005 HK1.4
β-Actin Antibody (C4) HRP WB 1:2000 Santa-Cruz sc-47778 HRP
InVivoMAb anti-mouse CD16/CD32 FcyRII blockade 1:500 BioX Cell BE0307 2.4G2
 APC/Cy7 Anti-Mouse CD45 Flow cytometry 1:500 Biologend 124308 10F.9G2
FITC anti-mouse/human CD45R/B220 Flow cytometry 1:501 Biologend 103206 RA3-682
InVivoMAb anti-mouse CD8a In vivo depletion 200 μg BioX Cell BE0117 YTS169.4
InVivoMAb anti-mouse IFNγ In vivo blockade 50 μg BioX Cell BE0055 XMG1.2
Yellow fluorescent protein (GFP) was used in living and fixed cells to assess cell viability.

Validation

All the primary antibodies we used were validated by manufacturers.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

MC38 cell lines were provided by D. Bartlett (University of Pittsburgh, Pittsburgh, PA). B16-OVA cells were provided by Dr. Yang-Xin Fu (UT Southwestern). B16F10 cell line was purchased from ATCC.

Authentication

All cell lines were authenticated according the ATCC cell line authentication test recommendations that included a morphology check by microscope, growth curve analysis and mycoplasma check.

Mycoplasma contamination

All cell lines are mycoplasma-free.

Commonly misidentified lines

(See ICLAC register)

No cell line listed by ICLAC was used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

Female Rag2-/- mice, 2C CD8+ T cell receptor (TCR)-Tg, CD11c-Cre and Zbtb46-DTR mice were purchased from Jackson laboratory. Female Ythdf1-/- mice and CD11c-CreMettl14f/f conditional knockout mice were generated in house. All mice were used at 6–12 weeks of age. All the mice were maintained under specific pathogen-free conditions and used in accordance with the animal experimental guidelines set by the Institute of Animal Care and Use Committee.

Wild animals

The study did not involve wild animals.

Field-collected samples

The study did not involve filed-collected samples.

Ethics oversight

All animal procedures used in this experiment were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee of University of Chicago.
Human research participants

Policy information about studies involving human research participants:

Population characteristics: Information about the patient sex, age, and tumor characteristics are given in Supplementary Table 2. 9 female and 13 male patients with age ranging from 42.1-86.8 and presented with adenocarcinoma colon cancer at various stages and locations were studied. Controlling for covariates was not necessary as the analyses correlate CD8 and YTHDF1 in stroma taken from the same patient.

Recruitment: Participants were recruited from the pool of patients at the University of Chicago. There may be self selection biases based on individuals who are able to seek care at the institution. The sample should be representative of the populations served by the institution.

Ethics oversight: All samples were obtained with informed consent under a protocol approved by the University of Chicago Institutional Review Board.

Flow Cytometry

Plots

Confirm that:
- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation: For flow cytometric analysis and cell sorting, tumors, lymph nodes and spleens were collected from mice and digested with 0.26U/ml Liberase TM and 0.25mg/ml DNase I at 37°C for 30min. Samples were then filtered through a 70μm cell strainer and washed twice with staining buffer. Cells were re-suspended in staining buffer (PBS with 2% FCS and 0.5 M EDTA).

Instrument: Cells were either analyzed on a BD Fortessa (BD) or sorted by AriaIIu (BD).

Software: Analysis of flow cytometry data was performed using Flowjo 7.0 (Treestar).

Cell population abundance: The purity of sorted cells was detected via Fortessa and samples with purity >90% were used.

Gating strategy: Cell populations were determined by A FSC-H/FSC-A gate. The boundaries were determined by the clear cell subpopulations and isotype controls.

 Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.