

Supplementary Information for

Palmitic Acid-Triggered B7H3 Palmitoylation Promotes Immune Escape

Zejun Rao¹, Changsheng Huang¹, Qi Wu¹, Mao Li¹, Anyi Liu¹, Tong Zhu¹, Wangshuo Yang¹, Lanlan Yin¹, Shengyu Zhu¹, Xiaowei She¹, Chengxin Yu¹, Lang Liu¹, Pengcheng Li¹, Yucong Bai¹, Dongjing Zhang¹, Tianci Xie¹, Xiang Liu¹, Lu Liu¹, Feng Xu¹, Guihua Wang^{1,2}, Junbo Hu^{1#}, Xuelai Luo^{1#}

1, GI Cancer Research Institute, Tongji Hospital, Huazhong University of Science and Technology, Wuhan, 430030, P. R. China;

2, State Key Laboratory for Diagnosis and Treatment of Severe Zoonotic Infectious Diseases, Huazhong University of Science and Technology Wuhan 430030, Hubei Province, China.

To whom corresponding authors:

Xuelai Luo, GI Cancer Research Institute, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, 430030, China. E-mail: luoxl@tjh.tjmu.edu.cn.

Junbo Hu, GI Cancer Research Institute, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, 430030, China. E-mail: jbh@tjh.tjmu.edu.cn.

Supplementary Information file Including:

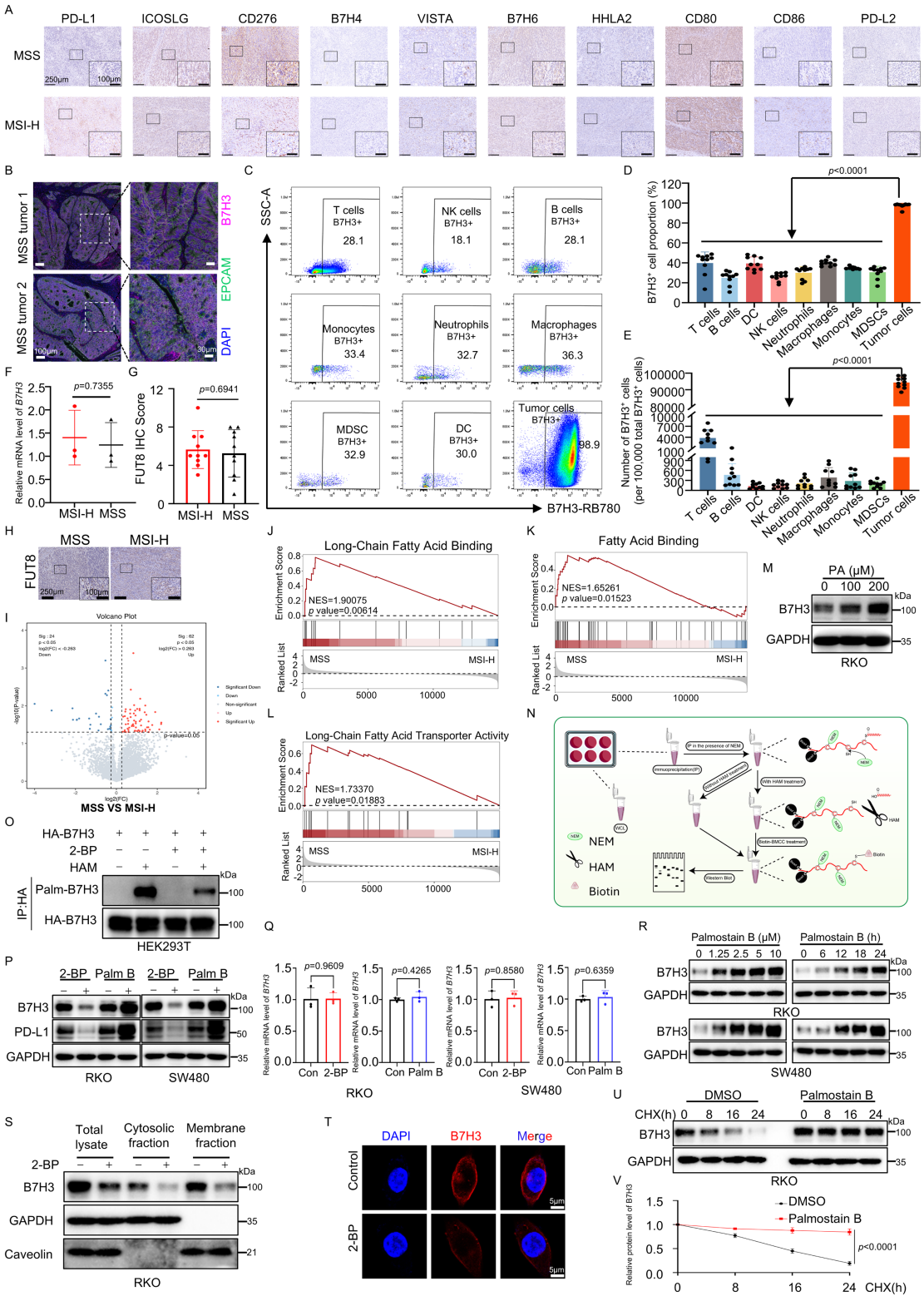
Supplementary Figures (S1-S12)

Supplementary Figure Legends (S1-S12)

Supplementary Tables (1-10)

Supplementary Figures and Figure Legends

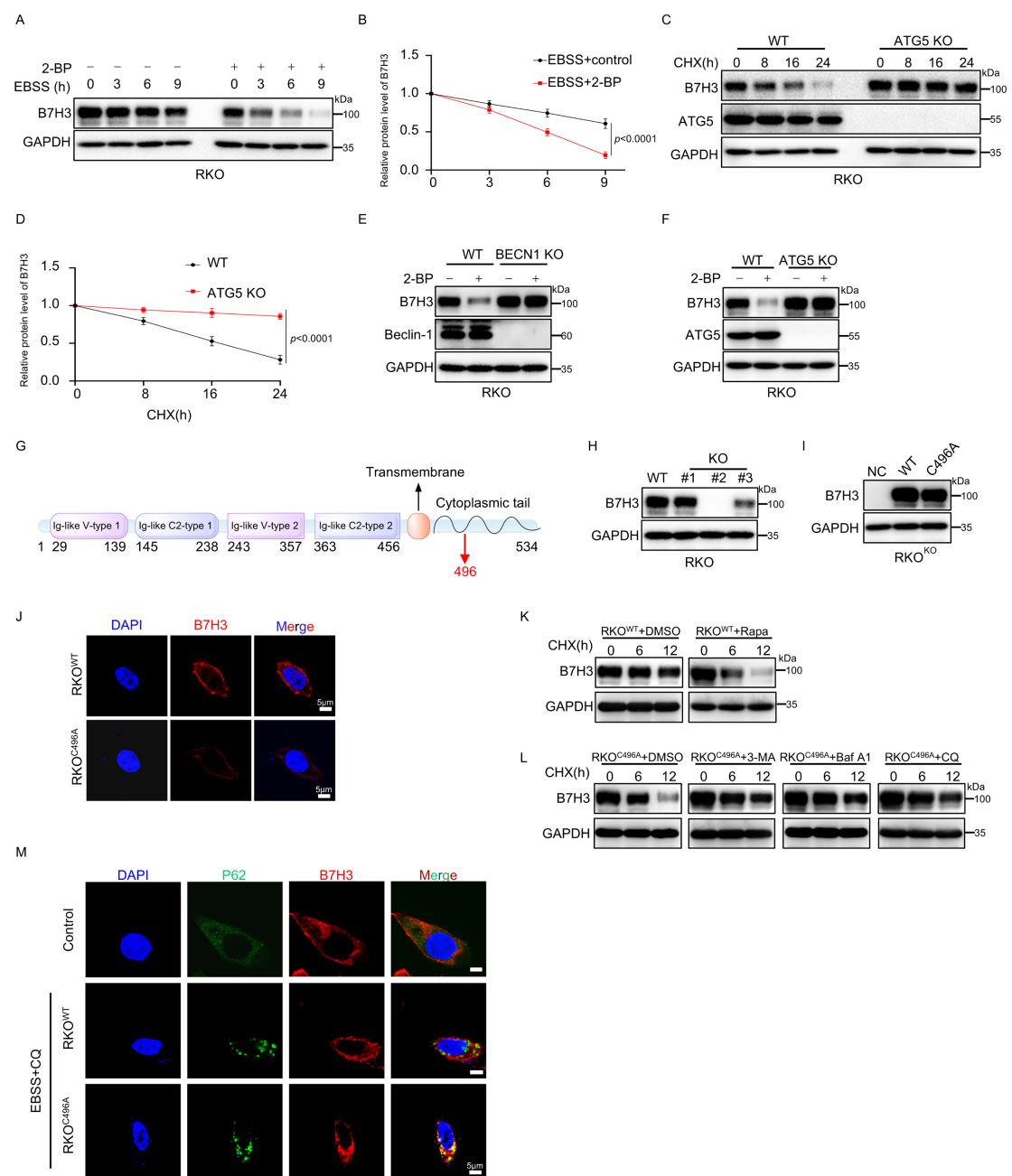
Fig. S1



Supplementary Fig. S1. Palmitic acid-triggered palmitoylation stabilizes B7H3 protein in MSS colorectal cancer.

A. Immunohistochemistry (IHC) images of B7 family staining (n = 10). Scale bars, 250 μ m (main images) and 100 μ m (insets). **B.** Immunofluorescence (IF) images showing co-localization of B7H3 with EPCAM in tumors (n = 10). Scale bars, 100 μ m (left) and 30 μ m (right). **(C to E).** Flow cytometry of B7H3 in tumor and immune cells (n = 10): representative plot (C), proportions in indicated populations (D), and B7H3⁺ cells normalized to 100,000 total cells (E). **F.** qRT–PCR analysis of B7H3 mRNA. (n=3). **(G and H).** FUT8 IHC staining scores (G) and representative images (H) (n = 10). Scale bars, 250 μ m (main images) and 100 μ m (insets). **I.** Volcano plot of differential metabolites between MSS and MSI-H tissues. **(J to L)** GSEA of RNA-seq data comparing MSS and MSI-H tissues. **M.** Immunoblot (IB) analysis of B7H3 after Palmitic acid (PA) treatment. **N.** Schematic of the acyl-biotin exchange (ABE) assay. **O.** ABE of B7H3 palmitoylation after DMSO or 2-BP (50 μ M) treatment. The samples derive from the same experiment but different gels for B7H3, and another for Palm-B7H3 were processed in parallel. **(P and Q).** IB analysis of B7H3 protein (P) and qRT–PCR analysis of B7H3 mRNA (Q) (n = 3) after 2-BP or Palmostain B (5 μ M). The samples derive from the same experiment but different gels for PD-L1, and another for B7H3 and GAPDH were processed in parallel. **R.** IB analysis of B7H3 after Palmostain B treatment at indicated doses/times. **S.** Cytoplasmic/membrane fractionation of RKO cells treated with DMSO or 2-BP, followed by IB. The samples derive from the same experiment but different gels for Caveolin, and another for B7H3 and GAPDH were processed in parallel. **T.** Immunofluorescence images of RKO cells treated with DMSO or 2-BP. Scale bars, 5 μ m. **(U and V).** CHX chase (100 μ g/mL) with Palmostain B: IB (U) and quantification (V) (n = 3). Data indicate the mean $\pm$ SD, by one-way ANOVA with Tukey’s test (D and E), unpaired 2-tailed Student’s t-test (F, G, and Q), and two-way ANOVA with Tukey’s test (V). n = 3 independent experiments (M, O–U). Source data are provided as a Source Data file.

Fig. S2

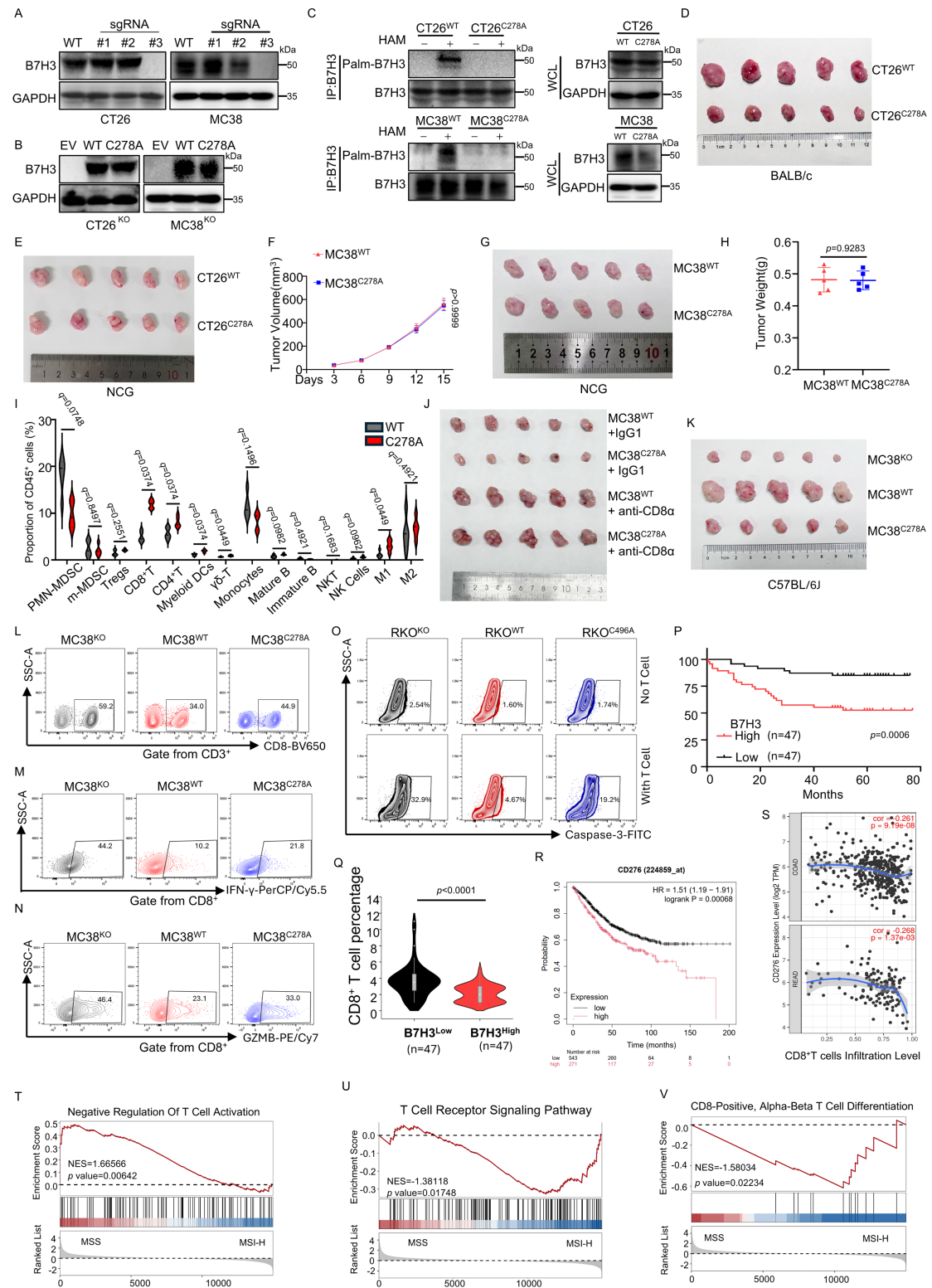


Supplementary Fig. S2. B7H3 C496 Palmitoylation suppresses SQSTM1/p62 mediated autophagic degradation.

(A and B). IB analysis of B7H3 after EBSS treatment with DMSO or 2-BP (50 μ M) (A) and quantification (B) (n = 3). (C and D). IB analysis of B7H3 stability after CHX chase (100 μ g/ml) in WT and ATG5 KO RKO cells (C) and quantification (D) (n = 3). (E and F). IB analysis of B7H3 in WT versus BECN1 KO (E) or ATG5 KO (F) RKO cells after DMSO or 2-BP (100 μ M, 24 h) treatment. G. Simplified schematic of the

B7H3 protein structure and localization of the palmitoylation site. **H.** Generation of stable B7H3 knockdown cell lines in RKO cells with the CRISPR/Cas9 system. **I.** Generation of wild-type (RKO^{WT}) and palmitoylation mutant (RKO^{C496A}) stable cell lines in RKO^{KO} cells. **J.** Representative confocal images of RKO^{WT} and RKO^{C496A}. Scale bars, 5µm. **K.** IB analysis of RKO^{WT} cells treated with rapamycin (Rapa, 200 nM) or DMSO for the indicated times. **L.** IB analysis of RKO^{C496A} cells treated with the DMSO, 3-methyladenine (3-MA, 5 mM), bafilomycin A1 (Baf A1, 0.2 µM), and chloroquine (CQ, 50 µM) for the indicated times. **M.** Representative confocal images of RKO^{WT} or RKO^{C496A} cells after EBSS and CQ (50 µM) for 8 hours. Scale bars, 5µm. n = 3 independent experiments (A, C, E, F, H–M). Data indicate the mean ± SD, by two-way ANOVA with Tukey's test (B and D). Source data are provided as a Source Data file.

Fig. S3

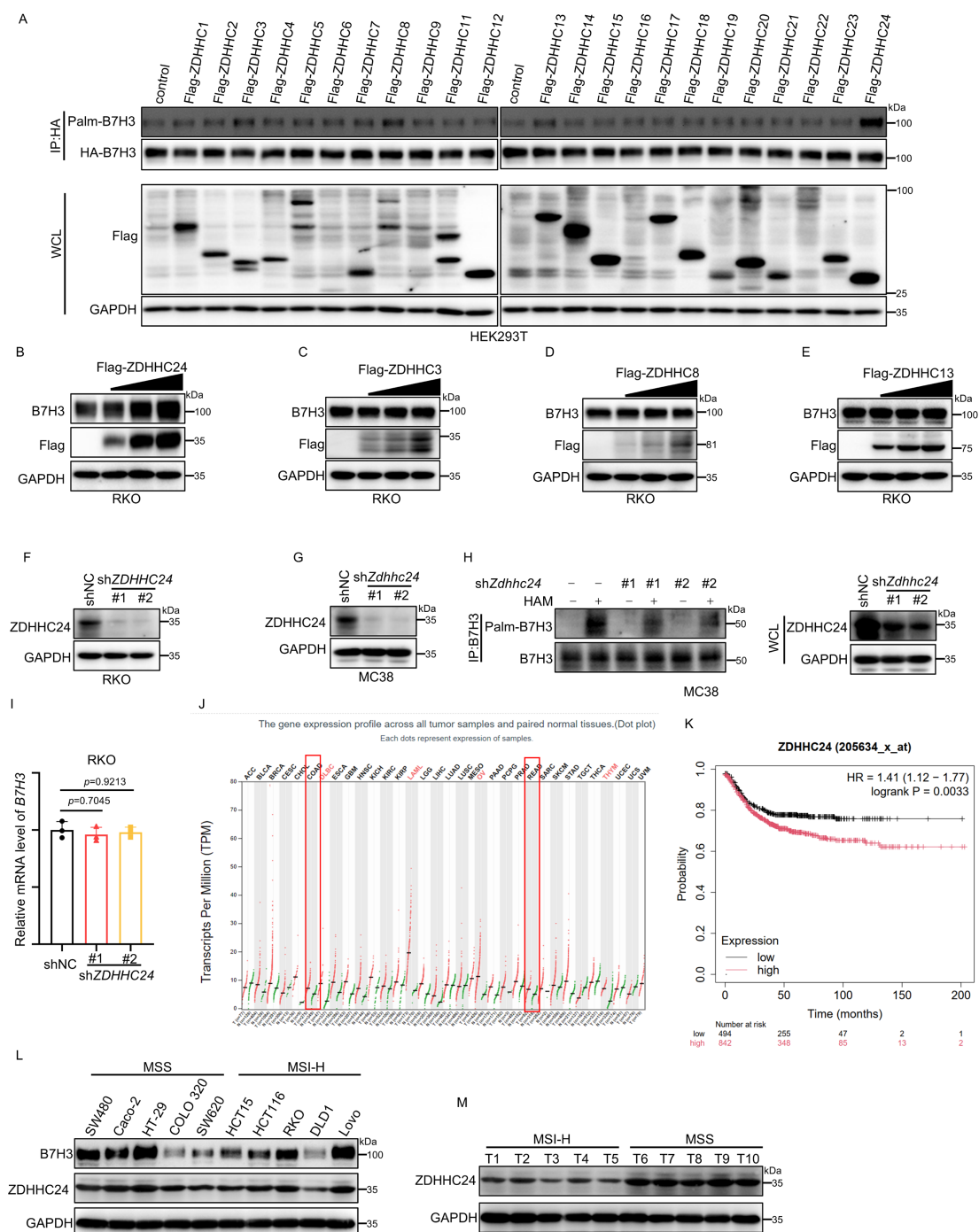


Supplementary Fig. S3. B7H3 Cys 496 Palmitoylation inhibits the antitumor activity of CD8⁺ T cells.

A. Generation of *B7h3* knockout CT26 and MC38 cell lines using CRISPR/Cas9 system.

B. Reconstitution of WT and palmitoylation deletion mutant (C278A) cell lines in CT26^{KO} and MC38^{KO} cells. The samples (A and B) derive from the same experiment but different gels for B7H3, and another for GAPDH were processed in parallel. **C.** ABE assay of B7H3 palmitoylation in reconstituted cells. The samples derive from the same experiment but different gels for B7H3, and another for Palm-B7H3 were processed in parallel. **(D and E).** CT26 tumor images from BALB/c (D) and NCG (E) mice (n = 5). **(F to H).** MC38^{WT} and MC38^{C278A} tumors in NCG mice (n = 5): tumor growth curves (F), tumor images (G), and tumor weights (H). **I.** Violin plots of immune cell subset infiltration in CT26^{WT} versus CT26^{C278A} tumors (Mann–Whitney U test with FDR correction; q values indicate adjusted p values. (n=5). **J.** Tumors from the CD8⁺ T-cell depletion experiment shown in Fig. 3I. (n=5). **K.** MC38^{KO}, MC38^{WT} and MC38^{C278A} tumors from C57BL/6J mice (n=5). **(L to N)** Representative flow cytometry plots of tumor-infiltrating CD8⁺ T cells (L) and IFN- γ ⁺ CD8⁺ T (M) or GZMB⁺ CD8⁺ T(N) from (K). **O.** Representative flow cytometry plot of caspase-3⁺ tumor cells after co-culture with activated human T lymphocytes. **P.** Kaplan–Meier overall survival of CRC tissue microarray patients stratified by B7H3 expression (n = 94; log-rank test). **Q.** Association between B7H3 expression and CD8⁺ T-cell infiltration in CRC tissue microarrays (n = 94). **R.** Kaplan–Meier Plotter analysis of overall survival in colon cancer patients stratified by B7H3 expression. **S.** TIMER analysis of the correlation between CD276 (B7H3) expression and CD8⁺ T-cell infiltration in colorectal cancer. **(T to V)** GSEA of RNA-seq data (Cohort 4, Fig. 1C) comparing MSS versus MSI-H colorectal cancer tissues. n = 3 independent experiments (A–C). Data indicate the mean $\pm$ SD, by two-way ANOVA with Tukey’s test (F) and by unpaired 2-tailed Student’s t-test (H and Q). Source data are provided as a Source Data file.

Fig. S4

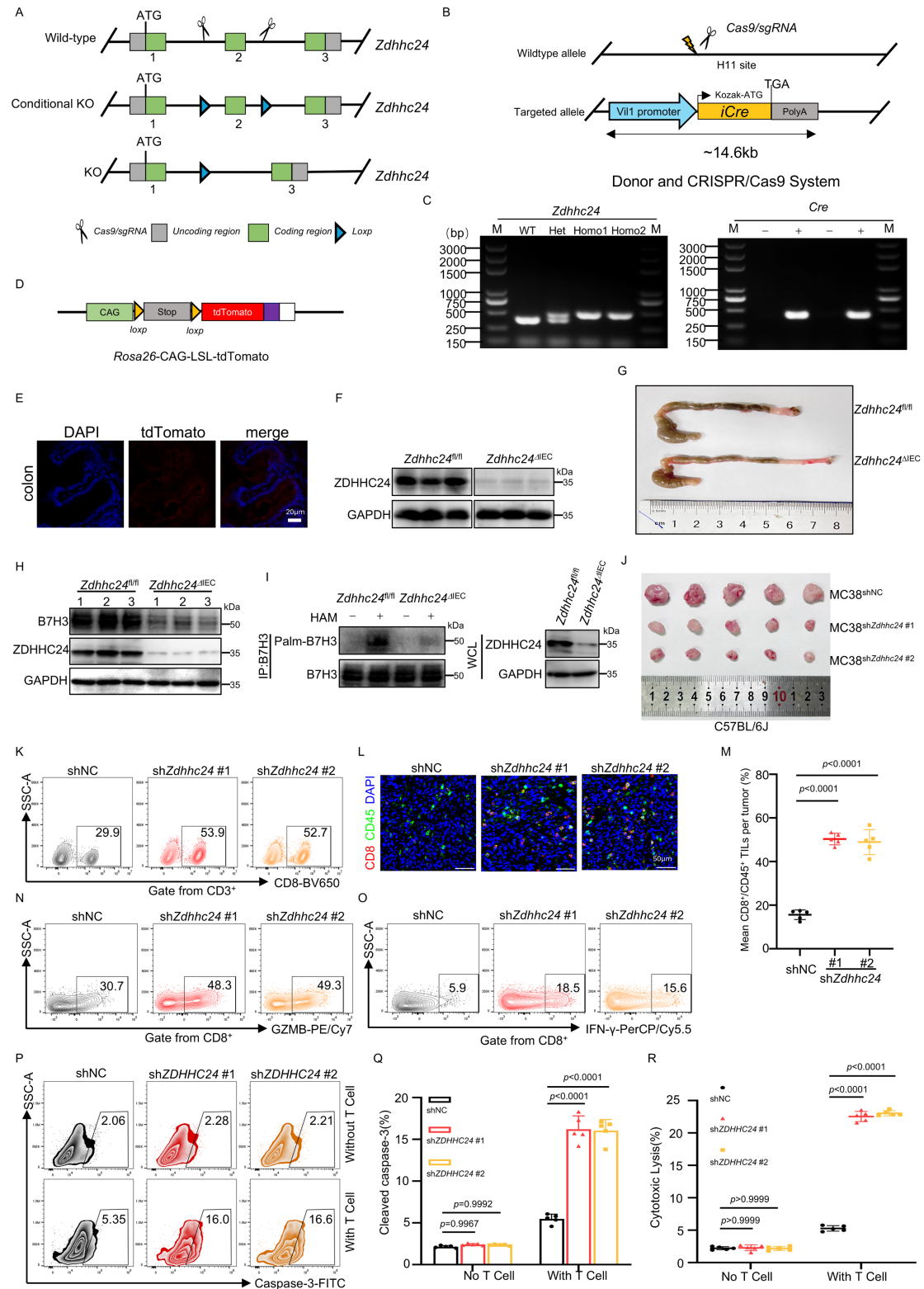


Supplementary Fig. S4. B7H3 C496 palmitoylation catalyzed by ZDHHC24.

A. ABE assay of B7H3 palmitoylation in HEK293T cells expressing HA-B7H3 and indicated DHHC family members. The samples derive from the same experiment but different gels for Flag, another for B7H3, another for Palm-B7H3, and another for GAPDH were processed in parallel. **(B to E).** IB analysis of B7H3 after overexpression of ZDHHC24 (B), ZDHHC3 (C), ZDHHC8 (D), or ZDHHC13 (E) in RKO cells. The

samples derive from the same experiment but different gels for Flag, and another for B7H3 and GAPDH were processed in parallel. **(F and G).** IB analysis of B7H3 in RKO (F) and MC38 (G) cells with control or *ZDHHC24/Zdhhc24* knockdown. The samples derive from the same experiment but different gels for ZDHHC24, and another for GAPDH were processed in parallel. **H.** ABE assay of B7H3 palmitoylation in MC38 cells with control or *Zdhhc24* knockdown. The samples derive from the same experiment but different gels for ZDHHC24, another for B7H3, another for Palm-B7H3, and another for GAPDH were processed in parallel. **I.** qRT-PCR analysis of *B7H3* mRNA in RKO cells with control or *ZDHHC24* knockdown. (n=3). **J.** GEPIA database analysis of B7H3 expression across cancers. **K.** Kaplan–Meier Plotter database analysis of relapse-free survival (RFS) in colon cancer patients stratified by ZDHHC24 expression. **L.** IB analysis of B7H3 and ZDHHC24 protein levels in MSS and MSI-H colorectal cancer cell lines. The samples derive from the same experiment but different gels for ZDHHC24, and another for B7H3 and GAPDH were processed in parallel. **M.** IB analysis of ZDHHC24 expression in patient-derived MSS and MSI-H colorectal cancer specimens. The samples derive from the same experiment but different gels for ZDHHC24, and another for GAPDH were processed in parallel. n = 3 independent experiments (A–I, L, and M). Data indicate the mean $\pm$ SD, by one-way ANOVA with Tukey’s test (I). Source data are provided as a Source Data file.

Fig. S5

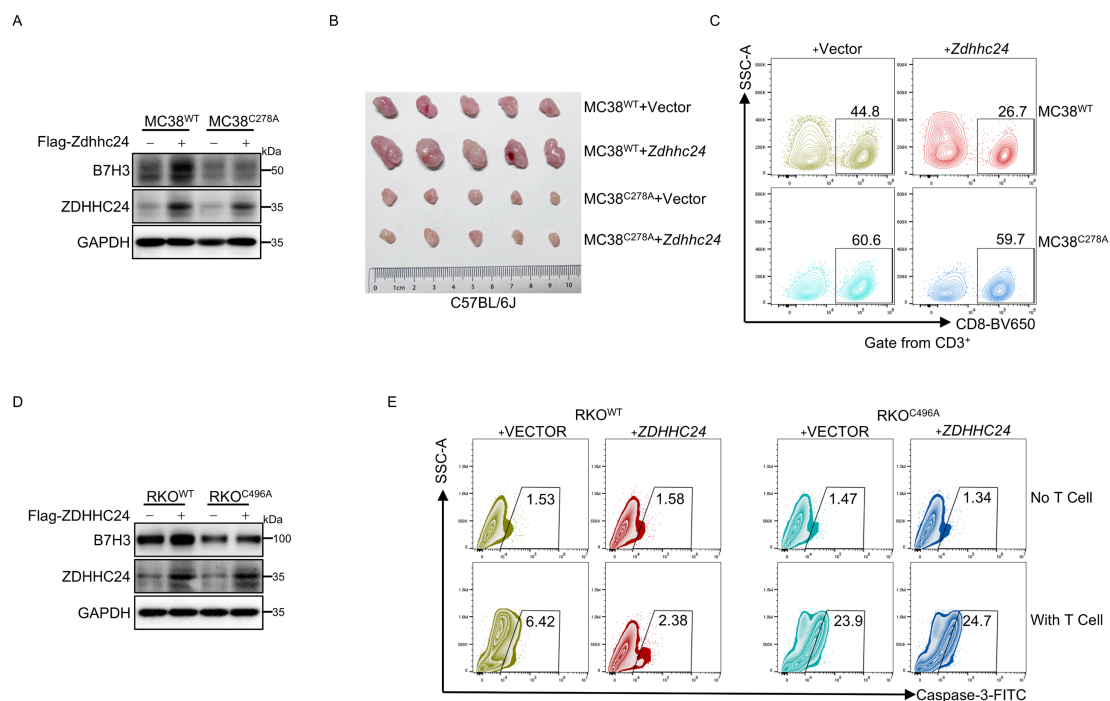


Supplementary Fig.S5. ZDHHC24 promotes tumor growth through inhibition of CD8⁺ T cell antitumor activity.

(A and B). Schematics of *Zdhhc24*^{fl/fl} (A) and *Villin-cre* (B) mice generation. C.

Genotyping of *Zdhhc24*^{fl/fl} and *Villin-cre* mice. **D.** Schematic of *Rosa26-CAG-LSL*-tdTomato reporter mice. **E.** Representative colonic immunofluorescence in progeny of *Villin-cre* and *Rosa26-CAG-LSL*-tdTomato mice. Scale bars, 20µm. **F.** IB analysis of ZDHHC24 in intestinal epithelial cells (IECs) from *Zdhhc24*^{fl/fl} and *Zdhhc24*^{ΔIEC} mice. (n = 3). The samples derive from the same experiment but different gels for ZDHHC24, and another for GAPDH were processed in parallel. **G.** Representative colon images from *Zdhhc24*^{fl/fl} and *Zdhhc24*^{ΔIEC} mice. **(H and I).** IB analysis of B7H3 protein (H) and ABE assay of B7H3 palmitoylation (I) in tumors from *Zdhhc24*^{ΔIEC} and *Zdhhc24*^{fl/fl} mice. The samples derive from the same experiment, with different gels for B7H3, ZDHHC24 and GAPDH in (H) and for Palm-B7H3, B7H3, ZDHHC24 and GAPDH in (I), all processed in parallel. **(J to O).** MC38 shNC, MC38 sh*Zdhhc24* (#1 and #2) cells were injected subcutaneously into C57BL/6J mice (n = 5). **J.** Excised tumors. **K.** Flow cytometry of tumor-infiltrating CD8⁺ T cells. **L.** Immunofluorescence of CD8⁺ T cells and CD45⁺ cells. Scale bars, 50 µm. **M.** Quantification of CD8⁺/CD45⁺ TILs from (L) (n = 5). Three fields per tumor were averaged to obtain one value. **N.** Flow cytometry of tumor-infiltrating GZMB⁺ CD8⁺ T cells. **O.** Flow cytometry of tumor-infiltrating IFN-γ⁺ CD8⁺ T cells. **(P to R)** RKO shNC, RKO shZDHHC24 (#1 and #2) cells were co-cultured with activated human T lymphocytes (n=5). **P.** Representative image showing the percentage of caspase-3⁺ in tumor cells by flow cytometry. **Q.** Statistical analysis shows the percentage of caspase-3⁺ in tumor cells by flow cytometry. **R.** Cell-free supernatants were collected and LDH release was measured to calculate percent cytotoxicity. n = 3 independent experiments (C, E, F, H and I). Data indicate the mean ± SD by one-way ANOVA with Tukey's test (M) and two-way ANOVA with Tukey's test (Q and R). Source data are provided as a Source Data file.

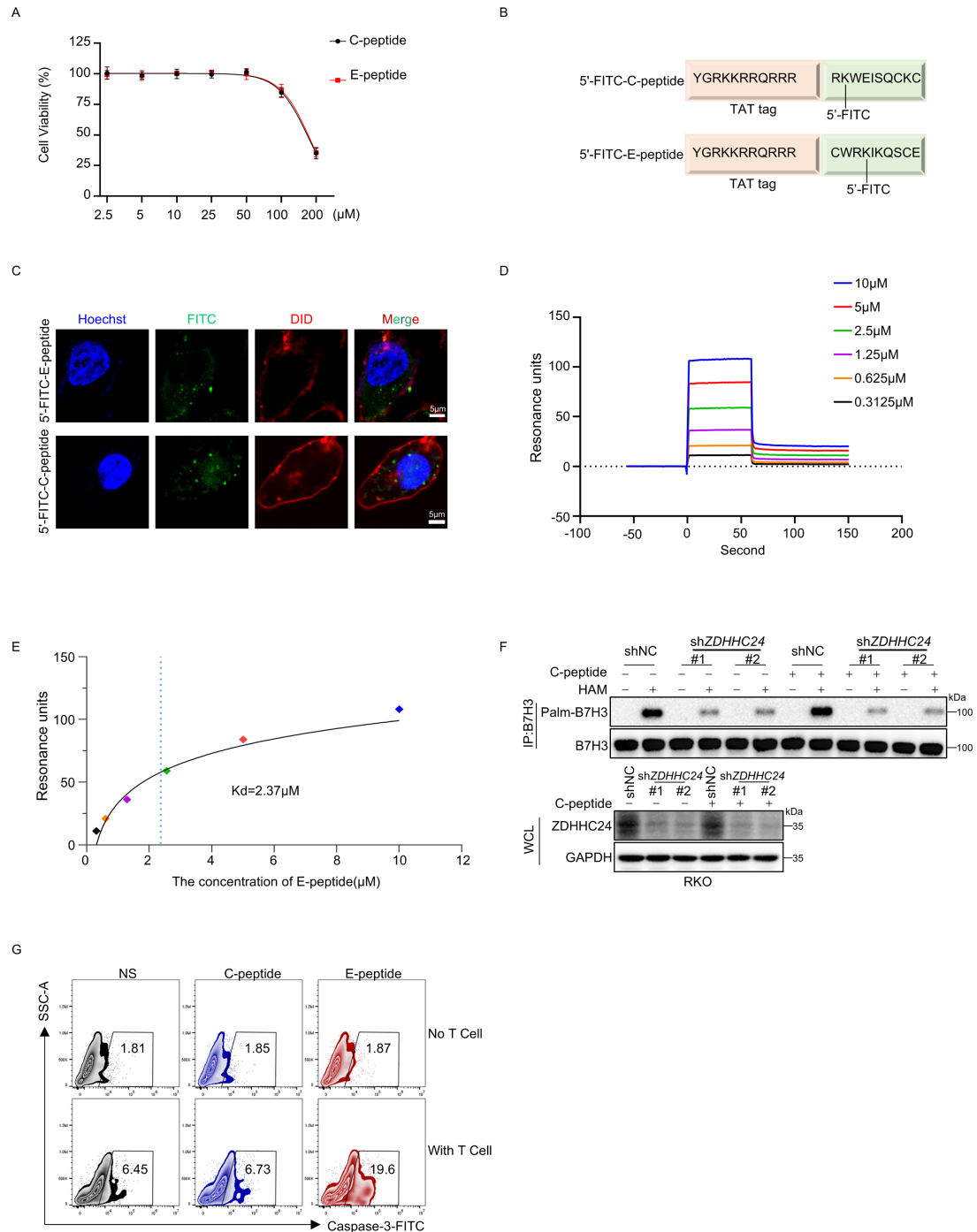
Fig. S6



Supplementary Fig.S6. ZDHHC24 inhibits the antitumor activity of CD8⁺ T cells by catalyzing B7H3 palmitoylation.

A. IB confirmation of ZDHHC24 overexpression in MC38^{WT} and MC38^{C278A}. The samples derive from the same experiment but different gels for ZDHHC24, and another for GAPDH and B7H3 were processed in parallel. **(B to C)** MC38^{WT}+Vector, MC38^{WT}+Zdhhc24, MC38^{C278A}+Vector, MC38^{C278A}+Zdhhc24 tumors in C57BL/6J mice (n=5). **B.** Subcutaneous grafted tumors images are shown after isolation from C57BL/6J mice (n=5). **C.** Representative images of tumor-infiltrating CD8⁺ T cells analyzed by flow cytometry. **D.** IB confirmation of ZDHHC24 overexpression in RKO^{WT} and RKO^{C496A} cells. The samples derive from the same experiment but different gels for ZDHHC24, and another for GAPDH and B7H3 were processed in parallel. **E.** RKO^{WT}+Vector, RKO^{WT}+ZDHHC24, RKO^{C496A}+Vector, and RKO^{C496A}+ZDHHC24 cells were co-cultured with activated human T lymphocytes (n=5). Representative image showing the percentage of caspase-3⁺ in tumor cells by flow cytometry. n = 3 independent experiments (A and D). Source data are provided as a Source Data file.

Fig. S7

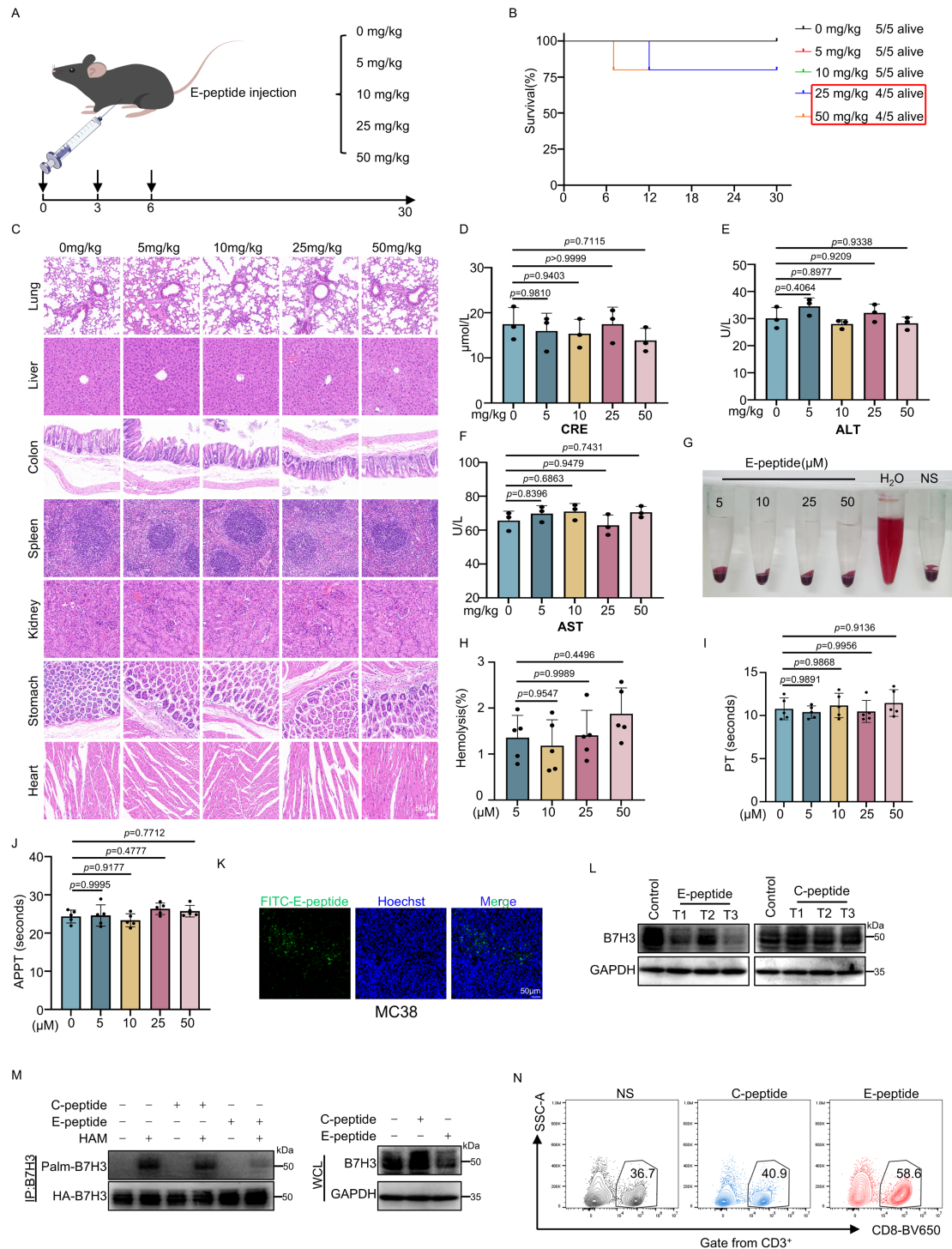


Supplementary Fig.S7. Synthesized peptides suppress CD8⁺ T responses in vitro by inhibiting palmitoylation of B7H3.

A. RKO cells were treated with different concentrations of E- or C- peptide and cell activity was analyzed by Cell Counting Kit-8 (CCK8). (n = 3). **B.** Schematic of 5' - FITC - labeled E- and C-peptide sequences. **C.** Representative confocal images of RKO cells treated with 5'-FITC-E- or 5'-FITC-C-peptide (10 μ M). Scale bars, 5 μ m. **D.**

Surface plasmon resonance (SPR) sensorgrams of E-peptide binding to immobilized ZDHHC24. **E.** Steady-state binding curve for the E-peptide–ZDHHC24 interaction (1:1 fit). **F.** ABE assay of B7H3 palmitoylation in RKO shNC and shZDHHC24 (#1, #2) cells after C-peptide (10 μ M) treatment. The samples derive from the same experiment but different gels for ZDHHC24, another for B7H3, another for Palm-B7H3, and another for GAPDH were processed in parallel. **G.** Co-culture of RKO cells treated with E- or C-peptide (10 μ M) with activated human T cells ($n = 5$): representative flow cytometry plot of caspase-3⁺ tumor cells. $n = 3$ independent experiments (C and F). Source data are provided as a Source Data file.

Fig. S8

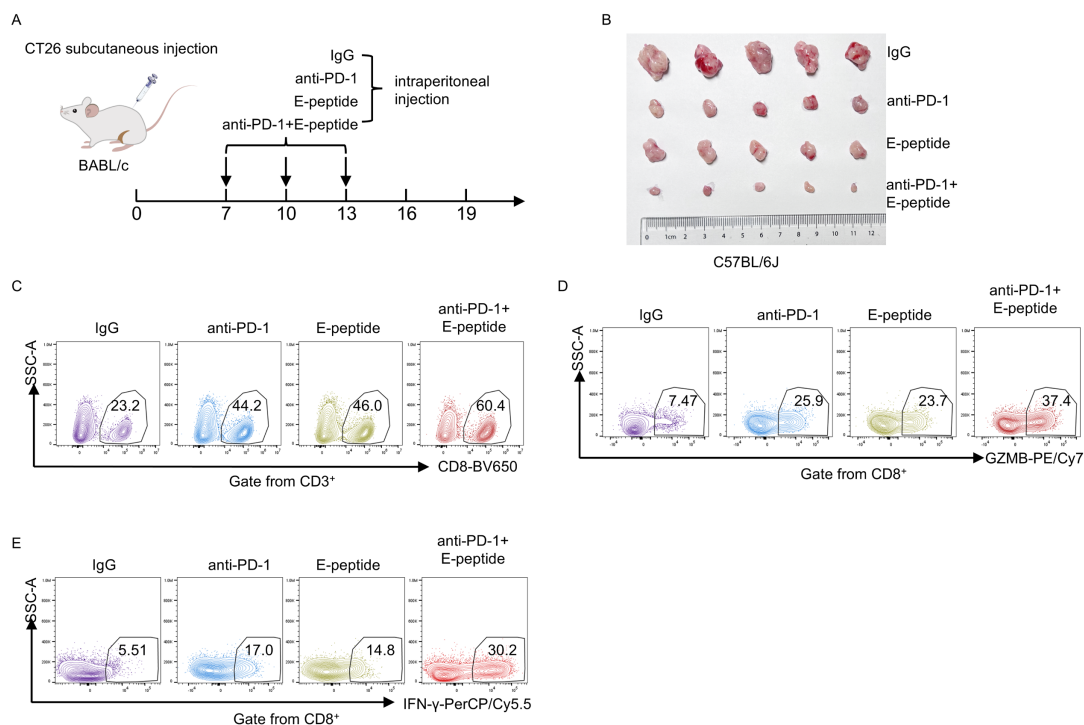


Supplementary Fig.S8. The synthesized E-peptide activates the antitumor immunity in vivo.

(A to C) C57BL/6J mice were treated with different doses of E-peptide: treatment scheme (A), survival curves (B), and representative H&E staining of major organs (lung, liver, colon, spleen, kidney, stomach, and heart) (C). (n = 5). Scale bars, 50μm. (D–F)

Serum biochemistry measurements from mice in (A): creatinine (CRE) (D), alanine transaminase (ALT) (E), and aspartate aminotransferase (AST) (F). (n = 3). **G.** Hemolysis assay of mouse red blood cells incubated with E-peptide at various concentrations at 37°C for 2 hours. **H.** Quantification of hemolysis percentage in (G). (n = 5). **I.** Prothrombin time (PT) assay conducted by incubating platelet-poor plasma with E-peptide and measuring clotting time after addition of PT reagent. (n = 5). **J.** Activated partial thromboplastin time (APTT) assay performed using peptide-treated plasma incubated with APTT reagent and CaCl₂ solution to assess clotting time. (n = 5). **K.** Representative fluorescence images of tumors from MC38-bearing mice after administration of 5'-FITC - E-peptide. Scale bars, 50μm. **(L and M).** IB analysis of B7H3 protein levels (L) and ABE assay of B7H3 palmitoylation (M) in tumor tissues after E- or C-peptide treatment. The samples derive from the same experiment but different gels for B7H3, another for GAPDH, and another for Palm-B7H3 (M) were processed in parallel. **N.** Representative flow cytometry plots of tumor-infiltrating CD8⁺ T cells from CT26 tumors shown in Fig. 8G. (n = 5). Data indicate the mean ± SD by one-way ANOVA with Tukey's test (D, E, F, H, I, and J). n = 3 independent experiments (K, L, and M). Source data are provided as a Source Data file.

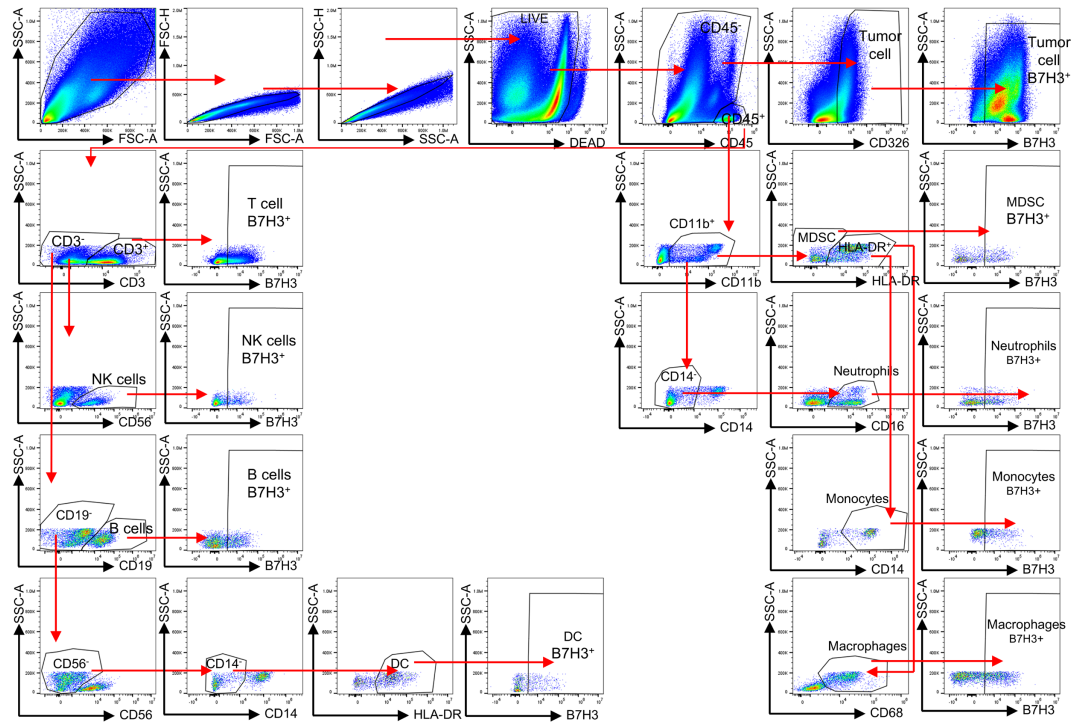
Fig. S9



Supplementary Fig.S9. Synergistic activation of antitumor CD8⁺ T cells by combined blockade of B7H3 palmitoylation and anti-PD-1.

A. Treatment scheme for CT26-luciferase tumors in BALB/c mice receiving IgG, anti-PD-1 (10 mg/kg), E-peptide (10 mg/kg), or the combination (n = 5). **B.** Representative tumor images from MC38-bearing C57BL/6J mice treated with IgG, anti-PD-1 (10 mg/kg), E-peptide (10 mg/kg), or the combination (n = 5). **(C to E).** Representative flow cytometry plots of tumor-infiltrating CD8⁺ T cells (C), GZMB⁺ CD8⁺ T cells (D), and IFN- γ ⁺ CD8⁺ T cells (E) from tumors in (B). Source data are provided as a Source Data file.

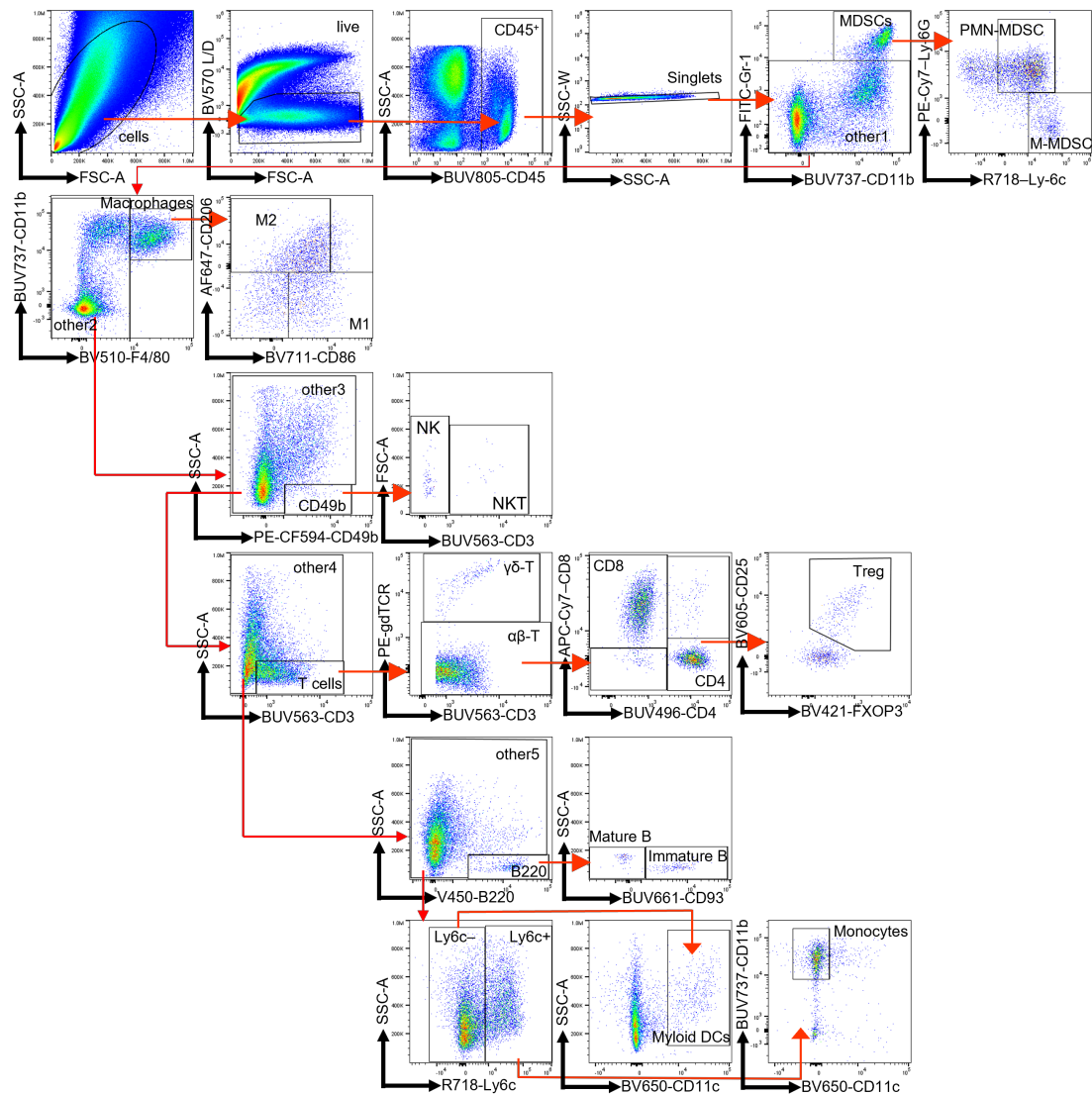
Fig. S10



Supplementary Fig.S10. Gating strategy for flow cytometric analysis of B7H3⁺ cells in different immune cell subsets and tumor cells.

This gating strategy corresponds to the data shown in Supplementary Fig. S1C–S1E.

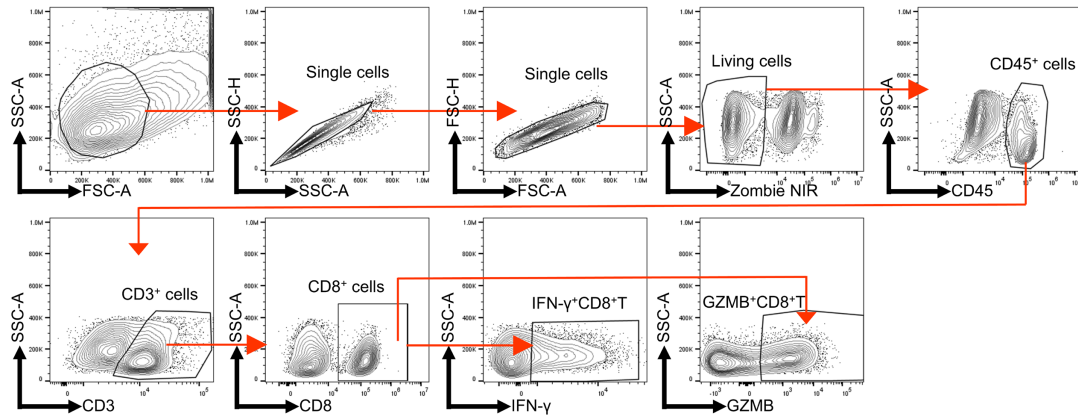
Fig. S11



Supplementary Fig.S11. Gating strategy for spectral flow cytometric analysis of immune cell subsets in CT26 tumors.

This gating strategy corresponds to the data shown in Fig. 3E–3H and Supplementary Fig. S3I.

Fig. S12



Supplementary Fig.S12. Gating strategy for flow cytometric analysis of tumor-infiltrating CD8⁺ T cells and effector molecule expression in tumors.

This gating strategy corresponds to the data shown in Fig. 3N, 3Q, 3R, Supplementary Fig. S3L–3N, Fig. 5L–5N, Supplementary Fig. S5K, S5N, S5O, Fig. 6C, 6F–6I, Supplementary Fig. S6C, Fig. 8H–8L, 8Q–8V, Supplementary Fig. S8N, Fig. 9J–9L and Supplementary Fig. S9C–9E.

Supplementary Table 1. Clinical information for Cohort 1

Cohort 1 Table S1

ID	Gender	Age	TNM stage	Microsatellite status
60014744442	Male	47	T3N0M0	MSI-H
60014740733	Male	46	T3N1M0	MSI-H
60014896199	Male	80	T3N0M0	MSI-H
60014990502	Male	38	T3N0M0	MSI-H
60015055092	Male	45	T3N0M0	MSI-H
60015381441	Female	50	T3N0M0	MSI-H
60015514332	Male	57	T3N0M0	MSI-H
60011248427	Male	62	T3N0M0	MSI-H
60015564831	Female	70	T4N1M0	MSI-H
60015596606	Female	64	T3N1M0	MSI-H
60015237125	Female	49	T4N1M0	MSS
60014850246	Male	66	T3N0M0	MSS
60014947349	Female	73	T4N1M0	MSS
60013892515	Male	44	T3N1M0	MSS
60015108075	Female	37	T3N2M1	MSS
60015311205	Male	72	T4N2M1	MSS
60015140598	Female	68	T3N2M0	MSS
60012226411	Female	49	T4N2M0	MSS
60015222849	Male	26	T4N2M0	MSS
60015370418	Male	44	T2N1M0	MSS

Supplementary Table 2. Clinical information for Cohort 2

Cohort 2 Table S2

ID	Gender	Age	TNM stage	Microsatellite status
60017795193	Female	55	T3N1M0	MSS
60017770904	Male	62	T3N0M1	MSS
60017760954	Female	52	T3N0M0	MSS
60017738159	Male	51	T3N1M0	MSS
60010978536	Male	52	T3N0M0	MSS
60017778109	Female	63	T3N1M0	MSS
60017735378	Male	73	T3N1M1	MSS
60011729686	Female	51	T3N1M0	MSS
60017801570	Female	86	T3N0M0	MSS
60017193050	Male	61	T3N0M0	MSS

Supplementary Table 3. Clinical information for Cohort 3

Cohort 3 Table S3

ID	Gender	Age	TNM stage	Microsatellite status
60011859987	Female	55	T3N1M0	MSI-H
60010895994	Male	68	T4N0M0	MSI-H
60011876253	Male	63	T3N1M0	MSI-H
9010108201	Female	67	T2N0M0	MSS
60013623934	Male	50	T3N0M0	MSS
60011867151	Female	62	T3N0M0	MSS

Supplementary Table 4. Clinical information for Cohort 4

Cohort 4 Table S4

ID	Gender	Age	TNM stage	Microsatellite status
60011809765	Male	55	T4N0M0	MSI-H
60011890440	Female	75	T3N0M0	MSI-H
60011549753	Male	67	T4N0M0	MSI-H
60011548779	Female	71	T3N1M0	MSI-H
1010887872	Female	73	T4N1M0	MSS
1010516079	Female	65	T4N1M1	MSS
60011639666	Male	73	T3N1M0	MSS
60011640460	Male	58	T3N1M0	MSS
60011738548	Male	62	T3N0M0	MSS

Supplementary Table 5. Clinical information for Cohort 5

Cohort 5 Table S5

ID	Gender	Age	TNM stage	Microsatellite status
60012147396	Male	54	T4N0M0	MSI-H
60012083816	Male	66	T4N1M0	MSI-H
60011999338	Male	51	T1N0M0	MSI-H
60011910228	Female	65	T4N0M0	MSI-H
60012017795	Male	70	T3N0M0	MSI-H
60012202234	Male	69	T3N1M0	MSI-H
60011982688	Female	77	T3N0M0	MSI-H
60012251567	Male	46	T3N0M0	MSI-H
60011992570	Female	58	T3N0M0	MSI-H
60010270758	Female	57	T4N1M0	MSI-H
60013085889	Female	62	T2N0M0	MSS
60012018560	Male	56	T4N1M0	MSS
60012119594	Male	65	T2N0M0	MSS
60011423601	Male	83	T3N0M0	MSS
60012043111	Female	70	T3N0M0	MSS
60011977469	Male	65	T3N0M0	MSS
60012161640	Female	65	T3N2M0	MSS
60011549965	Male	64	T3N1M1	MSS

Supplementary Table 6. The prime used to construct the plasmids

Plasmid	Forward Prime	Reverse Prime
B7H3 C496A	AAACAGAGCGCTGAGGAGGAG AATGCAGGAGCT	TCCTCAGCGCTCTGTTTGATCTTTCT CCAGCA

Supplementary Table 7. The sequence of shRNAs and sgRNAs

shRNAs	Sequence
shZDHHC24#1	TGCTCAACCTGCTGGGCAA
shZDHHC24#2	GCGTGGGCTTCGGCAACTA
shZDHHC24#3	GCTACCAATGCCAAAGCCA
shZdhhc24#1	GCAAAGTGTCTCTGGCCCA
shZdhhc24#2	CTAGTCTGGTTCTGGCCTT
shZdhhc24#1	GATGTGGGTCTTGTGACTT
sgRNAs	Sequence
B7H3 sgRNA #1	CTCAGGGTAGCCCCGGTAGC
B7H3 sgRNA #2	CAACGTGACCACGTTCGCAGA
B7H3 sgRNA #3	GCCTATGACATTCCCCCAG
<i>B7h3</i> sgRNA #1	CGCGTCCGAGTAACCGACGA
<i>B7h3</i> sgRNA #2	GTACCCCTCTCCACGCTCAC
<i>B7h3</i> sgRNA #3	ATCGAACAAGCCCCGCTCGT
ATG5 sgRNA	GTGCTTCGAGATGTGTGGTT
BECN1 sgRNA	ATTTATTGAAACTCCTCGCC
SQSTM1 sgRNA	TCAGGAGGCGCCCCGCAACA

Supplementary Table 8. The sequences of the primers for the quantitative RT-PCR

Gene	Forward Prime	Reverse Prime
B7H3	AGGGCAGCCTATGACATTCC	GGTCCTCAGCTCCTGCATTC
GAPDH	TCGGAGTCAACGGATTGGT	TGAAGGGGTCATTGATGGCA

Supplementary Table 9. The gRNA sequences used to generate *Zdhhc24^{fl/fl}* mice.

gRNA NO.	gRNA Sequence	PAM
5S3	AGTTAGTCTTAATATATTTG	TGG
3S3	GTGGGGATATTGGACCATT	AGG
5S4	TAATATATTTGTGGATGCTA	TGG
3S4	ATTAGGGGAGGTGGACCTAA	TGG

Supplementary Table 10. The sequences of the primer sequences for mouse-genotyping

Mouse	Forward Prime	Reverse Prime
<i>Zdhhc24^{fl/fl}</i>	GAAGAGTGAACAGGCCATCAAGTC	AGTCGCTGACCTTGTGGTTCTG
<i>Villin-cre</i>	GGGCAGTCTGGTACTTCCAAGCT	AGTTTCCAAACTCCAGGTGACAGG