

Compartment-specific PPAR δ activity controls the outcome of cancer–immune cell competition and immunotherapy efficacy

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Paul Bunk^{1,17}, Ilgin Ergin^{1,17}, Timothy Maher^{1,2,3,17}, Vyom Shah^{1,4,17}, Julia R. Bonney⁵, Lokesh B. S. Kumar¹, Charlie Chung^{1,2}, Nils Wellhausen⁶, Philip Moresco^{1,2,3}, Sarah Shao¹, Jill Habel¹, Vinay Mandati¹, Rachel Rubino¹, David E. Rivadeneira^{7,8}, John E. Wilkinson⁹, Laura Herrero^{10,11,12}, Peter M. K. Westcott¹, Samuel E. Weinberg¹³, Saar I. Gill^{6,14}, Nathalie Y. R. Agar^{5,15,16} & Semir Beyaz¹✉

Most patients with solid tumors fail to sustain antitumor immunity, in part because immune cells cannot outcompete cancer cells inside the immunosuppressive tumor microenvironment. Here, we show the lipid-sensing transcription factor PPAR δ is a compartment-specific determinant of cancer–immune fitness balance and immunotherapy efficacy. Activating PPAR δ in cancer cells promotes their growth and impairs antitumor immunity, whereas activating it in immune cells drives tumor suppression and overrides tumor-promoting effects of cancer-intrinsic activation. Mechanistically, PPAR δ enhances CD8⁺ T cell fitness against cancer through effector-associated chromatin remodeling and Cpt1a-mediated fatty acid oxidation. Therapeutically, PPAR δ -activated CD8⁺ T cells sensitize immunotherapy-resistant microsatellite stable metastatic colorectal cancer to checkpoint blockade in mice, and pharmacological or genetic PPAR δ activation enhances human chimeric antigen receptor T cell function. These findings identify compartment-specific PPAR δ activity as a targetable driver of immune cell fitness that can be harnessed to improve outcomes in immunotherapy-resistant tumors.

Tumors are ecological systems where cancer cells and immune cells share physical and metabolic space, depending on overlapping resources and adaptive programs for survival, proliferation, migration and function^{1–4}. Tumor progression may therefore reflect not only unchecked cancer cell growth, but also immune cell failure to maintain fitness under competitive pressure. Successful antitumor immunity may require immune cells to acquire sufficient metabolic, transcriptional and epigenomic competence to withstand the tumor microenvironment (TME) and outcompete cancer cells^{3–5}. Defining pathways that govern this competition could reveal strategies to strengthen antitumor immunity and improve immunotherapy efficacy.

This problem is especially relevant to colorectal cancer (CRC), among the most prevalent and deadly cancers, with incidence and mortality rising among younger individuals, becoming the leading cause of cancer-related deaths in men and the second in women under 50 in the United States⁶. CRC is classified by mismatch repair (MMR) status⁷. Most cases are microsatellite stable (MSS), are mismatch repair proficient (pMMR) and resist current immunotherapies, including immune checkpoint blockade (ICB) and chimeric antigen receptor (CAR) T cell therapy⁷. MSS metastatic colorectal cancer (mCRC) is especially lethal due to its poor response to current treatments, reflecting tumor-intrinsic and microenvironmental constraints that

limit immune cell infiltration, persistence and effector function^{7,8}. The therapeutic challenge is therefore not only removing inhibitory signals, but also equipping immune cells with the fitness to function within immune-evasive tumors.

PPAR δ is a lipid-sensing transcription factor governing fatty acid oxidation (FAO) in both cancer cells and immune cells. Yet, its precise role in each compartment remains unresolved. In cancer, genetic and pharmacologic PPAR δ activation promotes tumorigenesis^{9–15}, yet PPAR δ loss also increases polyp burden in mice, and ligand activation can attenuate carcinogenesis^{16,17}. Similarly, pan-PPAR or PPAR α /PPAR δ agonists enhance CD8⁺ T cell fitness, and PPAR δ supports CD8⁺ T cell memory^{18–21}, but PPAR δ has also been reported to restrain CD8⁺ T cell cytotoxicity and to promote suppressive immune states^{22–27}. This uncertainty extends to the downstream metabolic program. Cpt1a-mediated FAO is important for CD8⁺ T cell memory and persistence²⁸, but genetic deletion of Cpt1a later argued that it is dispensable for memory T cell formation²⁹. In cancer cells, Cpt1a-driven FAO is predominantly pro-tumorigenic, supporting tumor progression and resistance to immune-mediated killing, although its requirement is context dependent rather than absolute^{30–32}. These discrepancies point to a central question. Do the effects of PPAR δ on tumorigenesis and antitumor immunity depend on the compartment in which it is activated?

Here, using compartment-specific genetic models, we show that cancer-intrinsic PPAR δ activation promotes tumor growth, whereas immune-intrinsic activation drives tumor control. Immune-specific activation sensitizes immune-evasive, MSS mCRC to ICB and enhances human CAR-T cell fitness and antitumor function. Our findings identify compartment-specific PPAR δ activity as a targetable determinant of antitumor immunity and resolve previously conflicting reports of pro-tumorigenic and anti-tumorigenic effects.

Results

Immune-intrinsic PPAR δ overrides cancer PPAR δ in tumor outcome

To assess how PPAR δ activity influences cancer–immune cell competition, we generated gain-of-function (GOF) genetic models overexpressing the VP16/VP64–PPAR δ fusion protein (PPAR δ -GOF) in either cancer cells or immune cells. First, we overexpressed the PPAR δ -GOF in carcinogen-induced MC38 colorectal adenocarcinoma cell line (PPAR δ -cGOF), which generates microsatellite instable (MSI) tumors with high mutational burden and immune infiltration. Next, we generated *Vav1*-cre⁺ VP16–PPAR δ ⁺ mice that overexpress PPAR δ -GOF in all immune cells (PPAR δ -iGOF; Extended Data Fig. 1a). As reported^{11–15}, selective activation of PPAR δ in cancer cells promoted their growth in wild-type (WT) mice. Strikingly, immune cell-specific activation in PPAR δ -iGOF mice inhibited the growth of both control and PPAR δ -cGOF tumors (Fig. 1a and Extended Data Fig. 1b).

To dissect how cancer-intrinsic or immune-intrinsic PPAR δ activation influences the tumor microenvironment (TME), we performed single-cell RNA sequencing (scRNA-seq) and single-cell assay for transposase-accessible chromatin using sequencing (scATAC-seq; Extended Data Fig. 1c–m). Genes involved in survival, proliferation and immunosuppression were upregulated in PPAR δ -cGOF MC38 cancer cells in WT mice (Fig. 1b). In contrast, immune-intrinsic PPAR δ activation promoted expression of apoptosis, negative regulation of proliferation and immune recognition-related genes in MC38 cancer cells with active PPAR δ (cGOF + iGOF; Fig. 1c,d).

WT CD8⁺ T cells in PPAR δ -cGOF showed increased expression and chromatin accessibility at T cell dysfunction-associated loci (*Ctla4*, *Pdcd1*, *Havcr2*, *Lag3*). In contrast, PPAR δ -iGOF CD8⁺ T cells displayed lower expression and accessibility of dysfunction-associated genes, and the dysfunction-promoting effects of PPAR δ -cGOF tumors were ameliorated in PPAR δ -iGOF mice (Fig. 1e,f and Extended Data Fig. 1i). When PPAR δ was active in both compartments (cGOF + iGOF), gene expression and chromatin accessibility changes observed in CD8⁺ T cells

resembled those of PPAR δ -iGOF CD8⁺ T cells in WT tumors more than WT CD8⁺ T cells in PPAR δ -cGOF tumors (Extended Data Fig. 1j–m). Immune-intrinsic activation of PPAR δ can thus override growth benefits and immunosuppressive effects of cancer-intrinsic PPAR δ signaling, suggesting that PPAR δ activity within immune cells is a key determinant of cancer–immune cell competition resulting in tumor elimination.

Immune-intrinsic PPAR δ restricts immune-evasive tumors and metastasis

We used a colonoscopy-guided orthotopic model of immune-evasive MSS primary CRC by injecting genetically engineered *Apc*^{null}, *Kras*^{G12D}, *Tp53*^{null} (AKP) organoids into the colon submucosa³³, which recapitulates histopathological and immune-evasive features of human CRC. Immune-intrinsic PPAR δ activation restricted growth of orthotopic AKP tumors (Fig. 2a,b and Extended Data Fig. 2a,b) and MC38 tumors (Extended Data Fig. 2c–e) compared to WT controls.

To test whether immune-intrinsic PPAR δ activity influences mCRC, we orthotopically injected genetically engineered *Apc*^{knockdown}, *Kras*^{G12D}, *Tp53*^{null}, *Smad4*^{null} (AKPS) organoids into mouse colons, generating more aggressive, immune-evasive MSS tumors that spontaneously metastasize to the liver and other sites at high frequency^{33,34}. AKPS tumor growth was significantly dampened in PPAR δ -iGOF mice (Fig. 2c). Adoptive transfer of PPAR δ -iGOF splenocytes also restricted the growth of primary AKPS tumors and inhibited metastasis (Fig. 2d,e and Extended Data Fig. 2f–h). Similar results were obtained with B16-F10 melanoma and EO771 breast tumors (Fig. 2f,g and Extended Data Fig. 2i,j), indicating that these tumor-suppressive effects generalize to other immune-evasive cancers. To ask whether PPAR δ activity is also necessary for baseline antitumor immunity, we generated *Vav1*-cre⁺ PPAR δ ^{fl/fl} mice, which lack PPAR δ in all immune cells (PPAR δ -iKO; Extended Data Figs. 1a and 2k). PPAR δ loss did not significantly alter MC38 tumor growth (Extended Data Fig. 2l,m), likely due to compensation by other PPAR family members³⁵. These findings suggest that immune-intrinsic PPAR δ activation restricts tumor growth across different cancer types and stages.

PPAR δ activity enhances immune infiltration and tumor killing

We next asked if PPAR δ activation suppresses tumors by increasing immune infiltration and tumor killing. Immunofluorescence staining in immunogenic MSI MC38 tumors showed that immune-specific PPAR δ activation significantly increased tumor-infiltrating immune cells (TICs; Fig. 2h,i). Although primary and metastatic MSS CRC tumors show poor immune infiltration, PPAR δ activation in immune cells promoted immune infiltration and formation of immune cell networks ('immune hubs') around orthotopic MSS AKP and AKPS tumors (Fig. 2j–m and Extended Data Fig. 2n–q), which are linked to augmented antitumor immune responses³⁶. Immune-infiltrated MSS AKP tumors in PPAR δ -iGOF mice were also less fibrotic, indicating enhanced tumor clearance and resolution of tumor-related inflammation (Extended Data Fig. 2r,s).

Next, we sought to determine whether immune-intrinsic PPAR δ activation promotes tumor killing. Pharmacologic and genetic PPAR δ activation in immune cells significantly increased cytotoxicity against cancer cells in vitro (Fig. 2n). We developed a human autologous patient-derived organoid (PDO)–peripheral blood mononuclear cell (PBMC) three-dimensional (3D) co-culture assay using MSI and MSS CRC PDOs, which unlike cell-line-based models avoids allogeneic responses and captures cellular diversity and the genetic landscape of the patient's tumor. Treating autologous PBMCs with the PPAR δ agonist GW501516 before co-culture significantly enhanced cytotoxicity against both MSI and MSS mCRC PDOs (Fig. 2o–q). These findings suggest that immune-intrinsic PPAR δ activation enhances tumor infiltration and cytotoxicity, irrespective of MMR status, in both mouse and human PDOs.

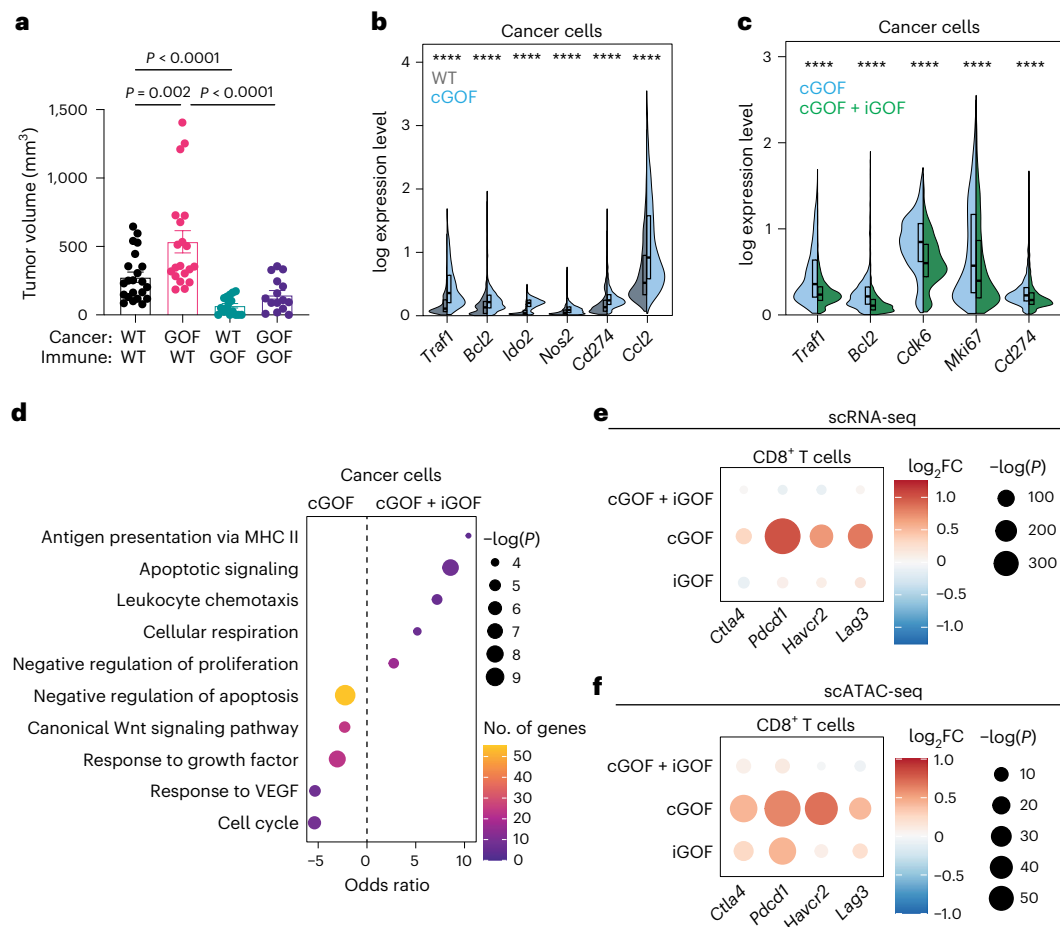


Fig. 1 | PPAR δ activity controls the outcome of cancer-immune cell competition. **a**, Endpoint tumor volume of subcutaneous MC38 control (WT) and PPAR δ -cGOF tumors (GOF) in WT and PPAR δ -iGOF (GOF) mice inoculated with 5×10^5 MC38 cells ($n = 22$ WT mice with control MC38, $n = 20$ WT mice with PPAR δ -cGOF MC38, $n = 16$ PPAR δ -iGOF mice with control MC38, $n = 15$ PPAR δ -iGOF mice with PPAR δ -cGOF MC38; from three independent experiments), $\pm$ s.e.m. **b,c**, Split violin plots demonstrating the expression levels of key genes involved in cancer cell survival, proliferation and cancer-mediated immunosuppression in control (WT) and PPAR δ -cGOF (cGOF) MC38 cancer cells in WT mice (**b**) and in PPAR δ -cGOF MC38 tumors in WT mice (cGOF) or PPAR δ -iGOF mice (cGOF + iGOF) (**c**). (WT mice with control MC38: $n = 1,796$ cells from two mice, WT mice with PPAR δ -cGOF MC38: $n = 5,082$ cells from two

mice, PPAR δ -iGOF mice with PPAR δ -cGOF MC38: $n = 4,516$ cells from two mice), **** $P \leq 0.0001$. **d**, Gene-set enrichment analysis of differentially expressed genes from scRNA-seq analysis of PPAR δ -GOF MC38 tumors from WT mice (cGOF) or PPAR δ -iGOF mice (cGOF + iGOF) as calculated using EnrichR. **e,f**, Dot plots depicting changes in expression and accessibility of T cell exhaustion-related genes within the CD8⁺ T cell cluster, based on scRNA-seq (**e**) and scATAC-seq (**f**) of tumor-infiltrating lymphocytes from PPAR δ -cGOF MC38 tumors in WT mice (cGOF), MC38 control tumors in PPAR δ -iGOF mice (iGOF) or PPAR δ -cGOF tumors in PPAR δ -iGOF mice (cGOF; iGOF), compared to MC38 control tumors in WT mice. Significance was assessed using an ordinary one-way analysis of variance (ANOVA) with a two-tailed Tukey's multiple-comparison test (**a**), a Wilcoxon rank-sum test (**b** and **c**), Fisher's exact test (**d**) and a *t*-test (**e** and **f**).

Immune-intrinsic PPAR δ drives TME remodeling and lymphocyte expansion

To delineate the effects of immune-intrinsic PPAR δ activation on the TME, we performed scRNA-seq on sorted TICs (Extended Data Figs. 3a–l and 4a–h). PPAR δ activation induced the expression of its bona fide target genes (Extended Data Figs. 3e and 4d) and reprogrammed the TME from an immunosuppressive to an immunostimulatory state in MSI MC38 and MSS AKPS tumors, associated with upregulation of genes involved in antigen presentation, survival, chemokine and interferon signaling across TIC clusters (Fig. 3a,b and Extended Data Figs. 3f,g and 4e–h). In a human TIC scRNA-seq dataset³⁷, PPAR δ activity signatures correlated with signs of antitumor immune activity (Fig. 3c,d and Extended Data Fig. 3m).

Clonal expansion of lymphocytes is essential for a strong, sustained and targeted antitumor response³⁸. PPAR δ activation increased the abundance of cytotoxic CD8⁺ T cells, CD4⁺ T cells and plasma B cells in AKPS tumors (Extended Data Fig. 3b,c). Single-cell immune profiling of T cell and B cell antigen receptors with gene expression

demonstrated lymphocytes in AKPS tumors in WT mice were not clonally expanded, consistent with the immune-evasive characteristics of MSS CRC⁷, whereas those from PPAR δ -iGOF mice showed T cell and B cell clonal expansion (Fig. 3e and Extended Data Fig. 3h–k). Cell–cell interaction modeling suggested enhanced interactions between clonally expanded CD8⁺ T and CD4⁺ T cells and other immune cells within the TME (Fig. 3f and Extended Data Fig. 3l). Thus, PPAR δ activation evokes a robust antitumor immune response in immune-evasive MSS CRC.

PPAR δ activation does not drive overt inflammatory toxicity

Inflammatory toxicity is a significant concern in immunotherapy. scRNA-seq showed that, unlike TICs, splenic immune cells did not upregulate inflammatory response genes, suggesting no overt systemic inflammation in PPAR δ -iGOF mice (Extended Data Fig. 5a–d). Histological examinations of mucosal and non-mucosal tissues, including the lung, spleen, heart, kidney, liver, small intestine and colon, revealed no signs of autoimmunity, abnormal immune activity

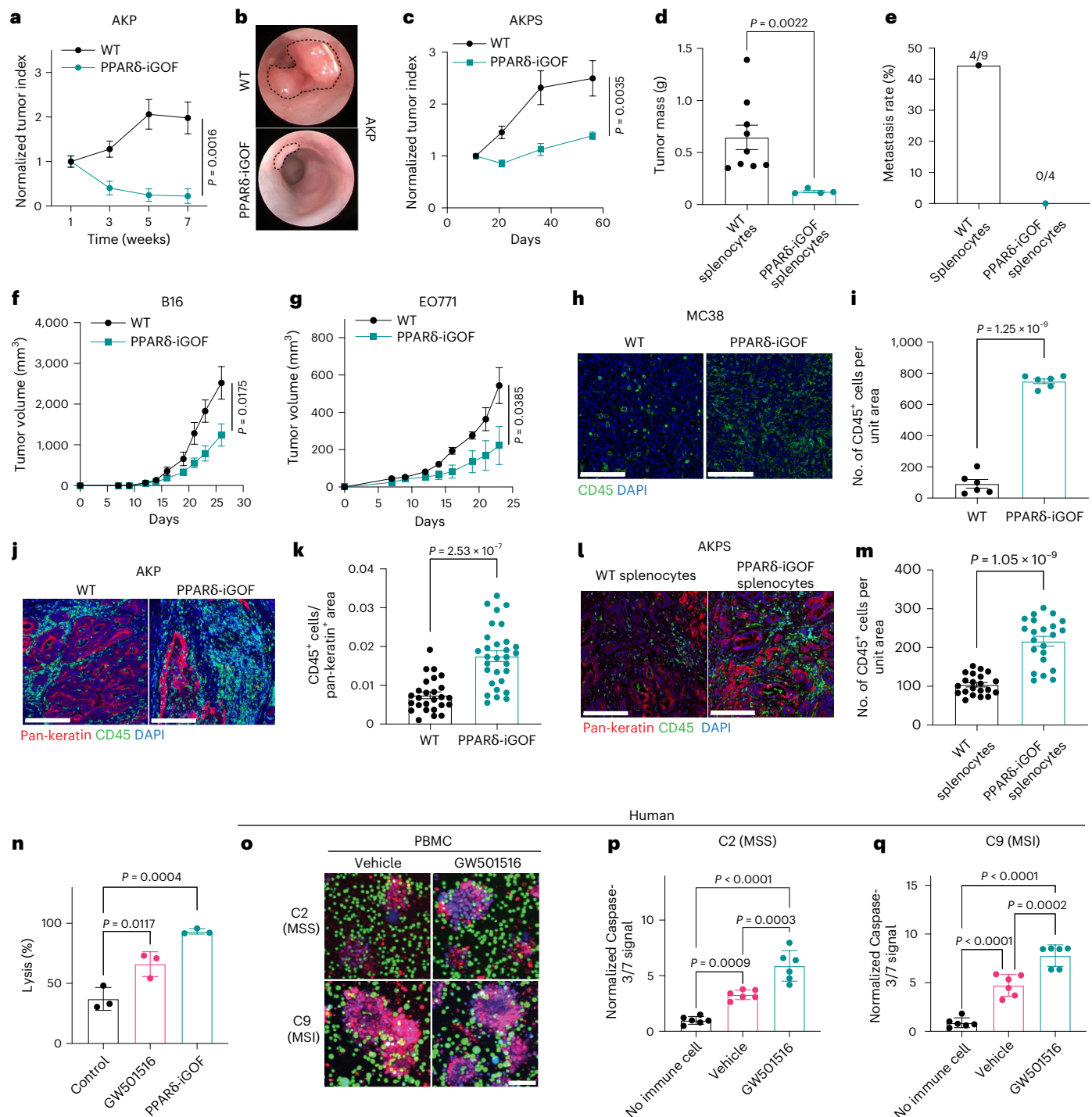


Fig. 2 | PPARδ activation enhances antitumor immunity in mice and autologous human co-cultures. **a, b**, AKP tumor growth (**a**) and representative colonoscopy images (**b**) at 5 weeks in WT and PPARδ-iGOF mice, (WT: $n = 7$ mice, PPARδ-iGOF: $n = 6$ mice), $\pm$ s.e.m. **c**, AKPS tumor growth in WT and PPARδ-iGOF mice (WT: $n = 6$ mice, PPARδ-iGOF: $n = 8$ mice), $\pm$ s.e.m. **d, e**, AKPS tumor weights (**d**) and rate of metastases (**e**) in WT mice that received 5×10^6 WT or PPARδ-iGOF splenocytes (WT: $n = 9$ mice, PPARδ-iGOF: $n = 4$ mice), $\pm$ s.e.m. **f, g**, B16-F10 (**f**) or EO771 (**g**) tumor growth in WT and PPARδ-iGOF mice (WT: $n = 8$ mice, PPARδ-iGOF: $n = 9$ mice), $\pm$ s.e.m. **h, i**, Representative immunofluorescence images (**h**) and quantification (**i**) of CD45⁺ cells in MC38 tumors from WT and PPARδ-iGOF mice. Scale bars (**h**), 150 μ m. ($n = 6$ areas from two mice per group), $\pm$ s.e.m. **j, k**, Representative immunofluorescence images (**j**) and quantification (**k**) of total CD45⁺ cells per total keratin⁺ area in AKP tumors from WT and PPARδ-iGOF mice. Scale bars (**j**), 150 μ m. (WT: $n = 26$ tumor areas, PPARδ-iGOF:

$n = 30$ tumor areas, from three mice per group), $\pm$ s.e.m. **l, m**, Representative immunofluorescence images (**l**) and quantification (**m**) of CD45⁺ cells in AKPS tumors from mice that received 5×10^6 WT or PPARδ-iGOF splenocytes. Scale bars (**l**), 150 μ m (WT splenocytes: $n = 21$ tumor areas from four mice, PPARδ-iGOF splenocytes: $n = 22$ tumor areas from three mice), $\pm$ s.e.m. **n**, MC38-luc lysis after co-culture with WT, PPARδ-iGOF or GW501516-treated mouse splenocytes at 5:1 effector ratio ($n = 3$ replicates per group, representative of two independent experiments), $\pm$ s.d. **o**, Representative images of apoptotic (blue) MSI and MSS CRC PDOs (red) co-cultured with autologous vehicle-treated or GW501516-treated PBMCs (green). Scale bar (**o**), 70 μ m. **p–q**, Normalized Caspase-3 dye signal on MSS (**p**) or MSI (**q**) CRC PDOs 16 h after co-culture start ($n = 6$ replicates per patient organoid), $\pm$ s.d. Significance was assessed using a two-tailed unpaired t -test (**a, c, f, g, i, k** and **m**), a Welch's t -test (**d**) and an ordinary one-way ANOVA with a two-tailed Tukey's test (**n, p** and **q**).

or oncogenic transformation (Extended Data Fig. 5e). Lifespans and body weights of PPAR δ -iGOF mice were comparable to age-matched and sex-matched control WT mice (Extended Data Fig. 5f,g). Splenic myeloid and lymphoid subsets were comparable in abundance between WT and PPAR δ -iGOF mice by flow cytometry (Extended Data Fig. 5h–q and Supplementary Fig. 1a). We challenged WT and PPAR δ -iGOF mice with dextran sodium sulfate (DSS) to induce acute colitis as a model of inflammatory disease. PPAR δ -iGOF and control WT mice had similar body weight loss and overall disease severity (Extended Data Fig. 5r–t). Thus, our data indicate that PPAR δ activation in immune cells does not trigger overt inflammatory toxicity in preclinical animal models tested, supporting its potential as a therapeutic strategy, while recognizing that longer-term safety studies will be required.

PPAR δ activity enhances function and abundance of tumor CD8 $^{+}$ T cells

Because cytotoxic CD8 $^{+}$ T cells are central effectors of tumor control, we asked whether PPAR δ activation improves their abundance and functional quality in tumors³⁹. scRNA-seq revealed that tumor-infiltrating PPAR δ -iGOF CD8 $^{+}$ T cells upregulated genes associated with cytotoxicity (*Prf1*, *Gzmk*), interferon signaling (*Ifng*, *Irf1*) and migration (*Cxcr3*) within MC38 and AKPS tumors, as well as tumor-draining lymph nodes (Fig. 3g,h and Extended Data Fig. 4h–k). In MC38 tumors, PPAR δ -iGOF CD8 $^{+}$ T cells also upregulated stem-like memory markers (*Tcf7*, *Lef1*, *Sell*; Extended Data Fig. 4h), in line with PPAR δ promoting T cell memory²¹. Clonally expanded PPAR δ -iGOF CD8 $^{+}$ T cells in AKPS tumors upregulated the expression of genes involved in cytotoxicity (*Ifng*, *Gzmk*, *Gzmb*; Fig. 3i). Consistently, flow cytometry of TICs showed that tumor-infiltrating PPAR δ -iGOF CD8 $^{+}$ T cells produced more interferon gamma (IFN γ) and tumor necrosis factor (TNF), with a significantly higher proportion of IFN γ -, TNF- and Gzmb-producing polyfunctional CD8 $^{+}$ T cells (Fig. 3j, Extended Data Fig. 4l,m and Supplementary Fig. 1b), which are associated with potent antitumor immunity and immunotherapy efficacy⁴⁰. Thus, PPAR δ induces increased CD8 $^{+}$ T cell activity within the TME.

High CD8 $^{+}$ T cell infiltration strongly correlates with better prognosis across many cancers, including CRC³⁹. CD8 $^{+}$ T cell abundance in MSI MC38 tumors (Fig. 3k,l) and MSS AKP or AKPS tumors (Fig. 3m,n and Extended Data Fig. 3b,c) was increased in PPAR δ -iGOF mice compared to WT controls, with numbers also significantly elevated in PPAR δ -cGOF tumors (Fig. 3k,l). These results suggest that immune-specific PPAR δ activation drives T cell infiltration and function within MSI and MSS tumors.

Using the ligand–target prediction algorithm NicheNet, we identified upregulation of the CXCL9/CXC10–CXCR3 axis in TICs from immune-evasive AKPS and immunogenic MC38 tumors in PPAR δ -iGOF mice (Fig. 3o and Extended Data Fig. 4n,o). CXCL9 and CXCL10 are IFN-inducible chemokines whose receptor CXCR3 is essential for directing effector CD8 $^{+}$ T cells to tumor sites⁴¹. PPAR δ activation increased *Cxcr3* expression in tumor-infiltrating CD8 $^{+}$ T cells

(Extended Data Fig. 4p–r) and depleting Cxcr3 $^{+}$ cells in PPAR δ -iGOF mice significantly increased tumor size compared to IgG-treated controls (Fig. 3p, Extended Data Fig. 4s and Supplementary Fig. 1c). These data indicate that Cxcr3 $^{+}$ cells, which are predominantly but not exclusively CD8 $^{+}$ T cells (Supplementary Fig. 1d), are required for the tumor suppression conferred by immune-intrinsic PPAR δ activity. In line with these findings in mouse models, PPAR transcriptional activity positively correlated with *IFNG*, *CXCR3* and *IRF7* expression in tumor-infiltrating CD8 $^{+}$ T cells from a human TIC dataset³⁷ (Fig. 3q,r and Extended Data Fig. 4t). These results establish that PPAR δ activation in CD8 $^{+}$ T cells promotes their infiltration and fitness within both MSI and MSS tumors.

CD8 $^{+}$ T cells mediate PPAR δ effects on antitumor immunity

Depleting CD8 $^{+}$ T cells in PPAR δ -iGOF mice bearing subcutaneous MC38 tumors accelerated tumor growth (Fig. 4a, Extended Data Fig. 6a and Supplementary Fig. 1e), indicating that they are necessary for PPAR δ -mediated antitumor immunity. To determine whether selective PPAR δ activation in CD8 $^{+}$ T cells is sufficient to promote antitumor immunity, we generated tamoxifen-inducible *ESR1*^{CreERT2}; *GFP*^{VP16}–PPAR δ ⁺ (PPAR δ -CD8^{GOF}) mice, which selectively activate PPAR δ in CD8 $^{+}$ T cells (Extended Data Fig. 6b–d). PPAR δ -CD8^{GOF} mice exhibited significantly reduced tumor growth, enhanced CD8 $^{+}$ T cell infiltration in the TME (Fig. 4b–e and Extended Data Fig. 6e) and increased abundance of polyfunctional IFN γ -, Gzmb- and TNF-producing CD8 $^{+}$ T cells (Fig. 4f, Extended Data Fig. 6f–k and Supplementary Fig. 1f). Adoptive transfer of CD8 $^{+}$ T cells from PPAR δ -iGOF mice into WT hosts before tumor inoculation suppressed tumor growth and enhanced infiltration of Gzmb-expressing CD8 $^{+}$ T cells (Fig. 4g–j and Extended Data Fig. 6l–p). Genetic or pharmacological activation of PPAR δ in polyclonal and antigen-specific CD8 $^{+}$ T cells enhanced their cytotoxicity (Fig. 4k and Extended Data Fig. 6q) and increased production of interleukin (IL)-2, IFN γ and TNF in vitro (Extended Data Fig. 6r–v). Thus, CD8 $^{+}$ T cells alone are sufficient to mediate the tumor-suppressive effects of PPAR δ activity in immune cells.

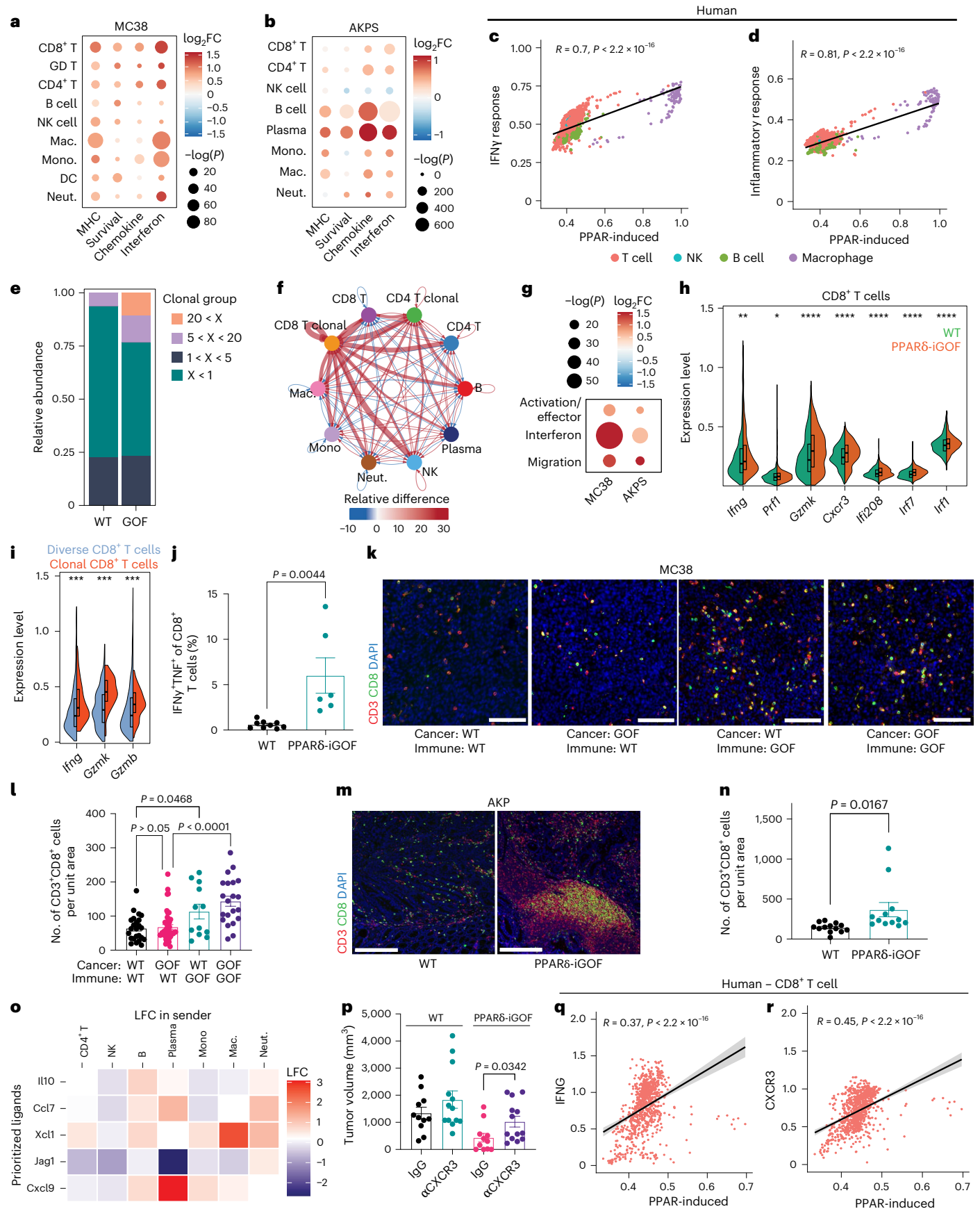
PPAR δ licenses effector-associated chromatin states in CD8 $^{+}$ T cells

PPAR δ can activate and inhibit transcription partly via co-activator and co-repressor complexes with chromatin-remodeling activities⁴². We assessed chromatin accessibility by scATAC-seq in TICs from AKPS tumors (Extended Data Fig. 7a–c). In intratumoral T cells, PPAR δ activation increased chromatin accessibility around effector-related gene loci including *Prf1*, *Gzmb*, *Ccl4* and *Klrg1* (Fig. 4l,m and Extended Data Fig. 7d,e). Regions with increased chromatin accessibility in CD8 $^{+}$ T cells from PPAR δ -iGOF showed motif enrichment for AP-1 transcription factors (Fig. 4n), which are implicated in T cell effector function, resistance to exhaustion and enhanced CAR-T cell function⁴³. These findings show that active PPAR δ promotes chromatin accessibility around regions associated with enhanced effector state.

Fig. 3 | PPAR δ activity promotes CD8 $^{+}$ T cell abundance and fitness in tumors.

a,b, Differential expression of immune signatures across PPAR δ -iGOF and WT MC38 (**a**) and AKPS (**b**) TICs. **c,d**, Pearson correlation coefficients (*R*) between ‘PPAR-induced’ and IFN γ response (**c**) or inflammatory response (**d**) in human melanoma TICs. **e**, T cell clonality in WT and PPAR δ -iGOF (GOF) AKPS TICs. **f**, Differential interactions between PPAR δ -iGOF and WT TICs calculated by CellChat. **g**, Differential expression between PPAR δ -iGOF and WT CD8 $^{+}$ T cells in MC38 and AKPS tumors. **h,i**, Differential expression in WT and PPAR δ -iGOF CD8 $^{+}$ T cells (**h**) or clonal and diverse PPAR δ -iGOF CD8 $^{+}$ T cells (**i**) in AKPS tumors (WT: 654 cells, PPAR δ -iGOF: 1,142 cells, diverse: 1,073 cells, clonal: 123 cells, from two mice per group). **P* ≤ 0.05, ***P* ≤ 0.01, ****P* ≤ 0.001, *****P* ≤ 0.0001. **j**, Intratumoral IFN γ /TNF $^{+}$ CD8 $^{+}$ T cells from WT and PPAR δ -iGOF (WT: *n* = 9 mice, PPAR δ -iGOF: *n* = 6 mice), $\pm$ s.e.m. **k–n**, Representative immunofluorescence images and quantification of CD3 $^{+}$ CD8 $^{+}$ cells in MC38

control (WT) and PPAR δ -cGOF tumors (**k** and **l**) or AKP tumors (**m** and **n**) from WT and PPAR δ -iGOF mice. Scale bars, 100 μ m. (WT mice with control MC38: *n* = 24 areas from five mice, WT mice with PPAR δ -cGOF MC38: *n* = 45 areas from eight mice, PPAR δ -iGOF mice with control MC38: *n* = 12 areas from three mice, PPAR δ -iGOF mice with PPAR δ -cGOF MC38: *n* = 21 areas from four mice, WT AKP: *n* = 13 areas from three mice, PPAR δ -iGOF AKP: *n* = 12 areas from three mice), $\pm$ s.e.m. **o**, Differential expression of NicheNet-prioritized ligands between PPAR δ -iGOF and WT AKPS TICs. **p**, WT and PPAR δ -iGOF MC38 tumor volume upon CXCR3 $^{+}$ cell depletion. (IgG: *n* = 11 mice per group, anti-CXCR3: *n* = 13 mice per group, from two independent experiments), $\pm$ s.e.m. **q,r**, Pearson correlation coefficient (*R*) between ‘PPAR-induced’ and *IFNG* (**q**) or *CXCR3* (**r**) z-scores in human melanoma CD8 $^{+}$ T cells, $\pm$ 95% confidence interval. Significance was assessed using a two-tailed unpaired *t*-test (**a**, **g**, **j**, **l** and **p**) and a Wilcoxon rank-sum test (**h** and **i**). LFC, log fold change.



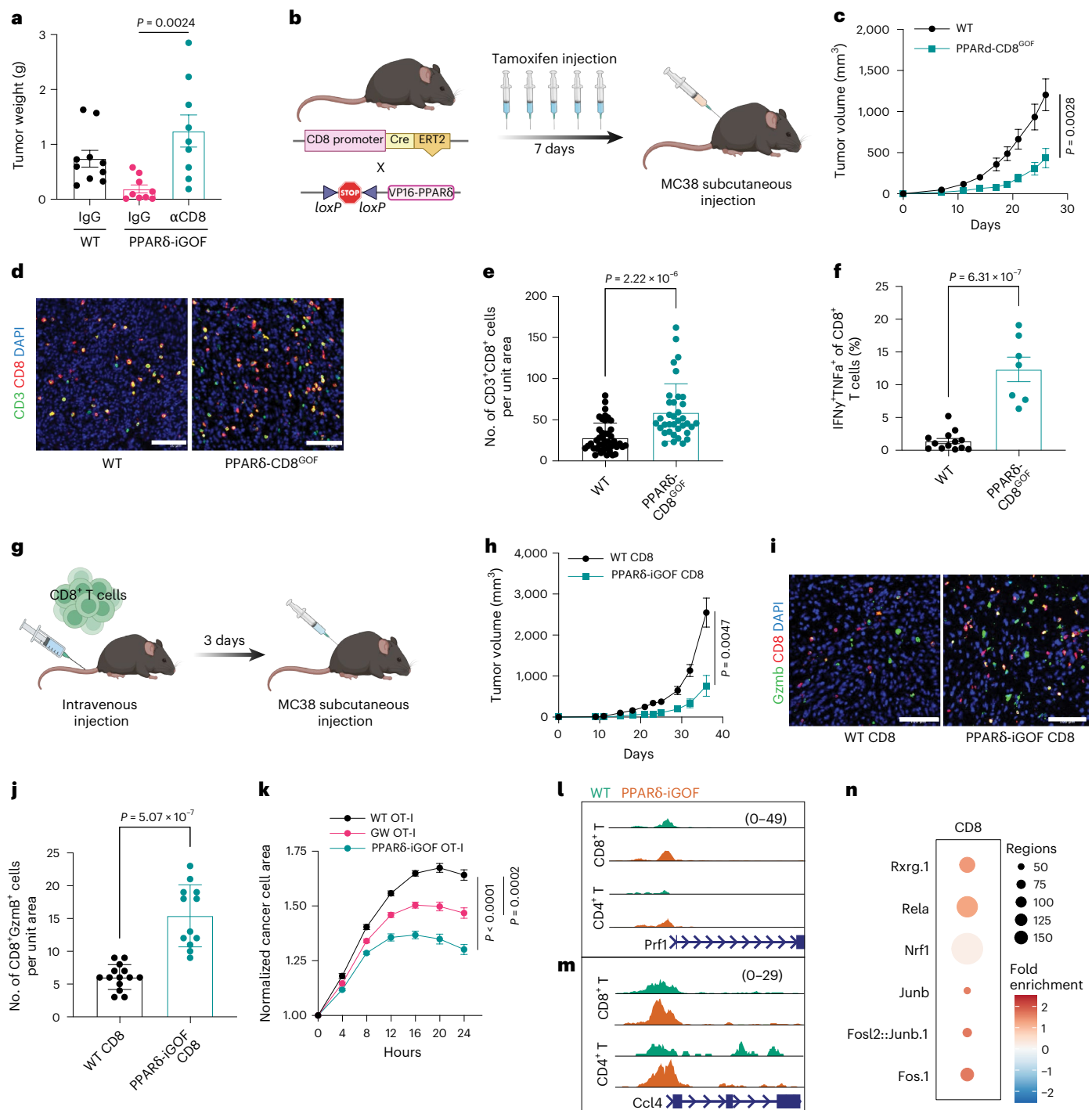


Fig. 4 | CD8⁺ T cells are necessary and sufficient for PPAR δ -mediated boost in antitumor immunity. **a**, MC38 tumor weights from WT or PPAR δ -iGOF mice upon CD8 α depletion. ($n = 9$ mice per group, except WT IgG: $n = 10$ mice), $\pm$ s.e.m. **b,c**, Schematic (**b**) and MC38 tumor growth (**c**) of tamoxifen-treated WT and PPAR δ -CD8^{GOF} mice. (WT: $n = 9$ mice, PPAR δ -CD8^{GOF}: $n = 10$ mice, from two independent experiments), $\pm$ s.e.m. **d,e**, Representative immunofluorescence images (**d**) and quantification (**e**) of CD3⁺CD8⁺ cells in MC38 from tamoxifen-treated WT and PPAR δ -CD8^{GOF} mice. Scale bars (**d**), 100 μ m. (WT: $n = 44$ areas from nine mice, PPAR δ -CD8^{GOF}: $n = 36$ areas from eight mice, from two independent experiments), $\pm$ s.d. **f**, Intratumoral IFN γ ⁺TNF α ⁺ CD8⁺ T cells from WT and PPAR δ -CD8^{GOF} mice (WT: $n = 13$ mice, PPAR δ -CD8^{GOF}: $n = 7$ mice), $\pm$ s.e.m. **g,h**, Schematic (**g**) and MC38 tumor growth (**h**) upon CD8⁺ T cell adoptive transfer (WT CD8⁺ T cells: $n = 9$ mice, PPAR δ -iGOF CD8⁺ T cells: $n = 5$ mice), $\pm$ s.e.m. **i,j**, Representative immunofluorescence images (**i**) and quantification (**j**)

of GzmB⁺CD8⁺ cells in MC38 tumors from mice adoptively transferred with WT or PPAR δ -iGOF CD8⁺ T cells. Scale bars (**i**), 100 μ m. (WT: $n = 14$ areas from three mice, PPAR δ -iGOF: $n = 12$ areas from three mice), $\pm$ s.d. **k**, Antigen-specific OT-I CD8⁺ T cell killing of MC38-Ova cells at an effector-to-target ratio of 1:1. ($n = 7$ replicates per group, representative of two independent experiments), $\pm$ s.e.m. **l,m**, Chromatin accessibility for *Prf1* (**l**) and *Ccl4* (**m**) in CD4⁺ and CD8⁺ T cell clusters from AKPS in PPAR δ -iGOF or WT mice. ($n = 19,223$ cells from two mice per group). **n**, Top upregulated motifs from scATAC-seq of CD8⁺ T cells from PPAR δ -iGOF and WT mice. Significance was assessed using an ordinary one-way ANOVA with two-tailed Tukey's test (**a**), a two-tailed unpaired *t*-test (**b**, **e**, **f**, **h** and **j**), a Welch's *t*-test (**k**) and a hypergeometric test using Signac (**n**). Images created in BioRender: **b**, Sirkali Suresh Kumar, L. <https://biorender.com/6l3p3cc> (2026); **g**, Ergin, I. <https://biorender.com/udhkhdu> (2026).

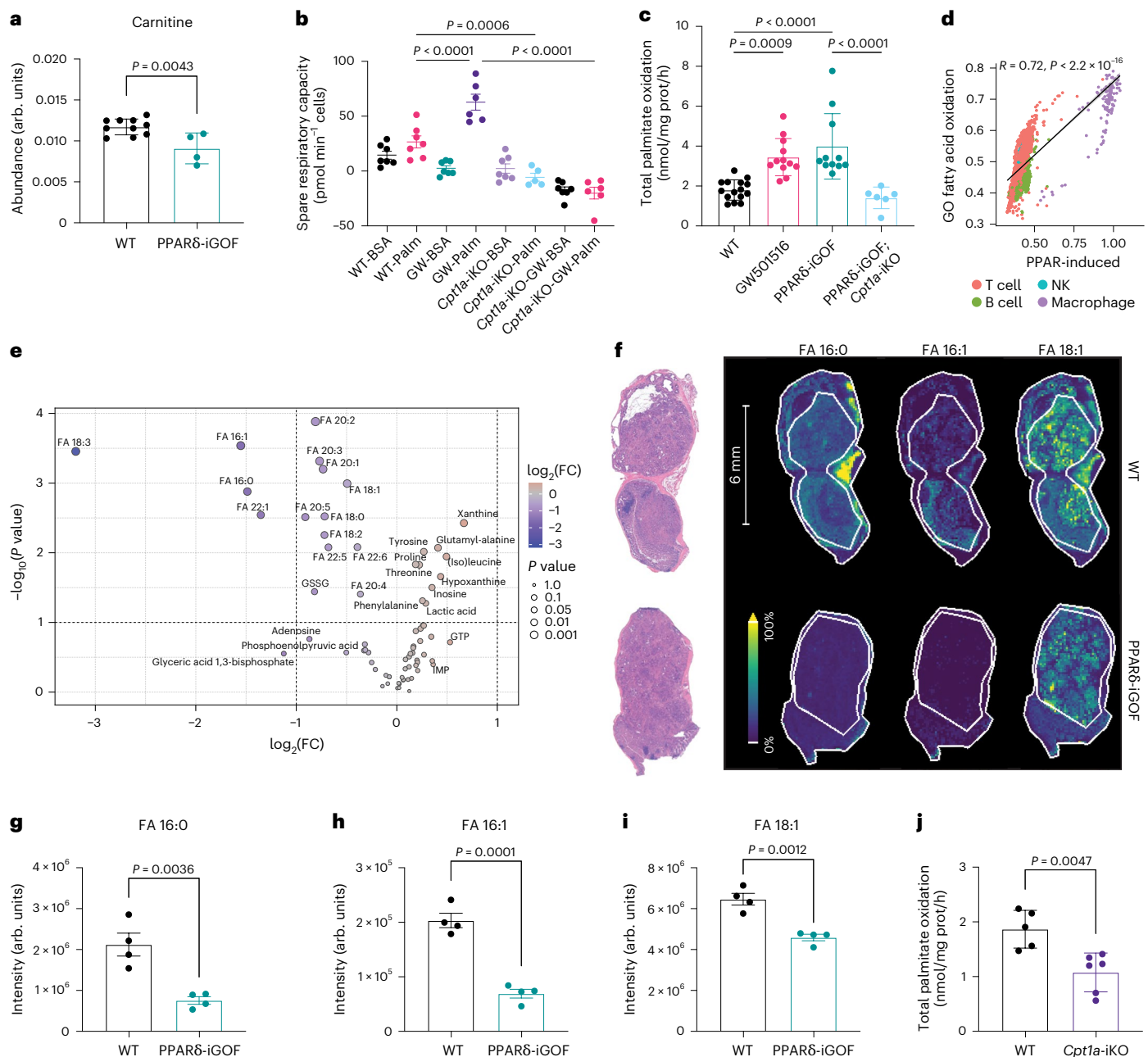


Fig. 5 | PPAR δ activation in CD8⁺ T cells reprograms lipid metabolism in tumors. **a**, Carnitine abundance in CD8⁺ T cells from WT or PPAR δ -iGOF mice, determined by high-performance liquid chromatography and high-resolution mass spectrometry and tandem mass spectrometry (WT CD8⁺ T cells: $n = 10$ mice, PPAR δ -iGOF CD8⁺ T cells: $n = 4$ mice), $\pm$ s.d. **b**, Spare respiratory capacity calculated by Agilent Seahorse XF palmitate oxidation stress test of mouse CD8⁺ T cells, isolated from WT or Cpt1a-iKO mice and treated with vehicle or GW501516 (GW) for 72 h ($n = 7$ technical replicates per group, except GW Palm: $n = 6$, Cpt1a-iKO GW Palm: $n = 6$, and Cpt1a-iKO Palm $n = 5$), $\pm$ s.e.m. **c**, Total ¹⁴C-palmitate oxidation of mouse CD8⁺ T cells, isolated from WT, PPAR δ -iGOF or PPAR δ -iGOF; Cpt1a-iKO mice and activated in vitro in the presence of vehicle or GW501516 for 72 h, (WT: $n = 15$ replicates, GW501516: $n = 12$ replicates, PPAR δ -iGOF: $n = 11$ replicates, PPAR δ -iGOF; Cpt1a-iKO: $n = 6$ replicates, from three independent experiments), $\pm$ s.d. **d**, Pearson correlation coefficient (R) between

'PPAR-induced' and 'GO FAO' z-scores in human melanoma TICs. **e**, Volcano plot showing $\log_2$ fold changes of metabolite abundance between AKPS tumors from WT and PPAR δ -iGOF mice determined by MALDI mass spectrometry imaging ($n = 4$ mice per group). **f–i**, Representative hematoxylin and eosin (H&E), MALDI mass spectrometry images and quantification of fatty acid (FA) 16:0 (palmitic acid) (**g**), 16:1 (palmitoleic acid) (**h**) and 18:1 (oleic acid) (**i**) abundance in AKPS tumors from WT and PPAR δ -iGOF mice. Tumor and healthy tissue are outlined in white ($n = 4$ mice per group), $\pm$ s.e.m. **j**, Total ¹⁴C-palmitate oxidation of mouse CD8⁺ T cells, isolated from WT or Cpt1a-iKO mice and activated in vitro for 72 h (WT: $n = 5$ replicates, Cpt1a-iKO: $n = 6$ replicates, representative of three independent experiments), $\pm$ s.d. Significance was assessed using a two-tailed unpaired t -test (**a**, **g–i** and **j**) and an ordinary one-way ANOVA with a two-tailed Tukey's multiple-comparison test (**b** and **c**).

PPAR δ reprograms CD8⁺ T cell metabolism through Cpt1a

Liquid chromatography–mass spectrometry (LC–MS) profiling of metabolites in PPAR δ -iGOF and WT CD8⁺ T cells revealed a decrease in carnitine levels (Fig. 5a and Extended Data Fig. 8a–c). This reduction

may suggest heightened activity of carnitine palmitoyltransferase 1a (Cpt1a), which utilizes carnitine to initiate mitochondrial FAO⁴⁴. Indeed, both pharmacological and genetic activation of PPAR δ boosted FAO in CD8⁺ T cells and cancer cells, evidenced by enhanced spare respiratory

capacity (Fig. 5b and Extended Data Fig. 8d), elevated Cpt1a expression (Extended Data Fig. 8e–g) and increased total ^{14}C -palmitate oxidation (Fig. 5c and Extended Data Fig. 8h,i). Additionally, PPAR δ transcriptional activity correlated with the FAO gene signature in human TICs (Fig. 5d). PPAR δ -activated CD8 $^{+}$ T cells increased metabolites involved in nucleotide metabolism (Extended Data Fig. 8j–m), consistent with studies linking Cpt1a-mediated FAO to de novo nucleotide synthesis and cell proliferation⁴⁵.

We performed MALDI mass spectrometry imaging for spatial metabolite quantification on AKPS tumors from WT and PPAR δ -iGOF mice. Most fatty acid species were significantly depleted in tumors from PPAR δ -iGOF mice compared to WT controls (Fig. 5e–i and Extended Data Fig. 8n–r), consistent with PPAR δ -activated immune cells outcompeting cancer cells for nutrients.

We generated *Vav1*-cre $^{+}$ *Cpt1a*^{fl/fl} mice to conditionally ablate Cpt1a in immune cells (Cpt1a-iKO; Extended Data Fig. 8s,t). Cpt1a loss reduced CD8 $^{+}$ T cell mitochondrial mass and activity (Extended Data Fig. 8u,v), as well as the oxygen consumption rate (OCR; Extended Data Fig. 8x). Cpt1a-iKO CD8 $^{+}$ T cells showed elevated carnitine (Extended Data Fig. 8b,c,y), consistent with impaired FAO. Indeed, ablation of Cpt1a decreased ^{14}C -palmitate oxidation in these cells (Fig. 5j). In compound PPAR δ -iGOF; Cpt1a-iKO mice, the absence of Cpt1a impaired the PPAR δ -driven enhancement of palmitate oxidation capacity (Fig. 5c). Thus, Cpt1a is essential for PPAR δ -mediated metabolic reprogramming of CD8 $^{+}$ T cells.

Cpt1a mediates PPAR δ antitumor and epigenetic effects

In MSI MC38 tumors Cpt1a deficiency in immune cells accelerated tumor growth (Fig. 6a and Extended Data Fig. 9a) and reduced CD8 $^{+}$ T cell infiltration (Extended Data Fig. 9b–e), indicating that loss of Cpt1a in immune cells blunts antitumor immunity. Cpt1a-iKO TICs showed dampened expression of genes related to migration (*Ccl6*, *Xcl1*), interferon response (*Irf5*, *Irf7*, *Ifit3*) and survival (*Bcl2*; Fig. 6b and Extended Data Fig. 9f–k). Furthermore, CD8 $^{+}$ T cell depletion led to comparable tumor growth between Cpt1a-iKO and WT mice, suggesting a potential functional role for Cpt1a in CD8 $^{+}$ T cell-mediated antitumor immunity (Fig. 6c and Extended Data Fig. 9l).

Compound PPAR δ -iGOF; Cpt1a-iKO mice developed larger tumors than PPAR δ -iGOF mice with intact *Cpt1a* but smaller than WT mice (Fig. 6d and Extended Data Fig. 9m), with reduced CD45 $^{+}$ immune and CD8 $^{+}$ T cell infiltration compared to PPAR δ -iGOF mice (Fig. 6e,f and Extended Data Fig. 9n). Consistently, scRNA-seq of PPAR δ -iGOF; Cpt1a-iKO and PPAR δ -iGOF CD8 $^{+}$ T cells showed that *Cpt1a* loss led to downregulation of gene signatures associated with cytokine response, proliferation, pro-inflammatory signaling, chemokine response, T cell activation and stem-like memory (Fig. 6g,h). These results indicate that Cpt1a-mediated FAO is in part required for PPAR δ -driven enhancement of antitumor immunity.

To assess whether enhanced FAO directly mediates the effects of PPAR δ , we generated Cpt1a-iGOF mice that express a mutant *Cpt1a* (Cpt1a^{M593S}) in immune cells, which encodes an enzyme resistant to inhibition by malonyl-CoA, its physiological inhibitor⁴⁴ (Extended Data Fig. 9o). CD8 $^{+}$ T cells from Cpt1a-iGOF mice showed enhanced rates of FAO, albeit lower than PPAR δ -iGOF CD8 $^{+}$ T cells (Extended Data Fig. 9p). Growth of MC38 tumors was similar in Cpt1a-iGOF and WT mice (Fig. 6i and Extended Data Fig. 9q). To determine whether active Cpt1a mediates PPAR δ -induced transcriptional or chromatin accessibility alterations, we performed scRNA-seq and scATAC-seq on CD45 $^{+}$ TICs. Cpt1a-iGOF CD8 $^{+}$ T cells exhibited downregulation of glycolysis-related genes (Extended Data Fig. 9r) and upregulation of stem-like memory markers (*Il7r*, *Sell*, *Tcf7*, *Lef1*), similar to MC38-infiltrating PPAR δ -iGOF CD8 $^{+}$ T cells, but lacked expression of effector and pro-inflammatory programs activated downstream of PPAR δ (Figs. 3h and 6j and Extended Data Fig. 4h). Chromatin accessibility changes mirrored these effects, with promoter

regions of memory-associated genes, such as *Sell*, *Lef1* and *Il7r*, opening in Cpt1a-iGOF while closing in Cpt1a-iKO (Fig. 6k,l and Extended Data Fig. 9s). Motifs of memory-associated transcription factors Lef1, Bcl11b, Foxo1 and Foxo3 were enriched in Cpt1a-iGOF CD8 $^{+}$ T cells (Fig. 6m). Together, these results suggest that Cpt1a-mediated FAO is necessary for, but not sufficient to fully recapitulate PPAR δ -driven antitumor immunity. Instead, active Cpt1a primarily supports T cell memory function. Because Cpt1a-iGOF cells achieve lower FAO rates than PPAR δ -iGOF cells, we cannot exclude that higher FAO would be sufficient to recapitulate the PPAR δ -mediated boost in effector function.

PPAR δ -activated CD8 $^{+}$ T cells sensitize MSS CRC to immunotherapy

Because current immunotherapies have limited effectiveness in MSS CRC⁷, we asked whether PPAR δ -enhanced CD8 $^{+}$ T cell fitness could render this refractory setting responsive. We transferred activated CD8 $^{+}$ T cells from either PPAR δ -iGOF or WT mice into congenic mice bearing established AKPS tumors, followed by treatment with anti-PD-1 (Fig. 7a). While tumors in mice receiving WT CD8 $^{+}$ T cells continued to grow and metastasize, those transferred with PPAR δ -iGOF CD8 $^{+}$ T cells exhibited significantly reduced tumor growth and metastasis following ICB (Fig. 7b–f and Extended Data Fig. 10a) and 50% of PPAR δ -iGOF CD8 $^{+}$ T cell recipients completely rejected their tumors by the end of the anti-PD-1 immunotherapy regimen (Fig. 7c). Despite these reductions in tumor burden and metastasis, overall survival did not significantly improve (Extended Data Fig. 10b).

Combined immune checkpoint inhibition has shown increased therapeutic efficacy in several cancer types, including MSI CRC^{7,8}. Indeed, combined anti-PD-1 and anti-CTLA4 ICB in AKPS-bearing mice receiving PPAR δ -iGOF CD8 $^{+}$ T cells led to significant survival benefits over mice receiving WT CD8 $^{+}$ T cells (Fig. 7g). These findings highlight the therapeutic potential of PPAR δ -activated CD8 $^{+}$ T cells in overcoming immunotherapy resistance in immune-evasive tumors such as MSS CRC.

PPAR δ activity boosts human CAR-T cell fitness and antitumor function

Because CAR-T cell efficacy in solid tumors is limited by poor persistence, dysfunction and metabolic stress⁴³, we next asked whether the PPAR δ fitness program could translate to human cellular immunotherapy. Treatment of human CAR-T cells targeting epidermal growth factor receptor (EGFR) with the PPAR δ agonist GW501516 augmented their cytotoxicity against CRC PDOs, irrespective of MMR status (Fig. 7h–j). We also engineered two distinct human epidermal growth factor receptor 2 (HER-2)-targeting CAR-T cell models to express VP64–PPAR δ protein (Extended Data Fig. 10c,d). In both HER-2 CAR-T cell designs, genetic activation of PPAR δ (PPAR δ -GOF) improved tumor cell killing in vitro compared to WT CAR-T cells (Fig. 7k and Extended Data Fig. 10e,f).

We characterized metabolic activity, gene expression and chromatin accessibility in HER-2-targeting CAR-T cells (Extended Data Fig. 10g–r). PPAR δ -GOF CAR-T cells showed increased ^{14}C -palmitate oxidation (Extended Data Fig. 10n) and upregulated canonical PPAR δ target genes and lipid-metabolism-related genes (*PDK4*, *PLIN2*, *ANGPTL4*, *CPT1A*, *CPT1B*; Extended Data Fig. 10h,k,l). This metabolic phenotype was accompanied by reduced expression of dysfunction-associated genes (*BTIA*, *TOX2*) and increased expression of genes associated with migration (*XCL1*, *CCL5*), interferon signaling (*IFIT3*, *IFITM3*) and T cell response (*GZMB*, *TNF*, *IL15*, *IRF8*; Fig. 7l and Extended Data Fig. 10h), with upregulation of gene signatures associated with effector function and cytotoxicity (Fig. 7m and Extended Data Fig. 10i).

PPAR δ -GOF CAR-T cells also showed increased promoter accessibility at PPAR δ target genes and T cell activation-related genes (Fig. 7n,o and Extended Data Fig. 10o–r). Mirroring our in vivo findings in mouse tumor-infiltrating T cells, chromatin accessibility was increased at

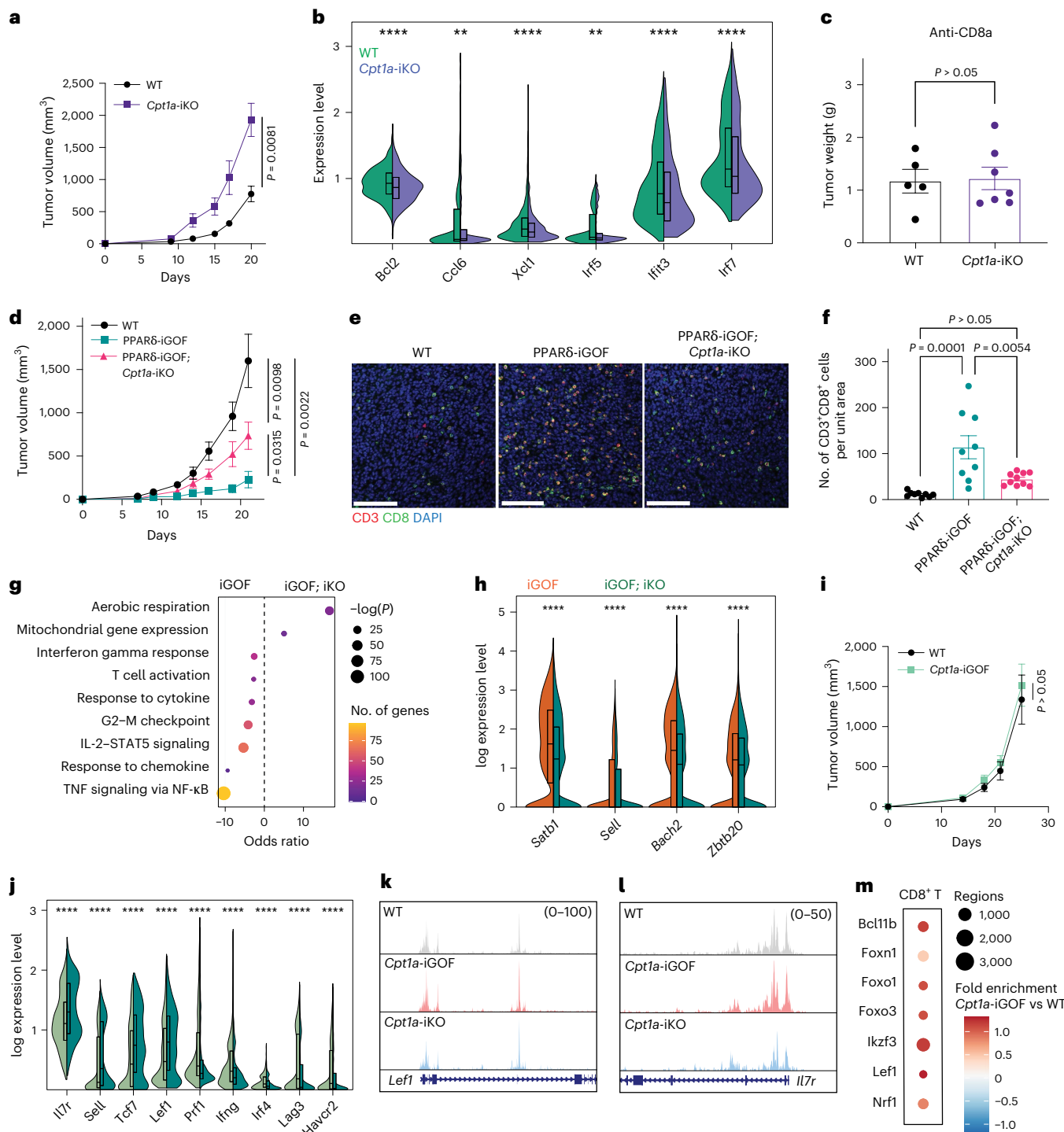


Fig. 6 | Immune-intrinsic PPAR δ –Cpt1a axis controls antitumor immunity.

a, MC38 tumor growth in WT and *Cpt1a*-iKO mice ($n = 7$ mice per group), $\pm$ s.e.m. **** $P \leq 0.0001$. **b**, Differential expression of candidate genes in WT and *Cpt1a*-iKO CD8⁺ T cells in MC38 tumors (WT: $n = 1,177$ cells, *Cpt1a*-iKO: $n = 516$ cells), ** $P \leq 0.01$, **** $P \leq 0.0001$. **c**, Endpoint MC38 tumor weights from WT and *Cpt1a*-iKO mice upon CD8a⁺ depletion. (anti-CD8a WT: $n = 5$ mice, anti-CD8a *Cpt1a*-iKO: $n = 7$ mice), $\pm$ s.e.m. $P > 0.05$. **d**, MC38 tumor growth of WT, PPAR δ -iGOF and PPAR δ -iGOF; *Cpt1a*-iKO mice. (WT: $n = 10$ mice, PPAR δ -iGOF: $n = 4$ mice, PPAR δ -iGOF; *Cpt1a*-iKO: $n = 11$ mice), $\pm$ s.e.m. $P = 0.00315$, $P = 0.0098$, $P = 0.0022$. **e, f**, Representative immunofluorescence images (**e**) and quantification (**f**) of CD3⁺CD8⁺ cells in MC38 from WT, PPAR δ -iGOF and PPAR δ -iGOF; *Cpt1a*-iKO mice. Scale bars (**e**), 100 μ m. ($n = 3$ tumor areas from three mice per group). **g**, Top enriched gene ontologies between CD8⁺ T cells from PPAR δ -iGOF; *Cpt1a*-iKO and PPAR δ -iGOF mice as calculated using EnrichR. **** $P \leq 0.0001$. **h**, Differential expression of memory-related genes in PPAR δ -iGOF; *Cpt1a*-iKO

and PPAR δ -iGOF CD8⁺ T cells in MC38 (PPAR δ -iGOF: $n = 10,379$ cells, PPAR δ -iGOF; *Cpt1a*-iKO: $n = 6,327$ cells, from two mice per group), **** $P \leq 0.0001$. **i**, MC38 tumor growth in WT and *Cpt1a*-iGOF mice (WT: $n = 6$ mice, *Cpt1a*-iGOF: $n = 9$ mice; representative of three independent experiments), $\pm$ s.e.m. $P > 0.05$. **j**, Differential expression in WT and *Cpt1a*-iGOF CD8⁺ T cells in MC38 tumors (WT: $n = 3,217$ cells, *Cpt1a*-iGOF: $n = 2,340$ cells, from two mice per group), **** $P \leq 0.0001$. **k, l**, Normalized chromatin accessibility for *Leif1* (**k**) and *Il7r* (**l**) in CD8⁺ T cell clusters from MC38 tumors in WT, *Cpt1a*-iGOF or *Cpt1a*-iKO mice. ($n = 30,247$ cells from two mice per group). **m**, Top upregulated motifs from scATAC-seq of CD8⁺ T cells from *Cpt1a*-iGOF compared to WT mice. ($n = 30,247$ cells from two mice per group). Significance was assessed using a two-tailed unpaired *t*-test (**a, c, d, g** and **i**), a Wilcoxon rank-sum test (**b, h** and **j**), an ordinary one-way ANOVA with two-tailed Tukey's test (**f**) and a hypergeometric test using Signac (**m**).

regions enriched for motifs of AP-1 transcription factors, which potentiate CAR-T cell function⁴³. Motif analysis also confirmed enrichment for PPAR δ and its heterodimerization partners, including RXR (Fig. 7p).

Finally, we transferred WT or PPAR δ -GOF CAR-T cells into NSG mice bearing established SK-OV-3 tumors. PPAR δ -GOF CAR-T cells significantly reduced tumor growth compared to WT CAR-T cells (Fig. 7q). Together, these data extend our mouse genetic findings to human cellular immunotherapy and show that PPAR δ activation enhances human CAR-T cell fitness, FAO, effector transcriptional programs, chromatin accessibility and antitumor function both in vitro and in vivo. These findings support immune cell-selective PPAR δ activation as a strategy to improve immunotherapy efficacy against treatment-resistant tumors.

Discussion

This study identifies the fitness balance between cancer cells and immune cells as a targetable determinant of immunotherapy efficacy. Since cancer and immune cells share an ecosystem and rely on overlapping programs to survive, expand and withstand stress^{1,3,4}, tumor outcome may depend, in part, on which compartment can better access and use these fitness programs. In this framework, a pathway that enhances fitness in both compartments may act as a context-dependent determinant of tumor outcome, promoting tumor growth in cancer cells and antitumor immunity in immune cells. We establish PPAR δ as a proof of concept for this principle and show that its effect on antitumor immunity is dictated by the compartment in which it acts.

Using cell-type-specific genetic models, we show that activating PPAR δ in cancer cells promotes tumor growth and immunosuppression, congruent with previous reports^{11–15}. In contrast, activating PPAR δ in immune cells enhances immune infiltration, clonal lymphocyte expansion, CD8⁺ T cell polyfunctionality and tumor control, suppressing tumors even when cancer cells also harbor active PPAR δ , indicating that selective reinforcement of the immune compartment can overcome a cancer cell fitness advantage driven by the same pathway. Redirecting such compartment-specific fitness determinants could therefore offer a strategy to overcome immune evasion and improve immunotherapy efficacy in treatment-resistant tumors such as MSS CRC.

Our data support a model in which PPAR δ enhances CD8⁺ T cell function through coordinated chromatin remodeling and lipid metabolic adaptation. PPAR δ increases chromatin accessibility at effector-associated and activation-associated loci and enriches AP-1 motifs in mouse tumor-infiltrating T cells and human CAR-T cells, a state linked to T cell effector function, exhaustion resistance and improved CAR-T activity⁴³. In parallel, PPAR δ increases Cpt1a-dependent FAO in mouse CD8⁺ T cells and human CAR-T cells. Spatial metabolomics further shows reduced abundance of fatty acids within tumors from mice with immune-intrinsic PPAR δ activation, consistent with altered lipid use and metabolic competition in the TME, although direct cell-to-cell substrate competition using isotope-resolved and cell-type-specific

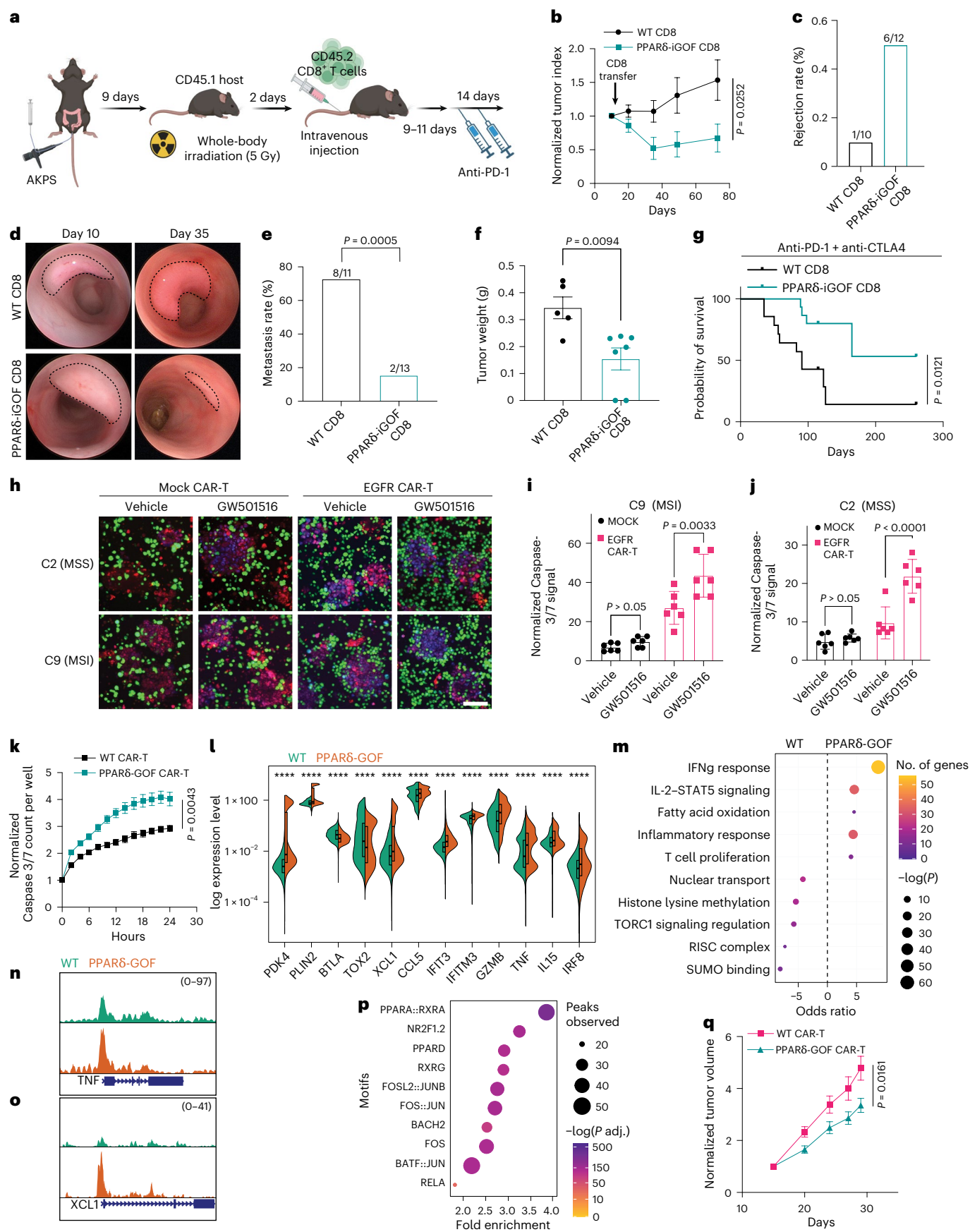
lipid tracing remains to be demonstrated^{1–3}. Thus, PPAR δ couples effector transcriptional programs with the metabolic fitness required for robust antitumor responses.

Genetically targeting Cpt1a refined this mechanism. Cpt1a loss decreased PPAR δ -induced FAO and impaired key aspects of the antitumor response, including CD8⁺ T cell infiltration and transcriptional programs associated with cytokine response and stem-like memory. Conversely, expression of a malonyl-CoA-insensitive Cpt1a gain-of-function mutant alone (Cpt1a-iGOF) increased FAO and preferentially promoted memory-associated transcriptional and chromatin features, consistent with the role of FAO in memory T cells²⁸, but did not significantly suppress tumor growth or recapitulate the full PPAR δ -dependent effector-associated chromatin and transcriptional programs. Thus, Cpt1a-mediated FAO supports components of PPAR δ -driven immune fitness but not the full phenotype. Instead, PPAR δ appears to integrate Cpt1a-dependent metabolic support with additional chromatin and transcriptional mechanisms. Because Cpt1a-iGOF CD8⁺ T cells did not reach the same FAO levels as PPAR δ -iGOF CD8⁺ T cells, we cannot exclude additional effects at higher FAO rates.

Our framework helps reconcile conflicting reports describing PPAR δ as pro-tumorigenic^{9,11–15,25}, tumor suppressive¹⁶, immunostimulatory and inhibitory^{20–23,25–27}. These ostensibly opposing observations are consistent with PPAR δ supporting both transcriptional activation and transcriptional repression, depending on ligand state, cell type, inflammatory context and duration of pathway engagement⁴². For example, prior studies showed that PPAR δ can limit T_H1 and T_H17 inflammation^{27,46}, and PPAR family members can transrepress inflammatory programs^{47–49}. Cen et al.²² recently reported that PPAR δ inhibition enhances CD8⁺ T cell cytotoxicity and proposed that PPAR δ restrains nuclear factor (NF)- κ B signaling. These findings differ from ours but were generated in distinct systems. They used whole-body knockout, native PPAR δ overexpression, and acute GW501516 exposure in T cell–cancer co-culture assays with an agonist present throughout, a setting that also activates immunosuppressive programs in cancer cells^{15,30}. In contrast, we activated PPAR δ genetically and selectively in immune, CD8⁺ T and CAR-T cells using a VP16/VP64 transactivator fusion, together with prolonged agonist preconditioning of immune cells before cytotoxicity assays in autologous, polyclonal or antigen-specific settings. These differences in activation state, compartment, context, kinetics and timing likely explain the divergent outcomes. Constitutively active PPAR δ is not equivalent to overexpression of unliganded PPAR δ , nor the molecular inverse of loss of endogenous PPAR δ . Unliganded PPAR δ may inhibit transcription through interactions with co-repressors or other transcription factors⁴². Our GOF approach imposes a ligand-independent VP16/VP64-driven activation program and does not preserve the full repertoire of native ligand-dependent activities, including potential transrepression, although we have not measured interaction with or repression of RelA/NF- κ B in our system. Gain and loss of PPAR δ function therefore need

Fig. 7 | PPAR δ activation enhances immunotherapy efficacy against immune-evasive tumors. a–f, Experimental design (a), AKPS tumor growth (b), rate of tumor rejection at day 35 (c), representative colonoscopy images (d), rate of metastasis (e) and tumor weights (f) at endpoint of AKPS-bearing congenic mice treated with WT or PPAR δ -iGOF CD8⁺ T cells along with anti-PD-1. (WT: $n = 10$ mice, PPAR δ -iGOF: $n = 12$ mice, except WT: $n = 12$ mice, PPAR δ -iGOF: $n = 13$ mice (e) and WT: $n = 5$ mice, PPAR δ -iGOF: $n = 7$ mice (f)), $\pm$ s.e.m. **g**, Survival of AKPS-bearing mice that received CD8⁺ T cells and combination immunotherapy. (WT: $n = 14$ mice, PPAR δ -iGOF: $n = 15$ mice). **h–j**, Representative images (h) and normalized Caspase-3 dye signal of apoptotic (blue) MSI (i) and MSS (j) CRC PDOs (red) after 16 h co-culture with vehicle-treated or GW501516-treated mock-transduced or EGFR-targeting CAR-T cells (green). Scale bar (h), 70 μ m. ($n = 6$ technical replicates per group), $\pm$ s.d. **k**, Normalized count of apoptotic SKBR3 cells during co-culture with version 2 WT or PPAR δ -GOF HER-2 CAR-T cells

($n = 7$ replicates per group), $\pm$ s.e.m. **l**, Differential expression in version 2 WT and PPAR δ -GOF HER-2 CAR-T cells (WT: $n = 24,227$ cells, PPAR δ -GOF: $n = 25,940$ cells), **** $P < 0.0001$. **m**, Top enriched gene ontologies between version 2 WT and PPAR δ -GOF HER-2 CAR-T cells. **n, o**, Normalized chromatin accessibility for *TNF* (n) and *XCL1* (o) version 2 WT and PPAR δ -GOF HER-2 CAR-T cells. ($n = 17,332$ cells). **p**, Top upregulated motifs from scATAC-seq of version 2 WT and PPAR δ -GOF HER-2 CAR-T cells. ($n = 17,332$ cells). **q**, Normalized SK-OV-3 tumor growth in NSG mice after treatment with version 2 human WT and PPAR δ -GOF HER-2 CAR-T cells ($n = 13$ mice each group, combined from two independent experiments), $\pm$ s.e.m. Significance was assessed using a two-tailed unpaired *t*-test (b, f and m), a *z*-test for proportions (c and e), a log-rank test (g), an ordinary one-way ANOVA with a two-tailed Tukey's test (i and j), a two-tailed Welch's *t*-test (k and q), a Wilcoxon rank-sum test (l) and a hypergeometric test using Signac (p). Images in a created in BioRender; Ergin, I. <https://biorender.com/cpsgiis> (2026).



not produce symmetric or opposing phenotypes. Consistent with this, immune-specific deletion of PPAR δ did not detectably alter baseline tumor growth in the model tested, possibly owing to redundancy with PPAR α or other compensatory regulators³⁵. Thus, endogenous PPAR δ may be dispensable for baseline antitumor immunity, whereas enforced activation reveals a therapeutic program that can be exploited in immune cells, consistent with studies showing PPAR δ activation supports memory CD8⁺ T cell formation and adoptive T cell therapy^{19–21}.

The same compartment and context dependency applies to the parallel debate over FAO, reported to promote^{18–20}, inhibit³² or have no effect on²⁹ immune cell function and antitumor immunity. This range partly reflects the specific immune context, whether memory or effector outputs are measured and whether FAO is perturbed genetically or pharmacologically, as genetic models have questioned the requirement for Cpt1a in memory by revealing off-target effects of the widely used FAO inhibitor etomoxir²⁹. Our data suggest Cpt1a-mediated FAO supports memory-associated and metabolic fitness-associated immune programs against cancer, whereas full effector function requires additional PPAR δ -dependent chromatin remodeling. Because PPAR δ -Cpt1a effects may differ in regulatory T cells²³, NK cells²⁵, myeloid cells²⁴, endothelial cells⁴⁵ and cancer cells^{13–15,30}, selectively engaging this axis in specific TME compartments warrants further investigation. Understanding these context-dependent effects will be essential for designing therapies that maximize antitumor immunity while minimizing potentially pro-tumorigenic actions.

Our study has therapeutic implications for immune-evasive cancers such as MSS CRC and for preventing metastasis, suggesting that resistance to ICB or CAR-T cell therapy can be overcome, at least in part, by improving the competitive capacity of T cells. Adoptive transfer experiments involved lymphodepleting irradiation, which may contribute to therapeutic outcome⁵⁰. However, control and PPAR δ -activated CD8⁺ T cell transfer groups were treated identically, supporting a CD8⁺ T cell-intrinsic contribution of PPAR δ activation to the improved response. In preclinical models, immune cell-specific PPAR δ activation did not cause overt systemic inflammation or exacerbated colitis for the observed duration. Nevertheless, longer-term safety studies across additional genetic backgrounds and inflammatory contexts will be required before clinical translation.

Overall, our study establishes that cancer–immune competition can be redirected by selectively enhancing immune cell activity of a shared cellular fitness program. Beyond PPAR δ , pathways that benefit cancer cells may also be exploitable for therapy if they are selectively and safely redirected to immune cells. Identifying such shared fitness determinants and selectively activating them in immune cells may provide a general strategy to overcome immune evasion and improve immunotherapy outcomes in treatment-resistant tumors.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41590-026-02685-2>.

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¹Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA. ²Graduate Program in Genetics, Stony Brook University, Stony Brook, NY, USA. ³Medical Scientist Training Program, Stony Brook University Renaissance School of Medicine, Stony Brook University, Stony Brook, NY, USA. ⁴Rutgers New Jersey Medical School, New Jersey, USA. ⁵Department of Neurosurgery, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA. ⁶Center for Cellular Immunotherapies, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA. ⁷Department of Surgery, Donald and Barbara Zucker School of Medicine Hofstra University, Northwell Health, Hempstead, NY, USA. ⁸Colorectal Surgery and Surgical Services, Huntington Hospital, Huntington, NY, USA. ⁹University of Michigan-Ann Arbor, Ann Arbor, MI, USA. ¹⁰Department of Biochemistry and Physiology, School of Pharmacy and Food Sciences, Universitat de Barcelona, Barcelona, Spain. ¹¹Institute of Biomedicine of the University of Barcelona (IBUB), Universitat de Barcelona, Barcelona, Spain. ¹²Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain. ¹³Department of Pathology, Northwestern University Feinberg School of Medicine, Chicago, IL, USA. ¹⁴Parker Institute for Cancer Immunotherapy at University of Pennsylvania, University of Pennsylvania, Philadelphia, PA, USA. ¹⁵Department of Radiology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA. ¹⁶Department of Cancer Biology, Dana-Farber Cancer Institute; Harvard Medical School, Boston, MA, USA. ¹⁷These authors contributed equally: Paul Bunk, Ilgin Ergin, Timothy Maher, Vyom Shah. ✉ e-mail: beyaz@csih.edu

Methods

Animal models

All mice were used in compliance with animal care guidelines from CSHL Institutional Animal Care and Use Committee at an AAALAC-accredited institution. Mice were housed on a 12-h light–dark cycle at 70–74 °F (21–23 °C) and 30–70% relative humidity, up to five sex-matched animals per cage, with ad libitum water and chow (Pico Lab Irradiated Rodent Diet, LD505330LB). C57BL/6J (B6; RRID: IMSR_JAX: 000664), congenic B6 CD45.1 (RRID: IMSR_JAX: 002014), NSG (RRID: IMSR_JAX: 005557) and *Ppard*^{fl/fl} (RRID: IMSR_JAX: 005897) mice were purchased from the Jackson Laboratory. *Vav1*-cre (Orkin Laboratory⁵¹), *Cp1a*^{fl/fl} (Carmeliet Laboratory⁴⁵), VP16–PPARδ (Yang Laboratory⁵²), *E81*^{CreERT2.GFP} (Vignali Laboratory⁵³) and CD45.1 OT-I (Fearon Laboratory⁵⁴) mice were provided by the indicated laboratories. Rosa26-CAG-LSL-CPT1-KI mice were generated by integrating a CAG-LSL-rat *Cpt1a*^{MS93S} sequence into the *Rosa26* locus of C57BL/6 embryonic stem cells, transferring engineered embryos into female mice, backcrossing resulting chimeric males to C57BL/6 females, and further backcrossing *Cpt1a*^{MS93S}-positive pups to C57BL/6 mice for a minimum of two generations. *Cpt1a*-iGOF mice were generated by crossing *Cpt1a*^{MS93S} animals with *Vav1*-cre animals. All experiments utilized age-matched and sex-matched controls between 8 and 12 weeks of age, if possible including littermate controls. Both male and female animals were used, and no sex-dependent differences were detected. Only female C57BL/6J or congenic B6 CD45.1 mice were used for cell transfer experiments to prevent graft-versus-host reactions.

Cell culture

MC38 (Kerafast, ENH204-FP, RRID: CVCL_B288) and all its derivative lines were cultured in DMEM/F-12 (Corning, 10-090-CV) supplemented with 10% fetal bovine serum (FBS; Corning, 35-016-CV), 1% penicillin–streptomycin (Corning, 30-001-CI), 10 mM HEPES (Thermo Fisher, 15630080) and 50 µg ml^{−1} gentamicin sulfate (Thermo Fisher, 15710064). B16-F10 (American Type Culture Collection (ATCC), CRL-6475, RRID: CVCL_0159), EO771 (Egeblad Laboratory, CSHL, RRID: CVCL_GR23), SK-BR-3 (ATCC, HTB-30, RRID: CVCL_0033) and SK-OV-3 (ATCC, HTB-77, RRID: CVCL_0532) cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin–streptomycin. All cell lines were cultured at 37 °C in a humidified 5% CO₂ incubator, were passaged every 2–3 days, tested negative for mycoplasma and were not independently authenticated.

Plasmid construction and lentiviral transduction

VP64-PPARd-Flag⁵⁵ and VP64-Flag were PCR amplified (NEB, M0492) from pLV-EF1A-VP64-PAPRd-Flag-EGFP (VectorBuilder) and pLV-EF1A-VP64-Flag-EGFP (VectorBuilder), cloned into pLV-EF1A-IRES-GFP (Vakoc Laboratory, CSHL), with the In-Fusion cloning system (Takara, 638948), and propagated in NEB Stable Competent *Escherichia coli* (NEB, C3040H) to generate VP64-PPARd-Flag (PPARδ-cGOF) and VP64-Flag (control MC38) cell lines (Supplementary Table 1). pLV-CMV-Ovalbumin-EF1A-mCherry (VectorBuilder) was used to generate MC38-Ova cells. Lentivirus was produced by co-transfecting a confluent 10-cm dish of HEK293T cells (Vakoc Laboratory, CSHL, RRID: CVCL_0063) with 10 µg lentiviral vector, 7.5 µg of psPAX2 (RRID: Addgene_12260), 5 µg of pMD2.G (RRID: Addgene_12259) and 80 µl 1 mg ml^{−1} of PEI (RnD, 7854). Virus was collected 36 h and 48 h after transfection. MC38 cells were spin transduced at 1,750 rpm for 45 min at room temperature with 20 µg ml^{−1} polybrene (Sigma, TR-1003) and sorted (Sony SH800S cell sorter) for GFP⁺ or mCherry⁺, choosing a viral dilution with 30% infection rate.

Subcutaneous tumor models

Mice were anesthetized with isoflurane, shaved at the injection site and injected subcutaneously in the right flanks with, depending on the experiment, 3 × 10⁵ or 5 × 10⁵ MC38 (parental cell line, PPARδ-cGOF

or control MC38), 2.5 × 10⁵ B16-F10 cells or 3 × 10⁶ SK-OV-3, or in the mammary fat pad with 2.5 × 10⁵ EO771 cells in 100 µl PBS. Tumors were measured with a caliper every 2–3 days by blinded investigators, until humane endpoints, defined as long diameter exceeding 20 mm, weight loss >15%, or declining body condition, including hunched posture, fur loss, reduced mobility or tachypnea. Tumor volume was calculated as 0.5 × *L* × *W*² with long (*L*) and short (*W*) diameter. PPARδ-CD8^{GOF} and respective control mice received five daily doses of 75 mg of tamoxifen per kilogram of body weight dissolved in corn oil (Sigma, C8267) intraperitoneally starting 12 days before tumor inoculation. Tumor-free mice were excluded from any further analysis. Mice were euthanized on day 12, day 25 or when reaching a humane endpoint.

Mouse intestinal organoid culture

AKP (*Apc*^{null}, *Kras*^{G12D}, *Tp53*^{null}) and AKPS (*Apc*^{null}, *Kras*^{G12D}, *Tp53*^{null}, *Smad4*^{null}) organoids were embedded in Matrigel (Corning, 356234) and cultured in minimal organoid media (Advanced DMEMF-12 (Gibco, 12634028) with N2 (Thermo Fisher, 17502048) and B27 (Thermo Fisher, 17504044)), and split using TrypLE Express (Thermo Fisher, 12604) every 3 days^{33,34}.

Colonoscopy-guided orthotopic CRC models

Orthotopic injections of CRC organoids and cell lines followed as previously described^{13,33,34}. Organoids were collected by gentle scraping and separated from Matrigel in Cell Recovery Solution (Corning, 354253) for 40 min at 4 °C. A fraction of organoids was dissociated in TrypLE Express (Thermo Fisher, 12604) for cell counting. Intact organoids were resuspended in minimal organoid media with 10% Matrigel, and 100 µl of solution containing 2.5 × 10⁶ cells for AKP organoids or 1 × 10⁶ cells for AKPS organoids injected into colon submucosa using a Hamilton syringe (7656-1) and a custom 33-gauge needle (Hamilton, custom made similar to 7803-05, 16 inch, Pt 4, Deg 12). Successful injections were confirmed by observing a mucosal bubble in the colon. MC38 cells (5 × 10⁶ cells per mouse in PBS with 10% Matrigel) were injected similarly. Tumor growth was followed by colonoscopy, with tumor index calculated by dividing the tumor diameter by the colon diameter, and the values were normalized to the respective week-1 tumor index. Mice were euthanized at specified time points or at humane endpoints, and metastasis to liver, lung or omentum was confirmed by mScarlet fluorescence (Electron Microscopy Sciences, Xite-GR). Mice with failed injections, indicated by absent colon tumors or tumors solely in the intraperitoneal space, were excluded.

DSS-induced colitis model

Acute colitis was induced with 3% wt/vol DSS in drinking water for 5 days, followed by 7 days of water. Mice were weighed daily and stool was assessed for consistency and blood (Hemocult, Beckman Coulter, 10012-018) in a blinded fashion. Severity was scored as the sum of weight loss (0–4), stool consistency (0–4) and rectal bleeding (0–4)^{56,57}.

Primary CD8⁺ T cell isolation, activation and culture

Spleens were mechanically disrupted, strained through a 40-µm cell strainer and centrifuged at 300g for 5 min. Red blood cells were lysed in ACK lysis buffer (CSHL, homemade) for 3 min. Splenic CD8⁺ T cells were isolated from the resulting splenocytes by negative magnetic selection (Stemcell Technologies, 19853). Around 1 × 10⁶ CD8⁺ T cells per ml were plated in either 96-well or 24-well plates pre-coated with 2 µg ml^{−1} anti-CD3 and anti-CD28 (BioLegend, 100340, RRID: AB_11149115, BioLegend, 102116, RRID: AB_11147170) or with Dynabeads Mouse T-Activator CD3/CD28 (Thermo Scientific, 11453D) and activated for 72 h at 37 °C in a humidified 5% CO₂ incubator in T cell media (RPMI (Corning, 10-040-CV), 10% FBS, 1% penicillin–streptomycin, 50 µM β-mercaptoethanol (MilliporeSigma, M3148-25ML) and 20 ng ml^{−1} recombinant mouse IL-2 (R&D systems, 402-ML-020)). Where indicated, 10 µM GW501516 (Enzo, ALX-420-032-M001) or 500 nM 4-hydroxytamoxifen (4-OHT) was added during activation.

Immunoblotting

Where indicated, nuclear and cytoplasmic fractions were prepared (Thermo Scientific, 78840). Protein was quantified by Pierce Rapid Gold BCA Protein Assay Kit (Thermo Scientific, A53227). Lysate was boiled in 2.5× Laemmli buffer and 10 µg or 20 µg resolved on NuPAGE 4–12% Bis-Tris 1.5-mm gels (Thermo Fisher, NP0335BOX). Cytoplasmic or whole-cell fractions were blotted with 1 µg ml⁻¹ anti-Cpt1a (Abcam, ab128568, RRID: AB_11141632), 1:1,000 dilution anti-tubulin (Cell Signaling Technology, 3873, RRID: AB_1904178) and 1:2,000 dilution anti-Beta actin (Cell Signaling Technology, 4967, RRID: AB_330288). Nuclear fraction A was blotted with 1:1,000 anti-PPARδ antibody (Cell Signaling Technology, 74076, RRID: AB_3661825), 1 µg ml⁻¹ anti-VP16 (Santa Cruz Biotechnology, sc-7545, RRID: AB_628443) and 1:1,000 dilution anti-HDAC2 (Cell Signaling Technology, 2540, RRID: AB_2116822). Detection used anti-rabbit-Horseradish peroxidase (BioLegend, 406401, RRID: AB_2099368) or anti-mouse-Horseradish peroxidase (BioLegend, 405306, RRID: AB_315009) at a 1:5,000 dilution with Pierce ECL Western Blotting Substrate (Thermo Fisher, 32106).

Preparation of FFPE tissues

Organs, tumors and Swiss-rolled small intestine and colon were fixed in 10% formalin for 24 h, washed in 70% ethanol and embedded in paraffin. Formalin-fixed paraffin-embedded (FFPE) sections (10-µm thick) were mounted on slides. H&E-stained slides were reviewed blinded by an expert pathologist.

Immunofluorescence staining

FFPE sections were deparaffinized using xylene, gradually rehydrated, antigen-retrieved, in Tris-EDTA buffer (10 mM Tris, 1 mM EDTA, 0.05% Tween-20, pH = 9) at 110 °C for 10 min in a pressure cooker, and blocked in SuperBlock (Thermo Fisher, 37515) 1 h at room temperature. Primary antibodies were applied overnight at 4 °C, followed by secondary antibodies for 1 h at room temperature. A list of antibodies used can be found in Supplementary Table 2. Sections were stained with 0.5 µg ml⁻¹ DAPI (Invitrogen, D3571) for 10 min, mounted in ProLong Diamond Antifade Mountant (Thermo Fisher, P36970) and imaged on a Zeiss LSM710 confocal microscope (Plan-Apochromat ×10/0.45 M27 Air lens or a ×20/0.8 M27 Air lens). Images were processed in Fiji (ImageJ2, version 2.3.0)⁵⁸. After z-projection and brightness/contrast adjustment in Fiji, images were quantified blinded in QuPath⁵⁹, using automatic positive cell detection, manual counting or both for cells, automatic pixel classification for pan-keratin-positive area, and manual annotation with polygon area calculation for tumor and immune hub areas.

For fresh-frozen tissue used in MALDI mass spectrometry, sections were mounted on Superfrost Plus slides (Fisher Scientific, 22-037-246), fixed in 4% paraformaldehyde for 20 min at room temperature, and permeabilized for 5 min in 0.1% detergent solution (Thermo Scientific, 28316). Anti-RFP (Rockland Immunochemicals, 600-401-379) at a 1:800 dilution in SuperBlock (Thermo Scientific, 37515) was incubated at 4 °C in the dark overnight, followed by donkey anti-rabbit AF555 (Thermo Scientific, A-31572) at a dilution of 1:1,000 in SuperBlock for 1 h at room temperature and conjugated anti-CD45 AF488 (Thermo Scientific, 53-0451-82) at a dilution of 1:100 in Superblock with Hoechst (Thermo Scientific, H3570, 1:10,000 dilution) overnight at 4 °C in the dark. Images were acquired on a Zeiss Axio Observer 7 with a ×20 objective and Zen 3.11 software.

Masson's trichrome staining

Fibrosis was scored blindly by an expert pathologist on Masson's trichrome-stained FFPE tumor slides, quantifying the blue channel using Aperio ImageScope color deconvolution. The total percentage of fibrosis was determined based on medium and strong blue pixels. The maximum score was 300.

Mouse co-culture experiments

Primary mouse CD8⁺ T cells were isolated, treated and activated as above and harvested, after 72 h. Splenocytes were expanded over 72 h using plate-bound CD3/CD28 antibodies, some treated with 10 µM of GW501516 (Enzo, ALX-420-032-M001). Both were co-cultured with 10,000 MC38-luc cells at effector-to-target ratios of 10:1, 5:1, 2:1, 1:1 and 0:1 in 96-well plates in T cell media. After 72 h, 50 µl luciferin working solution (450 µg ml⁻¹, GoldBio, LUCK-300) was added per well and luminescence (LUM) was read after 5 min. The percentage of lysis was calculated as $100 \times (1 - (LUM_{\text{sample}} - LUM_{\text{min}}) / (LUM_{\text{max}} - LUM_{\text{min}}))$. For antigen-specific killing, CD8⁺ T cells from OT-I mice were prepared as above, added at ratios of 1:2 and 1:1 to 3,000 MC38-Ova cells stained with Incucyte Cytolight Rapid Red dye (Sartorius, 4706) per well, and growth was followed for 24 h on an Incucyte Live Cell Analysis instrument and quantified based on Cytolight Rapid Red dye signal.

RNA isolation and RT-qPCR

Cell pellets were resuspended in 300 µl TRIzol (Thermo Fisher, 15596026) and RNA isolated with Direct-zol RNA Microprep (Zymo, R2062). cDNA generated from 500 ng RNA using the SuperScript IV VIL0 Master Mix (Thermo Fisher, 11756050) was assayed by real-time qPCR with the TaqMan Gene Expression Master Mix (Thermo Fisher, 4369016) and TaqMan probes (*Cd3e* (Mm00599683_m1), *Cd8a* (Mm01182107_g1), *Ifng* (Mm01168134_m1), *Tbp* (Mm00446973_m1), *Cpt1a* (Mm01231182_m1) and *Ppard* (Mm01305434_m1)).

PDO generation from individuals with CRC

De-identified human colon tumor samples were obtained from participants with informed consent at Huntington Hospital under Northwell Health Biospecimen Repository protocol 1810. Tissue was rinsed in cold Ca²⁺/Mg²⁺-free PBS, cut into small pieces and incubated for 20 min in PBS with 100 µg ml⁻¹ normocin (Invivogen, ant-nr-1), 50 µg ml⁻¹ gentamicin (Amresco, E737) and 1× penicillin–streptomycin (Thermo Fisher, 15070063). Pieces were washed in cold PBS and then dissociated for 45 min to 60 min in 1 mg ml⁻¹ Collagenase (Sigma-Aldrich, C9407) and 10 µM Y-27632 (Tocris, 1254). Mechanical trituration via pipetting was performed at 30 min. After digestion, cells were pelleted, resuspended for 5–10 min in TrypLE Express (Thermo Fisher, 12604039) and quenched with serum-free medium. Following centrifugation, pellets were resuspended in 70% Matrigel (Corning, 356231) in media. Droplets were placed onto prewarmed six-well plates and polymerized at 37 °C for 10 min before adding Advanced DMEM (Life Technologies, 12634028), 1× Glutamax (Life Technologies, 35050061), 10 mM HEPES (Thermo Fisher Scientific, 15630080), 50% WRN conditioned medium (Homemade), 1× B27 (Life Technologies, 12587010), 1× N2 (Life Technologies, 17502048), 10 mM Nicotinamide (Sigma-Aldrich, N0636), 1 mM N-acetyl cysteine (Sigma-Aldrich, A9165), 100 µg ml⁻¹ primocin (Invivogen, ant-pm-1), 10 µM SB202190 (Sigma-Aldrich, S7067), 10 µM Y-27632 (Tocris, 1254), 10 nM gastrin I (Sigma-Aldrich, G9020), 50 ng ml⁻¹ EGF (PeproTech, AF-100-15) and 500 nM A83-01 (Sigma-Aldrich, SML0788). Culture medium was changed every 4–5 days and PDOs were passaged at a 1:2 ratio every 7–10 days, by dissolving Matrigel in Cell Recovery Solution (Corning, 354253) for 1 h at 4 °C, dissociating pelleted organoids in TrypLE Express 5–15 min at 37 °C, quenching and reseeding in 70% Matrigel, with medium added after 5–10 min.

Autologous PDO-immune co-culture system

As previously described⁶⁰, autologous cryofrozen PBMCs were thawed, resuspended in human T cell media (ImmunoCult-XF T cell expansion medium (Stem Cell Technology, 10981) supplemented with hIL-2 (R&D Systems, 202-IL-010) and hIL-7 (R&D Systems, 207-IL-010)), expanded with ImmunoCult Human CD3/CD28 T Cell Activator (Stem cell technology, 10971) and treated with vehicle or 10 µM GW501516 (Enzo, ALX-420-032-M001) for 72 h. PDOs were grown for 2–3 days after passaging before co-culture. PDOs and PBMCs were stained with Cell Tracker Red

(Thermo Fisher, C34552) and Cell Tracker Green (Thermo Fisher, C7025), respectively. Cells were washed and 200,000 PBMCs were mixed with 50–100 PDOs in 45% organoid media/45% human T Cell media and 10% Matrigel with 500 nM Nucview 405 Blue Caspase-3 dye (Sigma-Aldrich, SCT104) and plated on a 96-well plate (Agilent, 204626-100). Images were acquired on the PerkinElmer UltraVIEW VoX and analyzed in Volocity 3D Image Analysis Software (RRID: SCR_002668) to calculate colocalization coefficient values⁶¹. After 16 h of co-culture, the complete-to-incomplete apoptosis ratio based on Caspase-3 dye was multiplied with coefficient values and normalized to no-immune cell controls.

Human CAR-T cell experiments

Mock-transduced and EGFR-targeting CAR-T cells (from M. Maus^{62,63}) were thawed, grown in T cell medium, and pretreated 72 h with vehicle or 10 μ M GW501516 (Enzo, ALX-420-032-M001). After washing, 100,000 CAR-T cells were co-cultured with 50–100 PDOs for 16 h, and then imaged and analyzed as above.

Version 1 HER-2-targeting CAR-T cells were generated and assayed by ProMab Biotechnologies. (RRID: SCR_012380). PPAR δ -GOF HER-2 CAR-T cells (Promab, PM-CAR1064, MNDU3-HER-2 scFv-CD8 TM-41BB-CD3z-PGK-GFP) had the GFP reporter replaced by VP64-human PPAR delta-FLAG sequence. Real-time cytotoxicity assays were performed against SK-OV-3 (RRID: CVCL_0532)⁶⁴. For bulk RNA-seq, CAR-T cells were thawed, cultured for 5 days, RNA was extracted as above, and libraries were prepared using NebNext Ultra II kit (New England BioLabs, E7760) before being sequenced on an Illumina NextSeq.

For version 2 HER-2 CAR-T cells, the anti-HER-2 scFv clone 4D5 was synthesized by GenScript and cloned into a second-generation CAR construct containing a 41BB costimulatory domain on a self-inactivating lentivirus expression vector (pTRPE). In the VP64-human PPAR δ CAR construct the VP64-PPAR δ fusion lay upstream of the CAR, separated via a P2A element. A total of 12×10^6 HEK293T cells were incubated 24 h at 37 °C in T150 culture flasks in complete RPMI-1640 (10% FBS and 1% penicillin–streptomycin) and transfected with Lipofectamine 3000 (129 μ l, Invitrogen), pTRP gag/pol (18 μ g), pTRP RSV-Rev (18 μ g), pTRP Coccal-G (3 μ g) and expression plasmid (14 μ g) in OptiMEM for 6 h at 37 °C. OptiMEM was replaced with complete RPMI-1640. The medium was collected after 48 h, concentrated by ultracentrifugation (25,000g for 2.5 h or 8,000g overnight at 4 °C), resuspended in cold medium and stored at –80 °C.

Version 2 HER-2-targeting CAR-T cells were made from CD4⁺ and CD8⁺ T cells isolated from healthy donor PBMCs (University of Pennsylvania Human Immunology Core) using CD4 (15062, Stem Cell Technologies) and CD8 (15063, Stem Cell Technologies) selection kits, combined at a 1:1 ratio, and activated using Dynabeads CD3/CD28 CTS (Gibco, Life Technologies) at a 3:1 bead-to-cell ratio in CTS Optimizer T cell expansion media (Gibco) with 5 ng ml^{–1} human IL-7 and IL-15 (PeproTech), 5% human Serum (Valley Biomedical), 1% penicillin–streptomycin (50 IU ml^{–1}), 1% HEPES and 1% Glutamax (Gibco, Life Technologies) at 37 °C in 5% CO₂. T cells were transduced at a multiplicity of infection of 3 at 24 h, debudded at 48 h and maintained for 8–10 days at 1×10^6 cells per milliliter before cryopreservation.

Version 2 CAR-T cells were thawed and cultured overnight (16 h) at 3×10^6 cells per ml in RPMI with 10% FBS, 1% Glutamax and 1% penicillin–streptomycin. Around 3,000 SK-BR-3 (RRID: CVCL_0033) cells stained with Incucyte Cytolight Rapid Red dye (Sartorius 4706) were plated per well in 96-well plates, and CAR-T cells were added the next day at 2:1 and 1:1 ratios, with the CAR-positive percentage normalized using untransduced T cells. Green Incucyte Caspase-3/Caspase-7 Dye was added (Sartorius, 4440), and growth was followed for 24 h on an Incucyte Live Cell Analysis instrument based on Caspase-3/Caspase-7 Green signal and Cytolight Rapid Red dye signal.

Sixteen days after subcutaneous SK-OV-3 tumors were implanted into NSG mice, CAR-T cells were thawed and cultured overnight (16 h) at 3×10^6 cells per ml in RPMI with 10% FBS, 1% Glutamax and 1%

penicillin–streptomycin. Animals were randomized into three groups with similar average tumor volumes, and injected with 5×10^5 untransduced T cells, WT CAR-T cells or PPAR δ -GOF CAR-T cells in 100 μ l PBS via tail vein injection on day 17, with the CAR-positive percentage normalized using untransduced T cells. Tumors were measured every 2–3 days, until humane endpoints were reached.

Tumor dissociation and tumor-infiltrating immune cell isolation

Tumors were minced and digested for 30–45 min at 37 °C with shaking in RPMI (Corning, 10-040-CV) together with 10% FBS, 2.5 mg ml^{–1} collagenase D (Sigma-Aldrich, 11088866001), 0.5 mg ml^{–1} liberase DL (Sigma-Aldrich, 5466202001) and 0.2 mg ml^{–1} DNase I (Sigma-Aldrich, D5025). The resulting cell suspension was filtered through 100- μ m cell strainers and used for downstream applications.

Tumor-draining lymph node dissociation

Inguinal lymph nodes were mechanically dissociated in fluorescence-activated cell sorting (FACS) buffer (PBS, 5% FBS, 2 mM EDTA), filtered through 40- μ m cell strainers and further processed for flow cytometry.

Flow cytometry staining for surface and intracellular markers

Cells were stained with fluorescently labeled surface antibodies (Supplementary Table 2) for 20 min on ice in FACS buffer. For intracellular staining, CD8⁺ T cells or digested tumors were stimulated with 50 ng ml^{–1} phorbol 12-myristate 13-acetate (Sigma-Aldrich, P1585-1MG), 1 μ g ml^{–1} ionomycin (Sigma-Aldrich, I9657-1MG) and brefeldin A (Thermo Fisher, 00-4506-51) for 5 h or 4 h, before staining with a fixable viability dye (Thermo Fisher, L34963 or L10119) and any surface antibodies. CD8⁺ T cells were permeabilized with Intracellular Fixation & Permeabilization Buffer Set (Thermo Fisher, 88-8824-00) and TICs with the eBioscience Foxp3/Transcription Factor Staining Buffer Set (Thermo Fisher, 00-5523-00) before intracellular staining. Mitochondria were stained with 100 nM MitoTracker Green FM (Thermo Fisher, M7514) or tetramethylrhodamine, ethyl ester (TMRE; Thermo Fisher, T669) in PBS 30 min at 37 °C. Samples were acquired on a BD Fortessa or Sony ID7000 and analyzed in FlowJo 10. Absolute cell numbers were calculated based on the acquired events and the ratio of acquired to total added CountBright Absolute Counting Beads (Invitrogen, C36950).

scRNA-seq

Tumor cell suspensions from day-12 subcutaneous MC38 tumors and day-22 orthotopic AKPS, naive splenocytes and 16-h CAR-T cell cultures were stained as above and live CD45⁺ cells sorted on a Sony SH800S cell sorter, with cancer cells sorted by GFP. In some experiments, sorted cells from three tumors per group were pooled before library preparation.

Libraries were prepared using a Chromium Controller (10x Genomics, 120223), with a Chromium Next GEM Single Cell 3' Gene Expression kit (10x Genomics, 1000268), a Chromium Next GEM Single Cell 5' Kit v2 (10x Genomics, 1000263) and a TCR Amplification Kit (10x Genomics, 1000254) at the CSHL Single Cell Biology Shared Resource. cDNA and libraries were checked for quality on an Agilent 2100 Bioanalyzer Instrument (RRID: SCR_018043), quantified by KAPA qPCR, and sequenced on NextSeq 500 or NextSeq 2000 (Illumina) instruments to approximately 20,000 reads per cell. The Cell Ranger pipeline (v6.0.0 10x Genomics) aligned FASTQs to the mouse (10x Genomics, gex-mm10-2020-A) or human (GRCh38) reference genome and produce digital gene-cell counts matrices with default parameters.

scRNA-seq data analysis

Quality control was performed as described^{65,66}. Cells with >10% mitochondrial transcripts, <250 feature counts or <500 expressed genes were excluded from further analysis. Seurat (v5.0.1)⁶⁷ was used for

normalization (SCTransform), selection of 5,000 most variable genes (SelectIntegrationFeatures), graph-based clustering and differential expression analysis, with all conditions integrated into a single dataset per experiment via PrepSCTIntegration, FindIntegrationAnchors and IntegrateData functions⁶⁸. MAGIC (RRID: SCR_006406) imputation was conducted to impute missing values and account for technical noise⁶⁹. UMAP clustering was performed on the top 25 principal components identified by RunPCA, using runUMAP, followed by FindNeighbors and FindClusters to perform clustering based on the Louvain algorithm at a resolution of 1 (ref. 69). Clusters were labeled based on CD45 tumor-infiltrating lymphocyte subtype signatures⁷⁰. Differential expression analysis between groups used FindMarkers with the MAST method to evaluate differences within the transcriptome⁷¹. TCR and BCR sequencing data were analyzed with scRepertoire (v2.0.0)⁷², retaining cells with productive alpha/beta or heavy/light chain pairings. Clonality was categorized as single (0–1 cell), small (2–5 cells), medium (6–20 cells) or large (21–100 cells), and data were integrated into Seurat using combineExpression(). Statistical significance of gene expression was tested with Wilcoxon's rank-sum test (wilcox.test) in stats (v4.1.0). Differential interactome modeling was performed using NicheNet (v2.0.5)⁷³ and CellChat (v2.1.0)⁷⁴. Gene-set enrichment analysis was conducted using EnrichR (v3.2)⁷⁵. scRNA-seq from 19 individuals with melanoma was tested for an association between PPAR signatures and lymphocyte gene expression patterns³⁷. Gene signatures were based on previous literature and differential expression in our scRNA-seq data ($\log_2\text{FC} > 0.25$ or < -0.25 and adjusted P value < 0.05 ; Supplementary Table 3), using Pearson correlation coefficient test to evaluate association.

scATAC-seq

Viable CD45⁺ cells were sorted on a Sony SH800S cell sorter, pelleted and lysed in nuclei lysis buffer (10 mM Tris-HCl pH 7.5, 146 mM NaCl, 1 mM CaCl₂, 21 mM MgCl₂, 0.03% Tween-20, 0.01% BSA, 1 mM dithiothreitol, 5% EZ Lysis Buffer (Sigma-Aldrich NUC101-1KT)). Nuclei were permeabilized with 0.01% digitonin and 0.1% Tween-20 in wash buffer (10 mM Tris-HCl pH 7.5, 146 mM NaCl, 1 mM CaCl₂, 21 mM MgCl₂, 2% BSA, 0.02% Tween-20), washed, and resuspended in Nuclei Buffer (10x Genomics, PN-2000207) and processed with Chromium ATAC v2 kit (10x Genomics, PN-1000390). Libraries were prepared according to User Guide CG000496, sequenced on a NextSeq 500 (Illumina) to approximately 20,000 reads per cell, and aligned to the mouse (mm10-2020-A) or human (GRCh38) genome using Cell Ranger ATAC (v2.1.0).

scATAC-seq data analysis

scATAC-seq data were processed in Signac⁷⁶. Cells with < 50 counts, nucleosome signal < 4 , or blacklist ratio > 0.05 were removed from analysis. Batch correction was performed with Harmony, and dataset normalization was implemented by applying term frequency-inverse document frequency normalization. UMAP analysis and clustering, as well as annotation, were performed as described above. Differential peak accessibility was examined using logistic regression with the FindMarkers function. Peaks were annotated using HOMER⁷⁷. Enriched motifs from differentially accessible peaks were identified with FindMotifs() against JASPAR2020 (ref. 78).

Bulk RNA-seq of CD8⁺ T cells in tumor-draining lymph nodes

Tumor-draining inguinal lymph nodes were processed as above, 50,000 CD8⁺ T cells were sorted by FACS, and RNA extraction and library preparation followed as above.

Bulk RNA-seq data analysis

Reads were trimmed with Trim Galore (v0.6.7), aligned to GRCm38.p6/Genecode annotation (release M24) or GRCh37/Genecode annotation using STAR (v2.7.2b)⁷⁹, quantified with Salmon (v1.5.2)⁸⁰, assembled with StringTie (v2.2.1)⁸¹ and quality assessed with RSeqQC (v3.0.1)⁸² and MultiQC (v1.9)⁸³. DEseq2 (v1.28)⁸⁴ fitted fixed batch and treatment

effects, calling transcripts differentially expressed above $\log_2(1)$ fold change and adjusted P value below 0.05. Heat maps used ggplot2 (v2.6.2)⁸⁵ and volcano plots used EnhancedVolcano (v1.8.0). Raw outputs were trimmed with Trim Galore (v0.6.7) and aligned to GRCm38.p6/Genecode annotation (release M24) or GRCh37/Genecode annotation using STAR (v2.7.2b)⁷⁹. Aligned counts were quantified using Salmon (v1.5.2)⁸⁰. StringTie was used for transcript assembly (v2.2.1)⁸¹. Read and alignment quality were analyzed with RSeqQC (v3.0.1)⁸² and summarized with MultiQC (v1.9)⁸³. Differential gene expression between control and experimental samples was assessed with DEseq2 (v1.28)⁸⁴, fitting a model with fixed effects for sequencing batch effects and treatment. Contrast for treatment was extracted and transcripts were considered differentially expressed with an absolute fold change greater than $\log_2(1)$ and adjusted P value of less than 0.05. For visualization purposes, heat maps were generated with ggplot2 (v2.6.2)⁸⁵, and volcano plots were generated with EnhancedVolcano (v1.8.0). Analysis code is available on GitHub (<https://github.com/Vyoming/A-metabolic-switch-that-boosts-immune-cell-fitness-against-cancer.git/>) and data are deposited in the Gene Expression Omnibus (GEO) under accession number GSE248008.

ELISA

After 72 h of activation, CD8⁺ T cell culture supernatants were collected, stored at -20°C and quantified using mouse TNF ELISA (R&D systems, MTA00B).

In vivo antibody-mediated cell depletions

Mice were intraperitoneally injected with 200 μg anti-CD8a (Bio X Cell, BE0061, RRID: AB_1125541), anti-CXCR3 (Bio X Cell, BE0249, RRID: AB_2687730), rat IgG2b isotype control (Bio X Cell, BE0090, RRID: AB_1107780) or Armenian hamster IgG isotype control (Bio X Cell, BE0091, RRID: AB_1107773) six times, on days $-1, 1, 5, 9, 13$ and 16 relative to subcutaneous tumor injection (day 0). Depletion was confirmed by flow cytometry on tail bleeds.

In vivo adoptive transfer experiments

For adoptive transfer of splenocytes, C57BL/6J mice underwent total body irradiation with a sublethal dose of 5-Gy^{86–89}. After 48 h, they received 5×10^6 splenocytes previously isolated^{86–89} from WT or PPAR δ -iGOF mice and cultured in RPMI (Corning, 10-040-CV) with 10% FBS, 1% penicillin-streptomycin, and 50 μM β -mercaptoethanol (MilliporeSigma, M3148-25ML). After 72 h, 2×10^6 AKPS cells were injected into the colon submucosa as above, and tumor growth was followed via colonoscopy.

C57BL/6J mice were sublethally irradiated using 5-Gy total body irradiation. After 48 h, mice received 3×10^6 naive splenic CD8⁺ T cells isolated from WT or PPAR δ -iGOF mice intravenously, and 72 h later they received 5×10^5 MC38 cells subcutaneously, after which tumor growth was followed.

Around 1×10^6 AKPS cells were injected into the colon submucosa of congenic B6 CD45.1 mice as above. Tumors were confirmed via colonoscopy on day 7 and mice were given 5-Gy total body irradiation on day 9. Around 3×10^6 CD8⁺ T cells from WT or PPAR δ -iGOF mice, stimulated for 72 h, were intravenously injected 48 h after mice were irradiated. After 9–11 days, when T cells had engrafted, mice received anti-PD-1 (200 μg , Bio X Cell, BE0146, RRID: AB_10949053) or anti-PD-1 and anti-CTLA4 (200 μg first dose, then 100 μg , Bio X Cell, BE0131, RRID: AB_10950184) intraperitoneally every 2–3 days for 14 days (7 doses) and tumor growth was followed via colonoscopy. Treatment groups were randomized for similar tumor size before treatment initiation, and mice with failed injections were excluded.

Metabolite measurements and analysis

Hydrophilic metabolite profiling was performed at the Metabolomics Core Facility, Robert H. Lurie Comprehensive Cancer Center, Northwestern University. Seventy-two-hour-activated CD8⁺ T cells were

resuspended at 5,000 cells per microliter in MS-grade 80% acetonitrile/20% H₂O solution, vortexed for 30 s, frozen at −80 °C, thawed and vortexed, frozen twice more, before samples were thawed on ice and centrifuged at 20,000g for 30 min at 4 °C. Supernatants were stored at −80 °C and analyzed using high-performance liquid chromatography and high-resolution mass spectrometry and tandem mass spectrometry⁹⁰. Metabolomics data were analyzed using the MetaboAnalyst 5.0 platform (<https://www.metaboanalyst.ca/>)⁹¹.

Extracellular flux analyses

Extracellular flux analysis of primary CD8⁺ T cells was performed using Seahorse XF Cell Mito Stress Test Kit (Agilent, 103015-100)⁹². Poly-D-Lysine-coated plates (Gibco, A3890401) were seeded with 125,000 activated CD8⁺ T cells per well and OCR recorded on Seahorse XFe96 analyzer (Agilent) after addition of 1.5 μM oligomycin, 1.5 μM FCCP and 0.5 μM rotenone–antimycin. For Seahorse XF Palmitate Oxidation Stress Test (Agilent, 103693-100), activated CD8⁺ T cells were starved overnight in RPMI containing 0.5 mM glucose (Agilent, 103577-100), 1 mM glutamine (Agilent, 103579-100), 1% FBS and 0.5 mM L-carnitine (Agilent), then assayed in RPMI containing 2 mM glucose and 0.5 mM L-carnitine, with palmitate-BSA or BSA control added. Next, 1.5 μM oligomycin, 6 μM FCCP and 0.5 μM rotenone–antimycin were injected into wells, and OCR was recorded over time. Results were normalized to nuclear DNA content per well based on Cyquant Cell Proliferation Assay kit (Thermo Fisher, C7026). Palmitate-BSA was made by reconstituting palmitic acid (Cayman, 57-10-3) in ethanol, adding 0.1 M NaOH to reach 12 mM, stirring for 30 min at 70 °C, adding 10% fatty acid-free BSA (Proliant Biologicals, 68700) to 3 mM, stirring for 1 h at 37 °C, filtering through a 0.22-μm filter and storing in glass containers (Thermo Fisher, B7999-2A) at −20 °C.

Radioactive palmitate oxidation experiments

FAO to CO₂ and acid-soluble products (ASPs) were measured as described⁹³. [1-¹⁴C]palmitate was conjugated to BSA by 1 g of fatty acid-free BSA (Proliant Biologicals, 68700) in 5.5 ml 0.9% NaCl at 40 °C, adding a clear solution of 6.97 mg cold palmitate in a dropwise manner (Sigma-Aldrich, P9767), which was previously dissolved in 1 ml of 0.1 N NaOH at 90 °C, followed by 2.5 ml of [1-¹⁴C]palmitic acid (PerkinElmer, NEC075H250UC). The final solution was filtered through a 0.45-μm filter and stored at −20 °C. CD8⁺ T cells activated over 72 h were washed with Krebs-Ringer bicarbonate buffer with 1 M HEPES (Thermo Fisher Scientific, 15630080; KRBH) and 0.1% BSA, incubated for 30 min at 37 °C in KRBH with 1% BSA, washed again, and 2 × 10⁶ cells were added per 25-cm² flask. One flask per condition was lysed in 500 μl RIPA Buffer (Thermo Fisher Scientific, 89900) for BCA protein quantification (Thermo Fisher Scientific, 23225). The remaining flasks were incubated for 3 h at 37 °C in 2 ml KRBH supplemented with 0.8 mM L-carnitine (Sigma-Aldrich, C0158), 2.5 mM or 25 mM glucose (Agilent, 103577-100), and 0.25 mM [1-¹⁴C]palmitate. These flasks were sealed with a rubber stopper holding a 3-cm piece of PVC tubing containing a 1-cm² piece of Whatman paper soaked in 0.1 N KOH. Reactions were stopped with 200 μl of perchloric acid (Sigma-Aldrich, 244252; 40% vol/vol) injected into each flask and left overnight at room temperature. Whatman papers holding bound ¹⁴CO₂ and 800 μl of the supernatant resulting from centrifugation of perchloric acid-treated medium at 14,000g for 10 min, were added into separate vials containing 5 ml of scintillation liquid (PerkinElmer, 6013321), left overnight at room temperature and measured by liquid scintillation counting. Oxidation results were expressed as nmol palmitate mg^{−1} protein h^{−1}, calculated using the following formula: ((CPM of sample – blank flask CPM) × 500) / (total CPM × mg protein × h), where CPM represents counts per minute the liquid scintillation counter detects, 500 is the total nanomoles of palmitate per flask, and total CPM indicates the counts resulting from measuring 200 μl of 2.5 mM [1-¹⁴C] palmitate stock solution in scintillation fluid. ‘mg protein’ represents the protein

content determined by BCA assay, and ‘h’ is the incubation time in hours. ASP results are expressed as: nmol of palmitate mg^{−1} protein h^{−1} = ((CPM of sample – blank flask CPM) × 500 × (2,200/800)) / (total CPM × mg protein × h). Where 500 is the total nanomoles of palmitate per flask, total CPM indicates the counts resulting from directly measuring 200 μl of 2.5 mM [1-¹⁴C] palmitate stock solution in scintillation fluid, 2,200/800 is the dilution factor, ‘mg protein’ represents the protein content determined by BCA assay, and ‘h’ is the incubation time. Total palmitate oxidation is the sum of both oxidation and ASP results.

MALDI mass spectrometry imaging

To perform spatial metabolomics⁹⁴, AKPS tumors from WT or PPARδ-iGOF mice were dissected at day 21, weighed, snap-frozen in liquid nitrogen, stored in −80 °C, and cryosectioned at 10 μm onto indium tin oxide-coated slides, with serial H&E-stained sections scanned at ×20 on a Zeiss Axio Observer 7. For small-molecule metabolite imaging, a 1,5-diaminonaphthalene hydrochloride matrix solution (4.3 mg ml^{−1} in a 4.5:5:0.5 mixture of HPLC-grade water, ethanol and 1 M hydrochloric acid, vol/vol/vol) was applied to an indium tin oxide-coated slide using a robotic sprayer (HTX Imaging) with the following parameters: four passes, 0.09 ml min^{−1} flow rate, 1,200 mm min^{−1} spray velocity, 75 °C spray nozzle temperature, 55 °C heated tray temperature, 10 psi nitrogen gas pressure, and 2-mm track spacing. For free fatty acid imaging, a 9-aminoacridine (9-AA) matrix was applied to the slide with a SublimMATE device from HTX imaging (10 mg in 1.5 ml methanol, 300 °C, 5 min), limiting phospholipid fragmentation and enabling free fatty acid detection⁹⁵.

Mass spectrometry imaging used a 15 T solarix XR Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer (Bruker Daltonics) in negative ion mode with 100 μm x and y spacing. Data was collected from *m/z* 46 to *m/z* 3,000 using a 0.2097 time-domain transient length, resulting in a resolving power of ~89,000 at *m/z* 351 for small-molecule metabolites and ~84,000 at *m/z* 345 for free fatty acids. Continuous accumulation of selected ions (CASI) was used with a 200-Da mass isolation window centered around *m/z* 180. Calibration was performed with an Agilent tune mix (Agilent Technologies) using the electrospray ionization source. Ion images were visualized and analyzed using SCI LS Lab Software Version 2026b (Bruker Daltonics).

Statistical analysis

Analyses were performed using GraphPad Prism 10 (version 10.6.1), with two-tailed unpaired *t*-tests, Dunnett’s or Tukey’s tests for multiple comparisons, log-rank tests for survival and z-tests for proportions. Data were assumed to be normally distributed, but this was not formally tested. Error bars indicate standard error of the mean or standard deviation, with statistical details explained in each figure legend. For bar plots in split violin plots, the center, minimum and maximum indicate the median, 25th percentile and 75th percentile, respectively. No statistical methods were used to predetermine sample sizes in our experiments, but our sample sizes are similar to those reported in previous publications^{13,15,21,25}. Statistical analyses for gene expression data were conducted in R.

Reporting summary

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

Data availability

Genomic data have been deposited in the GEO under the accession code [GSE248008](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE248008). Source data are provided with this paper. All other data are present in the article and its Supplementary Information.

Code availability

Analysis code is available in GitHub (<https://github.com/Vyoming/A-metabolic-switch-that-boosts-immune-cell-fitness-against-cancer.git/>).

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Author contributions

Conceptualization: S.B., I.E., T.M., P.B. and V.S. Methodology: S.B., P.B., T.M., I.E., L.H., J.R.B. and N.W. Investigation: I.E., P.B., T.M., J.R.B., N.W., L.B.S.S.K., C.C., P.M., S.S., J.H., S.E.W., V.M. and R.R. Formal analysis: V.S. and J.E.W. Writing—original draft: S.B., I.E., T.M., P.B. and V.S. Writing—review and editing: S.B., P.B. and T.M. Funding acquisition: S.B. Resources: D.E.R., L.H., P.M.K.W., S.I.G. and N.Y.R.A. Supervision: S.B.

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Competing interests

S.B. received research funding from Caper Labs, Elstar Therapeutics and Revitope Oncology for research unrelated to this study. S.B. is cofounder and advisor for Caper Labs. S.B., I.E., C.C., V.S., T.M. and P.B. are listed as inventors on a patent application related to activating metabolic switches in immune cells to boost antitumor immunity (63/460,971). N.Y.R.A. is a key opinion leader to Bruker Daltonics, has a collaboration with Thermo Finnigan, has service agreements with EMD Serono and iTeos Therapeutics and is founder of BondZ, SafeLinkZ and ChemLinkz. N.W. and S.I.G. are listed as inventors of patents related to CAR therapy. S.I.G. is a scientific cofounder and holds equity in Carisma Therapeutics and Interius Biotherapeutics. S.I.G. is a scientific advisor to Carisma, Cartography, Currus, Interius, Kite, NKILT and Mission Bio. The other authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to Semir Beyaz.

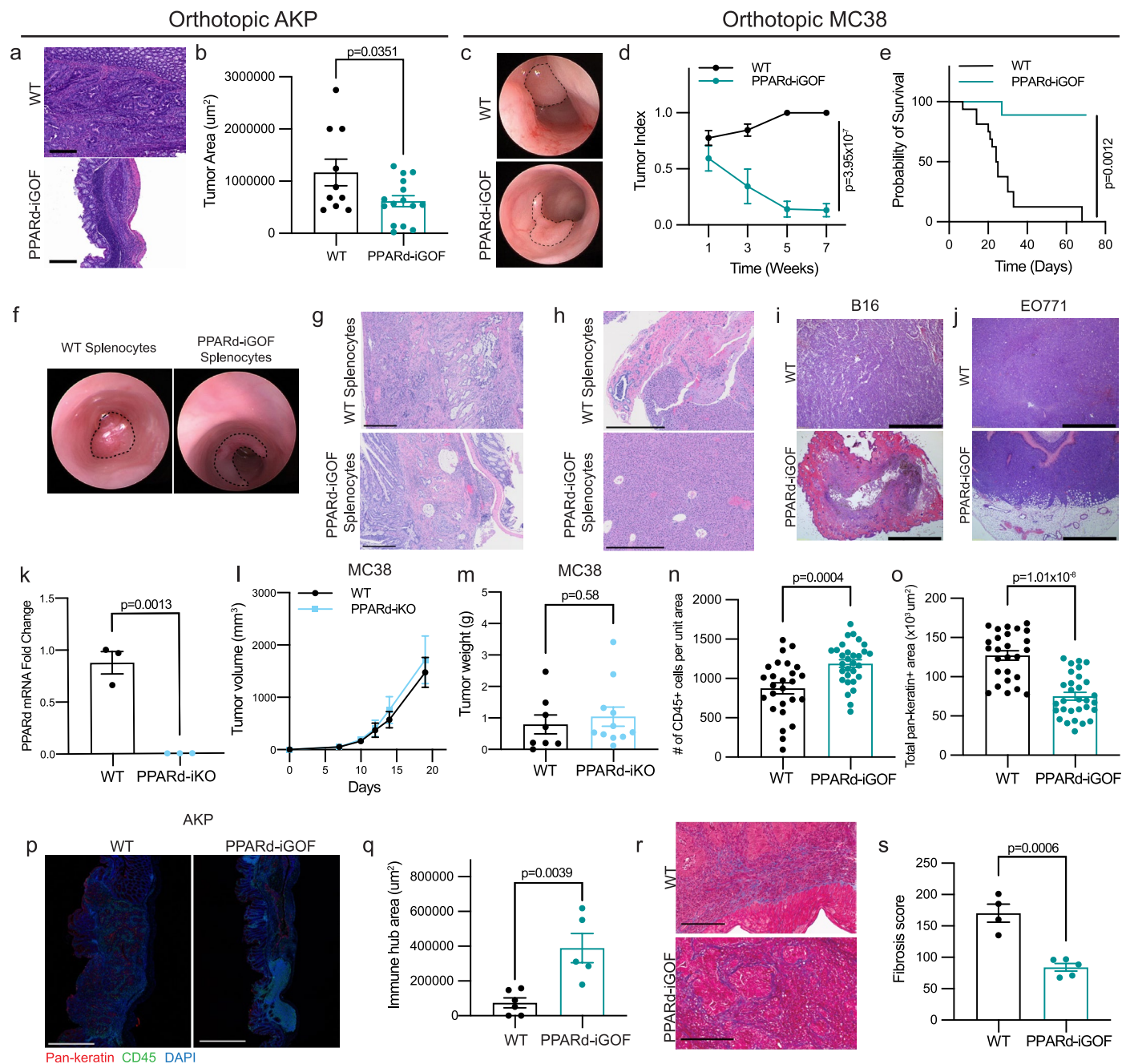
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Extended Data Fig. 1 | PPAR δ activation remodels gene expression and chromatin accessibility in cancer and immune cells. **a.** Anti-PPAR δ and anti-VP16 immunoblots of splenocytes from WT, PPAR $\delta^{-/-}$ and PPAR δ -iGOF mice from one experiment, *ns* indicates non-specific bands, *p* indicates PPAR δ band, and *V* indicates VP16 band **b.** H&E images of endpoint MC38 tumors from WT and PPAR δ -iGOF mice (representative of 4 independent experiments), scale bars=750 μ m **c.** Uniform Manifold Approximation and Projection (UMAP) visualization of scRNA-seq data analysis of WT or PPAR δ -cGOF MC38 tumors from WT or PPAR δ -iGOF mice. **d.** Expression of cell type marker genes across MC38 TICs. **e.** Proportions of different TICs in MC38 control tumors in WT mice (WT), PPAR δ -cGOF tumors in WT mice (cGOF), MC38 control tumors in PPAR δ -iGOF mice (iGOF), or PPAR δ -cGOF tumors in PPAR δ -iGOF mice (cGOF; iGOF) based on scRNA-seq data analysis. **f.** Uniform Manifold Approximation and Projection (UMAP) visualization of scATAC-seq data analysis of WT or PPAR δ -cGOF MC38 tumors isolated from WT or PPAR δ -iGOF mice. **g.** Accessibility of

cell type marker genes across tumor-derived cell clusters in MC38 tumors. **h.** Proportions of different immune cell clusters in MC38 control tumors in WT mice (WT), PPAR δ -cGOF tumors in WT mice (cGOF), MC38 control tumors in PPAR δ -iGOF mice (iGOF), or PPAR δ -cGOF tumors in PPAR δ -iGOF mice (cGOF; iGOF) based on scATAC-seq data analysis. **i.** scATAC-seq tracks depicting normalized chromatin accessibility for *Pdcd1*, *Lag3*, *Havcr2*, and *Tnfr* in CD8 + T cell clusters from MC38 control tumors in WT mice (WT), PPAR δ -cGOF MC38 tumors in WT mice (cGOF), MC38 control tumors in PPAR δ -iGOF mice (iGOF), or PPAR δ -cGOF tumors in PPAR δ -iGOF mice (cGOF + iGOF). (*n* = 79,810 cells from 2 mice per group). **j-m.** Pearson correlation and hierarchical clustering of significantly differentially expressed genes or accessible loci in CD8 + T cells from PPAR δ -cGOF tumors in WT mice (cGOF), MC38 control tumors in PPAR δ -iGOF mice (iGOF), or PPAR δ -cGOF tumors in PPAR δ -iGOF mice (cGOF+iGOF) compared to MC38 control tumors in WT mice (WT) based on (**j**, **k**) scRNA-seq or (**l**, **m**) data analysis.



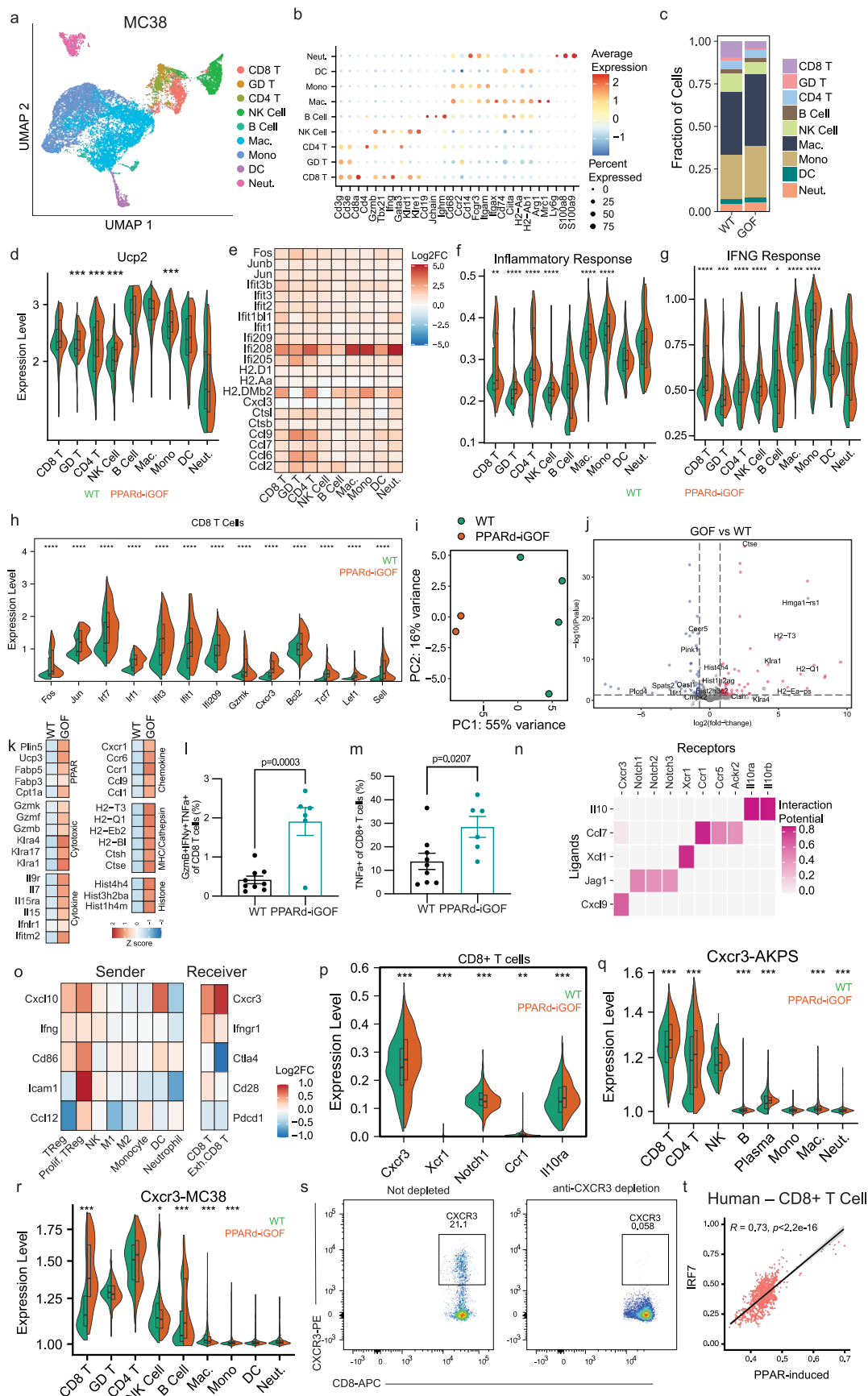
Extended Data Fig. 2 | Immune-intrinsic PPAR δ activation inhibits tumor growth. **a.** H&E images of orthotopic AKP tumors from WT and PPAR δ -iGOF mice representative of 2 independent experiments, scale bar=250 μ m. **b.** Quantification of total tumor area from AKP H&E images from WT and PPAR δ -iGOF mice. (WT: $n = 10$ areas from 5 mice, PPAR δ -iGOF: $n = 15$ areas from 7 mice), $\pm$ SEM. **c.** Colonoscopy images of 3-week orthotopic MC38 tumors in WT and PPAR δ -iGOF mice representative of 2 independent experiments. **d.** Orthotopic MC38 tumor growth in WT and PPAR δ -iGOF mice, (WT: $n = 8$ mice, PPAR δ -iGOF: $n = 5$ mice, representative of 2 independent experiments), $\pm$ SEM. **e.** Survival of WT and PPAR δ -iGOF mice with orthotopic MC38 tumors, (WT: $n = 16$ mice, PPAR δ -iGOF: $n = 9$ mice, from 2 independent experiments). **f.** Colonoscopy images of 3-week AKPs tumors in WT mice that were intravenously injected with 5×10^6 WT, or PPAR δ -iGOF splenocytes representative of 2 independent experiments. **g-h.** H&E images of AKPs tumors and (h) livers from mice that received WT splenocytes, or PPAR δ -iGOF splenocytes prior to tumor inoculation representative of 2 independent experiments, (g) scale bar=350 μ m, (h) scale bar=350 μ m. **i-j.** H&E

images of (i) subcutaneous B16-F10 and (j) orthotopic EO771 tumors from WT and PPAR δ -iGOF mice representative of 2 independent experiments each, scale bars=800 μ m. **k.** PPAR δ mRNA quantification of CD8+ T cells, ($n = 3$ biological replicates per group), $\pm$ SD. **l-m.** Subcutaneous MC38 tumor growth and (m) tumor weights of WT and PPAR δ -iKO mice (WT: $n = 7$ mice, PPAR δ -iKO: $n = 11$ mice), $\pm$ SEM. **n-o.** Quantification of (n) CD45+ cells per unit area and (o) pan-keratin+ area from immunofluorescence images of orthotopic AKP tumors from WT and PPAR δ -iGOF mice, (WT: $n = 26$ areas, PPAR δ -iGOF: $n = 30$ areas, from 3 mice per group), $\pm$ SEM. **p-q.** Representative immunofluorescence images and (q) quantification of immune hubs in AKP tumors from WT and PPAR δ -iGOF mice, scale bar=750 μ m, (WT: $n = 6$ sections from 3 mice, PPAR δ -iKO: $n = 5$ sections from 3 mice), $\pm$ SEM. **r.** Representative Masson's trichrome images of AKP tumors grown in WT and PPAR δ -iGOF mice representative of 1 independent experiment, scale bars = 200 μ m. **s.** Fibrosis scores of AKP tumors grown in WT and PPAR δ -iGOF mice, determined by Masson's trichrome stain. (WT: $n = 4$ mice, PPAR δ -iGOF: $n = 5$ mice), $\pm$ SEM. Two-tailed unpaired t-test (b, d, k-o, q, s), Log-rank test (e).



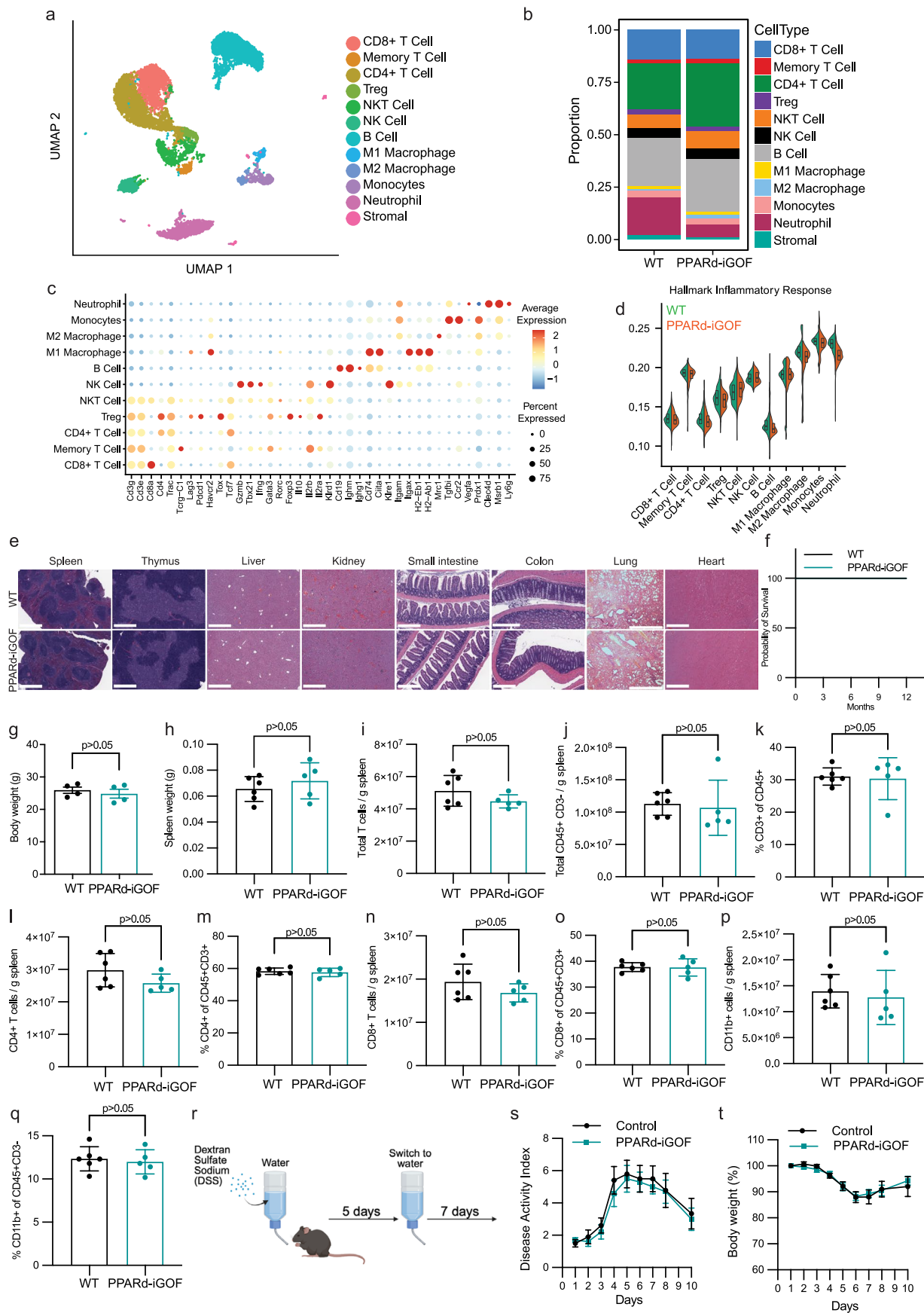
Extended Data Fig. 3 | Immune-intrinsic PPAR δ activation reprograms the tumor microenvironment of MSS CRC. **a.** Uniform Manifold Approximation and Projection (UMAP) visualization of scRNA-seq data analysis of day-22 orthotopic AKPS tumors isolated from WT and PPAR δ -iGOF mice. **b.** Proportions of AKPS TICs in WT and PPAR δ -iGOF (GOF). **c.** Population density difference across PPAR δ -iGOF AKPS TICs. **d.** Expression of immune cell type marker genes across AKPS TICs. **e.** Differential expression of *Angptl4* and *Ucp2* across WT (green) and PPAR δ -iGOF (orange) AKPS TICs, (WT: $n = 15,423$ cells, PPAR δ -iGOF: $n = 16,624$ cells), * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$. **f.** Differential expression across AKPS TICs in PPAR δ -iGOF mice. **g.** Differential expression of *Irfng* and *Irf7* across AKPS TICs in WT (green) and PPAR δ -iGOF (orange) mice, (WT: $n = 15,423$ cells, PPAR δ -iGOF: $n = 16,624$ cells), * $p \leq 0.05$, ** $p \leq 0.01$,

*** $p \leq 0.001$. **h.** Species diversity of T cells between WT and PPAR δ -iGOF (GOF) conditions, $\pm 95\%$ confidence interval. **i.** Relative abundance of clonal B cells in WT and PPAR δ -iGOF (GOF) AKPS TICs. **j.** Species diversity of B cells between WT and PPAR δ -iGOF (GOF) conditions, $\pm 95\%$ confidence interval. **k.** Split violin plots demonstrating the expression levels of key genes involved in B cell effector function in WT and PPAR δ -iGOF tumor infiltrating plasmablasts in AKPS tumors, (Diverse: $n = 920$ cells, Clonal: $n = 199$ cells), ** $p \leq 0.01$, *** $p \leq 0.001$. **l.** Differential interaction strength and number of interactions between PPAR δ -iGOF and WT as calculated using the CellChat package. **m.** Pearson Correlation Coefficients (R) between “PPAR-induced”, and “immune response” z-scores in human melanoma TICs. Wilcoxon rank-sum test (**e, g**).



Extended Data Fig. 4 | Immune-intrinsic PPAR δ activation reprograms the tumor microenvironment of MSI CRC. **a.** Uniform Manifold Approximation and Projection (UMAP) visualization of scRNAseq data analysis of MC38 TICs from WT and PPAR δ -iGOF mice **b.** Expression of immune cell type defining genes across MC38 TICs **c.** Proportions of different TICs in MC38 from WT and PPAR δ -iGOF (GOF) mice **d.** *Ucp2* expression across MC38 TICs, (WT: $n = 12,243$ cells, PPAR δ -iGOF: $n = 5076$ cells), *** $p \leq 0.001$ **e.** Top differentially expressed genes across PPAR δ -iGOF MC38 TICs **f-g.** (f) Hallmark inflammatory response and (g) IFN γ response z-scores across MC38 TICs, (WT: $n = 12,243$ cells, PPAR δ -iGOF: $n = 5076$ cells), * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$ **h.** Differential expression of key genes involved in T cell effector and memory function in WT and PPAR δ -iGOF MC38-infiltrating CD8 + T cells, (WT: $n = 1177$ cells, PPAR δ -iGOF: $n = 231$ cells) **** $p \leq 0.0001$ **i.** Principal component analysis (PCA) for bulk RNA sequencing of tumor-draining lymph node-derived CD8 + T cells **j-k.** Differential expression between tumor-draining lymph node-derived CD8 + T cells from WT and PPAR δ -iGOF (GOF) mice **l-m.** Flow cytometric quantification

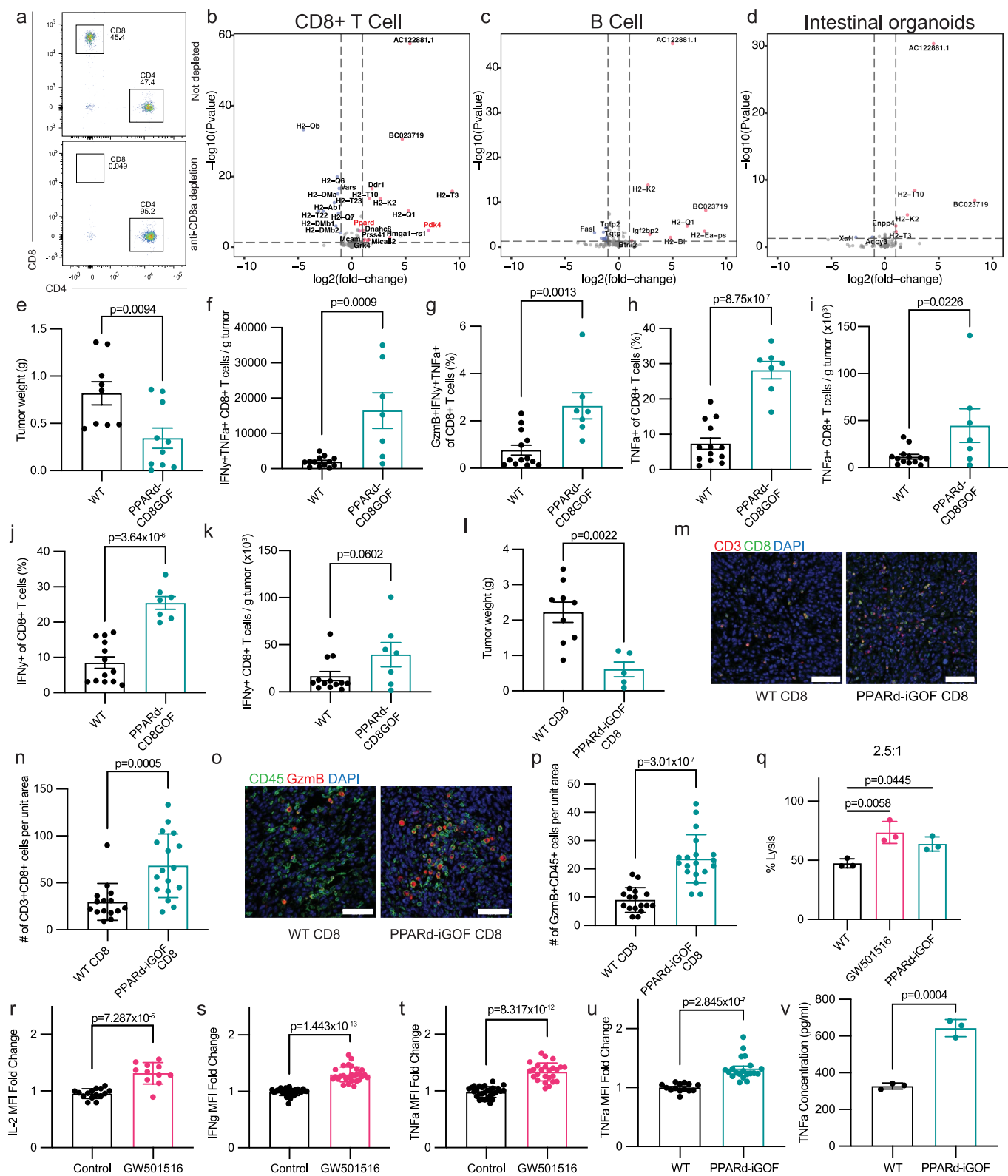
of intratumoral (l) Gzmb+ IFN γ + TNF + CD8 + T cells, (m) TNF + CD8 + T cells from WT and PPAR δ -iGOF mice, (WT: $n = 9$, PPAR δ -iGOF: $n = 6$ mice), $\pm$ SEM **n.** NicheNet-predicted interaction potential between PPAR δ -iGOF AKPS TICs. **o.** Differential expression of top prioritized ligands and receptors between PPAR δ -iGOF and WT across MC38 TICs. **p.** Differential expression of top NicheNet-predicted receptors between WT and PPAR δ -iGOF tumor infiltrating CD8 + T cells in AKPS tumors, (WT: $n = 654$ cells, PPAR δ -iGOF: $n = 1142$ cells), ** $p \leq 0.01$, *** $p \leq 0.001$ **q-r.** *Cxcr3* z-scores across tumor-infiltrating immune cell clusters in (d) AKPS and (e) MC38 tumors, ((q) WT: $n = 15,423$ cells, PPAR δ -iGOF: $n = 16,624$ cells; (r) WT: $n = 12,243$ cells, PPAR δ -iGOF: $n = 5076$ cells), * $p \leq 0.05$, *** $p \leq 0.001$ **s.** CXCR3 + CD8 + T cell depletion efficiency during *in vivo* CXCR3+ depletion experiments, cells were gated on single cells/alive/CD3 + NK1.1-/CD8 + **t.** Pearson Correlation Coefficient (R) between "PPAR-induced" and *IRF7* z-scores in human melanoma tumor-infiltrating CD8 + T cells, $\pm 95\%$ confidence interval. Wilcoxon rank-sum test (d, f, g, p), Two-tailed unpaired t-test (l, m).



Extended Data Fig. 5 | See next page for caption.

Extended Data Fig. 5 | Immune-intrinsic PPAR δ activation does not lead to overt inflammation. **a.** UMAP of scRNA-seq data of splenocytes from WT and PPAR δ -iGOF mice at steady state **b.** Proportions of different immune cell clusters in WT and PPAR δ -iGOF splenocytes at steady state, (WT: $n = 5627$ cells, PPAR δ -iGOF: $n = 6038$ cells) **c.** Expression of immune cell type defining genes across splenocyte clusters at steady state **d.** Hallmark inflammatory response z-scores across WT and PPAR δ -iGOF splenocyte clusters at steady state **e.** H&E staining of various tissues isolated from WT and PPAR δ -iGOF at steady state representative of 1 independent experiment, scale bars = 800 μ m except small intestine (400 μ m), colon (400 μ m), and lung (1000 μ m) **f.** Survival of WT and PPAR δ -iGOF littermates

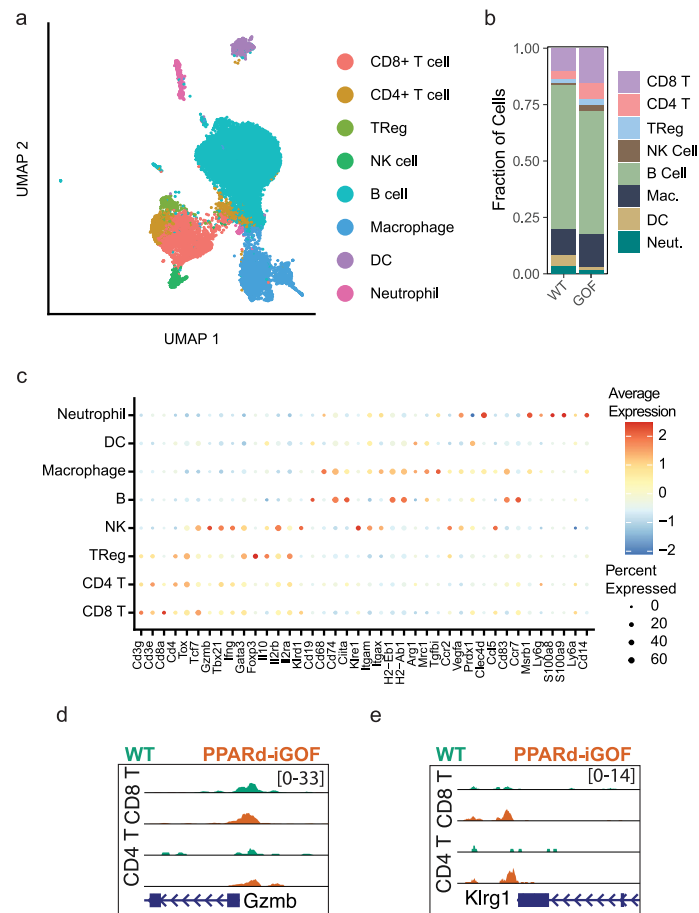
at steady state, ($n = 5$ mice per group) **g.** Body weights of litter-mate WT and PPAR δ -iGOF mice, ($n = 4$ mice per group), $\pm$ SEM **h.** Spleen weights of WT and PPAR δ -iGOF mice at steady state, (WT: $n = 6$ mice, PPAR δ -iGOF: $n = 5$ mice), $\pm$ SEM **i-q.** Quantification of WT and PPAR δ -iGOF splenocytes at steady state, (WT: $n = 6$ mice, PPAR δ -iGOF: $n = 5$ mice, representative of 2 independent experiments), $\pm$ SEM **r-t.** (r) Experimental design, (s) disease activity index and (t) body weight of WT and PPAR δ -iGOF mice during DSS-induced colitis, ($n = 10$ mice per group, representative of 2 independent experiments), $\pm$ SEM. Wilcoxon rank-sum test (**d**), Log-rank test (**f**), Two-tailed unpaired t-test (**g**, **h**, **i-q**, **s**, **t**). Images in **r** created in BioRender; Sirkali Suresh Kumar, L. (<https://biorender.com/f79y6ih>) (2026).



Extended Data Fig. 6 | See next page for caption.

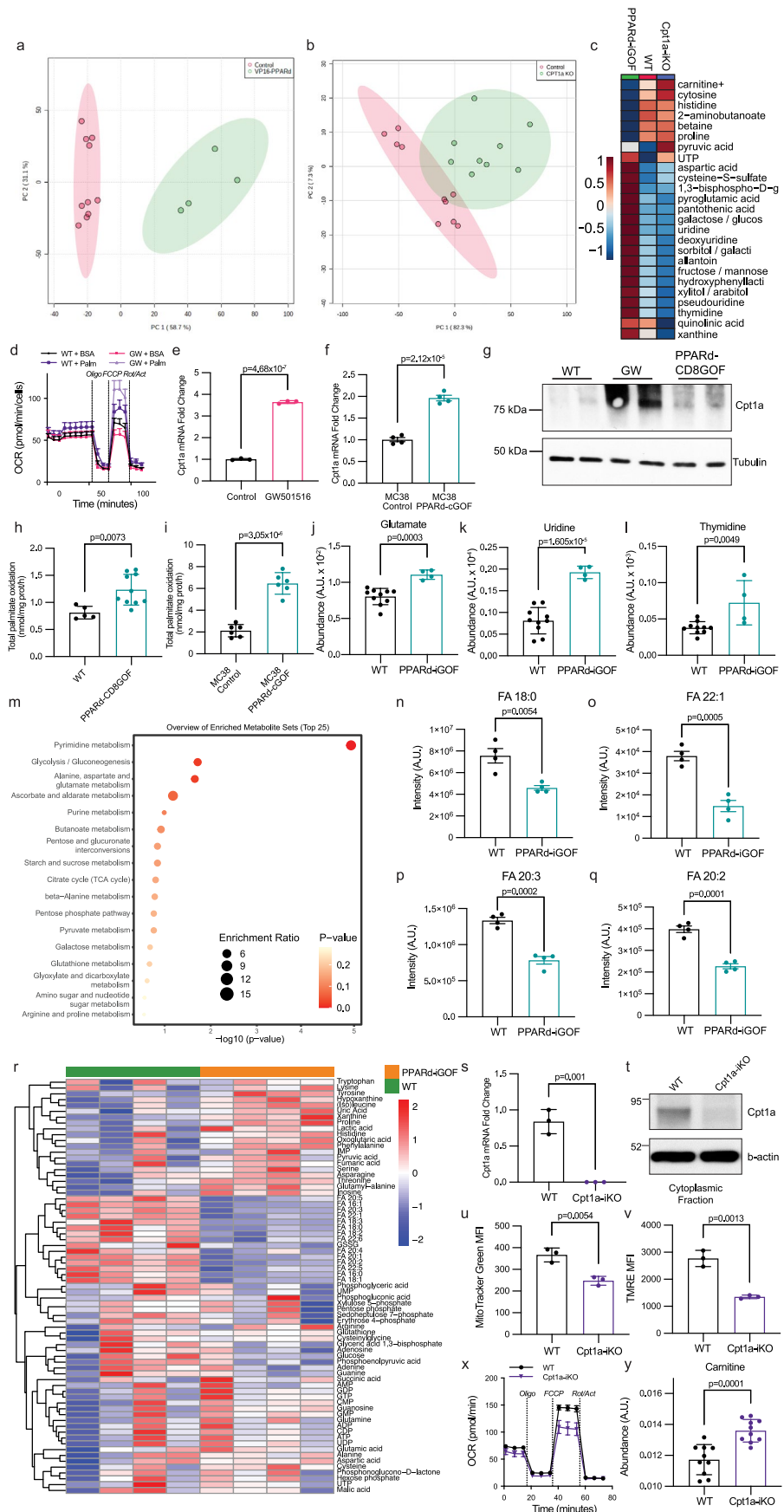
Extended Data Fig. 6 | PPAR δ activation enhances CD8⁺ T cell anti-tumor immunity. **a.** CD8⁺ T cell depletion efficiency during *in vivo* CD8a⁺ depletion experiments, cells were gated on single cells/alive/CD3⁺ **b-d.** Differential expression in 4-OHT treated **(b)** CD8⁺ T cells, **(c)** B cells, and **(d)** intestinal organoids from WT and PPAR δ -CD8GOF mice, with *Ppard* and its target *Pdk4* highlighted. **e.** MC38 tumor weights from tamoxifen-treated WT and PPAR δ -CD8GOF mice. (WT: $n = 9$ mice, PPAR δ -CD8GOF: $n = 10$ mice), $\pm$ SEM **f-k.** **(f)** Number of IFN γ + TNF + CD8⁺ T cells, **(g)** percentage of Gzmb⁺ IFN γ + TNF + CD8⁺ T cells, **(h)** percentage of TNF + CD8⁺ T cells, **(i)** number of TNF + CD8⁺ T cells, **(j)** percentage of IFN γ + CD8⁺ T cells, and **(k)** number of IFN γ + CD8⁺ T cells from MC38 tumors in WT and PPAR δ -CD8GOF mice, (WT: $n = 12$ mice, PPAR δ -CD8GOF: $n = 7$ mice), $\pm$ SEM **l.** MC38 tumor weights upon *in vivo* CD8⁺ T cell adoptive transfer. (WT CD8⁺ T cells: $n = 9$ mice, PPAR δ -iGOF CD8⁺ T cells: $n = 5$ mice), $\pm$ SEM **m-n.** **(m)** Representative immunofluorescence images and **(n)** quantification of CD3⁺ CD8⁺ cells in MC38 from mice that received WT or PPAR δ -iGOF CD8⁺ T cells prior to tumor inoculation. scale bar = 100 μ m, ($n = 4$ mice per group, 4 areas per tumor), $\pm$ SD **o-p.** **(o)** Representative immunofluorescence images

and **(p)** quantification of Gzmb⁺ CD45⁺ cells in MC38 from mice that received WT or PPAR δ -iGOF CD8⁺ T cells prior to tumor inoculation. scale bar = 100 μ m, ($n = 4$ mice per group, 4 areas per tumor), $\pm$ SD **q.** CD8⁺ T cell killing of MC38-luc cells after 72-hours co-culture at effector-to-target ratio 2.5:1, ($n = 3$ technical replicates per group, representative of 2 independent experiments), $\pm$ SD **r-t.** Quantification of **(r)** IL-2, **(s)** IFN γ and **(t)** TNF production by primary mouse CD8⁺ T cells that were treated with vehicle or GW501516 during *in vitro* T cell activation, (Vehicle: $n = 10$ biological replicates, GW501516: $n = 8$ biological replicates, 3 technical replicates each, from 3 independent experiments), $\pm$ SD **u.** Intracellular flow cytometry analysis of TNF production by primary mouse CD8⁺ T cells from WT and PPAR δ -iGOF mice, (WT: $n = 5$ biological replicates, PPAR δ -iGOF: $n = 7$ biological replicates, 3 technical replicates each, from 3 independent experiments), $\pm$ SEM **v.** TNF ELISA on CD8⁺ T cells from WT and PPAR δ -iGOF mice. ($n = 3$ mice per group, 3 technical replicates), $\pm$ SD. Two-tailed unpaired t-test (**f-l**, **n**, **p**, **h**), Two-tailed Dunnett's test (**q**), Two-tailed Welch's t-test (**r-v**).



Extended Data Fig. 7 | PPAR δ activation alters chromatin accessibility at T cell function-related loci. **a.** UMAP visualization of scATAC-seq data analysis of tumor-infiltrating immune cells isolated from AKPS tumors in WT and PPAR δ -iGOF mice **b.** Bar plots demonstrating the proportions of different immune cell clusters in AKPS tumors from WT and PPAR δ -iGOF mice **c.** Dot plot depicting the accessibility of immune cell type defining genes across

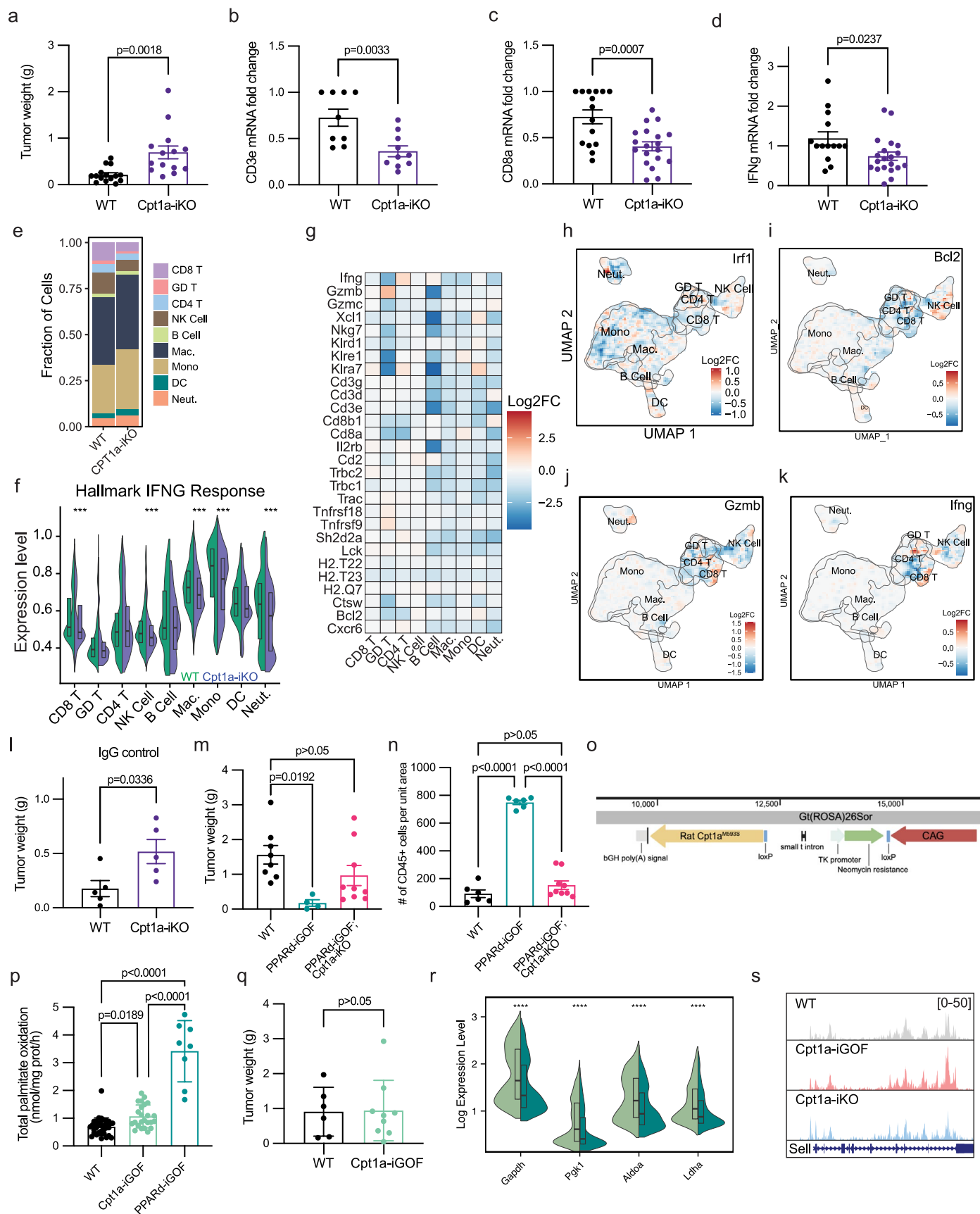
tumor-infiltrating immune cells from AKPS tumors **d-e.** scATAC-seq tracks depicting normalized chromatin accessibility for **(d)** *Gzmb* and **(e)** *Klrp1* in CD4+ and CD8+ T cell clusters from tumor-infiltrating immune cells from AKPS tumors in PPAR δ -iGOF or WT mice. Gene structure and direction of transcription are provided below the plots ($n = 19,223$ cells from 2 mice per group).



Extended Data Fig. 8 | See next page for caption.

Extended Data Fig. 8 | PPAR δ -Cpt1a axis drives metabolic reprogramming of CD8 + T cells. **a-b.** Principal component analysis (PCA) for hydrophilic metabolite abundance in **(a)** PPAR δ -iGOF (VP16-PPARd) or **(b)** Cpt1a-iKO (Cpt1a KO) CD8 + T cells compared to WT (control). **c.** Normalized metabolite abundance. (*WT: n = 10 mice, PPAR δ -iGOF: n = 4 mice, Cpt1a-iKO: n = 10 mice*) **d.** Oxygen consumption rate (OCR) during Agilent Seahorse XF Palmitate Oxidation Stress test of CD8 + T cells from WT mice and treated with vehicle or GW501516 (GW), (*BSA: n = 7 technical replicates per group, Palm: n = 6 technical replicates per group*), $\pm$ SEM **e-f.** Cpt1a mRNA quantification of **(e)** GW501516 treated CD8 + T cells and **(f)** MC38 control and MC38 PPAR δ -cGOF cells, (*CD8 + T cells: n = 3 biological replicates per group, MC38: n = 4 biological replicates per group*), $\pm$ SD **g.** Anti-Cpt1a immunoblot of 4-OHT treated CD8 + T cells from WT or PPAR δ -CD8GOF mice, with some WT cells also treated with PPAR δ agonist GW501516 (representative of 2 independent experiment). **h-i.** Total ^{14} C-palmitate oxidation of **(h)** activated CD8 + T cells from tamoxifen-treated WT or PPAR δ -CD8GOF mice and **(i)** MC38 control and MC38 PPAR δ -cGOF cells, (*WT CD8 + T cells: n = 5 replicates, PPAR δ -CD8GOF CD8 + T cells: n = 10 replicates, WT MC38: n = 6 replicates, PPAR δ -cGOF MC38: n = 6 replicates*), $\pm$ SD **j-l.** Abundance of **(j)**

glutamate, **(k)** uridine, and **(l)** thymidine in CD8 + T cells from WT or PPAR δ -iGOF mice (*WT: n = 10 mice, PPAR δ -iGOF: n = 4 mice*), $\pm$ SD **m.** Pathway enrichment analysis for top upregulated metabolites in PPAR δ -iGOF CD8 + T cells compared to WT. **n-q.** Metabolite abundance based on MALDI mass spectrometry imaging for fatty acids **(n)** 18:0 (stearic acid), **(o)** 22:1 (erucic acid), **(p)** 20:3 (dihomo- γ -linolenic acid), and **(q)** 20:2 (eicosadienoic acid), (*n = 4 mice per group*), $\pm$ SEM **r.** Metabolite abundance between AKPS tumors in PPAR δ -iGOF and WT mice as determined by MALDI mass spectrometry imaging, (*n = 4 mice per group*) **s.** qRT-PCR Cpt1a mRNA quantification of CD8 + T cells from WT and Cpt1a-iKO mice, (*n = 3 biological replicates per group*), $\pm$ SD **t.** Anti-Cpt1a immunoblot of splenocytes from WT and Cpt1a-iKO mice (representative of 1 independent experiment) **u-v.** **(u)** MitoTracker Green and **(v)** TMRE signal of CD8 + T cells from WT and Cpt1a-iKO mice, (*n = 3 mice per group, representative of 2 independent experiments*), $\pm$ SD **x.** Oxygen consumption rate (OCR) during Agilent Seahorse Mito Stress test of activated CD8 + T cells from WT and Cpt1a-iKO mice. (*n = 12 technical replicates per group*), $\pm$ SEM **y.** Abundance of carnitine in CD8 + T cells isolated from WT or Cpt1a-iKO mice, (*n = 10 mice per group*), $\pm$ SD. Two-tailed unpaired t-test (**e, f, h-l, n-q, s, u, v, y**).

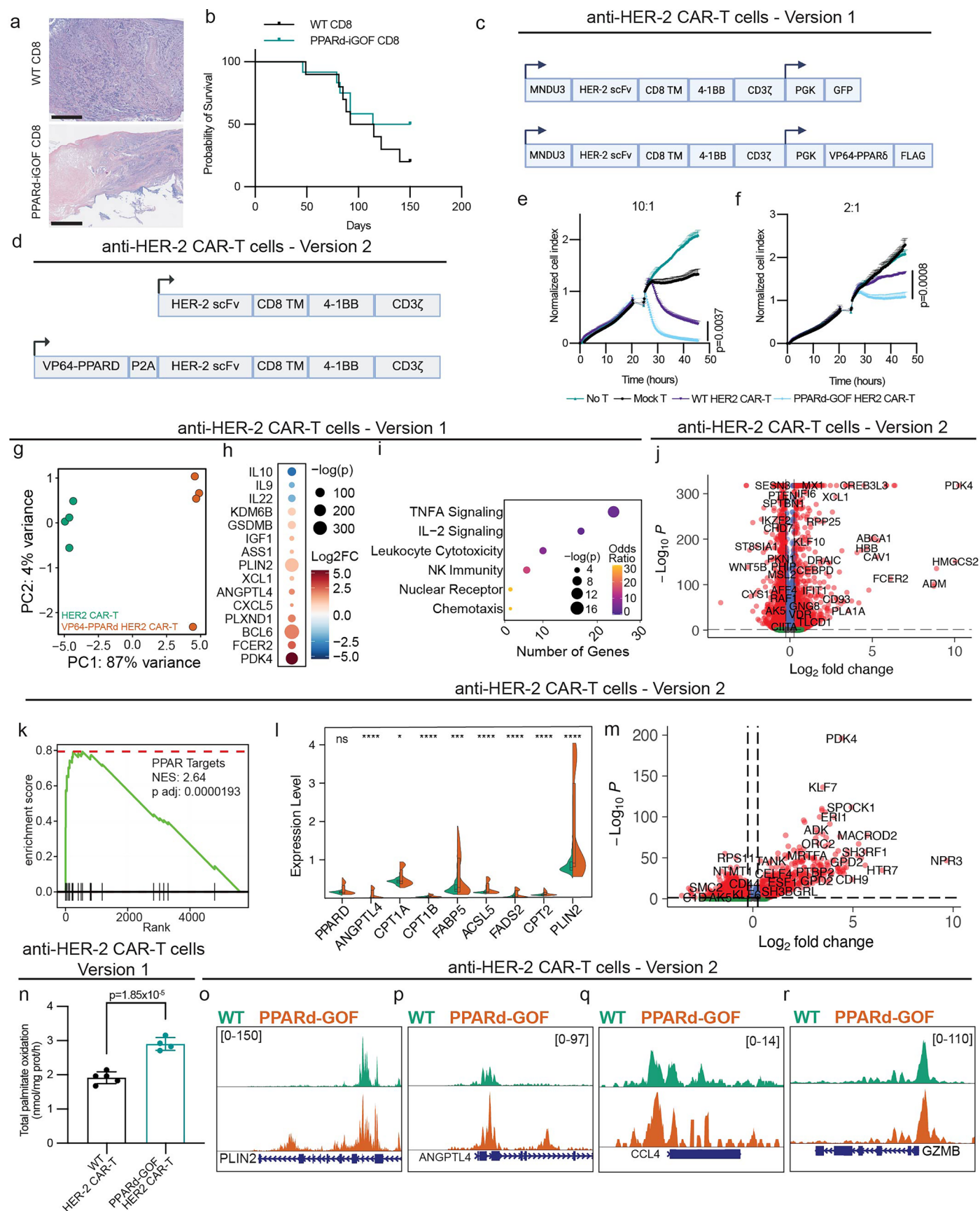


Extended Data Fig. 9 | See next page for caption.

Extended Data Fig. 9 | Immune-intrinsic Cpt1a loss- and gain-of-function

modulates anti-tumor immunity. **a.** MC38 tumor weights from WT and Cpt1a-iKO mice, (WT: $n = 15$ mice, Cpt1a-iKO: $n = 14$ mice, from 3 independent experiments), $\pm$ SEM **b-d.** **(b)** *Cd3e*, **(c)** *Cd8a*, and **(d)** *Ifng* mRNA quantification using qRT-PCR. (WT: $n = 9$ mice, Cpt1a-iKO: $n = 10$ mice, from 3 independent experiments), $\pm$ SEM **e.** Proportions of TICs in WT and Cpt1a-iKO mice. **f.** Hallmark IFNG response z-scores across WT and Cpt1a-iKO TICs, (WT: $n = 12,243$ cells, Cpt1a-iKO: $n = 10,684$ cells), $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$ **g.** Differential expression across all Cpt1a-iKO TICs. **h-k.** UMAP of **(h)** *Irf1*, **(i)** *Bcl2*, **(j)** *Gzmb*, and **(k)** *Ifng* gene expression $\log_2$ fold changes across Cpt1a-iKO TICs. **l.** MC38 tumor weights from WT and Cpt1a-iKO mice upon treatment with IgG control. ($n = 5$ mice per group), $\pm$ SEM **m.** MC38 tumor weights from WT, PPAR δ -iGOF, and PPAR δ -iGOF; Cpt1a-iKO mice, (WT: $n = 8$ mice, PPAR δ -iGOF: $n = 4$ mice, PPAR δ -iGOF; Cpt1a-iKO: $n = 9$ mice, from 2 independent experiments), $\pm$ SEM **n.** Quantification of CD45+ cells in MC38 tumors from WT, PPAR δ -iGOF, and PPAR δ -iGOF; Cpt1a-iKO

mice, (WT: $n = 6$ areas, PPAR δ -iGOF: $n = 6$ areas, PPAR δ -iGOF; Cpt1a-iKO: $n = 9$ areas, from 3 mice per group), $\pm$ SEM **o.** Schematic showing integration of CAG-LSL-Rat Cpt1a^{M593S} construct in Rosa26 locus. **p.** Total ¹⁴C-palmitate oxidation of activated CD8+ T cells from WT, Cpt1a-iGOF, or PPAR δ -iGOF mice, (WT: $n = 34$ replicates, Cpt1a-iGOF: $n = 24$ replicates, PPAR δ -iGOF: $n = 8$ replicates, combined from 4 independent experiments), $\pm$ SD **q.** MC38 tumor weights from WT and Cpt1a-iGOF mice, (WT: $n = 6$ mice, Cpt1a-iGOF: $n = 9$ mice, representative of 3 independent experiments), $\pm$ SEM **r.** Differential expression of key glycolysis-related genes MC38-infiltrating CD8+ T cells from WT and Cpt1a-iGOF mice, (WT: $n = 3217$ cells, Cpt1a-iGOF: $n = 2340$ cells), $****p \leq 0.0001$ **s.** Normalized chromatin accessibility for *Sell* in CD8+ T cells from MC38 tumors in WT, Cpt1a-iGOF, or Cpt1a-iKO mice. ($n = 30,247$ cells from 2 mice per group). Two-tailed unpaired t-test (**a-d**, **l**, **q**), Wilcoxon rank-sum test (**f**, **r**), Ordinary one-way ANOVA with two-tailed Tukey's multiple comparison test (**m**, **p**). Schematic in **o** created in BioRender. Sirkali Suresh Kumar, L. (<https://biorender.com/dv6slqe>) (2026).



Extended Data Fig. 10 | See next page for caption.

Extended Data Fig. 10 | PPAR δ activation in T cells enhances the efficacy of immunotherapy. a–b. (a) AKPS H&E images (*scale bar=500um*) and (b) survival of CD45.1 host mice receiving adoptive transfer of CD8⁺ T cells along with anti-PD1 immunotherapy, (*WT CD8: n = 10 mice, PPAR δ -iGOF CD8: n = 12 mice*) **c–d.** Construct design for (c) version 1 and (d) version 2 of WT and PPAR δ -GOF human HER-2 CAR-T cells. **e–f.** HER-2 CAR-T cell killing of SK-OV-3 cell at effector-to-target ratios (e) 10:1 and (f) 2:1, (*n = 3 technical replicates per group*), $\pm SD$ **g.** PCA plot of RNA-seq results of version 1 WT and PPAR δ -GOF HER-2 CAR-T cells. **h.** Differential expression between version 1 PPAR δ -GOF and WT HER-2 CAR-T cells. **i.** Top enriched gene ontologies between version 1 PPAR δ -GOF and WT HER-2 CAR-T cells as calculated using EnrichR. **j.** Differential expression between version 2 PPAR δ -GOF vs WT HER-2 CAR-T cells. **k.** Enrichment of PPAR δ targets in differentially expressed genes from scRNA-seq of PPAR δ -GOF vs WT HER-2 CAR T cells, (*n = 50,167 cells*) **l.** Differential expression of PPAR δ targets and

fatty acid oxidation-related genes in version 2 WT and PPAR δ -GOF HER-2 CAR T cells, (*WT: n = 24,227 cells, PPAR δ -GOF: n = 25,940 cells*), *****p* ≤ 0.05 , *****p* ≤ 0.0001 **m.** Differential accessible genes between version 2 PPAR δ -GOF and WT HER-2 CAR-T cells as determined by scATAC-seq **n.** Total ¹⁴C-palmitate oxidation of version 1 WT and PPAR δ -GOF HER-2 CAR-T cells, (*WT: n = 5 replicates, PPAR δ -GOF: n = 4 replicates*), $\pm SD$ **o–r.** scATAC-seq tracks depicting normalized chromatin accessibility for (o) *PLIN2*, (p) *ANGPTL4*, (q) *CCL4*, and (r) *GZMB* in version 2 WT and PPAR δ -GOF HER-2 CAR T cells, (*n = 17332 cells*). Ordinary one-way ANOVA with two-tailed Tukey's multiple comparison test (e, f), DESeq2 (h), Two-tailed unpaired t-test (i, n), Fischer's exact test (k), Wilcoxon rank-sum test (l). Schematics created in BioRender: c, Sirkali Suresh Kumar, L. <https://biorender.com/Oul7pkp> (2026); d, Sirkali Suresh Kumar, L. <https://biorender.com/ld9ktix> (2026).

Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- ☐ ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- ☐ ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☐ ☒ The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- ☒ ☐ A description of all covariates tested
- ☐ ☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- ☐ ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- ☐ ☒ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- ☒ ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- ☒ ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- ☐ ☒ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	Flow cytometry data was acquired using BD FACSDiva or ID7000 system software. Confocal images were acquired on a Zeiss LSM 710 using ZEN software.
Data analysis	Analysis code for genomic data is available on GitHub (https://github.com/Vyoming/A-metabolic-switch-that-boosts-immune-cell-fitness-against-cancer.git) and utilized the following softwares: Seurat (v5.1.0), Cell Ranger (v6.0.0), stats (v4.1.0), scRepertoire (v2.0.0), NicheNet (v2.0.5), CellChat (v2.1.0), EnrichR (v3.2), trim galore (v0.6.7), STAR (v2.7.2b), Salmon (v1.5.2), StringTie (v2.2.1), RSeqQC (v3.0.1), MultiQC (v1.9), DESeq2 (v1.28), Cell Ranger ATAC (v2.1.0), Signac, EnhancedVolcano (v1.8.0), and ggplot2 (v2.6.2). Experimental data was analyzed using Prism (v10.4.0), ImageJ2 (v2.3.0), QuPath (v0.4.4), and FlowJo (v10.10.0).

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 and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

All data referenced in the manuscript can be found in the main text or supplemental materials. Genomic data are publicly available at GEO (accession number GSE248008).

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	Organoid lines used as models for MSI (C9) and MSS (C2) where derived from a female (C9) and male (C2) patient respectively. No sex-specific analysis were conducted.
Reporting on race, ethnicity, or other socially relevant groupings	No race, ethnicity or other socially relevant groupings-based comparisons were done in this study.
Population characteristics	Human colon tissue samples were obtained from patients with informed consent undergoing surgical resection procedures at Huntington Hospital. Tissues were obtained anonymously from the surgical pathology services of the hospital based upon availability without bias in terms of sex and age.
Recruitment	Human colorectal cancer samples were obtained from patients with informed consent undergoing surgical resection procedures at Huntington Hospital. Patient-derived organoids were generated from such samples.
Ethics oversight	Northwell Health Biospecimen Repository (Protocol number: 1810).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

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](https://www.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	No statistical methods were used to pre-determine sample sizes in our experiments. Sample sizes were chosen in line with previous publications and preliminary data. For in vivo experiments, each group contained generally at least 5 mice. The sample size for each experiment and the number of independent experiments are indicated in the figure legends.
Data exclusions	No data was excluded from analysis, unless technical reasons rendered samples unusable. For in vivo experiments, failed injections were excluded from experiments prior to data analysis, as detailed in the method section.
Replication	Experiments were independently replicated as indicated in the figure legends and methods section.
Randomization	Mice were randomized with groups containing similar sized tumors for immunotherapy experiments. Beyond this, allocation to groups was dependent on genotype and therefore not determined by investigators.
Blinding	Tumor measurements and DSS colitis scoring was performed in a blinded manner. Immunofluorescence and histological analyses were performed in a blinded manner. Other quantitative data analysis such as flow cytometry was not consistently performed in a blinded fashion since such purely quantitative readouts were determined to be less susceptible to investigator bias.

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 and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

For immunoblotting: anti-Cpt1a antibody (Abcam Cat# ab128568, clone 8F6AE9, RRID:AB_11141632, dilution: 1ug/ml), anti-Beta actin (Cell Signaling Technology Cat# 4967, RRID:AB_330288, dilution: 1:2000), anti-PPAR δ antibody (Cell Signaling Technology Cat# 74076, RRID:AB_3661825, clone E8O3HR, dilution: 1:1000), anti-VP16 Antibody (Santa Cruz Biotechnology Cat# sc-7545, RRID:AB_628443, clone 1-21, dilution: 1 ug/ml), anti-HDAC2 (Cell Signaling Technology Cat# 2540, RRID:AB_2116822, dilution: 1:1000), anti-Tubulin (Cell Signalling Technology Cat# 3873, clone DM1A, RRID:AB_1904178, dilution: 1:1000), anti-rabbit-HRP (BioLegend Cat# 406401, RRID:AB_2099368, clone Poly4064, dilution: 1:5000), anti-mouse-HRP (BioLegend Cat# 405306, RRID:AB_315009, clone Poly4053, dilution: 1:5000)

For immunofluorescence staining: CD8a (Cell Signaling Technology Cat# 98941, Clone D4W2Z, RRID:AB_2756376, dilution 1:200), CD3 (Abcam Cat# ab11089, Clone CD3-12, RRID:AB_2889189, dilution 1:200), CD45 (Cell Signaling Technology Cat# 70257, Clone D3F8Q, RRID:AB_2799780, dilution 1:500), pan-keratin (Cell Signaling Technology Cat# 4545, Clone C11, RRID:AB_490860, dilution 1:250), granzyme B (Thermo Fisher Scientific Cat# 14-8822-82, Clone 16G6, RRID:AB_468530, dilution 1:800), Donkey anti-Rabbit AF488 (Thermo Fisher Scientific Cat# A32790, RRID:AB_2762833, dilution 1:500), Donkey anti-Rat AF488 (Thermo Fisher Scientific Cat# A-21208, RRID:AB_2535794, dilution 1:500), Donkey anti-Rabbit AF647 (Thermo Fisher Scientific Cat# A-31573, RRID:AB_2536183, dilution 1:500), Donkey anti-Rat AF647 (Thermo Fisher Scientific Cat# A48272, RRID:AB_2893138, dilution 1:500), Donkey anti-Mouse AF647 (Thermo Fisher Scientific Cat# A-31571, RRID:AB_162542, dilution 1:500)

For flow cytometry staining: CD45 – APC (BioLegend, 103112, Clone 30-F11, dilution: 1:200), CD3 – PE/Fire 810 (BioLegend, 100277, Clone 17A2, dilution: 1:200), CD8 – FITC (BioLegend, 100706, Clone 53-6.7, dilution: 1:200), CD11b – APC/Cy7 (BioLegend, 101226, Clone M1/70, dilution: 1:200), TNF α – BV711 (BioLegend, 506349, Clone MP6-XT22, dilution: 1:200), IFN γ – BV785 (BioLegend, 505838, Clone XMG1.2, dilution: 1:200), Granzyme B – Spark UV 387 (BioLegend, 396442, Clone QA18A28, dilution: 1:200), CD45 – APC/Cy7 (BioLegend, 103116, Clone 30-F11, dilution: 1:200), CD8 – AF700 (BioLegend, 100730, Clone 53-6.7, dilution: 1:200), IFN γ – APC (BioLegend, 505809, clone XMG1.2, dilution: 1:200), CD45 – PE (BioLegend, 103106, Clone 30-F11, dilution: 1:200), CD3 – FITC (BioLegend, 100306, Clone 145-2C11, dilution: 1:200), CD8 – Spark UV387 (BioLegend, 100797, Clone 53-6.7, dilution: 1:200), CD4 – BV605 (BioLegend, 100451, Clone GK1.5, dilution: 1:1000), NK1.1 – PE/Cy7 (BioLegend, 108713, Clone PK136, dilution: 1:200), CD3 – FITC (BioLegend, 100203, Clone 17A2, dilution: 1:200), CD8 – APC (BioLegend, 100711, Clone 53-6.7, dilution: 1:200), CXCR3 – PE (BioLegend, 126505, Clone CXCR3-173, dilution: 1:200), TNF α – APC (BioLegend, 506307, Clone MP6-XT22, dilution: 1:200), IFN γ – FITC (BioLegend, 505805, Clone XMG1.2, dilution: 1:200), IL-2 – PE (BioLegend, 503807, Clone JES6-5H4, dilution: 1:200)

For in vivo usage: anti-CD8a (Bio X Cell Cat# BE0061, RRID:AB_1125541), anti-CXCR3 (Bio X Cell Cat# BE0249, RRID:AB_2687730), rat IgG2b isotype control (Bio X Cell Cat# BE0090, RRID:AB_1107780), Armenian hamster IgG isotype control (Bio X Cell Cat# BE0091, RRID:AB_1107773), anti-PD1 (200ug, Bio X Cell Cat# BE0146, RRID:AB_10949053), anti-CTLA4 (200ug first dose, then 100ug, Bio X Cell Cat# BE0131, RRID:AB_10950184)

Validation

All antibodies have been validated by the manufacturer for the species and the application to be used for as described in the data sheets provided on the manufacturer website. Validation information can be found under the following links for the respective antibodies:

anti-Cpt1a antibody (Abcam Cat# ab128568, clone 8F6AE9, RRID:AB_11141632, dilution: 1ug/ml): <https://www.abcam.com/en-us/products/primary-antibodies/cpt1a-antibody-8f6ae9-ab128568>
 anti-Beta actin (Cell Signaling Technology Cat# 4967, RRID:AB_330288, dilution: 1:2000): https://www.cellsignal.com/products/primary-antibodies/beta-actin-antibody/4967?srltid=AfmBOook-whPo80e2Bvj_wjLmiqhKTRq41zX2KCHPFFm2gFgvfsa4urq
 anti-PPAR δ antibody (Cell Signaling Technology Cat# 74076, RRID:AB_3661825, clone E8O3HR, dilution: 1:1000): https://www.cellsignal.com/products/primary-antibodies/ppar-delta-e8o3h-rabbit-monoclonal-antibody/74076?srltid=AfmBOookwkdNckiiB1RUmvmDpy3VMtAhnpplcb_Xf9qAt2gLuo8rDLo
 anti-VP16 Antibody (Santa Cruz Biotechnology Cat# sc-7545, RRID:AB_628443, clone 1-21, dilution: 1 ug/ml): https://www.scbt.com/p/vp16-antibody-1-21?srltid=AfmBOopBJwSba0pwGM7Qk7JNhq_vNtqQIGtf5xrUVYxu51WVGUHhP-G
 anti-Tubulin (Cell Signalling Technology Cat# 3873, clone DM1A, RRID:AB_1904178, dilution: 1:1000): <https://www.cellsignal.com/products/primary-antibodies/alpha-tubulin-dm1a-mouse-monoclonal-antibody/3873?srltid=AfmBOoo5FlspcKQ2vJSPiAl-rSvO9Doly0LcDR7H5WXSoYVRjcPENdEa>
 anti-HDAC2 (Cell Signaling Technology Cat# 2540, RRID:AB_2116822, dilution: 1:1000): https://www.cellsignal.com/products/primary-antibodies/hdac2-antibody/2540?srltid=AfmBOopNbB2jKJdYp-F0dKFWQJhgFn_dv_bXH_FJBnZSyPB8Sc8t831
 anti-rabbit-HRP (BioLegend Cat# 406401, RRID:AB_2099368, clone Poly4064, dilution: 1:5000): <https://www.biolegend.com/en-us/products/hrp-donkey-anti-rabbit-igg-minimal-x-reactivity-2264>
 anti-mouse-HRP (BioLegend Cat# 405306, RRID:AB_315009, clone Poly4053, dilution: 1:5000): <https://www.biolegend.com/en-us/products/hrp-goat-anti-mouse-igg-minimal-x-reactivity-1395>

CD8a (Cell Signaling Technology Cat# 98941, Clone D4W2Z, RRID:AB_2756376, dilution 1:200): <https://www.cellsignal.com/products/primary-antibodies/cd8-alpha-d4w2z-rabbit-monoclonal-antibody/98941?srsltid=AfmBOooq-2DmUSfeYrPFU2E3w8ntVh02yQVD75BHRjDMiw0mQ1PI5dU1>

CD3 (Abcam Cat# ab11089, Clone CD3-12, RRID:AB_2889189, dilution 1:200): <https://www.abcam.com/en-us/products/primary-antibodies/cd3-antibody-cd3-12-ab11089>

CD45 (Cell Signaling Technology Cat# 70257, Clone D3F8Q, RRID:AB_2799780, dilution 1:500): https://www.cellsignal.com/products/primary-antibodies/cd45-d3f8q-rabbit-monoclonal-antibody/70257?srsltid=AfmBOoobUBJukqnaHVpv2UCerAiYC8L7D8_hf2dRKlJeTw30s8EGmfN

pan-keratin (Cell Signaling Technology Cat# 4545, Clone C11, RRID:AB_490860, dilution 1:250): https://www.cellsignal.com/products/primary-antibodies/pan-keratin-c11-mouse-monoclonal-antibody/4545?srsltid=AfmBOoohphYeCOW_CUGw4eou4aBZGO3yRwX4jz-r9f3b1sdphVuDJtG0

granzyme B (Thermo Fisher Scientific Cat# 14-8822-82, Clone 16G6, RRID:AB_468530, dilution 1:800): <https://www.thermofisher.com/antibody/product/Granzyme-B-Antibody-clone-16G6-Monoclonal/14-8822-82>

Donkey anti-Rabbit AF488 (Thermo Fisher Scientific Cat# A32790, RRID:AB_2762833, dilution 1:500): <https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A32790TR>

Donkey anti-Rat AF488 (Thermo Fisher Scientific Cat# A-21208, RRID:AB_2535794, dilution 1:500): <https://www.thermofisher.com/antibody/product/Donkey-anti-Rat-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21208>

Donkey anti-Rabbit AF647 (Thermo Fisher Scientific Cat# A-31573, RRID:AB_2536183, dilution 1:500): <https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-31573>

Donkey anti-Rat AF647 (Thermo Fisher Scientific Cat# A48272, RRID:AB_2893138, dilution 1:500) <https://www.thermofisher.com/antibody/product/Donkey-anti-Rat-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A48272TR>

Donkey anti-Mouse AF647 (Thermo Fisher Scientific Cat# A-31571, RRID:AB_162542, dilution 1:500) <https://www.thermofisher.com/antibody/product/Donkey-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-31571>

CD45 – APC - Clone 30-F11 (BioLegend, 103112): <https://www.biolegend.com/en-us/products/apc-anti-mouse-cd45-antibody-97>

CD3 – PE/Fire 810 - Clone 17A2 (BioLegend, 100277): <https://www.biolegend.com/en-ie/products/pe-fire-810-anti-mouse-cd3-antibody-21530>

CD8 – FITC - Clone 53-6.7 (BioLegend, 100706): <https://www.biolegend.com/en-us/products/fitc-anti-mouse-cd8a-antibody-153>

CD11b – APC/Cy7 - Clone M1/70 (BioLegend, 101226): <https://www.biolegend.com/fr-ch/products/apc-cyanine7-anti-mouse-human-cd11b-antibody-3930?GroupID=BLG10616>

TNFα – BV711 - Clone MP6-XT22 (BioLegend, 506349): <https://www.biolegend.com/fr-fr/products/brilliant-violet-711-anti-mouse-tnf-alpha-antibody-13622>

IFNγ – BV785 - Clone XMG1.2 (BioLegend, 505838): <https://www.biolegend.com/en-us/products/brilliant-violet-785-anti-mouse-ifn-gamma-antibody-7987>

Granzyme B – Spark UV 387 - Clone QA18A28 (BioLegend, 396442): <https://www.biolegend.com/en-us/products/spark-uv-387-anti-humanmouse-granzyme-b-recombinant-antibody-25184>

CD45 – APC/Cy7 - Clone 30-F11 (BioLegend 103116): <https://www.biolegend.com/en-us/products/apc-cyanine7-anti-mouse-cd45-antibody-2530>

CD8 – AF700 - Clone 53-6.7 (BioLegend, 100730): <https://www.biolegend.com/en-us/products/alexa-fluor-700-anti-mouse-cd8a-antibody-3387>

IFNγ – APC - XMG1.2 (BioLegend, 505809): <https://www.biolegend.com/en-us/products/apc-anti-mouse-ifn-gamma-antibody-993?GroupID=GROUP24>

CD45 - PE - Clone 30-F11 (BioLegend, 103106): <https://www.biolegend.com/en-us/products/pe-anti-mouse-cd45-antibody-100>

CD3 - FITC - Clone 145-2C11 (BioLegend, 100306): <https://www.biolegend.com/en-us/products/fitc-anti-mouse-cd3epsilon-antibody-23>

CD8 - Spark UV387 - Clone 53-6.7 (BioLegend, 100797): <https://www.biolegend.com/en-us/products/spark-uv-387-anti-mouse-cd8a-antibody-22117>

CD4 – BV605 - Clone GK1.5 (BioLegend, 100451): <https://www.biolegend.com/en-us/products/brilliant-violet-605-anti-mouse-cd4-antibody-10708?GroupID=BLG4745>

NK1.1 – PE/Cy7 – Clone PK136 (BioLegend, 108713): <https://www.biolegend.com/en-us/products/pe-cyanine7-anti-mouse-nk-1-1-antibody-2840>

CD3 – FITC – Clone 17A2 (BioLegend, 100203): <https://www.biolegend.com/en-us/products/fitc-anti-mouse-cd3-antibody-45>

CD8 – APC – Clone 53-6.7 (BioLegend, 100711): <https://www.biolegend.com/en-us/products/apc-anti-mouse-cd8a-antibody-150>

CXCR3 – PE – Clone CXCR3-173 (BioLegend, 126505): <https://www.biolegend.com/en-us/products/pe-anti-mouse-cd183-cxcr3-antibody-4592>

TNFα – APC – Clone MP6-XT22 (BioLegend, 506307): <https://www.biolegend.com/en-us/products/apc-anti-mouse-tnf-alpha-antibody-975>

IFNγ – FITC – Clone XMG1.2 (BioLegend, 505805): <https://www.biolegend.com/en-us/products/fitc-anti-mouse-ifn-gamma-antibody-995>

IL-2 – PE – Clone JES6-5H4 (BioLegend, 503807): <https://www.biolegend.com/en-us/products/pe-anti-mouse-il-2-antibody-954>

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

MC38 (Kerafast Cat# ENH204-FP, RRID:CVCL_B288) was purchased from Kerafast and all derivative lines were derived from this commercial stock. B16-F10 (ATCC Cat# CRL-6475, RRID:CVCL_0159), SK-BR-3 (ATCC Cat# HTB-30, RRID:CVCL_0033), and SK-OV-3 (ATCC Cat# HTB-77, RRID:CVCL_0532) were purchased from ATCC. EO771 cell line was provided by Egeblad laboratory (CSHL). HEK293T (RRID:CVCL_0063) cells were provided by the Vakoc Laboratory (CSHL).

Authentication

Cell lines were authenticated by the vendor and we used early passage in our experiments. No independent authentication was performed.

Mycoplasma contamination

Cell lines were regularly tested for mycoplasma contamination by the CSHL core facility and always tested negative.

Commonly misidentified lines
(See [ICLAC](#) register)

No commonly misidentified lines were used in this study.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals

Mice were housed on a 12-hour light/dark cycle at 70–74 °F (21–23 °C) and 30–70% relative humidity, up to five sex-matched animals per cage, with ad libitum water and chow (Pico Lab Irradiated Rodent Diet, LD505330LB). C57BL/6J (RRID:IMSR_JAX:000664), congenic B6 CD45.1 (RRID:IMSR_JAX:002014), NSG (RRID:IMSR_JAX:005557), and Ppardfl/fl (RRID:IMSR_JAX:005897) mice were purchased from Jackson Laboratory. Vav-cre [Orkin Laboratory,51], Cp1afl/fl [Carmeliet Laboratory,44], VP16-PPAR δ [Yang Laboratory,52], E8liCreERT2.GFP [Vignali Laboratory,53], and CD45.1 OT-I [Fearon Laboratory,54] mice were provided by indicated laboratories. Rosa26-CAG-LSL-CPT1-KI mice were generated by integrating a CAG-LSL-Rat CPT1AM593S sequence into the Rosa26 locus of C57BL/6 embryonic stem cells, transferring engineered embryos into female mice, backcrossing resulting chimeric males to C57BL/6 females, and further backcrossing Cpt1aM593S positive pups to C57BL/6 mice for a minimum of 2 generations. Cpt1a-iGOF mice were generated by crossing Cpt1aM593S animals with Vav-cre animals. All experiments utilized age- and sex-matched controls between 8 and 12 weeks of age, if possible including litter mate controls.

Wild animals

The study did not involve wild animals

Reporting on sex

Both male and female animals that were age-matched were used and no sex-dependent differences were detected, so male and female mice were analyzed together. Experiments with cell transfer into C57BL/6J (RRID:IMSR_JAX:000664) or congenic B6 CD45.1 (RRID:IMSR_JAX:002014) only utilized female recipient animals in order to prevent graft-versus-host reactions.

Field-collected samples

This study did not involve field-collected samples.

Ethics oversight

All mice were used in compliance with animal care guidelines from CSHL Institutional Animal Care and Use Committee (IACUC) at an AAALAC accredited institution.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks

No plants were used in this study.

Novel plant genotypes

No plants were used in this study.

Authentication

No plants were used in this study.

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

Cells were stained with fluorescently-labelled surface antibodies (Supplementary Table 1) for 20 minutes on ice in FACS buffer. For intracellular staining, CD8+ T cells or digested tumors were stimulated with 50 ng/ml PMA (SigmaAldrich, P1585-1MG), 1 ug/ml ionomycin (SigmaAldrich, I9657-1MG), and brefeldin A (Thermo Fisher, 00-4506-51) for 5 or 4 hours, before staining with a fixable viability dye (Thermo Fisher, L34963 or L10119) and any surface antibodies. CD8+ T cells were permeabilized with Intracellular Fixation & Permeabilization Buffer Set (Thermo Fisher, 88-8824-00) and TICs with the eBioscience™ Foxp3 / Transcription Factor Staining Buffer Set (Thermo Fisher, 00-5523-00) before intracellular staining. Mitochondria were stained with 100 nM MitoTracker™ Green FM (Thermo Fisher, M7514) or tetramethylrhodamine, ethyl

ester (TMRE) (Thermo Fisher, T669) in PBS 30 minutes at 37°C. Samples were acquired on a BD Fortessa or Sony ID7000 and analyzed in FlowJo 10. Absolute numbers were calculated based on acquired cell events and the ratio of acquired to total CountBright™ Absolute Counting Beads (Invitrogen, C36950) added to the sample.

Instrument

Flow cytometry data was acquired on a Becton Dickinson LSRII Cell Analyzer or Sony ID7000 spectral cell analyzer.

Software

Flow cytometry data was analyzed using FlowJo10

Cell population abundance

Any sort was performed with at least 95% purity.

Gating strategy

Lymphocytes were gated based on appropriate FSC-A/SSC-A values, followed by singlet discrimination and selection of viability dye negative (i.e. alive) cells.

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