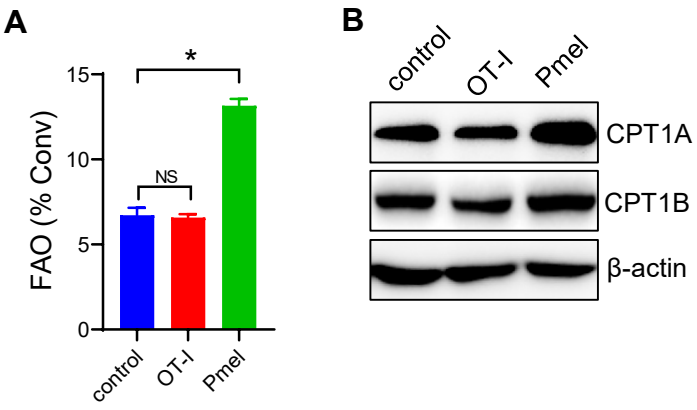
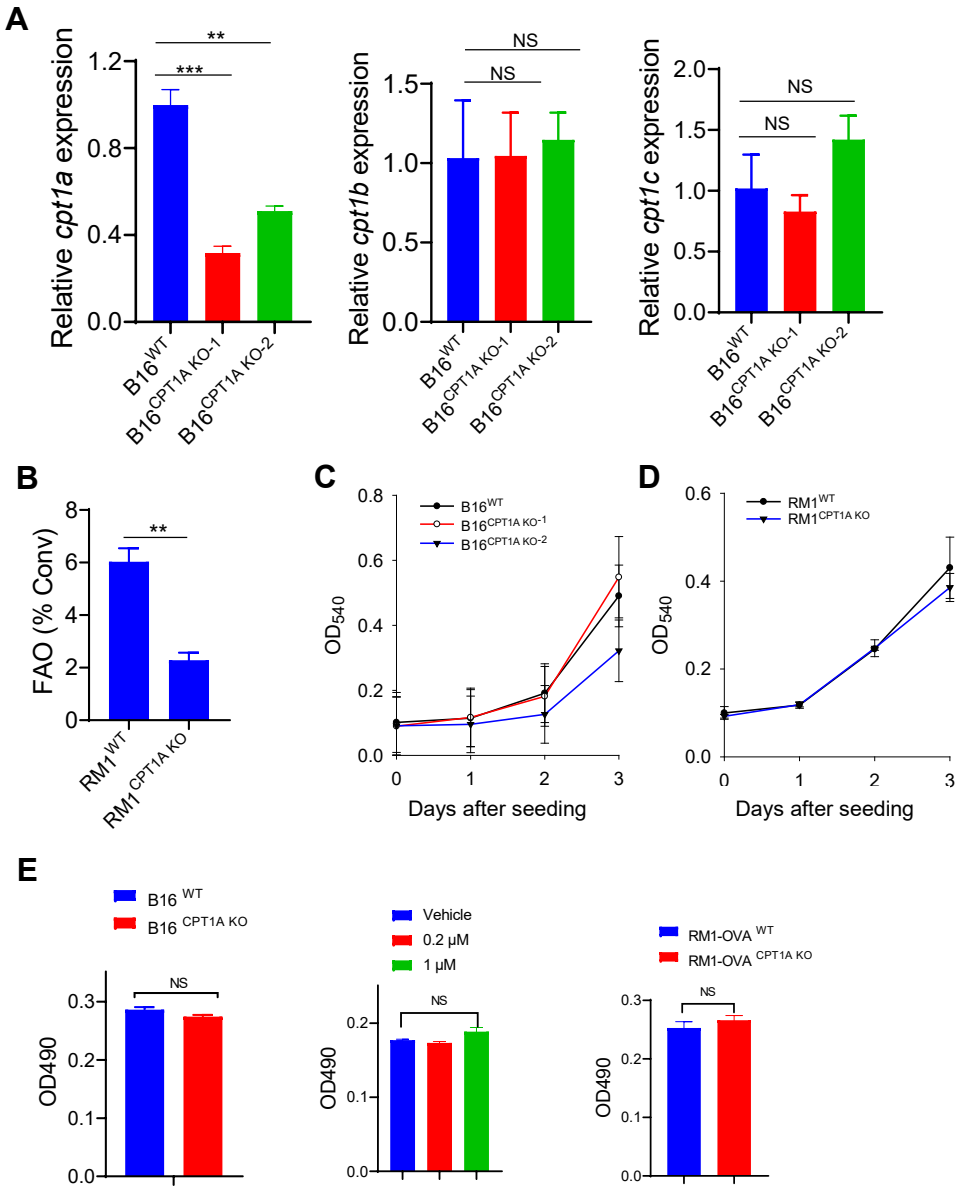
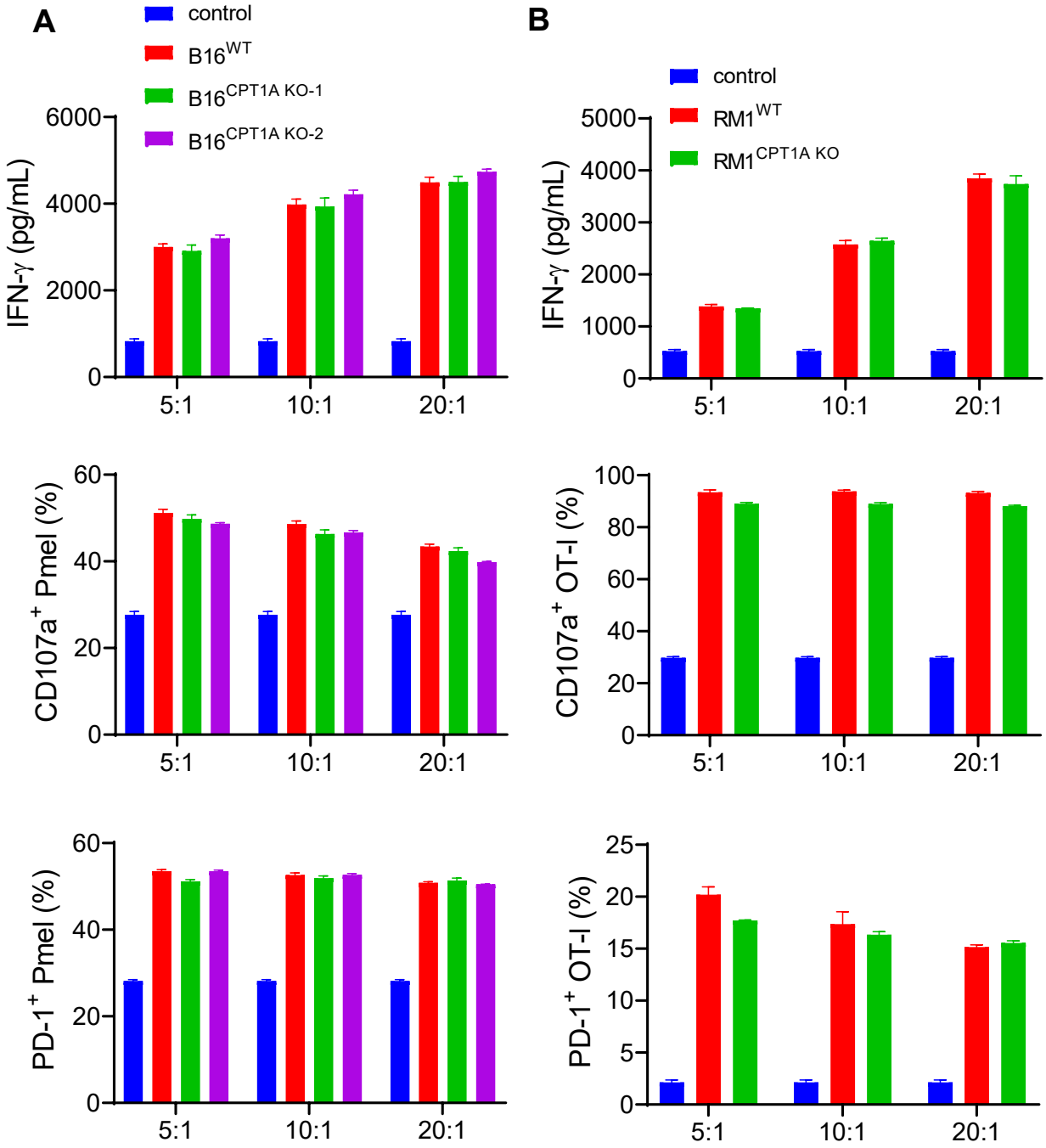


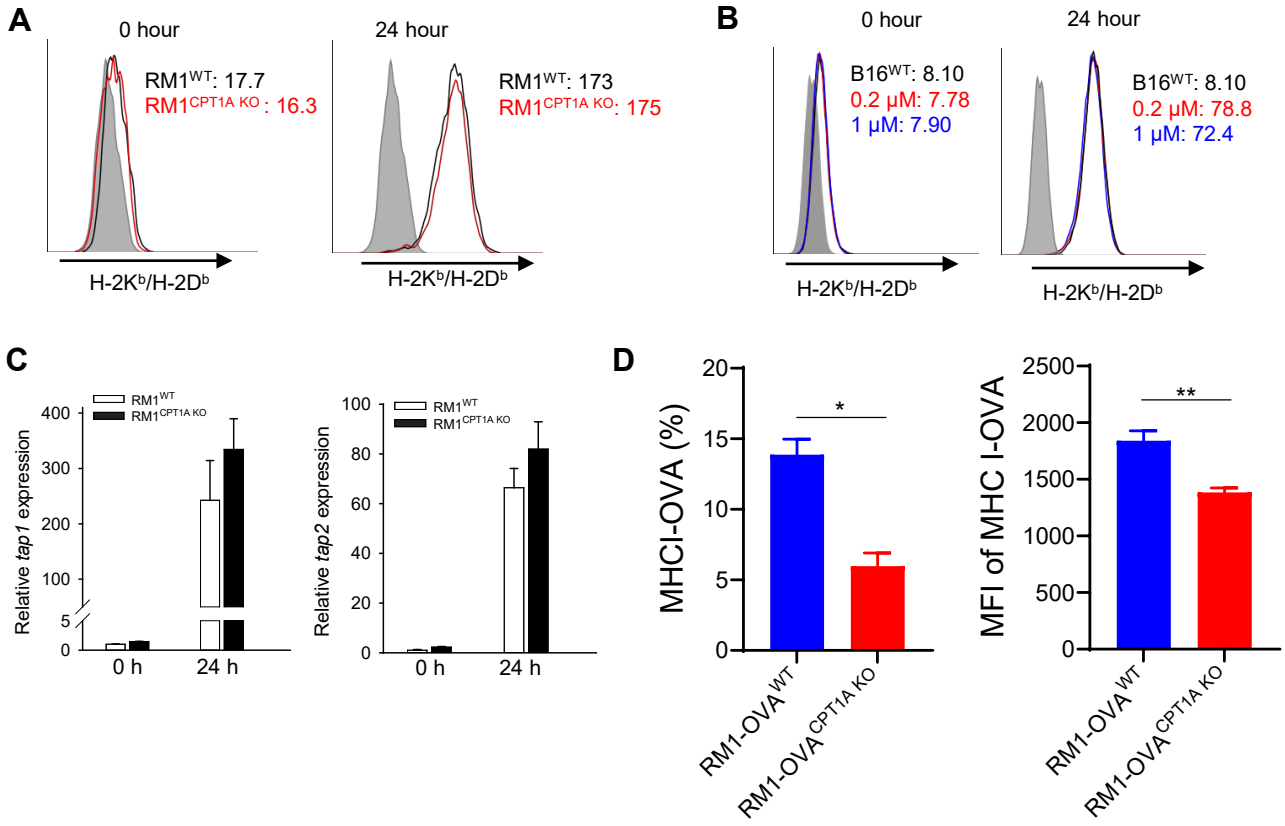
Supplementary Figure S1. Upregulation of FAO in cancer cells requires antigen-specific recognition by T cells. B16 melanoma cells were co-cultured with OT-I cells or Pmel cells for 6 hours. FAO activity in B16 cells were measured by quantifying the conversion of ^{3}H-palmitic acid to $^{3}H_2O$ following extensive wash to remove T cells (**A**). The expression of CPT1A and CPT1B in B16 cells was examined by immunoblotting (**B**). NS, not significant. *, $p < 0.05$.



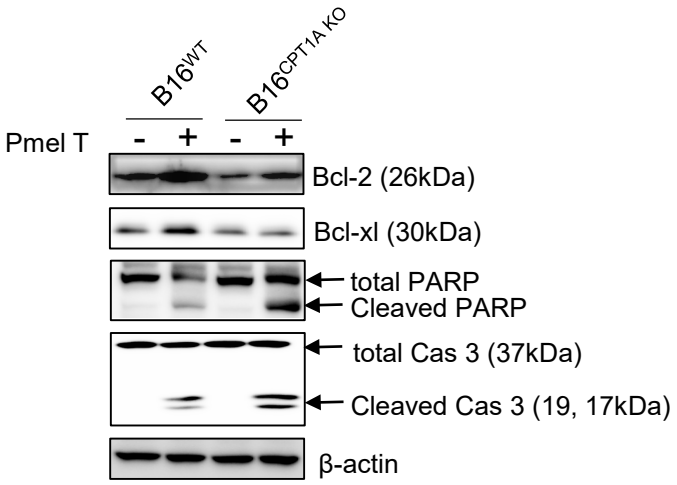
Supplementary Figure S2. Deletion of CPT1A does not affect tumor cell growth. Transcription of *cpt1a*, *cpt1b* and *cpt1c* in $B16^{WT}$ and $B16^{CPT1A\ KO}$ cells was determined by realtime PCR (**A**). FAO activities in $RM1^{WT}$ and $RM1^{CPT1A\ KO}$ cells were measured by quantifying the conversion of ^{3}H-palmitic acid to $^{3}H_2O$ (**B**). The growth of $B16^{WT}$/$B16^{CPT1A\ KO}$ cells (**C**) and $RM1^{WT}$/$RM1^{CPT1A\ KO}$ cells (**D**) was assessed by MTT assay. (**E**) LDH levels in $B16^{WT}$/$B16^{CPT1A\ KO}$ cells, $RM1^{WT}$/$RM1^{CPT1A\ KO}$ cells as well as in Etomoxir-treated cells were determined. Data are representative of at least two independent experiments. *, $p < 0.05$. **, $p < 0.01$. ***, $p < 0.001$. NS, not significant.



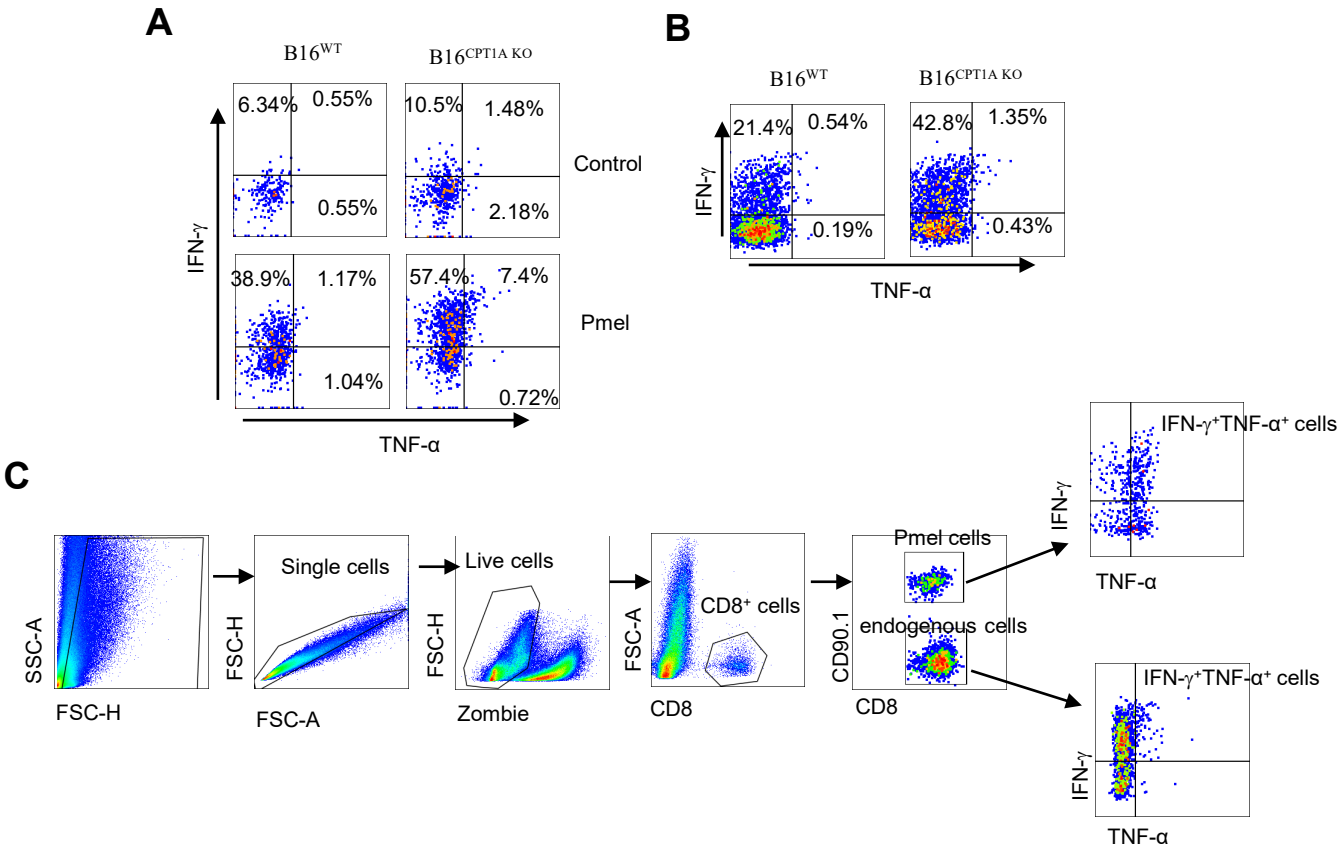
Supplementary Figure S3. Loss of CPT1a in cancer cells does not alter the functionality of tumor specific T cells. $B16^{WT}$/$B16^{CPT1A\ KO}$ (**A**) or $RM1^{WT}$/$RM1^{CPT1A\ KO}$ (**B**) tumor cells were co-cultured with antigen peptide activated Pmel/OT-I T cells at the indicated ratio in U-bottom 96-well plates for 24 hours. The levels of IFN-γ in the culture media were assayed by ELISA. Surface expression of CD107a and PD-1 was analyzed by flow cytometry. Data are representative of at least two independent experiments.



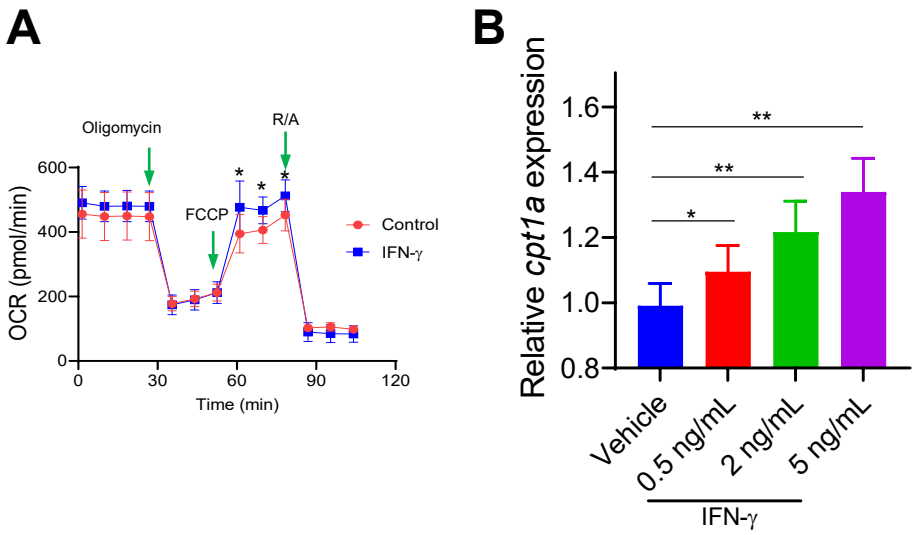
Supplementary Figure S4. CPT1A deletion does not affect tumor-intrinsic interferon signaling and tumor immunogenicity. RM1^{WT}/RM1$^{CPT1A\ KO}$ were cultured in the presence of IFN-γ (2 ng/mL) for 24 hours. Surface expression of MHC-I (H-2K^b/H-2D^b) was determined by flow cytometry (**A**). B16 cells were treated with indicated concentrations of Etomoxir for 24 h followed by stimulation with IFN-γ (2 ng/mL) for 24 hours. MHC-I (H-2K^b/H-2D^b) expression on B16 cells was determined by flow cytometry (**B**). RM1^{WT} or RM1$^{CPT1A\ KO}$ cells were stimulated by IFN-γ (2 ng/mL) followed by analysis of tap1/tap2 expression by realtime PCR (**C**). Surface expression of $OVA_{257-264}$ bound to H-2K^b of MHC class I on RM1^{WT} or RM1$^{CPT1A\ KO}$ cells was determined by flow cytometry (**D**). Data are representative of at least two independent experiments. *, $p < 0.05$. **, $p < 0.01$.



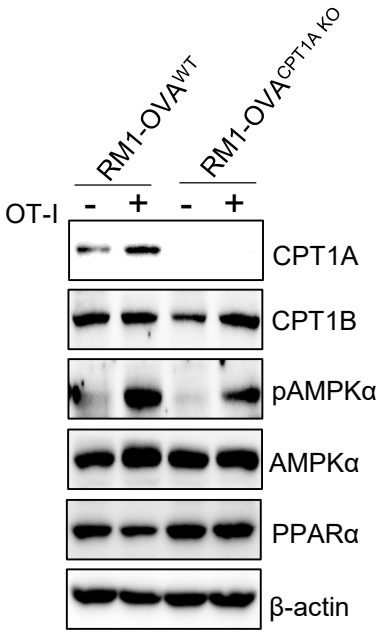
Supplementary Figure S5. Deletion of CPT1A enhances the activation of pro-apoptotic signaling pathways. $B16^{WT}$ or $B16^{CPT1A\ KO}$ cells were co-cultured with Pmel cells (tumor cell : T cell = 1 : 20) for 6 h followed by analysis of the activation of pro-apoptotic signaling pathways. Data are representative of at least two independent experiments.



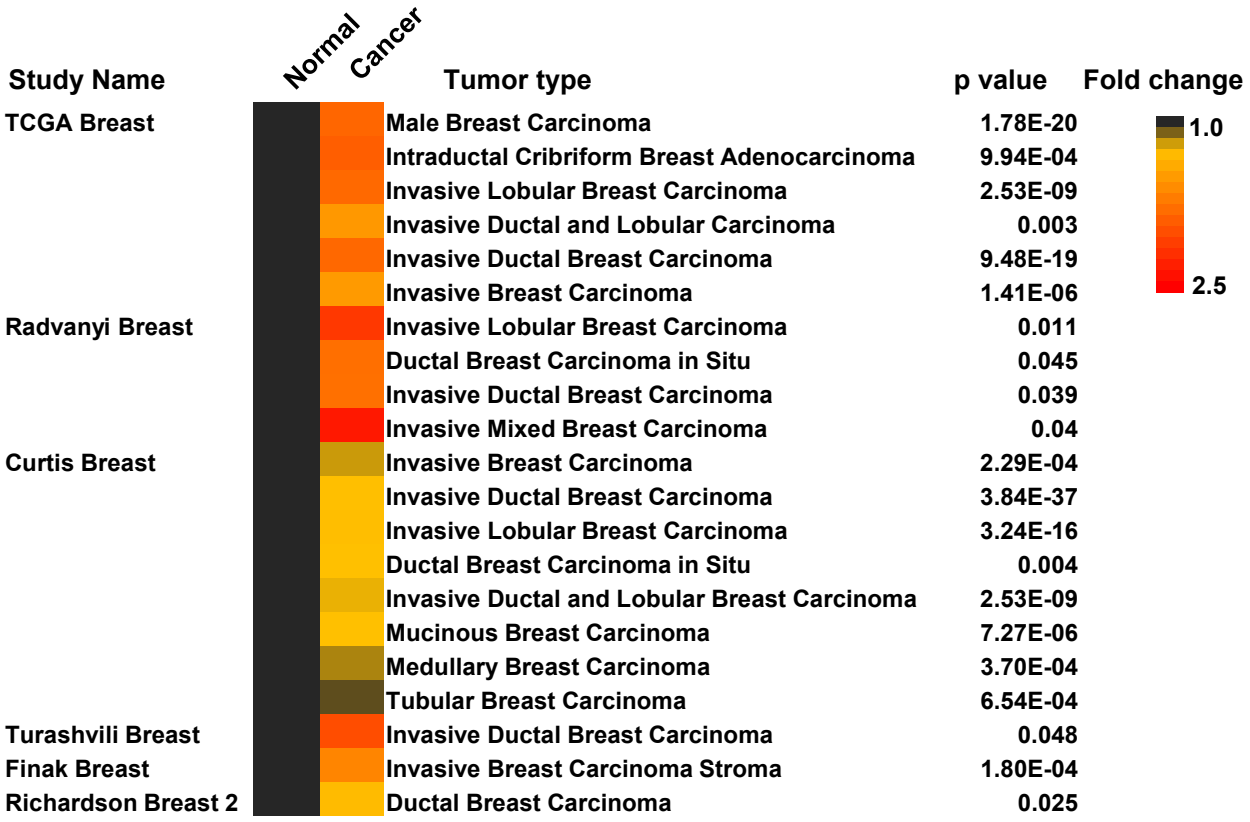
Supplementary Figure S6. Lack of CPT1A in tumors results in increased activation of CD8$^+$ T cells in vivo. A. B16^{WT} or B16$^{CPT1A\ KO}$ tumor-bearing C57BL/6 mice were treated with Pmel T cells on day 17 post tumor cell inoculation and tumor tissues were collected on day 21. The frequencies of endogenous CD8$^+$ T cells expressing IFN-γ and/or TNF-α were assessed by intracellular cytokine staining and flow cytometry analysis. **B.** C57BL/6 mice were established with experimental lung metastases by inoculating B16^{WT} or B16$^{CPT1A\ KO}$ cells (1×10^5 cells, i.v.) on day 0. Mice received Pmel T cells (10^7 cells, i.v.) on days 8 or left untreated. The frequency of IFN-γ$^+$TNF-α$^+$ CD8 T cells in the lungs was analyzed. **C.** Gating strategies used to determine the frequency of IFN-γ$^+$ TNF-α$^+$ CD8 T cells are shown.



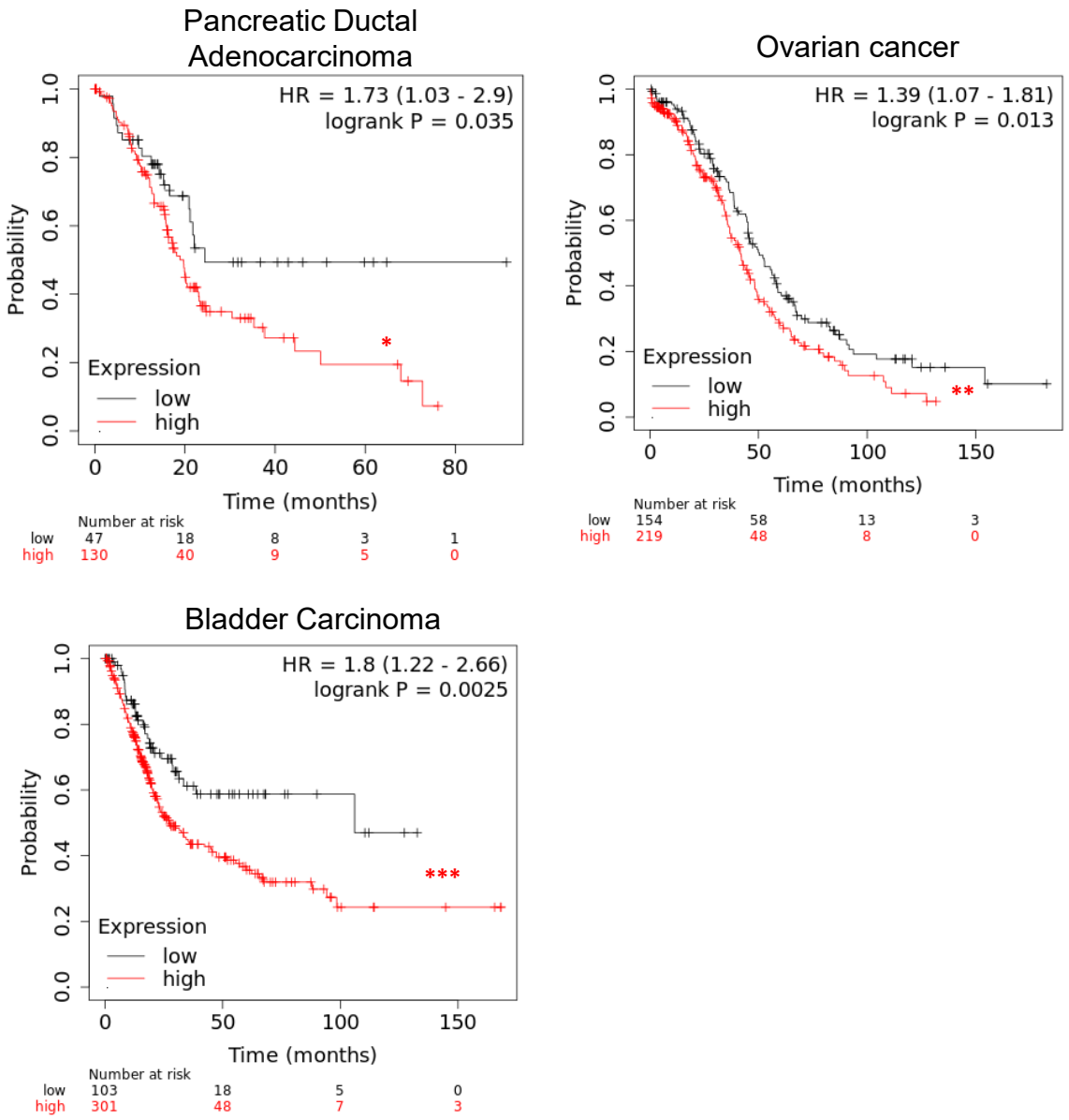
Supplementary Figure S7. IFN-γ upregulates FAO and gene transcription of *cpt1a* in murine melanoma cells. A. B16 tumor cells were treated with IFN-γ (0.5 ng/mL) for 24 hours. OCR was measured using XF-24 Extracellular Flux Analyzers). **B.** B16 cells were stimulated with indicated doses of IFN-γ for 24 hours. The transcriptional of *cpt1a* was examined using real-time qPCR. Data are representative of at least two independent experiments. *, $p < 0.05$. **, $p < 0.01$.



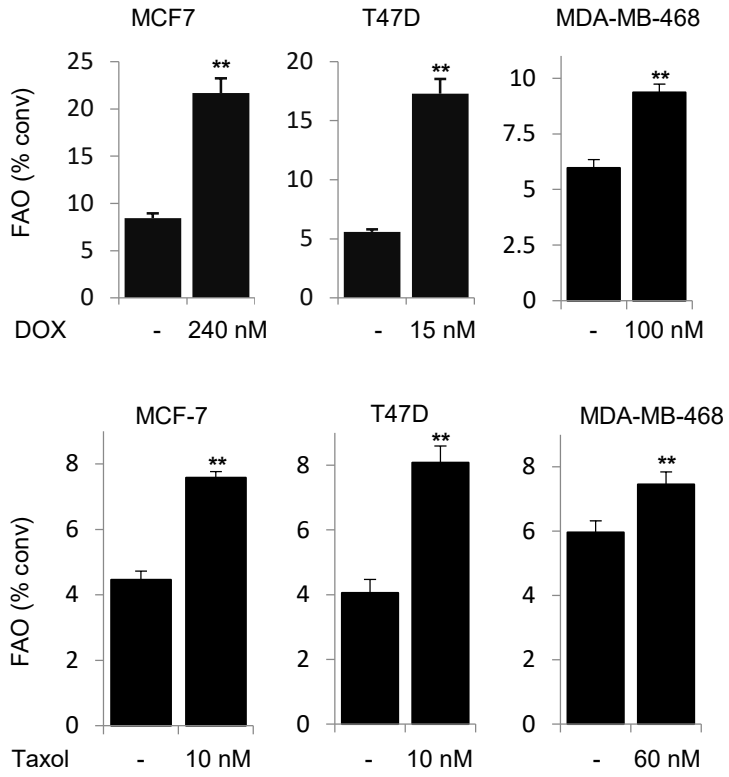
Supplementary Figure S8. T cell-induced upregulation of CPT1a in RM1 cells does not require cell-cell contact. RM1-OVA^{WT} or RM1-OVA$^{CPT1A\ KO}$ cells (5×10^5/well) were plated into the lower chambers of the trans-wells with 3 μm pore polycarbonate membrane inserts. OT-I cells (1×10^6/well) were plated onto the upper chambers. The levels of CPT1A, pAMPKα and PPARα in tumor cells were assayed by immunoblotting after 24 hours.



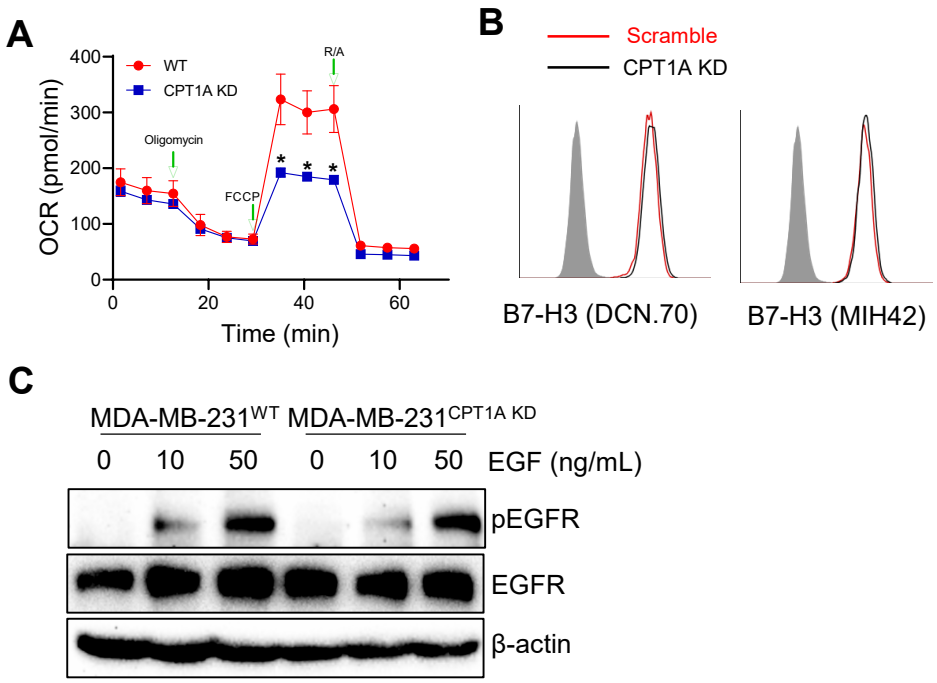
Supplementary Figure S9. CPT1A mRNA is overexpressed in human breast cancer. The publicly available data on Oncomine were used to analyze CPT1A mRNA expression in breast cancer. Shown was heat map of fold change in breast tumor samples versus normal tissues.



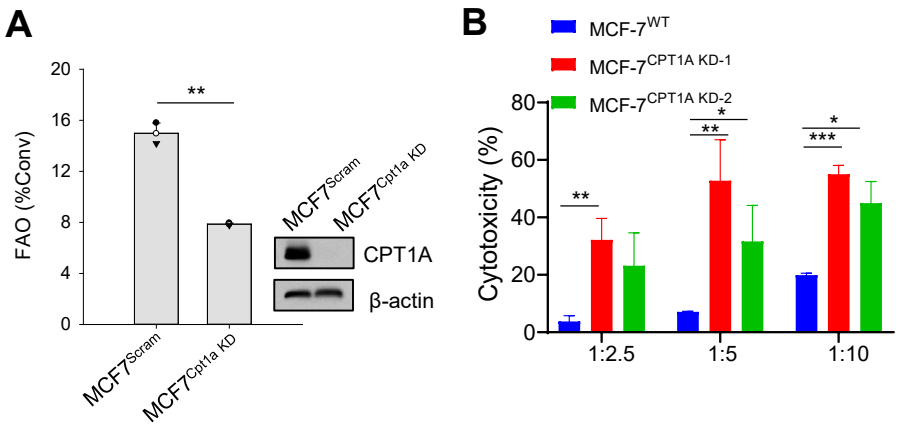
Supplementary Figure S10. CPT1A overexpression correlates with poor survival of cancer patients. Kaplan-Meier survival curves were utilized to assess the prognoses of patients diagnosed with Pancreatic Ductal Adenocarcinoma, Ovarian Cancer, or Bladder Carcinoma based on CPT1A expression levels. The patient data including RNA-seq information was obtained from the TCGA datasets. The Kaplan-Meier plotter [PAN-cancer] (http://kmplot.com) with the "auto select best cutoff" patient split method was conducted for this analysis.



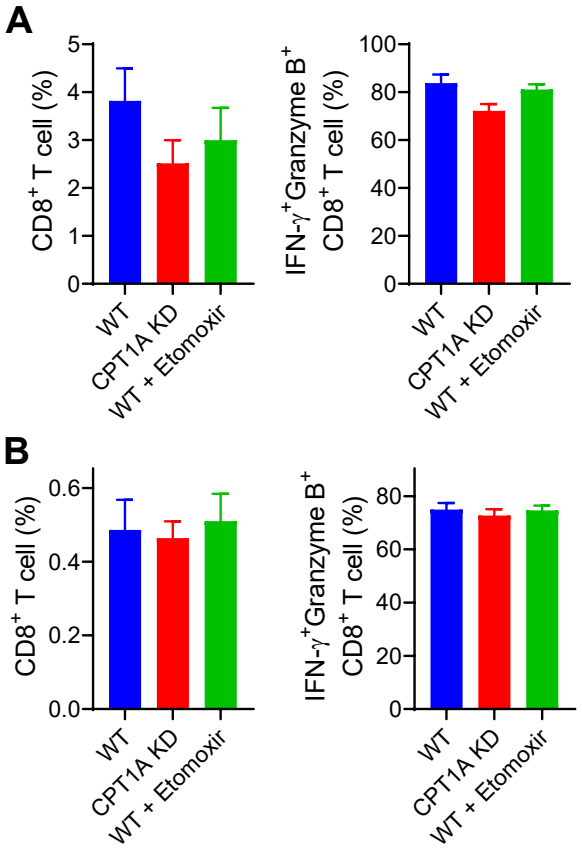
Supplementary Figure S11. Treatment with chemotherapeutic drugs elevates FAO in human breast cancer cell lines. MCF-7, T47D and MDA-MB-468 cells were treated with DOX or Taxol at the indicated concentrations for 24 hours. FAO was measured by quantifying the conversion of ^{3}H-palmitic acid to $^{3}H_2O$. Data are representative of at least two independent experiments. **, $p < 0.01$.



Supplementary Figure S12. CPT1A knockout does not alter the EGFR signaling in human breast cancer MDA-MB-231 cells. A. Downregulation of FAO in MDA-MB-231$^{CPT1A\ KD}$ cells was confirmed by measuring oxygen consumption rate (OCR) using Seahorse assays. $p < 0.05$. **B.** The expression of B7-H3 on MDA-MB-231^{Scram} or MDA-MB-231$^{CPT1A\ KD}$ cells was examined by flow cytometry analysis. **C.** MDA-MB-231^{Scram} or MDA-MB-231$^{CPT1A\ KD}$ cells were stimulated with recombinant human EGF for 30 min at the indicated concentrations. The phosphorylation of EGFR was determined by immunoblotting.



Supplementary Figure S13. Lack of CPT1A sensitizes human breast cancer cells to NK cell mediated killing. FAO in MCF7^{Scram} or MCF7$^{CPT1A\ KD}$ tumor cells was determined by quantifying the conversion of ^{3}H-palmitic acid to $^{3}H_2O$ (**A**). MCF7^{Scram} or MCF7$^{CPT1A\ KD}$ tumor cells were co-cultured with NK92 cells at the indicated ratios for 24 hours. Relative cytotoxicity was measured by LDH assays (**B**). Data are representative of at least two independent experiments. *, $p < 0.05$. **, $p < 0.01$. ***, $p < 0.001$.



Supplementary Figure S14. Inhibition of FAO does not alter the activation phenotype of CAR-T cells in vivo. NSG mice (n=5) with established MDA-MB-231^{Scram} or MDA-MB-231$^{CPT1A\ KD}$ tumors were treated with B7-H3 CAR-T cells (2×10^6 cells). A cohort of mice bearing WT tumors were also treated with Etomoxir plus CAR-T cells. The frequency and activation status of CAR-T cells in the peripheral blood (**A**) and spleen (**B**) were examined by flow cytometry 72 h after treatment.