Fig S1. Related to Figure 1

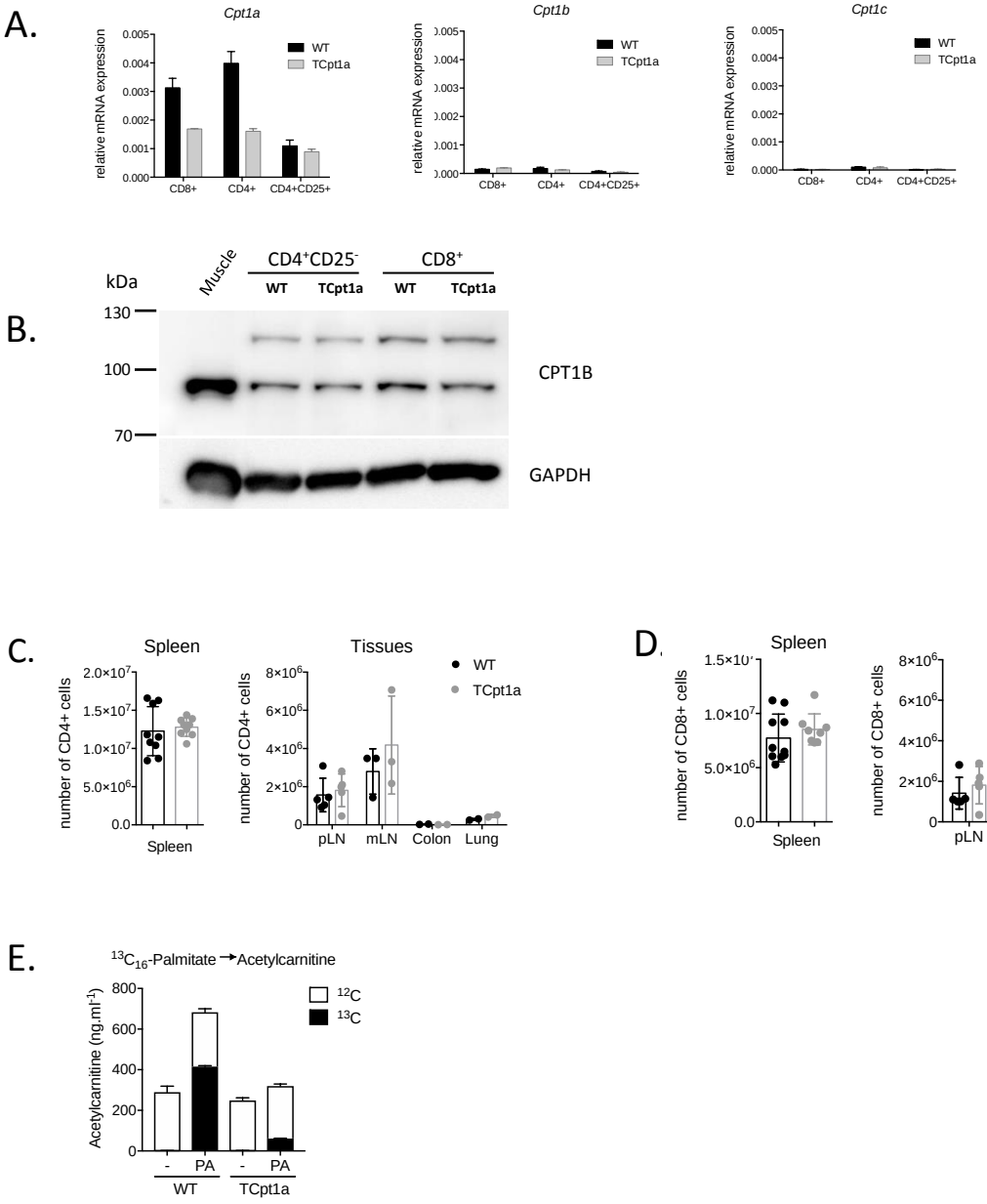

Figure S1

(A) mRNA expression of Cpt1 isoforms in sorted naïve cells from WT and TCpt1a mice. Results are representative of two independent experiments. (B) Quantification of CPT1B protein expression by WB in sorted T cells from naïve mice.

(C-D) T cell numbers in TCpt1a mice. Total amounts of CD4+ and (D) CD8+ cells in TCpt1a mice were analyzed by FACS in different organs (pLNs=axillar and inguinal lymph nodes, mLNs=mesenteric lymph nodes). Results show pooled data from 2 experiments.

(E) U-[^{13}C]-palmitate-derived acetyl-carnitine in CD4^{+}CD25^{-} T cells from WT and TCpt1a mice. Cells were incubated with 10ng/ml IL-7cells for 6hr in medium containing 100µM U-[^{13}C]-palmitate.

Fig S2. Related to Figure 2.

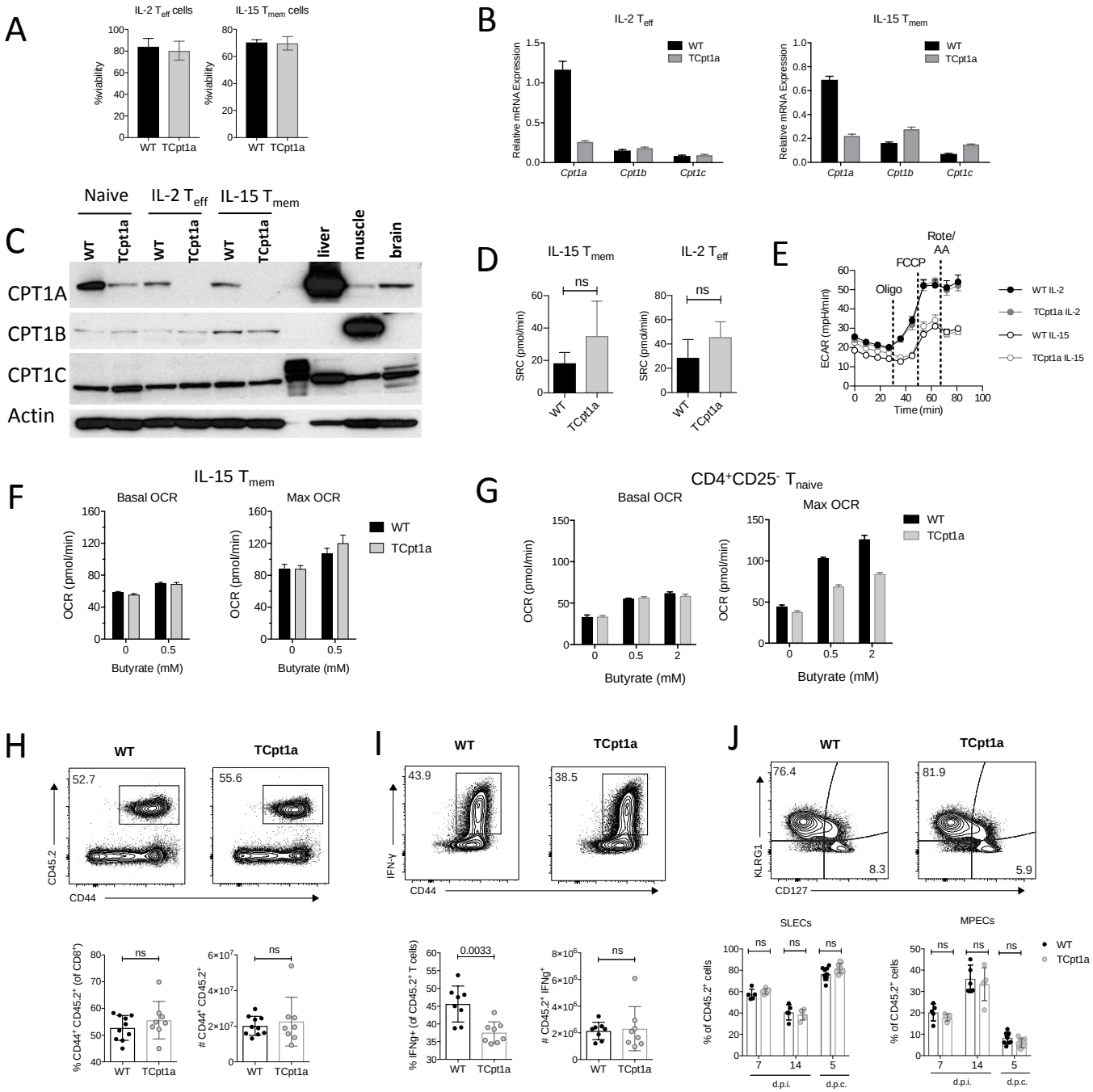

Figure S2

(A) Viability and (B) expression of Cpt1 mRNA in naïve CD8, IL-2 Teff, and IL-15 Tmem cells as determined by RT-qPCR. Transcript levels for the indicated genes were determined relative to OGDH mRNA levels and normalized to Cpt1a levels in WT naive CD8 T cells. (B) Immunoblot of Cpt1 isoforms for cells in (A). Protein extracts from mouse liver, muscle, and brain served as positive controls for Cpt1a, Cpt1b, and Cpt1c, respectively. (D-E) SRC (D) and ECAR (E) of WT and TCpt1a IL-15 Tmem and IL-2 Teff cells. Results are representative of two experiments. (F) OCR of in vitro generated IL-15 OT-I Tmem cells assayed with or without 0.5mM butyrate under basal conditions (left panel) and in response to FCCP (right panel). Graphs show mean ± SEM. (G) OCR of CD4+CD25- cells with or without butyrate under basal conditions (left panel) and in response to FCCP (right panel). Graphs show mean ± SEM. (H) Memory recall response of WT and TCpt1a CD8$^+$ OT-I cells. Shown is the cellularity (H), INF-**γ** production (I) and KLRG1/CD127 expression status (J) of OVA-specific CD45.2$^+$CD8$^+$ OT-I T cells 5 day post-rechallenge with LmOVA (5 d.p.c.). (J) also shows the KLRG1/CD127 expression status of OVA-specific CD45.2$^+$CD8$^+$ OT-I T cells in the blood 7 and 14 days post-primary infection. (d.p.i.) Short-lived effector cells (SLECs, KLRG1$^+$/CD127$^-$) Memory precursor effector cells (MPECs, KLRG1$^-$/CD127$^+$).

Fig S3. Related to Figure 3

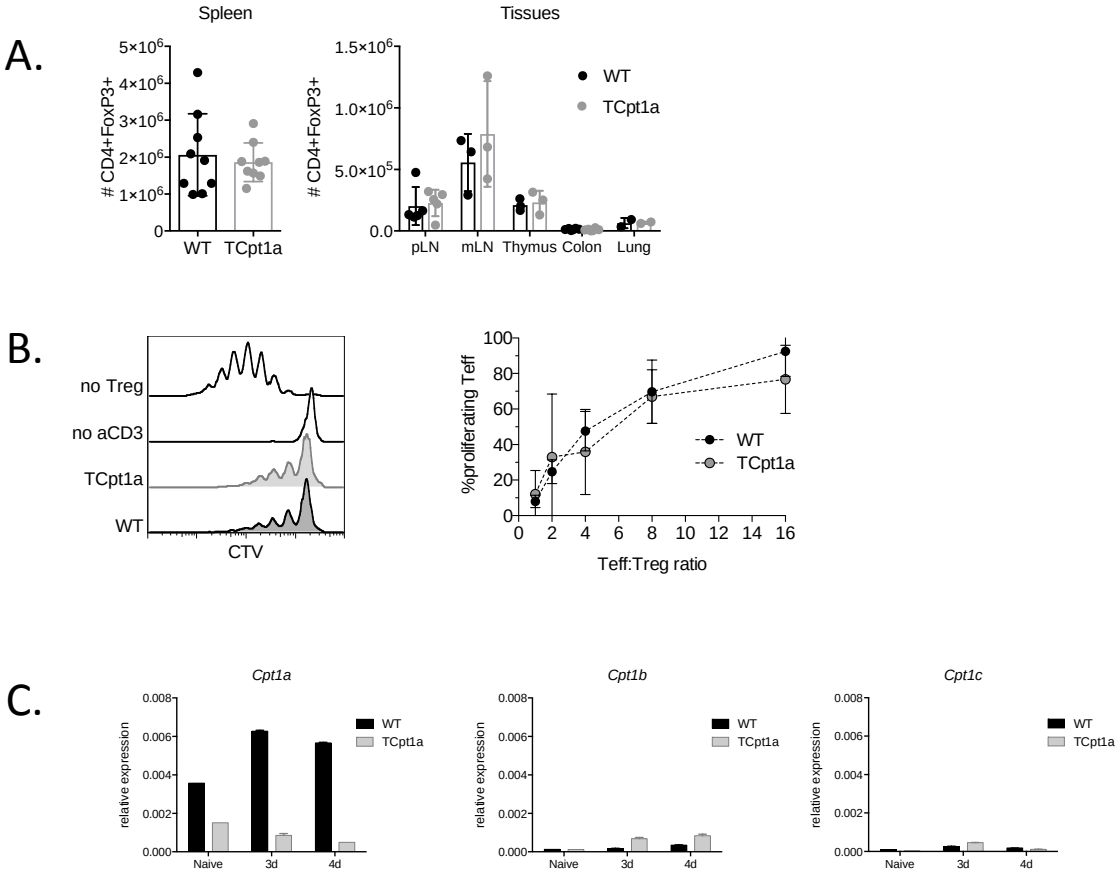

Figure S3

(A) Total amounts of Foxp3$^+$CD4+ cells in different organs from control and TCpt1a mice as determined by flow cytometry (pLNs=axillar and inguinal lymph nodes, mLNs=mesenteric lymph nodes). Results show pooled data from 2 experiments.

(B) CD4+CD25+ cells from WT and TCpt1a mice were co-cultured with CD45.1 naïve T cells, APCs and anti-CD3 antibody for 4 days and the proliferation of CD45.1 cells was evaluated using cell trace violet proliferation dye. Histogram shows one representative experiment and the graph on the right contains pooled data from 3 independent experiments

(C) Expression of Cpt1 isoforms in iTreg cultures, as determined by qPCR. These data are from 1 experiment.

Fig S4. Related to Figure 4

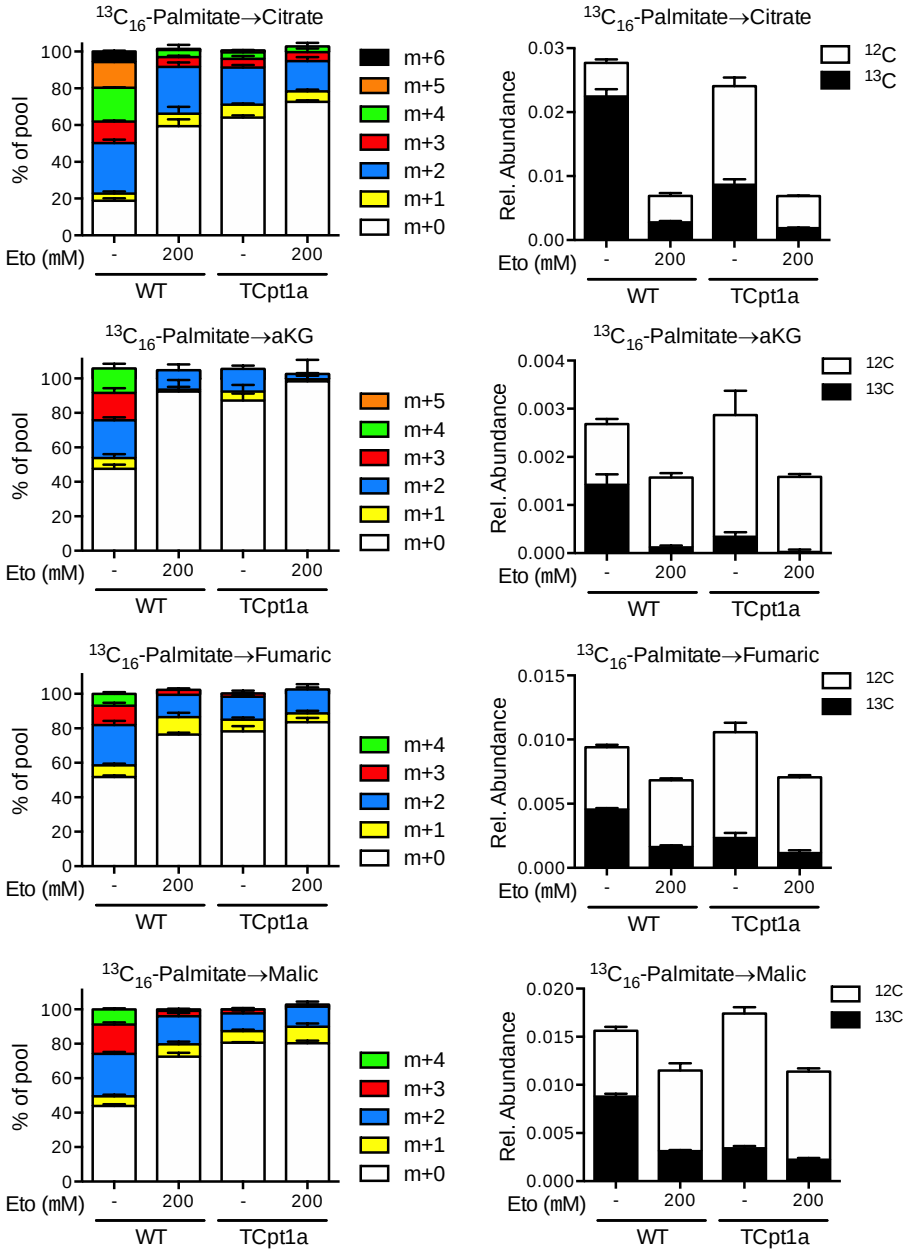

Figure S4: Metabolic fate of ^{13}C-palmitate in IL-15 Tmem cells cultured with etomoxir.

Mass isotopomer distribution (MID) of U-[^{13}C]-palmitate-derived TCA cycle intermediates in WT and TCpt1a in vitro generated IL-15 OT-I T_{mem} cells. T_{mem} cells were cultured for 24hr in medium containing U-[^{13}C]-palmitate with or without etomoxir (200 μM), and the ^{13}C isotopomer distribution was determined by GC-MS. The percent distribution of each isotopomer for their respective metabolite pool (left panels) as well as the relative abundance of unlabeled and U-[^{13}C]-palmitate-labeled (right panels) is shown. Data are normalized to cell number (mean ± SEM, n = 3).