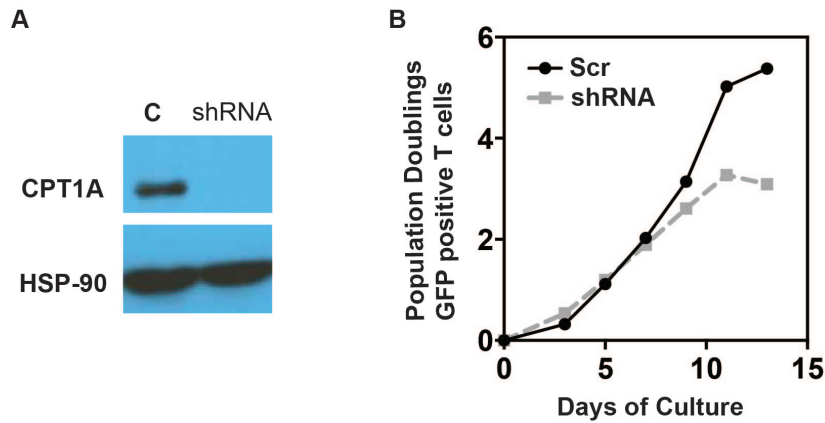


## Supplementary Information

### **The CPT1a inhibitor, etomoxir induces severe oxidative stress at commonly used concentrations**

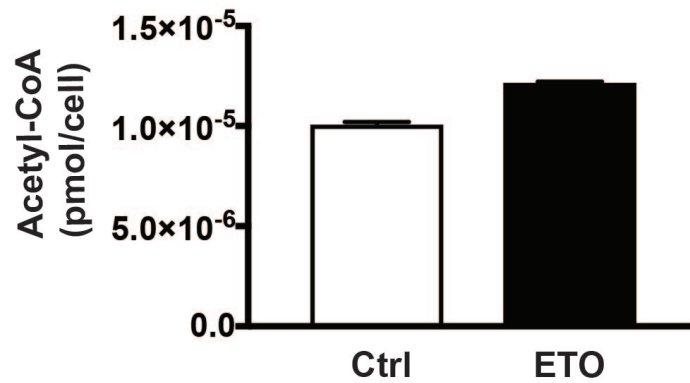
**Authors:** Roddy S. O'Connor<sup>1, 4</sup>, Lili Guo<sup>2</sup>, Saba Ghassemi<sup>1, 4</sup>, Nathaniel W. Snyder<sup>3</sup>, Andrew J. Worth<sup>2</sup>, Liwei Weng<sup>2</sup>, Yoonseok Kam<sup>5</sup>, Benjamin Philipson<sup>6</sup>, Sophie Trefely<sup>3</sup>, Selene Nunez-Cruz<sup>1, 4</sup>, Ian A. Blair<sup>2</sup>, Carl H. June<sup>1, 4</sup>, Michael C. Milone<sup>1, 4</sup>

<sup>1</sup>Center for Cellular Immunotherapies, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA. <sup>2</sup>Penn SRP center, Center of Excellence in Environmental Toxicology, and Department of Systems Pharmacology and Translational Therapeutics at the University of Pennsylvania, Philadelphia, PA. <sup>3</sup>A.J. Drexel Autism Institute, Drexel University, Philadelphia, PA. <sup>4</sup>Department of Pathology and Laboratory Medicine, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA. <sup>5</sup>Agilent Technologies Inc, Lexington, MA. <sup>6</sup>University of Pennsylvania School of Medicine, Philadelphia, PA.



### Supplementary Figure S1. CPT1A knockdown impairs T cell proliferation/survival

(A) After overnight stimulation with dynabeads, primary human T cells were infected with either shRNA against CPT1A or a control shRNA. These cells were expanded for 5 days and CPT1A expression was measured by immunoblot analysis. Relative protein loading was determined by immunoblotting for HSP-90. Representative data from 3 independent experiments are shown. (B) T cells were activated with dynabeads and infected with a bicistronic plasmid encoding a GFP transgene as well as shRNA against CPT1A. Cells were enumerated using flow cytometric, bead-based approaches described in the materials and methods. The number of population doublings is plotted as a function of time in the GFP+ subsets. Values are means  $\pm$  S.E.M. from 4 independent experiments. (\* $P < 0.05$  for control vs shRNA CPT1A).



**Supplementary Figure S2. Total cellular acetyl-CoA levels are unaffected by 50 $\mu$ M ETO.**

Primary human T cells were activated dynabeads and expanded for 7 days. Where indicated, activated T cells (day3) were treated with 50 $\mu$ M ETO throughout expansion. Cells were seeded in triplicate and harvested in 10% TCA solution. Total cellular acetyl-CoA concentrations were determined by LC-MS. Values are means  $\pm$  S.E.M. Representative data from two independent experiments with separate donors are shown.