

1 **Ketolysis is a metabolic driver of CD8⁺ T cell effector function through**
2 **histone acetylation**

3 Katarzyna M. Luda^{1,2}, Susan M. Kitchen-Goosen¹, Eric H. Ma¹, McLane J. Watson¹,
4 Lauren R. Duimstra¹, Brandon M. Oswald¹, Joseph Longo¹, Zhen Fu³, Zachary Madaj³,
5 Ariana Kupai⁴, Bradley M. Dickson⁴, Irem Kaymak¹, Kin H. Lau³, Shelby Compton¹, Lisa
6 M. DeCamp¹, Daniel P. Kelly⁵, Patrycja Puchalska⁶, Kelsey S. Williams¹, Connie M.
7 Krawczyk¹, Dominique Lévesque⁷, François-Michel Boisvert⁷, Ryan D. Sheldon⁸, Scott
8 B. Rothbart⁴, Peter A. Crawford^{6,9}, and Russell G. Jones^{1*}

9

10 ¹Department of Metabolism and Nutritional Programming, Van Andel Institute, Grand
11 Rapids, MI, USA; ²University of Copenhagen, Novo Nordisk Foundation Center for
12 Basic Metabolic Research, Blegdamsvej 3B, 2200 København, Denmark;
13 ³Bioinformatics and Biostatistics Core Facility, Van Andel Institute, Grand Rapids, MI,
14 USA; ⁴Department of Epigenetics, Van Andel Institute, Grand Rapids, MI, USA;
15 ⁵Cardiovascular Institute and Department of Medicine, Perelman School of Medicine at
16 the University of Pennsylvania, Philadelphia, PA, USA; ⁶Department of Medicine,
17 Division of Molecular Medicine, University of Minnesota, Minneapolis, MN, USA;
18 ⁷Department of Anatomy and Cell Biology, Université de Sherbrooke, Sherbrooke, QC,
19 Canada; ⁸Metabolomics and Bioenergetics Core Facility, Van Andel Institute, Grand
20 Rapids, MI, USA; ⁹Department of Biochemistry, Molecular Biology, and Biophysics,
21 University of Minnesota, Minneapolis, MN, USA.
22 *Corresponding author: Russell G. Jones, russell.jones@vai.org

23 **Abstract:**

24 **Environmental nutrient availability influences T cell metabolism, impacting T cell**
25 **function and shaping immune outcomes. However, the metabolic pathways**
26 **critical for optimal T cell responses remain poorly understood. Here, we identify**
27 **ketone bodies (KBs) – including β -hydroxybutyrate (β OHB) and acetoacetate**
28 **(AcAc) – as essential fuels supporting CD8 $^{+}$ T cell metabolism and effector**
29 **function. Ketolysis is an intrinsic feature of highly functional CD8 $^{+}$ T effector (Teff)**
30 **cells and β OHB directly increases CD8 $^{+}$ Teff cell IFN- γ production and cytolytic**
31 **activity. Using metabolic tracers, we establish that CD8 $^{+}$ Teff cells preferentially**
32 **use KBs over glucose to fuel the tricarboxylic acid (TCA) cycle *in vitro* and *in vivo*.**
33 **KBs directly boost the respiratory capacity of CD8 $^{+}$ T cells and TCA cycle-**
34 **dependent metabolic pathways that fuel T cell growth. Mechanistically, we find**
35 **that β OHB is a major substrate for acetyl-CoA production in CD8 $^{+}$ T cells and**
36 **regulates effector responses through effects on histone acetylation. Together,**
37 **our results identify cell-intrinsic ketolysis as a metabolic and epigenetic driver of**
38 **optimal CD8 $^{+}$ T cell effector responses.**

39

40 **One Sentence summary:**

41 Ketone bodies promote CD8 $^{+}$ T cell metabolism and effector function through
42 regulation of epigenetic programming

43

44

45 **Main text:**

46 Multi-cellular organisms have evolved protective strategies for defending against
47 pathogens such as viruses and bacteria and intrinsic threats such as cancer. Central to
48 host defense are CD8⁺ T cells, which are critical for pathogen clearance and protecting
49 the host from re-infection through long-lived immune memory (1, 2). One of the
50 fundamental biological programs supporting T cell effector function is cellular
51 metabolism, which generates energy and biosynthetic precursors essential for CD8⁺ T
52 effector (Teff) cell proliferation, survival, and production of effector molecules (i.e., IFN-
53 γ , TNF- α , and cytolytic factors) (3–5). CD8⁺ Teff cells undergo extensive metabolic
54 rewiring to arm themselves with the bioenergetic and biosynthetic capacity to
55 successfully protect the host (6, 7). However, Teff cell function is highly dependent on
56 nutrient availability (8–11), which in the whole organism is governed by diet and host
57 metabolism. Changes in host metabolic homeostasis triggered by infection—which can
58 include catabolic wasting (“cachexia”) and anorexia—can influence the pathogenesis
59 of the infection and affect disease tolerance (12, 13). In response to bacterial and viral
60 infections, disrupted feeding behavior and metabolic rewiring in tissues, such as the
61 liver, promote changes in host metabolism including lipolysis and production of ketone
62 bodies (14–17); however, how T cell-intrinsic metabolic programming synergizes with
63 changes in host metabolism during an immune response remains poorly defined. Here,
64 we set out to delineate cell-intrinsic and host metabolic factors critical for the formation
65 of highly functional CD8⁺ Teff cells.

66 Effective control of infection and cancer growth requires robust effector CD8⁺ T
67 cell responses, which are driven by TCR-dependent transcriptional, epigenetic, and

68 metabolic programs (2, 18–20). To define features of highly functional CD8⁺ T effector
69 (Teff) cells from setting of both cancer and infection, we conducted a meta-analysis of
70 three independent studies characterizing gene expression profiles of CD8⁺ T cells
71 responding to acute infection (*Listeria monocytogenes* (*Lm*) or *Lymphocytic*
72 *choriomeningitis virus* (LCMV), Armstrong strain), chronic infection (LCMV, Clone 13
73 (CL-13) strain), or syngeneic tumors (21–23). Pearson correlations (**Fig. 1a, S1a**) and
74 principal component analysis (PCA) among samples revealed clustering of samples
75 into 6 main cellular subtypes: 1) naïve (Tn), 2) Teff, 3) T memory (Tmem), 4) early cancer
76 T exhausted (Tex), 5) late cancer Tex, and 6) chronic virus Tex. To identify genes
77 preferentially associated with “functional” versus “dysfunctional” CD8⁺ T cells, we
78 compared gene expression patterns between Teff cells and late cancer- or chronic
79 viral-driven Tex cells (**Table S1**). Wald test statistics for each gene were generated for
80 each pairwise comparison (cancer and viral datasets) and median scores used to
81 identify genes associated with functional versus dysfunctional states (**Fig. 1b**). Rank
82 analysis revealed that genes associated with CD8⁺ T cell cytotoxicity (i.e., *Klrg1*, *Klf2*,
83 *Rora*, *Lef1*) were enriched in the functional Teff cell cohort, while dysfunctional CD8⁺ T
84 cells displayed enrichment in genes associated with T cell exhaustion (i.e., *Tox*, *Pdcd1*,
85 *Entpd1*, *Lag3*) (**Fig. 1b, Fig. S1b, Table S1**) and inflammatory signaling (**Fig. S1c**).
86 Given the central role of metabolism in supporting Teff cell responses (6, 24), we
87 hypothesized that specific metabolic pathways may contribute to CD8⁺ Teff cell
88 function. KEGG pathway analysis of the top ~4% of genes expressed by functional
89 CD8⁺ Teff cells revealed preferential enrichment of several metabolic pathways (**Fig.**

90 **1c).** Among these, the synthesis and degradation of ketone bodies was the most highly
91 enriched metabolic pathway in functional CD8⁺ T cells (**Fig. 1c**).

92 Ketone bodies (KBs), including β -hydroxybutyrate (β OHB) and acetoacetate
93 (AcAc), are alternative metabolic fuels critical for supporting bioenergetic metabolism
94 during periods of nutrient deprivation (25–27). KBs can also have fates beyond terminal
95 oxidation, including impact on cell signaling, direct and indirect effects on histone
96 post-translational modifications (PTMs), and inhibition of inflammatory processes (26,
97 28). The primary site of KB production in mammals is the liver, which generates AcAc
98 and β OHB from fatty acid-derived acetyl-CoA. KBs are subsequently transported to
99 extrahepatic tissues for terminal oxidation in the tricarboxylic acid (TCA) cycle. KBs are
100 metabolized to acetyl-CoA in a set of enzymatic reactions collectively called ketolysis.
101 Mitochondrial β OHB dehydrogenase (BDH1) converts D- β OHB to AcAc, which is in
102 turn converted to acetoacetyl-CoA (AcAc-CoA) by succinyl-CoA-3-oxaloacid CoA
103 transferase (SCOT, encoded by the *Oxct* genes) (**Fig. 1d**). AcAc-CoA is further
104 processed by thiolase acetyl-CoA acetyltransferase (ACAT) to generate two molecules
105 of acetyl-CoA, which can enter the TCA cycle (**Fig. 1d**). Gene expression analysis of
106 CD8⁺ T cells responding to *Lm* infection (29) revealed increased expression of mRNA
107 transcripts encoding several enzymes in the pathway (i.e., *Bdh1*, *Oxct1*, *Acat1/2*) in
108 Teff cells relative to Tn and Tmem cells (**Fig. 1d**). Proteomic analysis of CD8⁺ T cells
109 (30, 31) revealed a ~2-3-fold induction in BDH1 and SCOT in Teff cells following *in vitro*
110 activation (**Fig. S2a**), while Teff cells responding to *Lm* infection *in vivo* displayed

111 prominent increases in BDH1 expression (**Fig. S2b**). Thus, the transition from naïve to
112 effector T cell states is associated with increased expression of ketolytic enzymes.

113 We next asked if KBs could exert direct impact on CD8⁺ Teff cell function. CD8⁺
114 Teff cells activated in the presence of D-βOHB (5 mM) displayed increased capacity to
115 produce IFN-γ, reflected by both increased numbers of IFN-γ-producing cells and
116 increased IFN-γ protein expression per cell (as determined by increased mean
117 fluorescence intensity (MFI) of IFN-γ staining) (**Fig. 1e**). We recently reported that the
118 presence of physiologic carbon sources (PCS) enhances CD8⁺ T cell effector function,
119 including IFN-γ and granzyme production (32). Removing βOHB from PCS-
120 supplemented cell culture medium reduced IFN-γ production by CD8⁺ T cells, while
121 addition of βOHB alone was sufficient to increase IFN-γ levels, suggesting that βOHB
122 was the active metabolite in PCS acting to enhance IFN-γ production (**Fig. S3a**). Next,
123 we assessed the function of CD8⁺ T cells lacking BDH1 expression (via crossing of
124 *Bdh1*-floxed mice (33) to *Cd4-Cre* transgenic mice to generate conditional deletion of
125 BDH1 in mature T cells). BDH1-deficient (*Bdh1KO*) CD8⁺ Teff cells displayed reduced
126 IFN-γ production relative to control cells following *in vitro* activation (**Fig. S3b**), and
127 BDH1 was required for the βOHB-driven increase in IFN-γ production by CD8⁺ Teff
128 cells (**Fig. 1f**). In addition, *Bdh1KO* T cells displayed a 30-40% reduction in their ability
129 to lyse MC38 tumor cells (**Fig. 1g**), implicating ketolysis as a regulator of cytolytic
130 capacity.

131 Finally, we assessed the role of cell-intrinsic ketolysis in CD8⁺ Teff cell function
132 *in vivo* following infection with *Lm*-OVA, which induces robust expansion of IFN-γ-

133 producing CD8⁺ T cells (34). Given that both βOHB and AcAc are present in circulation
134 (35) and that either metabolite may mediate the effects of KBs on T cell function, we
135 generated ketolysis-deficient T cells via shRNA-mediated silencing of *Oxct1* in Bdh1KO
136 T cells (validation in **Fig. S4a**). OVA-specific OT-I⁺ CD8⁺ T cells (Control or Bdh1KO)
137 were transduced with control (*shFF*, targeting firefly luciferase) or *Oxct1*-targeting
138 retroviral vectors, transferred into congenic hosts, followed by infection with *Lm*-OVA.
139 CD8⁺ Teff cell responses were then analyzed 7 days post infection (dpi) (**Fig. S4b-c**).
140 Ketolysis-deficient OT-I T cells displayed increased expansion *in vivo* (**Fig. S4d**) but no
141 major change in effector or memory precursor subsets (**Fig. S4e**). However, similar to
142 our observation with *in vitro*-activated T cells (**Fig. 1f, S3b**), ketolysis-deficient CD8⁺ T
143 cells displayed lower IFN- γ production *in vivo* compared to control cells (**Fig. 1h**). To
144 identify the molecular mechanisms by which ketolysis drives CD8⁺ T cell effector
145 function, we performed transcriptomics on control and ketolysis-deficient OT-I T cells
146 isolated from the spleens of *Lm*-OVA-infected mice 7 dpi (**Table S2, Fig. S5a**).
147 Pathway analysis revealed enrichment of proliferative programs (i.e., cell cycle, E2F
148 targets)—consistent with increased expansion of ketolysis-deficient OT-I cells *in vivo*
149 (**Fig. S4d**)—and a loss of inflammatory signatures (i.e., IFN- α /IFN- γ response, allograft
150 rejection) in ketolysis-deficient T cells (**Fig. S5b-c**). Strikingly, gene set enrichment
151 analysis (GSEA) using gene signatures derived from single cell profiling of human T
152 cells (**Table S3**) (36) revealed a loss of the CD8⁺ cytotoxicity signature in ketolysis-
153 deficient T cells (**Fig. 1i**). Collectively, these findings suggest that cell-intrinsic ketolysis
154 is required for the development of CD8⁺ T cell functional programs.

155 Given that KBs play critical roles in organismal energy homeostasis, including
156 serving as oxidative fuels during states of low nutrient availability (26), we next
157 questioned whether KBs could function as a fuel source for CD8⁺ T cells. Using ¹³C-
158 based metabolic tracers, we found that D-[U-¹³C₄] β OHB was readily imported by
159 proliferating CD8⁺ Teff cells, saturating the intracellular β OHB pool within 2 minutes of
160 exposure (**Fig. 2a, S6a**). While previous work suggests that Tmem cells can undergo
161 ketogenesis (37), rapid and near complete ¹³C-enrichment of the β OHB pool from [U-
162 ¹³C₄] β OHB suggests the absence of ketogenesis from endogenous substrates in Teff
163 cells, which would dilute the total β OHB pool with unlabeled β OHB. Furthermore, as
164 expected, we did not observe contribution of [U-¹³C₆]glucose to intracellular β OHB in
165 Teff cells (**Fig. 2a**). CD8⁺ T cells display plasticity in metabolic fuel choice, which allows
166 them to operate and maintain effector function in diverse metabolic environments (5).
167 To compare the contribution of KB-derived carbon with other carbon sources for TCA
168 cycle metabolism, we cultured CD8⁺ Teff cells in the presence of ¹³C-labelled
169 substrates at concentrations commonly observed in plasma (38, 39) and assessed
170 contribution to TCA cycle-derived metabolites. Of all ¹³C-labeled substrates, [U-
171 ¹³C₄] β OHB-derived carbon contributed the highest enrichment to TCA cycle
172 intermediates (**Fig. 2b**), contributing to the citrate (M+2) pool 5-fold more effectively
173 than [U-¹³C₆]glucose (**Fig. S6b**), indicating that β OHB could contribute to TCA cycle
174 metabolism even under nutrient-replete conditions.

175 We continued our *in vitro* studies using KBs at 2 mM (unless stated otherwise) to
176 mimic the concentration of circulating β OHB achieved with therapeutic ketogenic diets

177 (40, 41). Both [$U-^{13}C_4$]βOHB and [$U-^{13}C_4$]AcAc significantly contributed to citrate and
178 malate synthesis even in the presence of physiologic glucose levels (5 mM) (**Fig. 2c**).
179 Of note, the ratio of malate (M+2) to citrate (M+2) labeling from ^{13}C -βOHB was
180 approximately 0.5, indicative of “non-canonical” TCA cycle activity (i.e., the citrate-
181 malate shuttle) (42) and potential use of citrate for other reactions beyond the TCA
182 cycle (i.e., export to the cytosol for acetyl-CoA production). To assess direct
183 competition between glucose and βOHB for TCA cycle metabolism, we designed a co-
184 labeling experiment where activated CD8⁺ Teff cells were cultured with [$U-^{13}C_6$]glucose
185 and [2,4- $^{13}C_2$]βOHB. In this setting, metabolic intermediates generated during the first
186 turn of the TCA cycle would be labelled M+1 from βOHB and M+2 from glucose (**Fig.**
187 **2d**). As expected, ^{13}C -labeled lactate (M+3) was generated exclusively from [$U-$
188 $^{13}C_6$]glucose and not [2,4- $^{13}C_2$]βOHB. (**Fig. 2d**); however, βOHB contributed
189 approximately 50% more carbon to the synthesis of TCA cycle
190 intermediates—particularly citrate—compared to glucose, despite being at lower molar
191 concentration (**Fig. 2d**). These observations are highly analogous to macrophages that
192 preferentially use AcAc over glucose for TCA cycle metabolism (35). Collectively, these
193 data confirm that KBs are metabolic substrates for CD8⁺ T cells, and that βOHB is
194 preferentially used by T cells over glucose to supply TCA cycle metabolism, even under
195 nutrient-replete conditions in which glucose is abundant.

196 Given the contribution of βOHB and AcAc to TCA cycle metabolism, we next
197 examined the impact of KB metabolism on T cell bioenergetics. Short-term exposure
198 (2h) of activated CD8⁺ Teff cells to either βOHB (**Fig. 2e**) or AcAc (**Fig. 2f**) modestly

199 increased their basal oxygen consumption rate (OCR) and ATP production from
200 OXPHOS (**Fig. S6c**). However, the maximal respiratory rate of CD8⁺ T cells was greatly
201 increased by KBs (2-fold and 1.5-fold increases for β OHB and AcAc, respectively),
202 which corresponded to a significant increase in their maximal ATP production rate from
203 OXPHOS (**Fig. 2e-f**). Collectively, these data indicate that KBs directly augment
204 mitochondrial ATP production by boosting maximal respiratory capacity.

205 Finally, we used *in vivo* ^{13}C metabolite infusions to evaluate whether T cells use
206 KBs as a fuel source *in vivo*. We transferred Thy1.1⁺ OT-I CD8⁺ T cells into congenic
207 recipient mice, infected the mice with *Lm*-OVA one day later, and then infused the mice
208 for 2 h at 2 dpi (during proliferative expansion) or 6 dpi (peak of T cell response) with D-
209 [$^{13}\text{C}_4$] β OHB or [U- $^{13}\text{C}_6$]glucose prior to CD8⁺ T cell isolation and metabolomic analysis
210 (**Fig. 2g**) (43, 44). With our [U- $^{13}\text{C}_4$] β OHB infusion strategy, we achieved ~75%
211 enrichment of fully labeled (M+4) ^{13}C -labeled β OHB in circulation at both 2 and 6 dpi
212 (**Fig. S7a-b**), with β OHB plasma levels achieving similar levels as observed in ketogenic
213 diet settings (~2 mM). Moreover, we detected M+4 ^{13}C - β OHB in *Lm*-OVA-specific CD8⁺
214 T cells after only 2 h of infusion (**Fig. S7b**), corresponding to ~80-85% labelling of the
215 total β OHB pool (**Fig. 2h**), indicating rapid import of β OHB by CD8⁺ Teff cells *in vivo*.
216 While we achieved good enrichment (~40%) of fully labeled (M+6) ^{13}C -glucose in the
217 circulating glucose pool (**Fig. S7c**), we observed no contribution of glucose to β OHB
218 production in T cells (**Fig. 2h**) or circulating β OHB levels in plasma (**Fig. S7d**). Similar to
219 our observations *in vitro* (**Fig. 2d**), infused [U- $^{13}\text{C}_4$] β OHB readily labeled TCA cycle and
220 TCA cycle-derived metabolites in *Lm*-OVA-specific CD8⁺ T cells *in vivo* (**Fig. 2i**).

221 Higher-order labeling patterns for [$U-^{13}C_4$]βOHB in citrate (i.e., M+3, M+4) suggests that
222 βOHB carbon contributed to several turns of the TCA cycle within the short 2 h infusion
223 period (**Fig. 2j**). Finally, we observed several key trends when we compared *in vivo*
224 utilization of [$U-^{13}C$]βOHB to [$U-^{13}C_6$]glucose by CD8⁺ T cells. When normalized to
225 circulating infusion rates, both βOHB and glucose displayed similar contribution of ¹³C-
226 carbon to the TCA cycle (M+2 citrate, malate and aspartate) during early T cell
227 expansion *in vivo* (2 dpi); however, while glucose utilization by T cells declined by the
228 peak of the T cell response to *Lm*-OVA (6 dpi), [¹³C₄]βOHB still readily labeled
229 intermediates of the TCA cycle (**Fig. S7e-f**), suggesting preferred use of βOHB as a
230 TCA cycle fuel during the peak effector phase of the T cell response. Collectively, these
231 data indicate that βOHB is utilized as a preferred fuel by CD8⁺ Teff cells both *in vitro*
232 and *in vivo* during an active immune response, even when glucose is present.

233 Given our results that ketolysis can fuel CD8⁺ T cell metabolism, we
234 hypothesized that the contribution of ketones to mitochondrial metabolism and acetyl-
235 CoA production may underlie the effects of KBs on T cell function. The bioenergetic
236 potential from KBs comes from both the dehydrogenation of βOHB to AcAc by BDH1,
237 which directly generates NADH, and the production of acetyl-CoA that can enter the
238 TCA cycle and fuel downstream ATP production by the electron transport chain (**Fig.**
239 **3a**). Consistent with this, we found that BDH1 was required for processing of [$U-$
240 ¹³C₄]βOHB but not [$U-^{13}C_4$]AcAc into citrate (**Fig. 3b**). As expected, targeting the
241 downstream ketolytic enzyme SCOT (via silencing of *Oxct1*) strongly diminished
242 production of citrate from both [$U-^{13}C_4$]βOHB and [$U-^{13}C_4$]AcAc (**Fig. 3c**). Surprisingly,

243 using a Seahorse Bioanalyzer, we observed that both the increased respiratory
244 capacity (**Fig. 3d**) and β OHB-induced boost in both basal and maximal ATP production
245 from OXPHOS (**Figs. 3e, S8**) was dependent on BDH1 but not on SCOT: β OHB was
246 still able to increase ATP production from OXPHOS in SCOT-deficient T cells (**Fig. 3e**).
247 Collectively, these data indicate that in CD8 $^{+}$ Teff cells BDH1 is a critical regulator of
248 both NADH production for OXPHOS and citrate synthesis for downstream metabolic
249 reactions.

250 We next used SCOT-knockdown T cells to identify the metabolic pathways
251 downstream of ketolysis in CD8 $^{+}$ T cells. Using the competitive [$U-^{13}C_6$]glucose and [$U-$
252 $2,4-^{13}C_2$] β OHB labeling strategy outlined in **Fig. 2d**, we observed loss of β OHB
253 incorporation into TCA cycle intermediates, as well as acetyl-CoA (M+1) and
254 downstream M+1 acetylated metabolites (i.e., Ac-spermidine, Ac-methionine) in
255 *shOxct1*-expressing T cells (**Fig. S9**), mechanistically linking ketolysis to acetyl-CoA
256 production in T cells. To further characterize the metabolic substrates contributing to
257 acetyl-CoA production, we cultured activated CD8 $^{+}$ T cells in the presence of
258 competitive uniformly ^{13}C -labeled fuels at their physiologic concentrations (as in **Fig.**
259 **2b**) (32). Strikingly, over 50% of the intracellular acetyl-CoA (M+2) pool was derived
260 from [$U-^{13}C_4$] β OHB, compared to less than 10% from [$U-^{13}C_6$]-glucose, in CD8 $^{+}$ T cells
261 (**Fig. 3f**). The production of acetyl-CoA from β OHB was dependent on cell-intrinsic
262 ketolysis, as [$U-^{13}C_4$] β OHB-dependent acetyl-CoA M+2 synthesis was decreased in
263 both Bdh1KO and ketolysis-deficient (Bdh1KO plus SCOT-knockdown) T cells (**Figs.**
264 **3f, S10a**).

265 Further analysis revealed that [$U-^{13}C_4$] β OHB-dependent acetylation of
266 intracellular metabolites, including Ac-carnitine, were significantly reduced in both
267 Bdh1KO (**Fig. 3g**) and SCOT-knockdown (**Fig. S10b**) CD8 $^+$ T cells. We also observed
268 significant levels of [$U-^{13}C_4$] β OHB-derived Ac-carnitine (M+2) in CD8 $^+$ T cells from *Lm*-
269 OVA-infected animals infused with [$U-^{13}C_4$] β OHB at 6 dpi (**Fig. S10c**), providing
270 evidence that β OHB is used to generate acetyl-CoA in T cells *in vivo*. Finally, we
271 examined whether β OHB was used for *de novo* fatty acid synthesis, one of the critical
272 biosynthetic pathways supported by cytosolic acetyl-CoA in proliferating cells (45, 46).
273 Consistent with the high contribution of β OHB to the acetyl-CoA pool in T cells (**Fig.**
274 **3f**), we found that [$U-^{13}C_4$] β OHB carbon was incorporated into the fatty acid palmitate
275 in a BDH1-dependent manner (**Fig. 3h**) and that [$U-^{13}C_4$] β OHB was preferred over [$U-$
276 $^{13}C_6$] β glucose for lipid synthesis in T cells (**Fig. S11**). Collectively, these results establish
277 β OHB as a major, and previously unappreciated, substrate for acetyl-CoA synthesis in
278 CD8 $^+$ T cells.

279 We next investigated the mechanistic impact of β OHB-dependent acetyl-CoA
280 production on CD8 $^+$ Teff cell function. Consistent with the effect of β OHB boosting
281 IFN- γ production by CD8 $^+$ T cells (**Figs. 1e-f**), we found that β OHB-treated CD8 $^+$ T cells
282 displayed increased IFN- γ mRNA compared to controls, which required expression of
283 BDH1 (**Fig. 4a**). Ketolysis-deficient CD8 $^+$ T cells cultured *in vitro* displayed reduced
284 expression of mRNAs coding for IFN- γ and the cytotoxic protein granzyme B (GzMB)
285 (**Fig. 4b**). Similarly, ketolysis-deficient CD8 $^+$ Teff cells responding to *Lm*-OVA at 7 dpi
286 displayed reduced expression of several effector-associated genes (i.e., *Gzmk*, *Gzma*,

287 *Cx3cr1, Klr1g1*) (**Fig. 4c**), correlating with their reduced cytotoxicity signature *in vivo*
288 (**Fig. 1i**). Together, these data indicate that KBs impact Teff cell function in part through
289 transcriptional regulation of effector genes.

290 Chromatin remodeling following T cell activation helps to stabilize effector gene
291 expression and reinforce CD8⁺ T cell effector functions (18, 24). Histone lysine (K)
292 acetylation—specifically acetylation of lysine 27 on histone H3 (H3K27Ac)—is
293 associated with chromatin accessibility and transcriptional activation (47), and is
294 important for effector gene expression by CD8⁺ T cells (48). By immunoblotting of
295 isolated histones, we observed reduced global histone H3 lysine acetylation in
296 activated ketolysis-deficient CD8⁺ T cells (**Fig. 4d**). In addition, treatment of *in vitro*-
297 activated CD8⁺ T cells with β OHB (5mM) was sufficient to globally increase histone H3
298 acetylation at several lysine residues compared to controls, including H3K14Ac and
299 H3K27Ac (**Fig. 4e, S12**). β OHB has been implicated in epigenetic programming
300 through its activity as a Class I histone deacetylase (HDAC) inhibitor or through direct
301 β -hydroxybutylation of histones (49, 50). However, the effect of β OHB on H3K27Ac
302 levels was reduced in Bdh1KO T cells (**Fig. 4f**), suggesting that ketolysis was directly
303 driving changes in histone acetylation.

304 Given the major contribution of β OHB to the acetyl-CoA pool in T cells (**Fig. 3f**),
305 we hypothesized that β OHB-derived acetyl-CoA was being used to acetylate histones.
306 To test this directly, we cultured *in vitro* activated CD8⁺ T cells with [U-¹³C₄] β OHB and
307 measured the enrichment of ¹³C-labelled acetyl-groups on histone H3 via mass
308 spectrometry (**Fig. 4g-h**). Carbon from [U-¹³C₄] β OHB was enriched on histone H3K27 in

309 control but not BDH1-deficient T cells (**Fig. 4g**), establishing ketolysis as an obligate
310 step for β OHB-dependent H3K27 acetylation in CD8 $^{+}$ T cells. We also observed
311 enrichment of [$^{13}\text{C}_4$] β OHB carbon in acetyl groups from multiple lysine residues on
312 histone H3 (K18, K23, K36, K115) and H4 (K5, K8, K12, K16) in control but not BDH1-
313 deficient T cells (**Fig. 4h**). Next, we used quantitative chromatin immunoprecipitation
314 coupled to next generation sequencing (ChIP-seq) (51) to assess the impact of BDH1-
315 deficiency on H3K27Ac modifications in T cells at genome-scale resolution. Overall, we
316 observed a global reduction of H3K27Ac levels in activated BDH1-knockout T cells
317 compared to controls (**Fig. S13a**). Notably, we observed reduced levels of H3K27Ac at
318 the promoters of effector gene loci—including *Ifng*, *Gzmb*, *Prf1*, and *Tbx21* (*Tbet*)—in
319 BDH1-deficient T cells compared to controls (**Fig. 4i, S13b**). Interestingly, H3K27Ac
320 enrichment at genetic loci associated with CD8 $^{+}$ T cell identity and signal transduction
321 (i.e., *Cd3e*, *Cd8a*, *Zap70*) were similar between control and BDH1-deficient T cells (**Fig.**
322 **S13b**). Collectively, we conclude from these data that KB-dependent acetyl-CoA
323 production supports CD8 $^{+}$ T cell effector function in part by directly altering
324 acetylation-dependent epigenetic programming at effector gene loci.

325 Infection-induced anorexia and increased KB production are host metabolic
326 adaptations to pathogen challenge with poorly defined impact on immune responses.
327 Here, we identify cell-intrinsic ketolysis as a non-redundant metabolic feature of CD8 $^{+}$
328 T cells that boosts effector function by altering T cell metabolic and epigenetic
329 programming. Our data establish KBs as potent bioenergetic substrates for CD8 $^{+}$ T
330 cells—preferred over glucose for oxidation—that increase mitochondrial respiratory

331 capacity and fuel TCA cycle-dependent biosynthesis both *in vitro* and *in vivo*. We
332 speculate that ketolysis evolved as a metabolic program to preserve T cell effector
333 function during periods of starvation or inflammation-driven changes in feeding
334 behavior (52). While immunomodulatory effects of KBs on immune cell populations
335 have been documented (17, 35, 53–55), our results point towards ketolysis-driven
336 acetyl-CoA synthesis as a key mechanism underlying the immunomodulatory effects
337 β OHb on T cell function. We establish that β OHb is a major source of acetyl-CoA
338 production in CD8 $^{+}$ T cells, which directly contributes to histone acetylation, and
339 promotes permissive H3K27 acetylation at effector gene loci (i.e., *Ifng*, *Gzmb*, *Tbx21*) in
340 activated CD8 $^{+}$ T cells. Our data highlight ketolysis as a potential target for advancing T
341 cell-based immunotherapies. Such therapeutic manipulation could be achieved by
342 altering systemic concentrations of circulating KBs through the diet (i.e., ketogenic
343 diet, ketone ester supplementation) (56, 57), and may benefit CD8 $^{+}$ T cell function when
344 conventional fuels such as glucose are limiting.

345

346 **References and Notes**

347 1. M. A. Williams, M. J. Bevan, Effector and memory CTL differentiation. *Annual
348 Review of Immunology* (2007), ,
349 doi:10.1146/annurev.immunol.25.022106.141548.

350 2. M. Philip, A. Schietinger, CD8+ T cell differentiation and dysfunction in cancer.
351 *Nature Reviews Immunology*. **22**, 209–223 (2022).

352 3. E. L. Pearce, M. C. Poffenberger, C. H. Chang, R. G. Jones, Fueling immunity:
353 Insights into metabolism and lymphocyte function. *Science* (1979) (2013), ,
354 doi:10.1126/science.1242454.

355 4. L. A. J. O'Neill, R. J. Kishton, J. Rathmell, A guide to immunometabolism for
356 immunologists. *Nature Reviews Immunology* (2016), , doi:10.1038/nri.2016.70.

357 5. N. M. Chapman, H. Chi, Metabolic adaptation of lymphocytes in immunity and
358 disease. *Immunity*. **55**, 14–30 (2022).

359 6. M. D. Buck, R. T. Sowell, S. M. Kaech, E. L. Pearce, Metabolic Instruction of
360 Immunity. *Cell* (2017), , doi:10.1016/j.cell.2017.04.004.

361 7. N. J. MacIver, J. C. Rathmell, *Curr Opin Immunol*, in press,
362 doi:10.1016/J.COI.2017.06.002.

363 8. C.-H. H. Chang, J. D. Curtis, L. B. Maggi Jr., B. Faubert, A. v Villarino, D.
364 O'Sullivan, S. C.-C. Huang, G. J. W. van der Windt, J. Blagih, J. Qiu, J. D. Weber,
365 E. L. E. J. L. J. Pearce, R. G. Jones, E. L. E. J. L. J. Pearce, L. B. Maggi, B.
366 Faubert, A. v Villarino, D. O'Sullivan, S. C.-C. Huang, G. J. W. van der Windt, J.
367 Blagih, J. Qiu, J. D. Weber, E. L. E. J. L. J. Pearce, R. G. Jones, E. L. E. J. L. J.

368 Pearce, E. L. E. J. L. J. Pearce, Posttranscriptional control of T cell effector
369 function by aerobic glycolysis. *Cell.* **153**, 1239–1251 (2013).

370 9. R. I. Klein Geltink, J. Edwards-Hicks, P. Apostolova, D. O’Sullivan, D. E. Sanin, A.
371 E. Patterson, D. J. Puleston, N. A. M. Lighthart, J. M. Buescher, K. M. Grzes, A. M.
372 Kabat, M. Stanczak, J. D. Curtis, F. Hässler, F. M. Uhl, M. Fabri, R. Zeiser, E. J.
373 Pearce, E. L. Pearce, Metabolic conditioning of CD8+ effector T cells for adoptive
374 cell therapy. *Nature Metabolism* (2020), doi:10.1038/s42255-020-0256-z.

375 10. J. Blagih, F. Coulombe, E. E. Vincent, F. Dupuy, G. Galicia-Vazquez, E.
376 Yurchenko, T. C. Raissi, G. J. W. van der Windt, B. Viollet, E. L. Pearce, J.
377 Pelletier, C. A. Piccirillo, C. M. Krawczyk, M. Divangahi, R. G. Jones, G. Galicia-
378 Vázquez, E. Yurchenko, T. C. Raissi, G. J. W. van der Windt, B. Viollet, E. L.
379 Pearce, J. Pelletier, C. A. Piccirillo, C. M. Krawczyk, M. Divangahi, R. G. Jones,
380 The energy sensor AMPK regulates T cell metabolic adaptation and effector
381 responses in vivo. *Immunity.* **42**, 41–54 (2015).

382 11. C. M. Cham, G. Driessens, J. P. O’Keefe, T. F. Gajewski, Glucose deprivation
383 inhibits multiple key gene expression events and effector functions in CD8+ T
384 cells. *European Journal of Immunology* (2008), doi:10.1002/eji.200838289.

385 12. J. S. Ayres, Immunometabolism of infections. *Nature Reviews Immunology*
386 (2020), doi:10.1038/s41577-019-0266-9.

387 13. C. Wilhelm, J. Surendar, F. Karagiannis, Enemy or ally? Fasting as an essential
388 regulator of immune responses. *Trends in Immunology.* **42**, 389–400 (2021).

389 14. H. Baazim, M. Schweiger, M. Moschinger, H. Xu, T. Scherer, A. Popa, S. Gallage,
390 A. Ali, K. Khamina, L. Kosack, B. Vilagos, M. Smyth, A. Lercher, J. Friske, D.

391 Merkler, A. Aderem, T. H. Helbich, M. Heikenwälder, P. A. Lang, R. Zechner, A.

392 Bergthaler, CD8+ T cells induce cachexia during chronic viral infection. *Nature*

393 *Immunology* (2019), doi:10.1038/s41590-019-0397-y.

394 15. A. Wang, S. C. Huen, H. H. Luan, S. Yu, C. Zhang, J. D. Gallezot, C. J. Booth, R.

395 Medzhitov, Opposing Effects of Fasting Metabolism on Tissue Tolerance in

396 Bacterial and Viral Inflammation. *Cell.* **166**, 1512-1525.e12 (2016).

397 16. A. Lercher, A. Bhattacharya, A. M. Popa, M. Caldera, M. F. Schlapansky, H.

398 Baazim, B. Agerer, B. Gürtl, L. Kosack, P. Májek, J. S. Brunner, D. Vitko, T.

399 Pinter, J. W. Genger, A. Orlova, N. Pikor, D. Reil, M. Ozsvár-Kozma, U. Kalinke,

400 B. Ludewig, R. Moriggl, K. L. Bennett, J. Menche, P. N. Cheng, G. Schabbauer,

401 M. Trauner, K. Klavins, A. Bergthaler, Type I Interferon Signaling Disrupts the

402 Hepatic Urea Cycle and Alters Systemic Metabolism to Suppress T Cell

403 Function. *Immunity* (2019), doi:10.1016/j.immuni.2019.10.014.

404 17. F. Karagiannis, K. Peukert, L. Surace, M. Michla, F. Nikolka, M. Fox, P. Weiss, C.

405 Feuerborn, P. Maier, S. Schulz, B. Al, B. Seeliger, T. Welte, S. David, I.

406 Grondman, A. H. de Nooijer, P. Pickkers, J. Lukas Kleiner, M. Moritz Berger, T.

407 Brenner, C. Putensen, B. COVIMMUNE Consortium, H. Kato, N. Garbi, M. G.

408 Netea, K. Hiller, K. Placek, C. Bode, C. Wilhelm, Impaired ketogenesis ties

409 metabolism to T cell dysfunction in COVID-19 (2022), doi:10.1038/s41586-022-

410 05128-8.

411 18. A. N. Henning, R. Roychoudhuri, N. P. Restifo, Epigenetic control of CD8+ T'cell

412 differentiation. *Nature Reviews Immunology.* **18**, 340–356 (2018).

413 19. S. M. Gray, S. M. Kaech, M. M. Staron, The interface between transcriptional and
414 epigenetic control of effector and memory CD8+ T-cell differentiation.
415 *Immunological Reviews*. **261**, 157–168 (2014).

416 20. S. M. Kaech, W. Cui, Transcriptional control of effector and memory CD8+ T cell
417 differentiation. *Nature Reviews Immunology* (2012), , doi:10.1038/nri3307.

418 21. K. Man, S. S. Gabriel, Y. Liao, R. Gloury, S. Preston, D. C. Henstridge, M.
419 Pellegrini, D. Zehn, F. Berberich-Siebelt, M. A. Febbraio, W. Shi, A. Kallies,
420 Transcription Factor IRF4 Promotes CD8+ T Cell Exhaustion and Limits the
421 Development of Memory-like T Cells during Chronic Infection. *Immunity*. **47**,
422 1129-1141.e5 (2017).

423 22. M. Philip, L. Fairchild, L. Sun, E. L. Horste, S. Camara, M. Shakiba, A. C. Scott,
424 A. Viale, P. Lauer, T. Merghoub, M. D. Hellmann, J. D. Wolchok, C. S. Leslie, A.
425 Schietinger, Chromatin states define tumour-specific T cell dysfunction and
426 reprogramming. *Nature*. **545**, 452–456 (2017).

427 23. K. E. Pauken, M. A. Sammons, P. M. Odorizzi, S. Manne, J. Godec, O. Khan, A.
428 M. Drake, Z. Chen, D. R. Sen, M. Kurachi, R. A. Barnitz, C. Bartman, B. Bengsch,
429 A. C. Huang, J. M. Schenkel, G. Vahedi, W. N. Haining, S. L. Berger, E. J.
430 Wherry, Epigenetic stability of exhausted T cells limits durability of reinvigoration
431 by PD-1 blockade. *Science* (1979). **354**, 1160–1165 (2016).

432 24. F. Franco, A. Jaccard, P. Romero, Y. R. Yu, P. C. Ho, Metabolic and epigenetic
433 regulation of T-cell exhaustion. *Nature Metabolism*. **2**, 1001–1012 (2020).

434 25. P. Puchalska, P. A. Crawford, Multi-dimensional Roles of Ketone Bodies in Fuel
435 Metabolism, Signaling, and Therapeutics. *Cell Metabolism*. **25**, 262–284 (2017).

436 26. P. Puchalska, P. A. Crawford, Metabolic and Signaling Roles of Ketone Bodies in
437 Health and Disease. *Annual Review of Nutrition*. **41**, 49–77 (2021).

438 27. F. C. George, Fuel metabolism in starvation. *Annu Rev Nutr*. **26**, 1–22 (2006).

439 28. H. bin Ruan, P. A. Crawford, Ketone bodies as epigenetic modifiers. *Current
440 Opinion in Clinical Nutrition and Metabolic Care*. **21**, 260–266 (2018).

441 29. J. A. Best, D. A. Blair, J. Knell, E. Yang, V. Mayya, A. Doedens, M. L. Dustin, A.
442 W. Goldrath, P. Monach, S. A. Shinton, R. R. Hardy, R. Jianu, D. Koller, J.
443 Collins, R. Gazit, B. S. Garrison, D. J. Rossi, K. Narayan, K. Sylvia, J. Kang, A.
444 Fletcher, K. Elpek, A. Bellemare-Pelletier, D. Malhotra, S. Turley, J. A. Best, V.
445 Jovic, D. Koller, T. Shay, A. Regev, N. Cohen, P. Brennan, M. Brenner, T.
446 Kreslavsky, N. A. Bezman, J. C. Sun, C. C. Kim, L. L. Lanier, J. Miller, B. Brown,
447 M. Merad, E. L. Gautier, C. Jakubzick, G. J. Randolph, F. Kim, T. N. Rao, A.
448 Wagers, T. Heng, M. Painter, J. Ericson, S. Davis, A. Ergun, M. Mingueneau, D.
449 Mathis, C. Benoist, Transcriptional insights into the CD8(+) T cell response to
450 infection and memory T cell formation. *Nat Immunol*. **14**, 404–412 (2013).

451 30. A. J. M. Howden, J. L. Hukelmann, A. Brenes, L. Spinelli, L. v. Sinclair, A. I.
452 Lamond, D. A. Cantrell, Quantitative analysis of T cell proteomes and
453 environmental sensors during T cell differentiation. *Nat Immunol*. **20**, 1542–1554
454 (2019).

455 31. E. H. Ma, M. J. Verway, R. M. Johnson, D. G. Roy, M. Steadman, S. Hayes, K. S.
456 Williams, R. D. Sheldon, B. Samborska, P. A. Kosinski, H. Kim, T. Griss, B.
457 Faubert, S. A. Condotta, C. M. Krawczyk, R. J. DeBerardinis, K. Marsh, M. J.
458 Richer, V. Chubukov, T. P. Roddy, R. G. Jones, K. M. Stewart, M. J. Richer, V.
459 Chubukov, T. P. Roddy, R. G. Jones, K. Marsh, M. J. Richer, V. Chubukov, T. P.
460 Roddy, R. G. Jones, Metabolic Profiling Using Stable Isotope Tracing Reveals
461 Distinct Patterns of Glucose Utilization by Physiologically Activated CD8+ T
462 Cells. *Immunity*. **51**, 856-870.e5 (2019).
463 32. I. Kaymak, K. M. Luda, L. R. Duimstra, E. H. Ma, J. Longo, M. S. Dahabieh, B.
464 Faubert, B. M. Oswald, M. J. Watson, S. M. Kitchen-Goosen, L. M. DeCamp, S.
465 E. Compton, Z. Fu, R. J. DeBerardinis, K. S. Williams, R. D. Sheldon, R. G.
466 Jones, Carbon source availability drives nutrient utilization in CD8+ T cells. *Cell
467 Metabolism* (2022), doi:10.1016/J.CMET.2022.07.012.
468 33. J. L. Horton, M. T. Davidson, C. Kurishima, R. B. Vega, J. C. Powers, T. R.
469 Matsuura, C. Petucci, E. D. Lewandowski, P. A. Crawford, D. M. Muoio, F. A.
470 Recchia, D. P. Kelly, The failing heart utilizes 3-hydroxybutyrate as a metabolic
471 stress defense. *JCI Insight*. **4** (2019), doi:10.1172/JCI.INSIGHT.124079.
472 34. M. D. Martin, S. A. Condotta, J. T. Harty, V. P. Badovinac, Population dynamics
473 of naive and memory CD8 T cell responses after antigen stimulations in vivo. *J
474 Immunol.* **188**, 1255–65 (2012).
475 35. P. Puchalska, S. E. Martin, X. Huang, J. E. Lengfeld, B. Daniel, M. J. Graham, X.
476 Han, L. Nagy, G. J. Patti, P. A. Crawford, Hepatocyte-Macrophage Acetoacetate
477 Shuttle Protects against Tissue Fibrosis. *Cell Metabolism*. **29**, 383-398.e7 (2019).

478 36. P. A. Szabo, H. M. Levitin, M. Miron, M. E. Snyder, T. Senda, J. Yuan, Y. L.
479 Cheng, E. C. Bush, P. Dogra, P. Thapa, D. L. Farber, P. A. Sims, Single-cell
480 transcriptomics of human T cells reveals tissue and activation signatures in
481 health and disease. *Nat Commun.* **10** (2019), doi:10.1038/S41467-019-12464-3.

482 37. H. Zhang, K. Tang, J. Ma, L. Zhou, J. Liu, L. Zeng, L. Zhu, P. Xu, J. Chen, K. Wei,
483 X. Liang, J. Lv, J. Xie, Y. Liu, Y. Wan, B. Huang, Ketogenesis-generated β -
484 hydroxybutyrate is an epigenetic regulator of CD8+ T-cell memory development.
485 *Nature Cell Biology.* **22**, 18–25 (2020).

486 38. J. R. Cantor, M. Abu-Remaileh, N. Kanarek, E. Freinkman, X. Gao, A. Louissaint,
487 C. A. Lewis, D. M. Sabatini, Physiologic medium rewires cellular metabolism and
488 reveals uric acid as an endogenous inhibitor of UMP synthase HHS Public
489 Access. *Cell.* **169**, 258–272 (2017).

490 39. S. M, I. S, N. K, T. M, S. H, S. T, MMMDB: Mouse Multiple Tissue Metabolome
491 Database. *Nucleic Acids Res.* **40** (2012), doi:10.1093/NAR/GKR1170.

492 40. E. C. Lien, A. M. Westermark, Y. Zhang, C. Yuan, Z. Li, A. N. Lau, K. M. Sapp, B.
493 M. Wolpin, M. G. vander Heiden, Low glycaemic diets alter lipid metabolism to
494 influence tumour growth. *Nature 2021* 599:7884. **599**, 302–307 (2021).

495 41. V. Gouirand, T. Gicquel, E. C. Lien, E. Jaune-Pons, Q. da Costa, P. Finetti, E.
496 Metay, C. Duluc, J. R. Mayers, S. Audebert, L. Camoin, L. Borge, M. Rubis, J.
497 Leca, J. Nigri, F. Bertucci, N. Dusetti, J. L. Iovanna, R. Tomasini, G. Bidaut, F.
498 Guillaumond, M. G. vander Heiden, S. Vasseur, Ketogenic HMG-CoA lyase and

its product β -hydroxybutyrate promote pancreatic cancer progression. *The EMBO Journal*. **41** (2022), doi:10.15252/embj.2021110466.

42. P. K. Arnold, B. T. Jackson, K. I. Paras, J. S. Brunner, M. L. Hart, O. J. Newsom, S. P. Alibekoff, J. Endress, E. Drill, L. B. Sullivan, L. W. S. Finley, A non-canonical tricarboxylic acid cycle underlies cellular identity. *Nature* 2022 **603**:7901. **603**, 477–481 (2022).

43. E. H. Ma, M. J. Verway, R. M. Johnson, D. G. Roy, M. Steadman, S. Hayes, K. S. Williams, R. D. Sheldon, B. Samborska, P. A. Kosinski, H. Kim, T. Griss, B. Faubert, S. A. Condotta, C. M. Krawczyk, R. J. DeBerardinis, K. Marsh, M. J. Richer, V. Chubukov, T. Roddy, R. G. Jones, Metabolic Profiling Using Stable Isotope Tracing Reveals Distinct Patterns of Glucose Utilization by Physiologically Activated CD8+ T Cells. *Immunity*. **51**, 856-870.e5 (2019).

44. R. D. Sheldon, E. H. Ma, L. M. DeCamp, K. S. Williams, R. G. Jones, Interrogating in vivo T-cell metabolism in mice using stable isotope labeling metabolomics and rapid cell sorting. *Nature Protocols* (2021), doi:10.1038/s41596-021-00586-2.

45. M. G. Heiden Vander, S. Y. Lunt, T. L. Dayton, B. P. Fiske, W. J. Israelsen, K. R. Mattaini, N. I. Vokes, G. Stephanopoulos, L. C. Cantley, C. M. Metallo, J. W. Locasale, Metabolic Pathway Alterations that Support Cell Proliferation. *Cold Spring Harbor Symposia on Quantitative Biology*. **76**, 325–334 (2011).

46. F. Röhrlig, A. Schulze, The multifaceted roles of fatty acid synthesis in cancer. *Nat Rev Cancer*. **16**, 732–749 (2016).

521 47. C. E. Barnes, D. M. English, S. M. Cowley, Acetylation & Co: an expanding
522 repertoire of histone acylations regulates chromatin and transcription. *Essays in*
523 *Biochemistry*. **63**, 97 (2019).

524 48. J. K. Northrop, R. M. Thomas, A. D. Wells, H. Shen, Epigenetic Remodeling of
525 the IL-2 and IFN - γ Loci in Memory CD8 T Cells Is Influenced by CD4 T Cells .
526 *The Journal of Immunology* (2006), doi:10.4049/jimmunol.177.2.1062.

527 49. Z. Xie, D. Zhang, D. Chung, Z. Tang, H. Huang, L. Dai, S. Qi, J. Li, G. Colak, Y.
528 Chen, C. Xia, C. Peng, H. Ruan, M. Kirkey, D. Wang, L. M. Jensen, O. K. Kwon,
529 S. Lee, S. D. Pletcher, M. Tan, D. B. Lombard, K. P. White, H. Zhao, J. Li, R. G.
530 Roeder, X. Yang, Y. Zhao, Metabolic Regulation of Gene Expression by Histone
531 Lysine β -hydroxybutyrylation. *Mol Cell*. **62**, 194 (2016).

532 50. T. Shimazu, M. D. Hirschey, J. Newman, W. He, K. Shirakawa, N. le Moan, C. A.
533 Grueter, H. Lim, L. R. Saunders, R. D. Stevens, C. B. Newgard, R. v. Farese, R.
534 de Cabo, S. Ulrich, K. Akassoglou, E. Verdin, Suppression of oxidative stress by
535 β -hydroxybutyrate, an endogenous histone deacetylase inhibitor. *Science* (1979).
536 **339**, 211–214 (2013).

537 51. B. M. Dickson, R. L. Tiedemann, A. A. Chomiak, E. M. Cornett, R. M. Vaughan, S.
538 B. Rothbart, A physical basis for quantitative ChIP-sequencing. *J Biol Chem*.
539 **295**, 15826–15837 (2020).

540 52. K. Troha, J. S. Ayres, Metabolic Adaptations to Infections at the Organismal
541 Level. *Trends in Immunology*. **41**, 113–125 (2020).

542 53. E. L. Goldberg, I. Shchukina, J. L. Asher, S. Sidorov, M. N. Artyomov, V. D. Dixit,
543 Ketogenesis activates metabolically protective $\gamma\delta$ T cells in visceral adipose
544 tissue. *Nat Metab.* **2**, 50–61 (2020).

545 54. Y. H. Youm, K. Y. Nguyen, R. W. Grant, E. L. Goldberg, M. Bodogai, D. Kim, D.
546 D'Agostino, N. Planavsky, C. Lupfer, T. D. Kanneganti, S. Kang, T. L. Horvath, T.
547 M. Fahmy, P. A. Crawford, A. Biragyn, E. Alnemri, V. D. Dixit, The ketone
548 metabolite β -hydroxybutyrate blocks NLRP3 inflammasome–mediated
549 inflammatory disease. *Nature Medicine* 2015 21:3. **21**, 263–269 (2015).

550 55. S. Hirschberger, L. Gellert, D. Effinger, M. Muenchhoff, M. Herrmann, J.-M.
551 Briegel, B. Zwißler, S. Kreth, Ketone Bodies Improve Human CD8+ Cytotoxic T-
552 Cell Immune Response During COVID-19 Infection. *Frontiers in Medicine*. **9**
553 (2022), doi:10.3389/FMED.2022.923502/FULL.

554 56. A. Kennedy, A high-fat, ketogenic diet induces a unique metabolic state in mice.
555 *Am. J. Physiol. Endocrinol. Metab.* **292**, E1724–E1739 (2007).

556 57. P. N. Thai, C. v. Miller, M. T. King, S. Schaefer, R. L. Veech, N. Chiamvimonvat,
557 D. M. Bers, E. N. Dedkova, Ketone Ester D- β -Hydroxybutyrate-(R)-1,3 Butanediol
558 Prevents Decline in Cardiac Function in Type 2 Diabetic Mice. *J Am Heart Assoc.*
559 **10** (2021), doi:10.1161/JAHA.120.020729.

560 58. S. A. Mookerjee, A. A. Gerencser, D. G. Nicholls, M. D. Brand, Quantifying
561 intracellular rates of glycolytic and oxidative ATP production and consumption
562 using extracellular flux measurements. *J Biol Chem.* **292**, 7189–7207 (2017).

563

564 **Acknowledgements**

565 **General:** We acknowledge Drs. Ralph DeBerardinis, Julian Lum, Sara Nowinski, and
566 members of the Jones and Krawczyk laboratories for scientific discussions
567 contributing to this manuscript. We thank Teresa Leone, Matthew Vos, Jeanie
568 Wedberg, and Michelle Minard for administrative assistance. We thank members of the
569 VAI Core Facilities (Metabolomics and Bioenergetics, Genomics, Bioinformatics and
570 Biostatistics, and Flow Cytometry) for technical assistance.

571 **Funding:** MJW is supported by a National Cancer Institute (NCI) T32 training grant
572 (T32CA251066-01A1). JL is supported by a Van Andel Institute (VAI) Metabolism &
573 Nutrition (MeNu) Program Pathway-to-Independence Award and Canadian Institutes of
574 Health Research (CIHR) Fellowship (MFE-181903). DPK is supported by the National
575 Institutes of Health (NIH, R01HL128349 and R01HL151345). CMK is supported by the
576 National Institute of Allergy and Infectious Diseases (NIAID, R21AI153997) and VAI.
577 FMB is a FRQS Senior scholar (award number 281824) and is supported by the
578 National Sciences and Engineering Research Council of Canada (RGPIN-2018-05414).
579 SBR is supported by the National Institute of General Medical Sciences (NIGMS,
580 R35GM124736) and VAI. PAC and PP are supported by the National Institute of
581 Diabetes and Digestive and Kidney Diseases (NIDDK, DK091538) and National Institute
582 on Aging (NIA, AG069781). RGJ is supported by the Paul G. Allen Frontiers Group
583 Distinguished Investigator Program, NIAID (R01AI165722), and VAI.
584 **Author Contributions:** Conceptualization, KML and RGJ; Experimental Design, KML,
585 EHM, DPK, PP, CMK, FMB, RDS, SBR, PAC, and RGJ; Investigation, KML, SMKG,

586 EHM, MJW, JL, LRD, BMO, AK, IK, LMD; Data Analysis, KML, SKMG, EHM, MJW, JL,
587 ZF, ZM, BMO, BMD, MJW, BF, KSW, RDS, and RGJ; Writing – Original Draft, KML,
588 KSW, and RGJ; Writing – Editing, KML, CMK, SBR, PAC, and RGJ; Visualization, ZF
589 and KSW; Supervision, RGJ; Funding Acquisition, RGJ.

590 **Competing Interests:** RGJ is a scientific advisor for Agios Pharmaceuticals and
591 Servier Pharmaceuticals and is a member of the Scientific Advisory Board of
592 Immunomet Therapeutics.

593 **Data and Materials Availability:**

594 RNA sequencing data used for meta analysis in Figure 1 are available at NCBI GEO
595 (accession numbers (GSE86881, GSE89307, GSE84820)). RNA sequencing data on
596 ketolysis-deficient T cells from **Figure 1** are available at NCBI GEO (accession:
597 GSE212048; token: clepicoczzcffop). The mass spectrometry proteomics data have
598 been deposited to the ProteomeXchange Consortium via the PRIDE partner repository
599 with the dataset identifier PXD036292 (access via username:
600 reviewer_pxd036292@ebi.ac.uk; password: sKA2ueMn). Bioenergetics data analysis
601 was based on protocols developed by Mookerjee and Brand (58) which is available for
602 download at <https://russelljoneslab.vai.org/tools>. All the data are available in the main
603 text or supplemental figures and tables. Additional information and request for
604 resources and reagents should be directed to and will be made available by the
605 corresponding author, Russell G. Jones (russell.jones@vai.org).

606

607 **Supplementary Materials:**

608 Materials and Methods

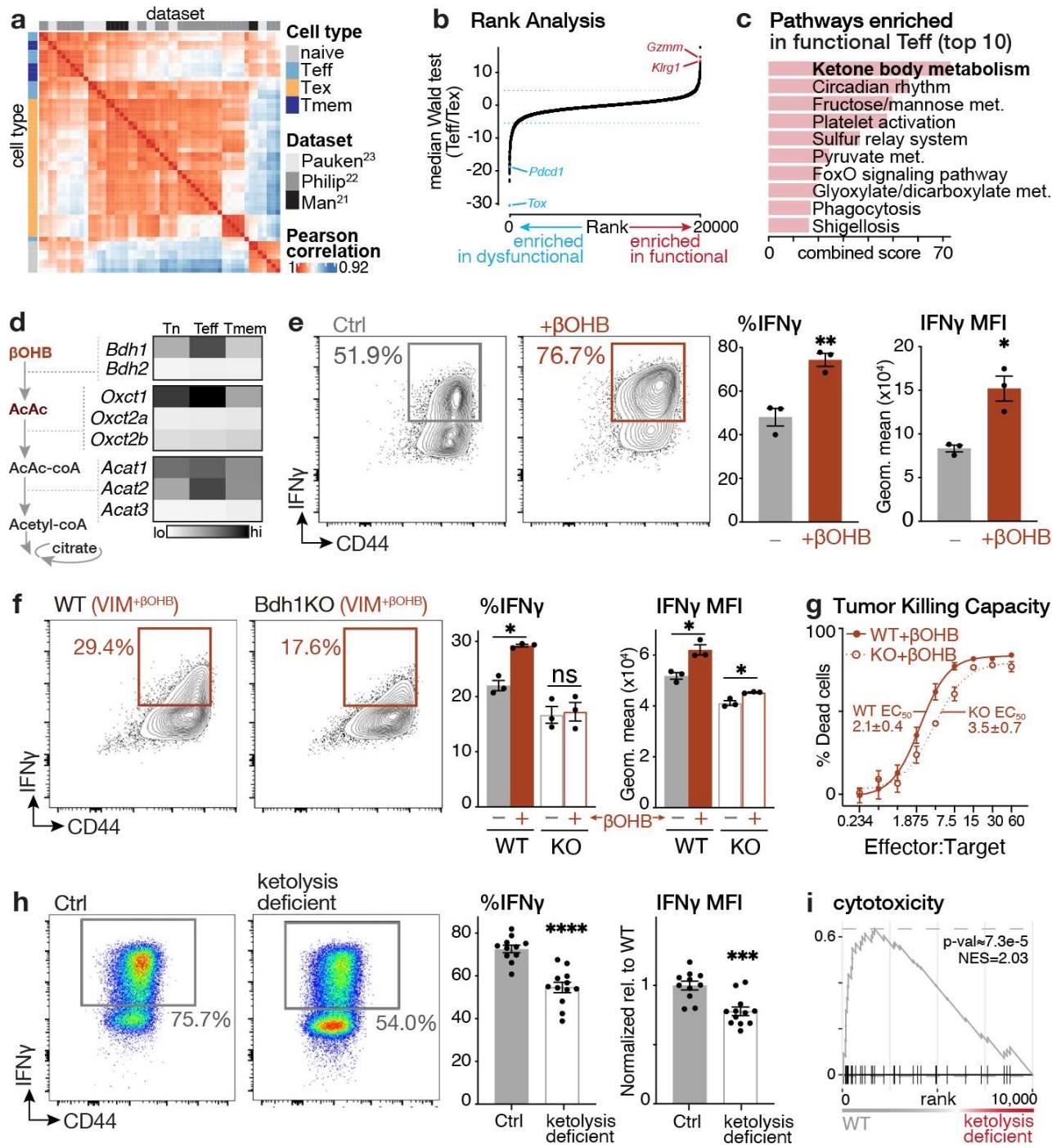
609 Figures S1 to S13

610 Table S4 to S5

611

612 **Other Supplementary Materials for this manuscript include the following:**

613 Tables S1 to S3 (.xlsx)



614

615

616 **Figure 1**

617

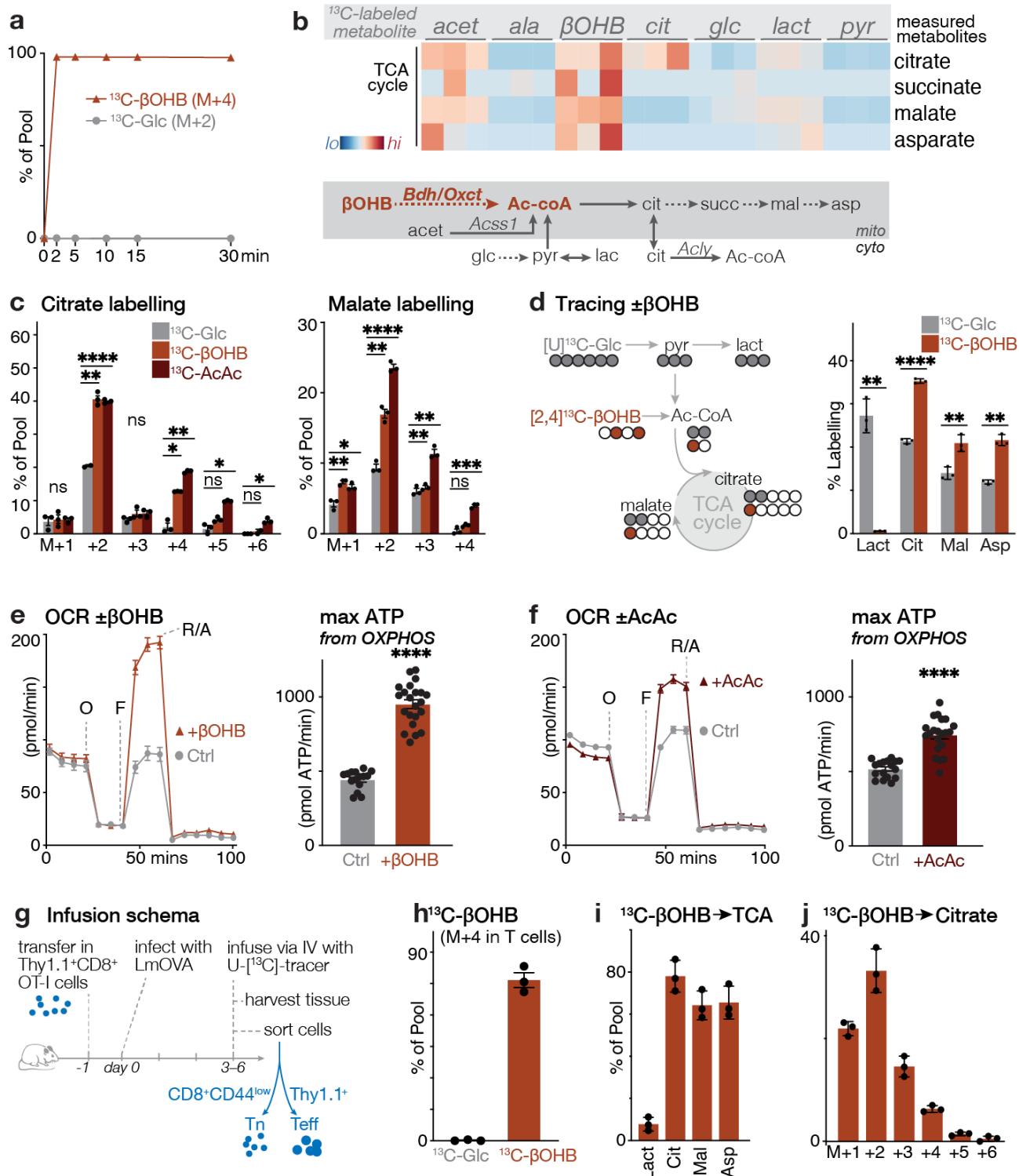
618

619 **Figure 1. Ketolysis is a metabolic feature of functional CD8⁺ effector T cells**

620 **(a)** Pearson correlation-driven similarity matrix analysis of gene expression profiles of
621 CD8⁺ T cell states (naïve, Teff – effector T cells, Tex – exhausted T cells, Tmem –
622 memory T cells). Analysis was conducted on RNA-seq datasets from three
623 independent studies characterizing gene expression profiles of antigen-specific CD8⁺ T
624 cells from acute infection (LCMV Armstrong, *Lm*), chronic infection (LCMV CL-13), and
625 cancer (hepatocellular carcinoma) models *in vivo*. **(b)** Rank analysis of genes enriched
626 in dysfunctional and in functional CD8⁺ T cell states. Median Wald test statistics for
627 DEG between Teff and Tex populations (Teff/Tex) were calculated based on cancer
628 and virus response datasets. **(c)** Pathway analysis of the top 10 KEGG pathways
629 enriched in functional Teff cells from (b). **(d)** Heatmap of ketolytic gene expression in
630 OT-I CD8⁺ naïve (Tn) or OT-I CD8⁺ Teff and Tmem Ocells triggered in response to *Lm*-
631 OVA infection (Teff, 2 dpi; Tmem, 30 dpi). A schematic of the enzymes involved in
632 ketolysis is shown. **(e)** IFN- γ production by CD8⁺ T cells cultured in IMDM in the
633 presence (+ β OHB) or absence (Ctrl) of 5 mM β OHB. *Left*, Representative flow
634 cytometry plots for CD44 versus IFN- γ expression by CD8⁺ T cells 3 days post
635 activation. *Right*, Bar graph showing the percentage of IFN- γ ⁺ T cells and the geometric
636 mean fluorescence intensity (MFI) for IFN- γ ⁺ T cells cultured with (+) or without (-)
637 β OHB. Data represent the mean \pm SEM (n=3). **(f)** IFN- γ production by wild type (WT)
638 and Bdh1-deficient (Bdh1KO) CD8⁺ T cells cultured in VIM containing or lacking 5 mM
639 β OHB. *Left*, Representative flow cytometry plots for CD44 versus IFN- γ expression by
640 WT and Bdh1KO CD8⁺ T cells cultured with β OHB 3 days post activation. *Right*, Bar

641 graph showing the percentage of IFN- γ ⁺ T cells and the geometric mean fluorescence
642 intensity (MFI) for IFN- γ ⁺ T cells cultured with (+) or without (-) β OHB. Data represent
643 the mean \pm SEM (n=3). (g) *In vitro* killing assay for WT and Bdh1KO T cells cultured in
644 the presence of β OHB. Shown is the percentage of dead MC38-OVA tumor cells after
645 48 h of co-culture with activated WT or Bdh1KO OT-I CD8⁺ cells at the indicated
646 effector:target (E:T) ratio. The E:T ratio required to kill 50% tumor cells (EC₅₀) for each
647 genotype is indicated (mean \pm SEM, n=3). (h) IFN- γ production by control (Ctrl) and
648 ketolysis-deficient OT-I CD8⁺ cells isolated from *Lm*-OVA-infected mice (7 dpi). *Left*,
649 Representative CD44 versus IFN- γ expression for control and ketolysis-deficient OT-I
650 cells. *Right*, Percentage of IFN- γ ⁺ OT-I T cells and MFI of IFN- γ expression (normalized
651 to Ctrl cells) for OT-I cells isolated from the spleen of *Lm*-OVA-infected mice 7 dpi
652 (mean \pm SEM, n=11-12/group). (i) Gene set enrichment analysis (GSEA) of CD8⁺ T cell
653 cytotoxicity genes in control versus ketolysis-deficient OT-I T cells responding to *Lm*-
654 OVA (7 dpi, n=3 biological replicates/group). Statistical significance and normalized
655 enrichment scores (NES) are indicated. *, p < 0.05; **, p < 0.01; ***, p < 0.001; ****, p <
656 0.0001; ns, not significant.

657



658

659 **Figure 2**

660

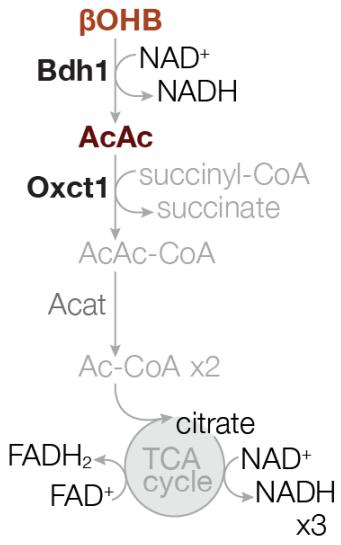
661 **Figure 2. Ketone bodies are physiologic fuels for CD8⁺ effector T cells**

662 **(a)** Timecourse of β OHB uptake and cell-intrinsic β OHB production from glucose in
663 activated CD8⁺ T cells. Shown is the contribution of ${}^1[{}^{13}\text{C}_4]\beta\text{OHB}$ (M+4, from exogenous
664 $[{}^{13}\text{C}_4]\beta\text{OHB}$) or $[\text{U}-{}^{13}\text{C}_6]\text{glucose}$ -derived β OHB (M+2) to the overall intracellular β OHB pool
665 over time (mean \pm SEM, n=3). **(b)** Heatmap representing relative contribution of ${}^{13}\text{C}$ from
666 indicated ${}^{13}\text{C}$ -labelled substrates into TCA cycle-derived metabolites. Shown is a
667 schematic depicting the contribution of different carbon sources to TCA cycle
668 metabolism, with enzymes and enzyme reactions localized to the cytosol and
669 mitochondrion indicated. **(c)** Mass isotopologue distribution (MID) for $[\text{U}-{}^{13}\text{C}_6]\text{glucose}$ -,
670 $[{}^{13}\text{C}_4]\beta\text{OHB}$ -, and $[{}^{13}\text{C}_4]\text{AcAc}$ -derived carbon into citrate and malate for activated CD8⁺
671 Teff cells after 2 h of culture (n=3/group). **(d)** Direct comparison of TCA cycle labeling
672 from β OHB and glucose using competitive tracing. Activated CD8⁺ Teff cells were
673 cultured in medium containing 5 mM $[\text{U}-{}^{13}\text{C}_6]\text{glucose}$ and 2 mM $[2,4-{}^{13}\text{C}_2]\beta\text{OHB}$ for 2 h
674 and the relative contribution of ${}^{13}\text{C}$ -label from $[\text{U}-{}^{13}\text{C}_6]\text{glucose}$ (M+2) or $[2,4-{}^{13}\text{C}_2]\beta\text{OHB}$
675 (M+1) to TCA cycle metabolite pools is shown (mean \pm SEM, n=3). Lactate labeling from
676 $[\text{U}-{}^{13}\text{C}_6]\text{glucose}$ (M+3) or $[2,4-{}^{13}\text{C}_4]\beta\text{OHB}$ (M+1) is shown as a control. **(e-f)** Bioenergetic
677 profile of *in vitro*-activated CD8⁺ T cells cultured with **(e)** 2 mM β OHB or **(f)** 2 mM AcAc
678 (mean \pm SEM, n=20–24/group). *Left*, OCR plots for activated CD8⁺ T cells over time for
679 β OHB and AcAc. Time of addition of oligomycin (O), fluoro-carbonyl cyanide
680 phenylhydrazone (FCCP, F), and rotenone and antimycin A (R/A) are indicated. *Right*,
681 maximal ATP production rates from OXPHOS following addition of β OHB and AcAc. T
682 cells that received no exogenous substrates (Ctrl) are indicated. **(g)** Schematic of

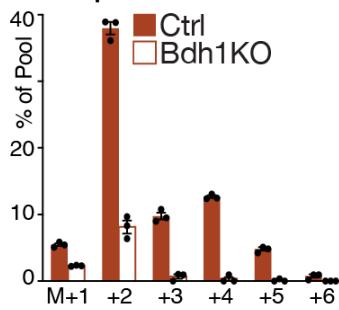
683 experimental set up for ^{13}C infusions in *Lm*-OVA-infected mice using $[^{13}\text{C}_4]\beta\text{OHB}$ and $[\text{U}-$
684 $^{13}\text{C}_6]\text{glucose}$. **(h)** Relative contribution of infused $[\text{U}-^{13}\text{C}_6]\text{glucose}$ or $[^{13}\text{C}_4]\beta\text{OHB}$ to the
685 intracellular βOHB (M+4) pool in *Lm*-OVA-specific OT-I T cells 6 dpi (mean \pm SEM, n =
686 3). ^{13}C metabolite enrichment was normalized relative to $[\text{U}-^{13}\text{C}_6]\text{glucose}$ (M+6) or
687 $[^{13}\text{C}_4]\beta\text{OHB}$ (M+4) levels in serum. **(i-j)** Enrichment of infused $[^{13}\text{C}_4]\beta\text{OHB}$ -derived carbon
688 into intracellular metabolites in *Lm*-OVA-specific OT-I T cells 6 dpi (mean \pm SEM, n = 3).
689 **(i)** Total ^{13}C enrichment from $[^{13}\text{C}_4]\beta\text{OHB}$ in lactate, citrate, malate, and aspartate. **(j)** MID
690 of $[^{13}\text{C}_4]\beta\text{OHB}$ -derived carbon in intracellular citrate. ^{13}C metabolite enrichment was
691 normalized relative to $[^{13}\text{C}_4]\beta\text{OHB}$ (M+4) levels in serum. *, $p < 0.05$; **, $p < 0.01$; ***, $p <$
692 0.001; ****, $p < 0.0001$; ns, not significant.

693

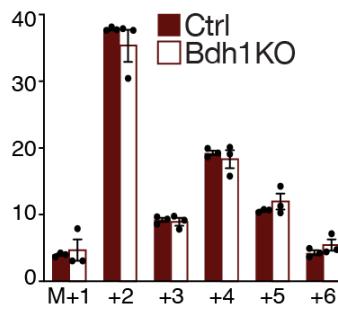
a Mitochondrial ketolysis



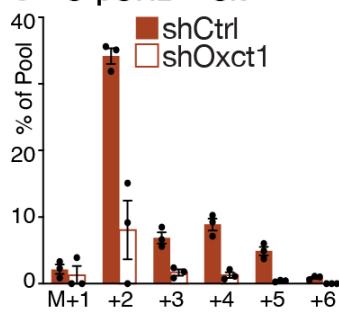
b ^{13}C - β BOHB \rightarrow Cit



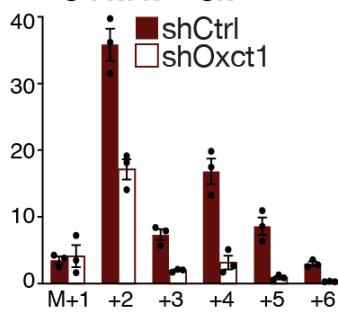
^{13}C -AcAc \rightarrow Cit



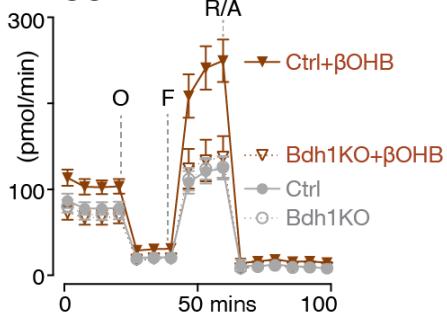
c ^{13}C - β BOHB \rightarrow Cit



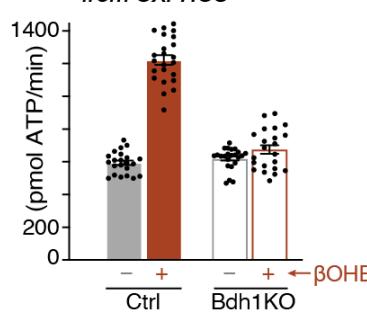
^{13}C -AcAc \rightarrow Cit



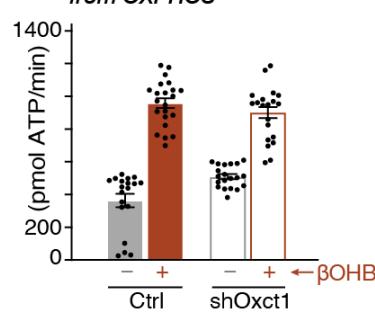
d OCR



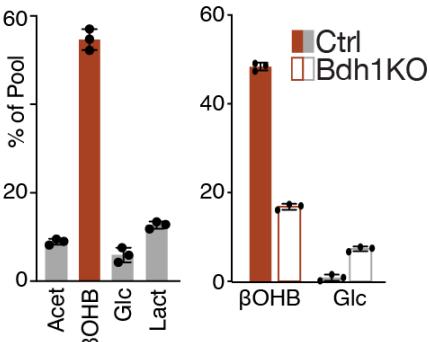
e max ATP from OXPHOS



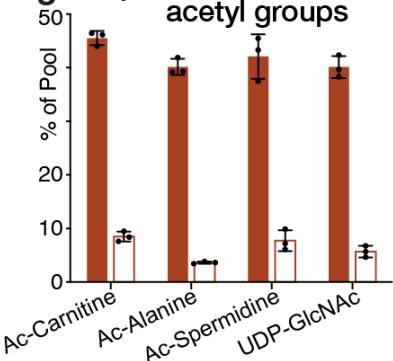
max ATP from OXPHOS



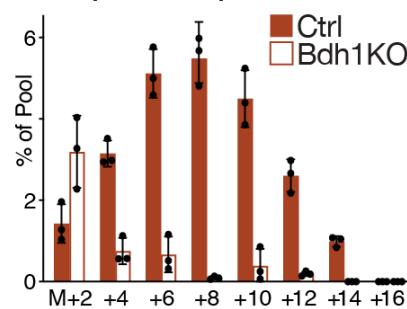
f ^{13}C -tracer \rightarrow Ac-CoA



g ^{13}C - β BOHB \rightarrow acetyl groups



h ^{13}C - β BOHB \rightarrow lipids



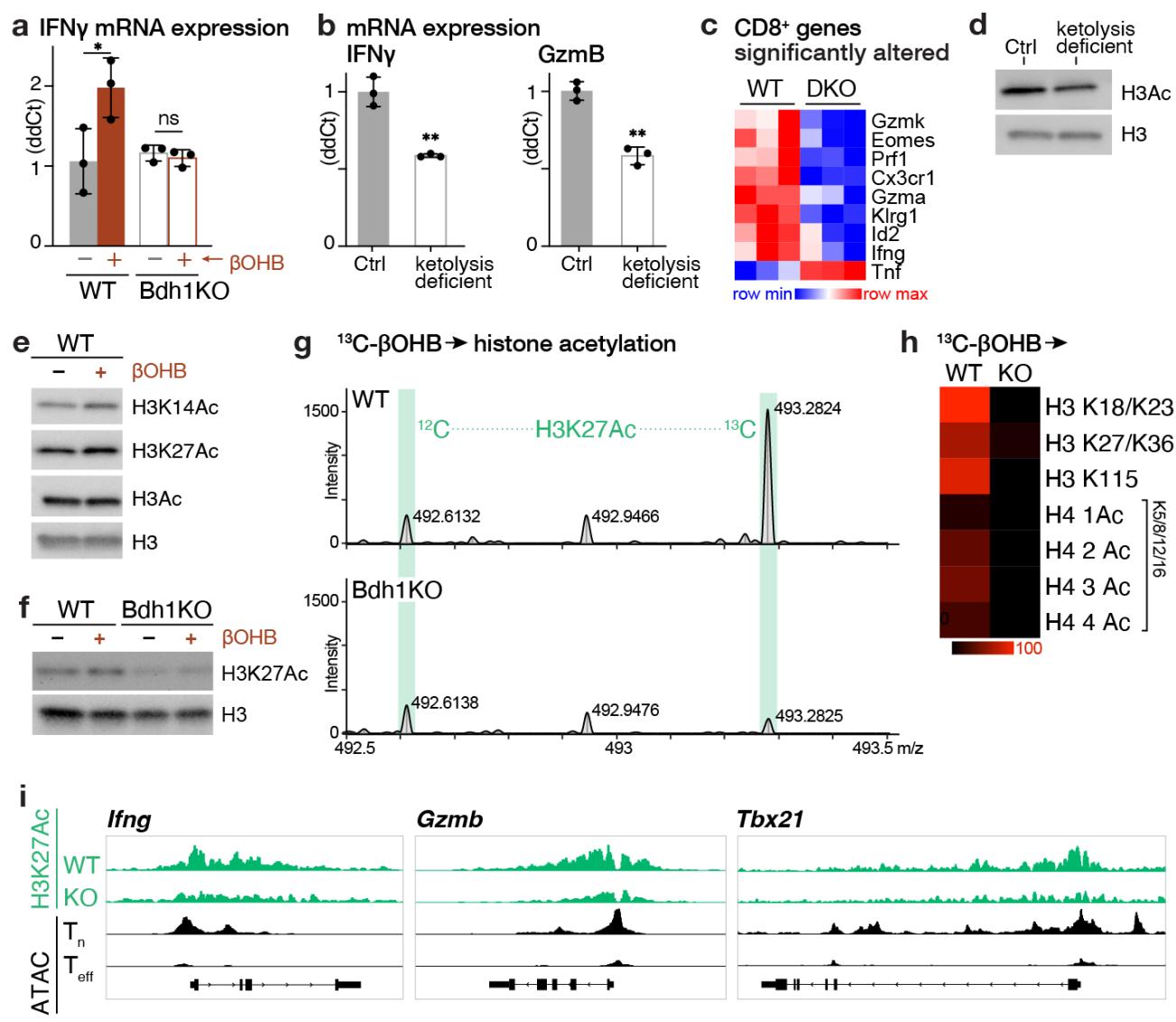
694

695 **Figure 3**

696

697 **Figure 3. Cell-intrinsic ketolysis regulates CD8⁺ effector T cell bioenergetics and**
698 **acetyl-CoA production.**

699 **(a)** Illustration of the mitochondrial ketolysis pathway. **(b)** Labeling of 2 mM [¹³C₄]βOHB
700 (left) or [¹³C₄]AcAc (right) into intracellular citrate in activated control (Ctrl) or Bdh1-
701 deficient (Bdh1KO) CD8⁺ T cells after 2 h of culture (mean ± SEM, n=3/group). **(c)**
702 [¹³C₄]βOHB (left) or [¹³C₄]AcAc (right) labeling into intracellular citrate in CD8⁺ T cells
703 expressing control (*shCtrl*) or Oxct1-targeting (*shOxct1*) shRNAs (mean ± SEM,
704 n=3/group). Cells were cultured with ¹³C substrates for 2 h as in (b). **(d-e)** Bioenergetic
705 profile of ketolysis deficient CD8⁺ T cells. **(d)** OCR plot over time for activated control
706 (Ctrl) or Bdh1KO CD8⁺ T cells cultured for 2h without or with 2 mM βOHB. Time of
707 addition of oligomycin (O), FCCP (F), and rotenone and antimycin A (R/A) are indicated.
708 **(e)** Maximal ATP production rates from OXPHOS in activated Bdh1KO (left) or *shOxct1*-
709 expressing (right) CD8⁺ T cells and respective controls (Ctrl) following addition of 2 mM
710 βOHB. **(f)** Metabolic production of acetyl-CoA in CD8⁺ T cells. *Left*, Fractional enrichment
711 of [¹³C₂]acetate, [¹³C₄]βOHB, [¹³C₆]glucose, and [¹³C₃]lactate in the intracellular acetyl-CoA
712 (M+2) pool of activated CD8⁺ T cells following 24 h of culture (mean ± SEM, n=3). *Right*,
713 Fractional enrichment of [¹³C₄]βOHB and [¹³C₆]glucose carbon into the acetyl-CoA (M+2)
714 pool in control (Ctrl) and Bdh1KO CD8⁺ T cells after 24 h of culture (mean ± SEM, n=3).
715 **(g)** Fractional enrichment of [¹³C₄]βOHB carbon in acetylated (M+2) metabolites from
716 control (Ctrl) and Bdh1KO CD8⁺ T cells after 24 h of culture (mean ± SEM, n=3). **(h)** MID
717 of [¹³C₄]βOHB carbon in palmitate for activated control (Ctrl) and Bdh1KO CD8⁺ T cells
718 after 24 h of culture (mean ± SEM, n=3).



719

720 **Figure 4**

721

722 **Figure 4. Ketolysis promotes histone acetylation of CD8⁺ T cell effector genes.**

723 **(a)** Relative abundance of *Ifng* mRNA transcripts in activated wild type (WT) and Bdh1KO
724 CD8⁺ T cells cultured for 3 days in the presence (+) or absence (-) of 5 mM β OHB (mean
725 \pm SEM, n=3/group). **(b)** Relative abundance of *Ifng* and *Gzmb* mRNA transcripts in
726 activated control (Ctrl) and ketolysis-deficient OT-I CD8⁺ T cells (mean \pm SEM, n=3). **(c)**
727 Heatmap of differentially expressed effector gene transcripts ($p<0.05$) altered in control
728 (WT) and ketolysis-deficient (DKO) OT-I CD8⁺ cells isolated from *Lm*-OVA-infected mice
729 7 dpi (n=3/group). **(d)** Immunoblot of acetylated histone H3 in lysates from activated
730 control (Ctrl) and ketolysis-deficient OT-I CD8⁺ cells. Total H3 levels are shown as a
731 control for protein loading. **(e)** Immunoblot of global histone H3 acetylation (H3Ac) and
732 specific acetylation at Lys14 (H3K14Ac) and Lys27 (H3K27Ac) in activated control (WT)
733 T cells. **(f)** Immunoblot for H3 and H3K27Ac levels in activated control (WT) versus
734 Bdh1KO CD8⁺ cells cultured for 24 h with (+) or without (-) 5 mM β OHB. **(g)**
735 Representative MS spectra of histone H3 (peptide 27-40) from activated control (WT) or
736 Bdh1KO CD8⁺ T cells cultured with [¹³C₄] β OHB for 24 h. Peaks corresponding to
737 unlabelled (¹²C) and ¹³C-labeled H3K27Ac peptides are highlighted in green. **(h)** Heatmap
738 quantifying levels of [¹³C₄] β OHB-dependent acetylation in histones H3 and H4 from
739 activated control (WT) or Bdh1KO CD8⁺ T cells cultured with [¹³C₄] β OHB for 24 h. For
740 histone H4, the number of acetylated lysine residues on peptides containing
741 Lys5/8/12/16 are quantified individually. **(i)** Data tracks for H3K27Ac peak enrichment at
742 *Ifng*, *Gzmb*, and *Tbx21* gene loci for control (WT) and Bdh1KO CD8⁺ T cells activated for
743 24 h with plate-bound anti-CD3 and anti-CD28 antibodies. H3K27Ac peak enrichment

744 is shown in green, with ATAC-seq tracks highlighting regions of chromatin accessibility
745 in *in vivo* Tn and Teff cells (from (22)) shown in black. The data are representative of
746 duplicate samples. *, $p < 0.05$; **, $p < 0.01$.

1 **Supplementary Materials for**

2

3 **Ketolysis is a metabolic driver of CD8⁺ T cell effector function through**
4 **histone acetylation**

5 Katarzyna M. Luda^{1,2}, Susan M. Kitchen-Goosen¹, Eric H. Ma¹, McLane J. Watson¹,
6 Lauren R. Duimstra¹, Brandon M. Oswald¹, Joseph Longo¹, Zhen Fu³, Zachary Madaj³,
7 Ariana Kupai⁴, Bradley M. Dickson⁴, Irem Kaymak¹, Kin H. Lau³, Shelby Compton¹, Lisa
8 M. DeCamp¹, Daniel P. Kelly⁵, Patrycja Puchalska⁶, Kelsey S. Williams¹, Connie M.
9 Krawczyk¹, Dominique Lévesque⁷, François-Michel Boisvert⁷, Ryan D. Sheldon⁸, Scott
10 B. Rothbart⁴, Peter A. Crawford^{6,9}, and Russell G. Jones^{1*}

11

12 *Corresponding author: Russell G. Jones, russell.jones@vai.org

13

14 **This PDF file includes:**

15 Materials and Methods

16 Figures S1 to S13

17 Tables S4 to S5

18 Supplemental references

19

20 **Other Supplementary Materials for this manuscript include the following:**

21 Tables S1 to S3 (.xlsx)

22

23 **Materials and Methods**

24

25 **Mice**

26 C57BL/6, CD90.1 (Thy1.1⁺), B6.SJL-*Ptprc^a* *Pepc^b*/BoyJ, Tg(TcraTcrb)1100Mjb (OT-I),
27 and *Cd4-Cre* mice were purchased from The Jackson Laboratory. *Bdh1*-floxed animals
28 were generated by Daniel Kelly (1). Mice were bred and maintained under specific
29 pathogen-free conditions at VAI under approved protocols. Genotyping was performed
30 using tail biopsies using defined primer sets (see **Table S4**). Experiments were
31 performed using mice (of both sexes) between 8 and 20 weeks of age.

32

33 **T cell purification and culture**

34 For mouse T cell isolation, naïve CD8⁺ T cells were purified from spleen and peripheral
35 lymph nodes by negative selection (StemCell Technologies, Vancouver, BC) as
36 previously described (2). Cells were cultured in Iscove's Modified Delbecco's Medium
37 (IMDM) or Van Andel Institute-modified Iscove's Medium (VIM) (3) supplemented with
38 10% dialyzed FBS (Wisent, St. Bruno, QC), penicillin-streptomycin (Invitrogen), and 2-
39 ME (Sigma-Aldrich, St. Louis, MO). Unless stated otherwise, CD8⁺ T effector (Teff) cells
40 were generated by activation in standard IMDM containing 25 mM glucose and 6 mM
41 L-glutamine. For physiologic culture conditions, Seahorse experiments, and ¹³C tracing
42 experiments, CD8⁺ Teff cells were culture in medium containing 5 mM glucose and 0.5
43 mM L-glutamine, respectively. Physiologic carbon sources (PCS) were added to
44 culture medium (IMDM or VIM) as indicated at the following concentrations: acetate

45 (400 μ M), β OHB (850 μ M), citrate (215 μ M), lactate (3 mM), and pyruvate (150 μ M) (3).
46 *In vitro*-activated CD8 $^{+}$ Teff cells were generated by stimulating naïve CD8 $^{+}$ T cells (1 \times
47 10 6 cells/mL) with plate-bound anti-CD3 ϵ (clone 2C11) and anti-CD28 (clone 37.51)
48 antibodies (eBioscience, San Diego, CA) for 3 days. For extracellular flux analysis,
49 stable isotope labelling, and histone isolation procedures activated CD8 $^{+}$ Teff cells were
50 re-cultured (5 \times 10 6 cells/well in 6-well plates) for up to 48h in IMDM containing 50
51 U/mL IL-2 (PeproTech, Rocky Hill, NJ) prior to use in assays. For retroviral transduction
52 experiments, CD8 $^{+}$ Thy1.1 $^{+}$ OT-I T cells (wild type or *Bdh1*-deficient) were transduced
53 with retrovirus 24 hours post activation and expanded for 2 additional days in IMDM
54 containing IL-2 as previously described (2, 4). Transduced T cells were FACS sorted
55 and cultured overnight prior to adoptive transfer into naïve Thy1.2 $^{+}$ C57BL/6 hosts.

56

57 **Adoptive transfer and infection with *L. monocytogenes* (Lm-OVA)**

58 Mice were immunized intravenously with a sublethal dose of recombinant attenuated
59 *Listeria monocytogenes* expressing OVA (Lm-OVA, 2 \times 10 6 CFU) as previously described
60 (5, 6). For adoptive transfer experiments 6-7 days post infection (dpi) using non-
61 transduced or transduced cells, 5 \times 10 3 CD8 $^{+}$ OT-I T cells (Thy1.1 $^{+}$ or CD45.2 $^{+}$) were
62 injected intravenously into C57BL/6 mice (Thy1.2 $^{+}$ CD45.2 $^{+}$ or CD45.1 $^{+}$), followed by *Lm*-
63 OVA infection 1 day later. Splenocytes were isolated from mice 7 dpi and analyzed for
64 the presence of OVA-specific CD8 $^{+}$ T cells by Thy1.1 or CD45.2 staining and cytokine
65 production analyzed by intracellular cytokine staining (ICS) following peptide re-
66 stimulation (OVA₂₅₇₋₂₆₄) as previously described (5, 6). For metabolic analysis of *Lm*-OVA-
67 specific Thy1.1 $^{+}$ OT-I T cells *in vivo* using ¹³C-labeled metabolites, Thy1.2 $^{+}$ C57BL/6 mice

68 received 2.5×10^6 or 5×10^4 Thy1.1 $^+$ OT-I T cells for analysis at 2 and 6 dpi, respectively.
69 *Lm*-OVA-specific CD8 $^+$ OT-I T cells were isolated from the spleen of infected mice by
70 positive selection using the EasySep mouse CD90.1 positive selection kit (StemCell
71 Technologies) as previously described (2, 7).

72

73 **Flow cytometry**

74 Single cell suspensions from the spleen were surface stained with a cocktail of
75 fluorescently labelled antibodies listed in **Table S5**. Cell viability was assessed by using
76 Fixable Viability Dye eFluor 780 (eBioscience) according to manufacturer's protocols. To assess
77 cytokine production, splenocytes were plated in the presence of PMA (50ng/ml) and
78 Ionomycin (50ng/ml and Merck) for 2h and with Brefeldin A (5ug/ml, Biolegend) added
79 for the last 2h of stimulation prior surface staining. After restimulation, cells were surface
80 stained, fixed, and permeabilized using FoxP3/Transcription Factor Staining Buffer Set
81 (eBioscience), followed by processing for intracellular staining using fluorescently labelled
82 antibodies. For analysis of antigen-specific response to *LmOVA*, splenocytes harvested
83 7 dpi were stimulated with OVA257-264 peptide using previously published protocols (5,
84 6). Flow cytometry was performed on Cytoflex (Beckman Coulter) or Aurora Cytek
85 cytometers and cell sorting on Astrios (Beckman Coulter) or BD FACSaria Fusion cell
86 sorters. Data analysis was performed using FlowJo software (Tree Star).

87

88 **Extracellular flux analysis**

89 T cell oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were
90 measured using a Seahorse XF96 Extracellular Flux Analyzer following established

91 protocols (2). Activated and IL-2-expanded T cells (1.5×10^5 /well) were cultured in XF
92 medium containing 5mM glucose and 0.5mM glutamine, following centrifugation onto
93 poly-D-lysine-coated XF96 plates, and cellular bioenergetics assessed at 5 minute
94 intervals following the sequential addition of oligomycin (2.0 μ M), fluoro-carbonyl cyanide
95 phenylhydrazone (FCCP, 2.0 μ M), rotenone/antimycin A (2 μ M), and monensin (10mM).
96 Data were normalized to cell number. Where indicated, β OHB (2 mM) and AcAc (2 mM)
97 were added to cell culture for 2 h prior to Seahorse analysis and added to Seahorse
98 medium over the assay period. Bioenergetics data analysis was based on protocols
99 developed by Mookerjee and Brand (8), which is available for download at
100 <https://russelljoneslab.vai.org/tools>.

101

102 **Acetoacetate synthesis**

103 [$^{12}\text{C}_4$]-ethyl acetoacetate (Merck) and [$^{13}\text{C}_4$]-ethyl-acetoacetate (Cambridge Isotope labs)
104 were hydrolysed by mixing 1ml of ethyl acetoacetate with 8 ml 1M NaOH at 60°C by
105 stirring for 30 min. Hydrolyzed samples were placed on ice and adjusted to pH 7.5 using
106 50% HCl. Subsequently, samples were frozen at -80°C. The concentration of
107 synthesized acetoacetate was evaluated using the Autokit Total Ketone Bodies Assay
108 (Fujifilm Wako) and used for *in vitro* assays.

109

110 **Stable isotope labeling (SIL) and metabolomics**

111 SIL experiments with *in vitro*-activated T cells using liquid chromatography (LC) or gas
112 chromatography (GC) coupled to mass spectrometry (MS) were conducted as

113 previously described (2, 3). In brief, naïve CD8⁺ T cells were activated as above
114 washed, in IMDM or VIM containing 10% dialyzed FBS, and re-cultured (2.5 × 10⁶
115 cells/well in 24-well plates) for indicated times in medium containing ¹³C-labeled
116 metabolites (Cambridge Isotope Laboratories) at the following concentrations: [U-
117 ¹³C₆]glucose, 5 mM; [U-¹³C₂]acetate, 400 μM; [U-¹³C₄]AcAc, 2 mM; [U-¹³C₃]alanine, 200
118 μM; [U-¹³C₄]βOHB, 0.85 or 2 mM; [¹³C₆]citrate, 215 μM; [¹³C₃]lactate, 3 mM;
119 [¹³C₃]pyruvate, 150 μM. Cells were transferred from tissue culture plates to falcon tubes
120 and centrifuged at 500g 4°C for 3 min. The cell pellet was washed with ice-cold saline
121 and centrifuged before being snap frozen on dry-ice and stored at -80°C. Metabolites
122 were extracted by modified Bligh-Dyer extraction (9) by the addition of ice-cold
123 methanol (A456, Fisher Scientific) directly to frozen cells, to which one volume of
124 chloroform (A456, Fisher Scientific) was added. The sample was vortexed for 10 s,
125 incubated on ice for 30 min, and then 0.9 parts of LC-MS grade water (W6-4, Thermo
126 Fisher Scientific) was added. The samples were vortexed vigorously and centrifuged at
127 maximum speed to achieve phase separation. The top layer containing polar
128 metabolites was aliquoted into a fresh tube and dried in a speedvac for LC-MS
129 analysis. The bottom layer was retained for fatty acid methyl-ester measurement.

130 For LC-MS analysis, metabolite extracts were resuspended in 50 μL of 60%
131 acetonitrile (A955, Fisher Scientific) and analyzed by high resolution accurate mass
132 spectrometry using an ID-X Orbitrap mass spectrometer (Thermo Fisher Scientific)
133 coupled to a Thermo Vanquish Horizon liquid chromatography system. 2 μL of sample
134 volume was injected on column. Chromatographic separations were accomplished
135 with Acuity BEH Amide (1.7 μm, 2.1 mm x 150 mm) analytical columns (#176001909,

136 Waters, Eschborn, Germany) fitted with a pre-guard column (1.7 μ m, 2.1mm x 5 mm;
137 #186004799, Waters) using an elution gradient with a binary solvent system. Solvent A
138 consisted of LC/MS grade water (W6-4, Fisher), and Solvent B was 90% LC/MS grade
139 acetonitrile (A955, Fisher). For negative mode analysis, both mobile phases contained
140 10 mM ammonium acetate (A11450, Fisher Scientific), 0.1% (v/v) ammonium
141 hydroxide, and 5 μ M medronic acid (5191-4506, Agilent Technologies). For positive
142 mode analysis, both mobile phases contained 10 mM ammonium formate (A11550,
143 Fisher), and 0.1% (v/v) formic acid (A11710X1, Fisher). For both negative and positive
144 mode analyses the 20-min analytical gradient at a flow rate of 400 μ L/min was: 0–1.0
145 min ramp from 100% B to 90% B, 1.0–12.5 min from 90% B to 75% B, 12.5–19 min
146 from 75% B to 60% B, and 19–20 min hold at 60% B. Following every analytical
147 separation, the column was re-equilibrated for 20 min as follows: 0–1 min hold at
148 65% B at 400 μ L/min, 1–3 min hold at 65% B and ramp from 400 μ L/min to 800 μ L/min,
149 3–14 min hold at 65% B and 800 μ L/min, 14–14.5 min ramp from 65% B to 100% B at
150 800 μ L/min, 14.5–16 min hold at 100% B and increase flow from 800 μ L/min to 1200
151 μ L/min, 16–18.4 min hold at 100% B at 1200 μ L/min, 18.4–19.5 min hold at 100% B
152 and decrease flow from 1200 μ L to 400 μ L/min, 19.5–20 min hold at 100% B and 400
153 μ L/min. The column temperature was maintained at 40°C. The H-ESI source was
154 operated at spray voltage of 2500 V(negative mode)/3500 V(positive mode), sheath
155 gas: 60 a.u., aux gas: 19 a.u., sweep gas: 1 a.u., ion transfer tube: 300°C, vaporizer:
156 300°C. For isotopically labelled experimental replicates, high resolution MS¹ data was
157 collected with a 20-min full-scan method with m/z scan range using quadrupole
158 isolation from 70 to 1000, mass resolution of 120,000 FWHM, RF lens at 35%, and

159 standard automatic gain control (AGC). Unlabelled control samples were used for data
160 dependent MS² (ddMS²) fragmentation for compound identification and annotation via
161 the AquireX workflow (Thermo Scientific). In this workflow, first blank and experimental
162 samples are injected to generate exclusion and inclusion lists, respectively, followed by
163 iterative sample injections for ddMS² fragmentation where triggered ions are added to
164 the exclusion list for subsequent injections. ddMS² data was collected using MS1
165 resolution at 60,000, MS² resolution at 30,000, intensity threshold at 2.0 x 10⁴, and
166 dynamic exclusion after one trigger for 10 s. MS² fragmentation was completed first
167 with HCD using stepped collision energies at 20, 35, and 50% and was followed on the
168 next scan by CID fragmentation in assisted collision energy mode at 15, 30, and 45%
169 with an activation Q of 0.25. Both MS² scans used standard AGC and a maximum
170 injection time of 54ms. The total cycle time of the MS¹ and ddMS² scans was 0.6 s.

171 Full scan LC-MS data were analyzed in Compound Discoverer (v 3.2, Thermo
172 Scientific). Compounds were identified by chromatography specific retention time of
173 external standards and MS² spectral matching using the mzCloud database (Thermo
174 Scientific).

175 TCA cycle intermediates were measured via GC-MS following LC-MS analysis.
176 Briefly, following LC/MS extracts were dried and derivatized with 30 µL of
177 methoxyamine (11.4 mg/mL) in pyridine and 70 µL of MTBSFA+1%TMCS as described
178 previously (10). In addition, GC-MS was used to evaluate incorporation of ¹³C into de
179 novo synthesized fatty acids from metabolic precursors using fatty acid methyl-esters
180 (FAMEs). The bottom organic fraction from the Bligh-Dyer extraction (above) was
181 aliquoted, dried in a speedvac, and FAMEs were generated as described previously

182 (11). GC-MS analysis of both TBDMS derivatives and FAMEs were conducted on an
183 Agilent 7890/5977b GC/MSD equipped with a DB-5MS+DG (30 m x 250 μ m x 0.25 μ m)
184 capillary column (Agilent J&W, Santa Clara, CA, USA) was used. Data were collected
185 by electron impact set at 70 eV. A total of 1 μ L of the derivatized sample was injected
186 in the GC in split mode (1:2 or 1:4) with inlet temperature set to 280°C, using helium as
187 a carrier gas with a column flow rate of 1.2 mL/min. The oven program for all
188 metabolite analyses started at 95°C for 1 min, increased at a rate of 40°C/min until
189 118°C and held for 2 min, then increased to 250°C at a rate of 12°C/min, then increased
190 to 320°C at a rate of 40°C/min and finally held at 320°C for 7 min. The source
191 temperature was 230°C, the quadrupole temperature was 150°C, and the GC-MS
192 interface at 285°C. Data were acquired both in scan mode (50–800 m/z) and 2 Hz.

193 MassHunter software (v10, Agilent Technologies) was used for peak picking and
194 integration of GC-MS data. Peak areas of all isotopologues for a molecular ion of each
195 compound in both labeled experimental and unlabeled control samples were used for
196 mass isotopologue distribution analysis via a custom algorithm developed at VAI. This
197 algorithm uses matrices correcting for natural contribution of isotopologue enrichment
198 were generated for each metabolite as described previously (12, 13).

199

200 ***In vivo* ^{13}C tracer infusions**

201 *In vivo* infusions of *Lm*OVA-infected mice were conducted using previously described
202 protocols (2, 7). Briefly, mice were anesthetized using isoflurane and infused
203 intravenously with ^{13}C tracers over a 2 h period. [$\text{U-}^{13}\text{C}_6$]glucose infusions were
204 performed as previously described, using a stock concentration of 100 mg/ml, an initial

205 bolus dose of 120 μ L and infusion rate of 2.5 μ L/min for 2 h for a 20 g mouse. For
206 infusions of sodium D-[U-¹³C₄] β OHB, mice received an initial bolus of 0.487 mg per gram
207 of mouse, followed by infusion of 0.05 μ L/min/g bodyweight using a 1.5 M stock
208 concentration for 2 hours. Subsequently, mice were cervically dislocated, plasma was
209 harvested via cardiac puncture, centrifuged at 500g 4°C for 3 min, and snap frozen in
210 liquid nitrogen. Spleens were collected and processed for Thy1.1⁺ T cell isolation by
211 magnetic bead isolation as previously described (7). Isolated T cells were snap frozen
212 on dry ice and subsequently processed for metabolomic analysis as described.

213

214 **RNA isolation, sequencing, and qPCR analysis**

215 Total RNA was isolated from murine T cells via RNeasy Kit (Qiagen) with DNase
216 digestion (Qiagen) following manufacturer's instructions. For quantitative PCR (qPCR)
217 analysis, total RNA was reverse transcribed using a High Capacity cDNA Reverse
218 Transcriptase kit (Life Technologies) and qPCR performed using SYBR green (Bio-
219 Rad). Results were normalized to *Stk11* mRNA levels and wild type controls using
220 standard ddCt methods. RNA preparation and library construction for RNA sequencing
221 was conducted by the VAI Genomics Core as previously described (14). Libraries were
222 sequenced using a NovaSeq 6000 (Illumina) using 50 bp paired-end sequencing (5x10⁷
223 reads/sample). Gene-set enrichment analysis (GSEA) on RNA-seq data was conducted
224 using the gage function and non-parametric Kolmogorov-Smirnov test from the GAGE
225 (version 2.22.0) R Bioconductor package (15). RNA-seq data files are available at NCBI
226 GEO (accession: GSE212048).

227 For the meta-analysis in **Figure 1**, raw sequences from RNA-sequencing of
228 CD8⁺ T cells from three previously published studies (GEO accessions: GSE89307,
229 GSE84820, and GSE86881) were downloaded. Adaptor sequences and low-quality
230 reads were trimmed using Trim Galore (v0.6.0) (16). Trimmed reads were aligned to the
231 mm10 reference genome using STAR (v2.7.8) (17). Count tables of all samples were
232 then imported into limma (v3.48.3) (18), with a batch variable included in the design
233 matrix to account for the different study designs and sequencing platforms. Pearson
234 correlations among samples were calculated using batch corrected, variance
235 stabilization transformed counts using DESeq2 (v1.32) (19), and the Pearson
236 correlation matrix was used to generate a heatmap. Batch corrected counts were also
237 used to conduct principal component analysis (PCA) using DESeq2. Differential gene
238 expression analyses of two main comparisons (comparison 1: cancer Tex cells vs. Teff
239 cells; comparison 2: virus Tex cells vs. Teff cells) were conducted on raw counts using
240 DESeq2, with a covariate to adjust for batch, and Benjamini-Hochberg adjusted p-
241 values to maintain a 5% false discovery rate. The median of the two Wald test statistics
242 for each gene, one from each of the two comparisons, were used for gene rankings.
243 Gene ontology (GO) terms for each gene was retrieved from BioMart using R package
244 biomaRt (v2.48.4) (20, 21). Lastly, for each comparison, genes were sorted by their fold
245 change from highest to lowest (using Teff cells as reference in both comparisons) and
246 gene set enrichment analysis (GSEA) (22) was conducted for each comparison
247 independently using clusterProfiler (v4.0.5) (23). Gene sets C2, C5, C6, C7, and
248 Hallmark for mm10 was retrieved from Molecular Signatures Database using R
249 package MSigDB (7.4.1).

250

251 **Immunoblotting**

252 Cells were lysed in modified Laemmli lysis buffer (240 mM Tris/HCl pH 6.8, 40%
253 glycerol, 8% SDS, 5% b-ME) supplemented with protease and phosphatase inhibitors
254 (Roche/Sigma-Aldrich). A Pierce BCA Protein Assay Kit (Thermo Fisher Scientific,
255 Waltham, MA, USA) was used to quantify protein from whole cell lysates. Lysates were
256 resolved by SDS-PAGE, transferred to nitrocellulose, and incubated with primary
257 antibodies to Bdh1, Oxct1, α -tubulin or β -actin, and HRP-conjugated secondary
258 antibodies. Histone proteins were extracted from T cells using a Histone Extraction Kit
259 (Abcam) following manufacturer's instructions. Lysates containing 0.5-2 μ g of protein
260 were resolved on a 4-20% SDS-PAGE gel and transferred to PVDF
261 membrane. Membranes were incubated with primary antibodies overnight at 4°C, and
262 then incubated with secondary antibody for 1hr at room temperature prior to
263 exposure. Primary antibodies are listed in **Table S5**.

264

265 **Proteomic analysis of histone proteins**

266 For proteomic analysis of histones, activated CD8 $^{+}$ T cells were cultured in VIM
267 medium (5 mM glucose, 0.5 mM glutamine) containing 5 mM standard [$^{12}\text{C}_4$] β OHB or
268 [$^{13}\text{C}_4$] β OHB for 24 h. Histone isolation was carried out using the same methodology for
269 immunoblotting, with the addition of 2 ice-cold PBS washes. Histone extracts were
270 lyophilized and resuspended in 50 μ L of 8M urea with 10 mM HEPES-KOH pH 7.5.
271 Proteins were reduced by adding dithiothreitol (DTT) to a final concentration of 5 mM

272 and by heating at 95°C for 2 minutes, followed by a 30 minute incubation at room
273 temperature. The alkylation of the proteins was carried out by adding chloroacetamide
274 (Sigma-Aldrich, Saint-Louis) to a final concentration of 7.5 mM followed by a 20 minute
275 incubation in the dark at room temperature. The urea concentration was then diluted to
276 a final concentration of 2 M by adding 150 µL of 50 mM ammonium bicarbonate
277 (NH_4HCO_3) (Sigma-Aldrich, Saint-Louis). Digestion was started by adding 1 µg of
278 Pierce MS-grade trypsin (Thermo Fisher Scientific, Waltham) and incubated overnight
279 at 30°C with shaking. Sample was then acidified by adding Trifluoroacetic acid (TFA)
280 (Sigma-Aldrich, Saint-Louis) to a final concentration of 0.2%.

281 The peptides were purified with ZipTip 100-µL micropipette tips containing a C18
282 column, according to the manufacturer's protocol (EMD Millipore, Burlington, VT) and
283 eluted with 300 µL of 50% ACN/1% FA buffer in a new low-binding microtube. The
284 peptides were then concentrated by centrifugal evaporator at 60°C until complete
285 drying (~3 h) and then resuspended in 50 µL of 1% FA buffer. Peptides were assayed
286 using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA) and
287 read at an absorbance of 205 nm. The peptides were then transferred to a glass vial
288 (Thermo Fisher Scientific) and stored at –20 °C until analysis by mass spectrometry.

289 For LC-MS analysis, 250 ng of peptides were injected into an HPLC (nanoElute,
290 Bruker Daltonics) and loaded onto a trap column (Acclaim PepMap100 C18 column,
291 0.3 mm id x 5 mm, Dionex Corporation) with a constant flow of 4 µL/min, then eluted
292 onto an analytical C18 Column (1.9 µm beads size, 75 µm x 25 cm, PepSep). Peptides
293 were eluted over a 2-hour gradient of ACN (5-37%) in 0.1% FA at 400 nL/min while
294 being injected into a TimsTOF Pro ion mobility mass spectrometer equipped with a

295 Captive Spray nano electrospray source (Bruker Daltonics). Data was acquired using
296 data-dependent auto-MS/MS with a 100-1700 m/z mass range, with PASEF enabled
297 with several PASEF scans set at 10 (1.17 seconds duty cycle) and a dynamic exclusion
298 of 0.4 min, m/z dependent isolation window and collision energy of 42.0 eV. The target
299 intensity was set to 20,000, with an intensity threshold of 2,500.

300 Raw data files were analyzed using MaxQuant (version 2.0.3.0) and the Uniprot
301 human proteome database (21/03/2020, 75,776 entries). The settings used for the
302 MaxQuant analysis (with TIMS-DDA type in group-specific parameters) were: 4
303 miscleavages were allowed; the minimum peptide length was set to 5; enzyme was
304 Trypsin (K/R not before P); fixed modification was carbamidomethylation on cysteine;
305 variable modifications were methionine oxidation, protein N-terminal acetylation (¹²C,
306 ¹³C), lysine (K) acetylation (¹²C, ¹³C) and protein carbamylation (K, N-terminal). A mass
307 tolerance of 20 ppm was used for both precursor and fragment ions. Identification
308 values "PSM FDR", "Protein FDR" and "Site decoy fraction" were set to 0.05. Minimum
309 peptide count was set to 1. Both the "Second peptides" and "Match between runs"
310 options were also allowed. Compass DataAnalysis (version 5.3, Bruker Daltonics) was
311 further used to represent both spectral and graphical MS1 results for specific peptides.

312

313 **Chromatin immunoprecipitation sequencing (ChIP-seq) analysis**

314 ChIP-seq analysis of H3K27Ac enrichment in CD8⁺ T cells was conducted using the
315 sans spike-in quantitative ChIP (siQ-ChIP) method as previously described (24, 25).
316 Naïve CD8⁺ T cells (5 x 10⁶) were activated with anti-CD3 and anti-CD28 antibodies for
317 24 h, then rinsed once with 10 mL of D-PBS (Gibco, 14190136) followed by cross-

318 linking in suspension for 5 min in 10 mL of 0.75% formaldehyde (Pierce, 28906) in D-
319 PBS at room temperature. Prior to every liquid removal step, cells were centrifuged at
320 800 RCF for 3 min. Formaldehyde was removed, and cells were quenched for 5 min
321 with 10 mL of 750 mM Tris pH 10-10.5. Cells were washed twice with 10 mL of D-PBS,
322 snap-frozen in liquid nitrogen, and stored -80°C. Cells were then lysed under hypotonic
323 conditions (1 mL of 20 mM Tris-HCl pH 8, 85 mM KCl, 0.5% NP- 40 (1 tablet of
324 protease inhibitor (Roche, 11836170001) per 5 mL of buffer)) for 30 min on ice. Nuclei
325 were collected by centrifugation at 1300 RCF for 5 min, lysed by resuspension in nuclei
326 lysis buffer (150 μ L 50 mM Tris-HCl pH 8, 150 mM NaCl, 2 mM EDTA, 1% NP-40, 0.5%
327 sodium deoxycholate, 0.1% SDS (1 tablet of protease inhibitor per 5 mL of buffer)), and
328 passed through a 27-gauge needle (BD #309623 Lot: 0227218). Lysates were diluted
329 to 500 μ L by addition of binding buffer (25 mM HEPES pH 7.5, 100 mM NaCl, 0.1%
330 NP-40). 5 μ L of RNase A/T1 (Thermo Scientific, EN0551) was added, and the sample
331 was incubated at 37°C for 25 min. Next, CaCl₂ was added to a final concentration of 40
332 mM, followed by the addition of 75 U of micrococcal nuclease (Worthington
333 Biochemical) and incubated at 37°C for 5 min. MNase was quenched by the addition of
334 40 mM EDTA, and the total volume was brought to 1.2 mL with binding buffer. Next,
335 insolubilities were removed by centrifugation at max speed (about 21,000 RCF) at 4°C
336 for 5 min, and the supernatant containing soluble chromatin was collected. At this
337 stage, 5 μ L of chromatin was measured using the Qubit dsDNA HS Assay Kit
338 (Invitrogen, Q32851).

339 Samples were diluted with binding buffer to ensure similar chromatin
340 concentrations and to match IP conditions. 50 μ L of chromatin was set aside for input

341 for each sample. For each IP, 25 μ L of Protein A coated magnetic beads (Invitrogen,
342 10008D) were washed once with binding buffer and incubated with either 0, 2.5, or 10
343 μ L of H3K27Ac antibody (Active Motif #39133, lot: 16119013). Total volume of bead
344 plus antibody was brought to 200 μ L using binding buffer and were rotated at room
345 temperature for 30 min. For comparison of PTM between samples, we performed one
346 biological replicate of all samples on the same day and made a master mix of bead
347 plus antibody, scaling up all components by the number of samples. For example, for 2
348 samples with technical replicate points, bead and antibody amounts were scaled by x
349 4.2 to account for dead volume. Buffer containing antibody was removed, and bead
350 plus antibody were resuspended in 200 μ L of soluble chromatin followed by 30 min
351 rotation at room temperature. Unbound chromatin was removed, and beads were
352 vortexed for 10 s with 500 μ L of binding buffer. Buffer was removed, and bound
353 material was eluted from beads by vortexing for 10 s in 133 μ L of elution buffer (25 mM
354 HEPES pH 7.5, 100 mM NaCl, 1% SDS, and 0.1% NP-40). At this time, the input was
355 brought to 133 μ L by the addition of 83 μ L elution buffer. Proteinase K (Invitrogen,
356 25530015) was added to a final concentration of 15 μ M and the sample was incubated
357 overnight at 37°C. The following morning, each DNA sample was purified using
358 MinElute PCR Kit (Qiagen, 28004) and eluted in 30 μ L of Buffer EB. Five μ L of DNA was
359 quantified by Qubit dsDNA HS Assay Kit. The remaining 25 μ L of DNA was frozen at -
360 20°C until library preparation.

361 Libraries were sequenced using a NextSeq 500 (Illumina) using 75 bp paired-
362 end sequencing (5×10^7 reads/sample for IPs, 1×10^8 reads/sample for input). Next
363 generation sequencing (NGS) data were aligned to the mm10 genome. Bed files

364 generated by the NGS alignment were processed using the latest siQ-ChIP release
365 (found at <https://github.com/BradleyDickson/siQ-ChIP>) and siQ-ChIP quantification
366 performed as previously described (24). Responses were computed automatically by
367 the siQ-ChIP software as the ratio of area under overlapping peaks for any pair of
368 tracks being compared. Individual ChIP tracks were visualized using IGV (26). Input
369 files for siQ-ChIP and resulting output files and gnuplot script for plotting will be
370 published in NCBI GEO.

371

372 **Statistics**

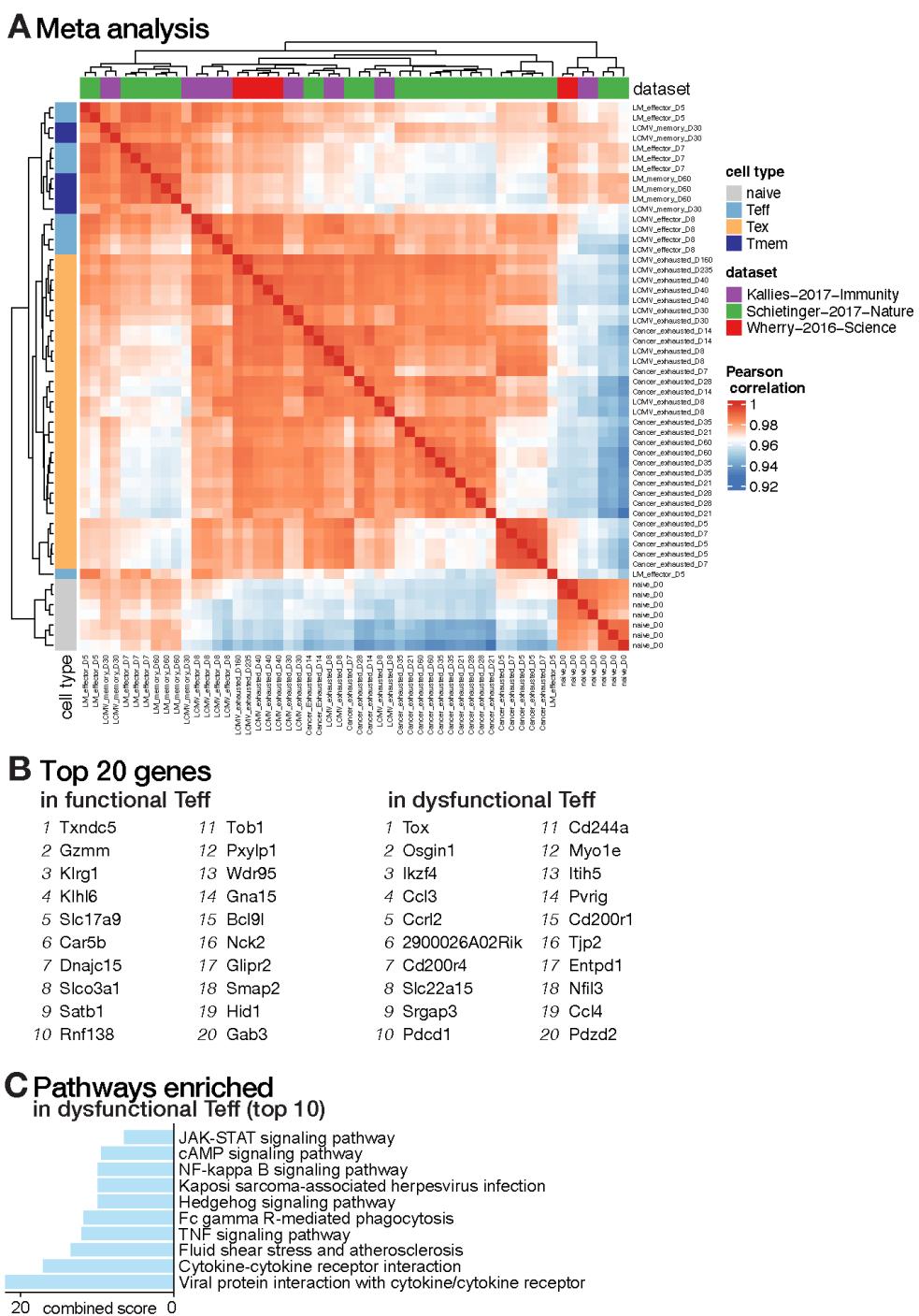
373 Statistical analysis was assessed by GraphPad Prism software (GraphPad) by
374 Student's t-test. Statistical significance is indicated in all figures by the following
375 annotations: *, $p < 0.05$; **, $p < 0.001$; ***, $p < 0.0001$; ****, $p < 0.00001$.

376

377

378

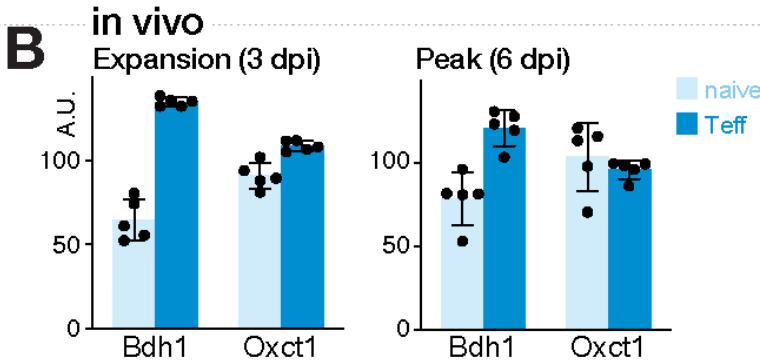
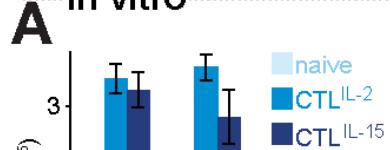
379 **Figure S1**



381 **Figure S1, related to Figure 1. Gene expression analysis of *in vivo* generated CD8⁺**
382 **T cell differentiation states.** (a) Full Pearson correlation-driven similarity matrix analysis
383 of gene expression profiles of CD8⁺ T cell states (naïve, Teff – effector T cells, Tex –
384 exhausted T cells, Tmem – memory T cells) from **Figure 1a**. Analysis was conducted on
385 RNA-seq datasets from three independent studies characterizing gene expression
386 profiles of antigen-specific CD8⁺ T cells from acute infection (LCMV Armstrong, *Lm*),
387 chronic infection (LCMV CL-13), and cancer (hepatocellular carcinoma) models *in vivo*.
388 (b) List of top 20 genes in functional (*left*) and dysfunction (*right*) Teff cells from rank
389 analysis of median Wald test statistics in **Figure 1b**. for DEG between Teff and Tex
390 populations (Teff/Tex) were calculated based on cancer and virus response datasets. (c)
391 KEGG pathway analysis of the top pathways enriched in dysfunctional Teff from **Figure**
392 **1b.**

393 **Figure S2**

Protein expression in vitro



394

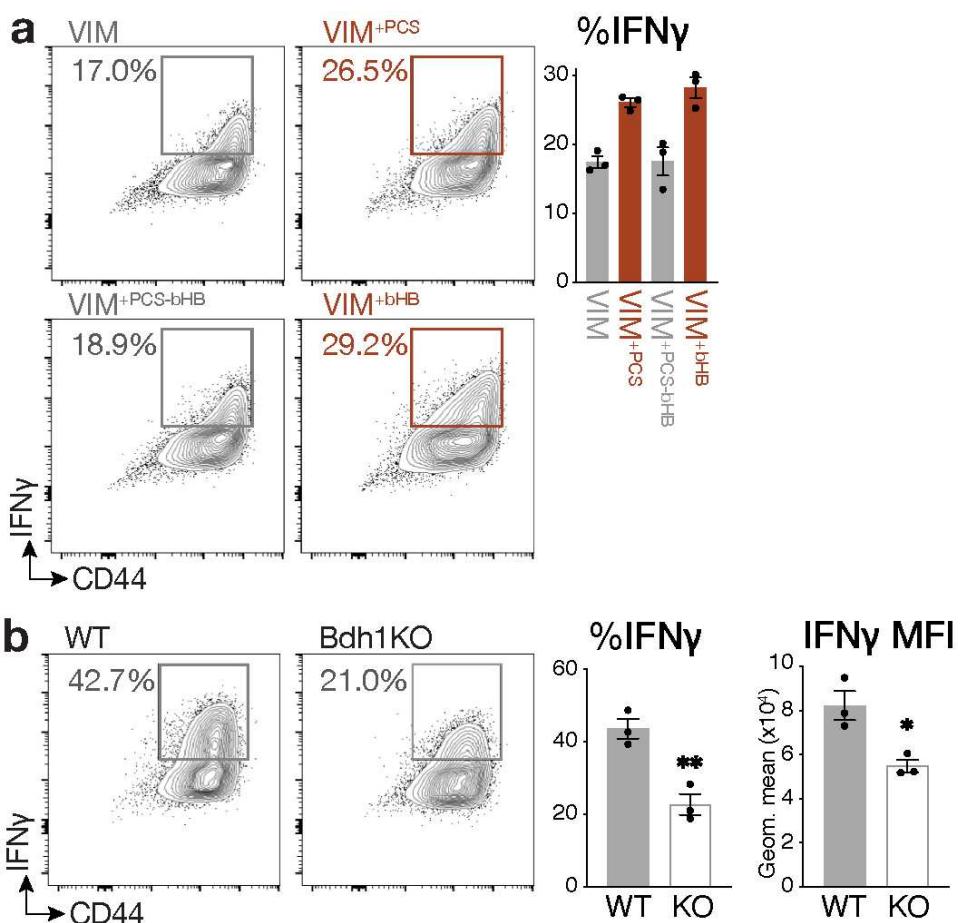
395 **Figure S2, related to Figure 1. Comparison of ketolytic enzyme expression in CD8⁺**

396 **effector T cells. (a)** Bar graphs depicting abundance of BDH1 and SCOT (Oxct1)
397 proteins extracted from *in vitro* cultured naïve, cytotoxic (CTL $^{IL-2}$), and memory-like (CTL $^{IL-}$
398 15) CD8⁺ T lymphocytes. Data were obtained from the ImmPRes proteomics resource
399 (www.immpres.co.uk). Data represent the mean \pm SEM (n=3). **(b)** Bar graphs depicting
400 abundance of Bdh1 and SCOT/Oxct1 proteins extracted from OT-I CD8⁺ cells or
401 bystander naïve CD8⁺ T cells isolated from *Lm*-OVA infected mice at 3 (T cell expansion
402 phase) or 6 (peak of T cell expansion) days post infection (dpi). Data represent the mean
403 \pm SEM (n=5). A.U., arbitrary unit.

404

405

406 **Figure S3**



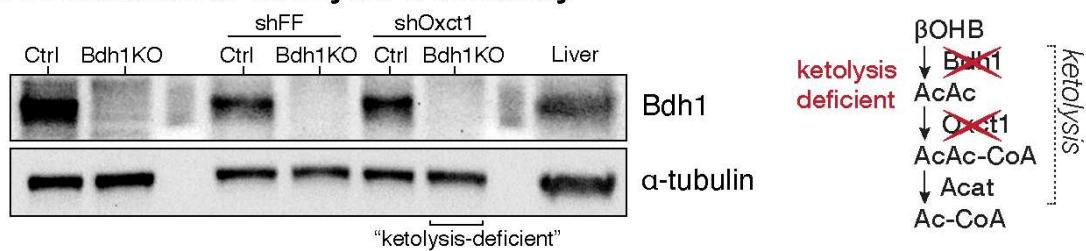
408 **Figure S3, related to Figure 1. Impact of βOHB and Bdh1-deficiency on IFN-γ**
409 **production in CD8⁺ Teff cells. (a)** IFN-γ production by CD8⁺ T cells cultured in VIM, VIM
410 containing physiologic carbon sources (PCS), VIM containing PCS minus 5 mM βOHB,
411 and VIM with 5 mM βOHB. *Left*, Representative flow cytometry plots for CD44 versus
412 IFN-γ expression by CD8⁺ T cells 3 days post activation. *Right*, Bar graph showing the
413 percentage of IFN-γ⁺ T cells in each culture. Data represent the mean \pm SEM (n=3). **(b)**
414 Intracellular IFN-γ production by control (wild type, WT) and *Bdh1*-deficient (Bdh1KO)
415 CD8⁺ T cells activated for 3 days in IMDM. *Left*, Representative flow cytometry plots for
416 CD44 versus IFN-γ expression by WT and Bdh1KO CD8⁺ T cells 3 days post activation.

417 *Right*, Bar graph showing the percentage of IFN- γ^+ T cells and the geometric mean
418 fluorescence intensity (MFI) for IFN- γ^+ T cells. Data represent the mean \pm SEM (n=3). *, p
419 < 0.05; **, p < 0.01.

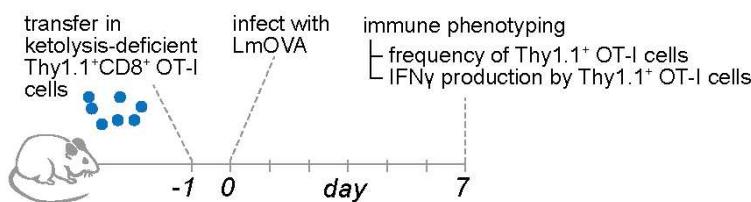
420

421 **Figure S4**

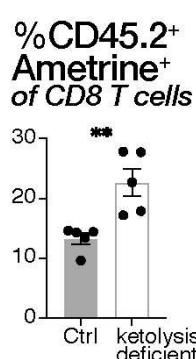
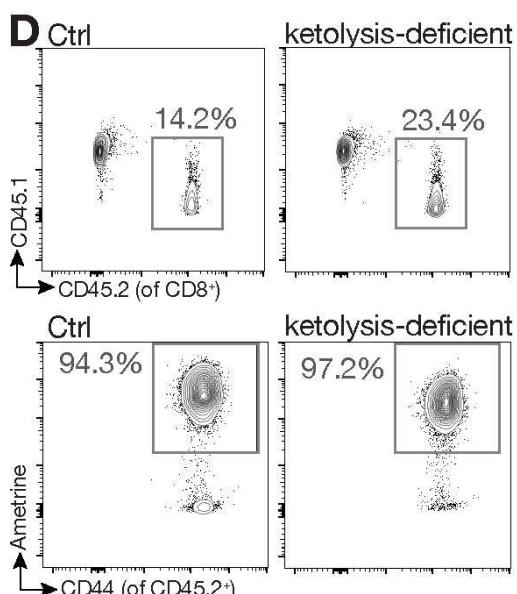
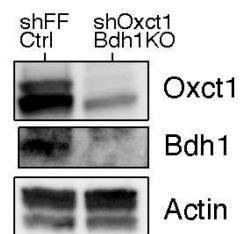
A Validation of ketolysis-deficiency



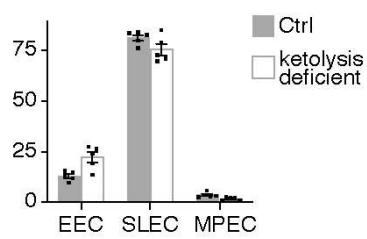
B Infection model



C Validation of sorted cells



E effector cells
% of CD45.2⁺ Ametrine⁺



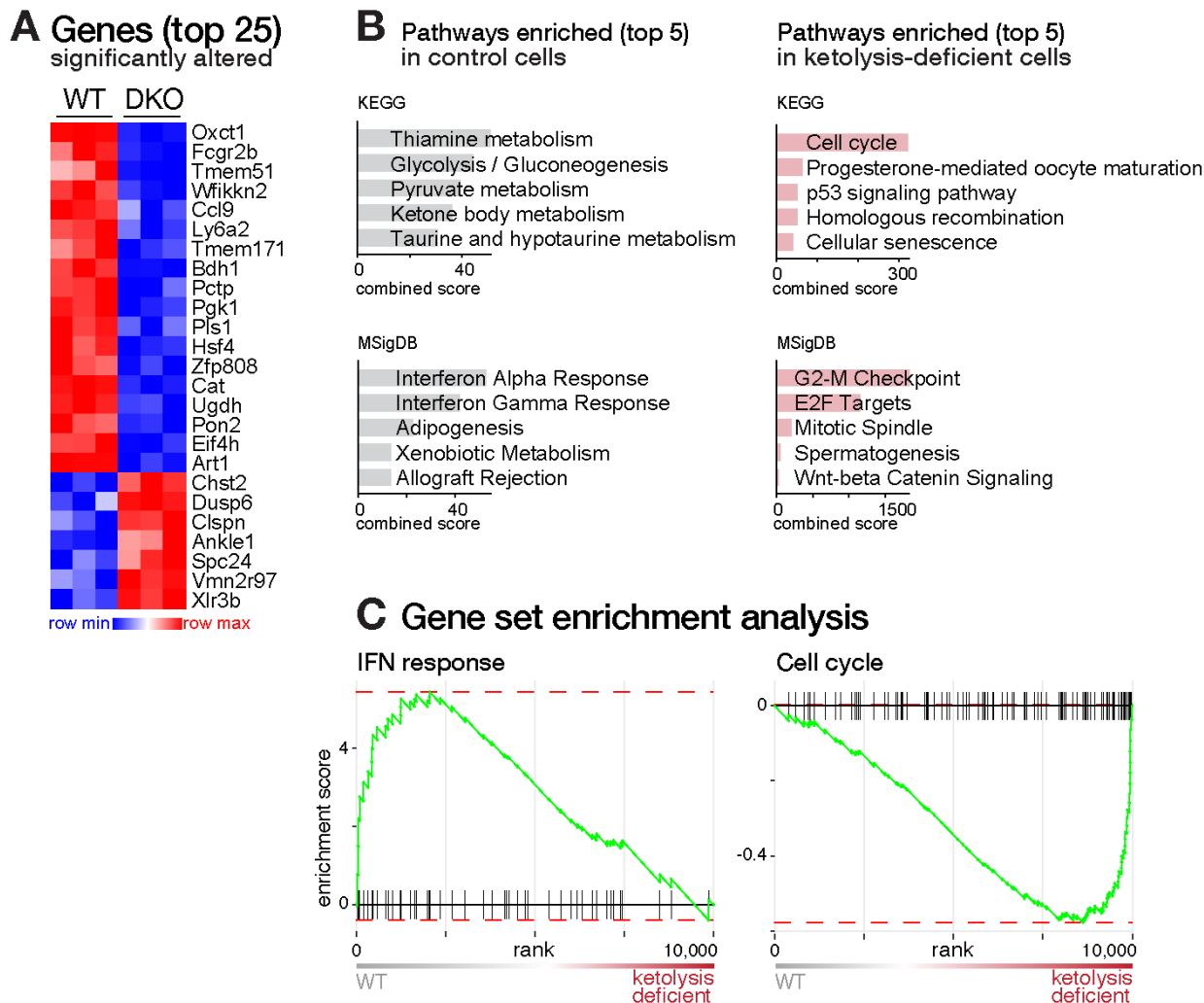
422

423 **Figure S4, related to Figure 1. Validation of ketolysis-deficient CD8⁺ effector T cells**

424 **and their *in vivo* response to Lm-OVA. (a) Validation of ketolysis-deficient T cells. Left,**
 425 **immunoblot of BDH1 protein expression in activated control (Ctrl) or Bdh1KO (Cd4-**
 426 **Cre;Bdh1^{fl/fl}) T cells transduced with control (shFF, firefly luciferase) or SCOT-targeting**
 427 **(shOxct1) retroviral vectors. Right, schematic depicting intervention strategy for**

428 generation of ketolysis-deficient T cells. **(b)** Schematic depicting the experimental setup
429 of the adoptive OT-I T cell transfer model coupled to *Lm*-OVA infection used for immune
430 phenotyping at 7 dpi. **(c)** Validation of *Oxct1*-silencing in ketolysis-deficient T cells.
431 Immunoblot of SCOT and BDH1 expression in CD8⁺ T cells from control T cells
432 expressing shRNA control vector (*shFF*;Ctrl) versus *Bdh1KO* cells transduced with
433 SCOT-targeting (*shOxct1*) retroviral vector (*shOxct1*;Bdh1KO). Actin levels are shown as
434 a loading control. **(d)** Response of splenic OT-I CD8⁺ cells to *Lm*-OVA 7 dpi as described
435 in **(b)**. *Left*, Representative flow cytometry plots for CD45.1 (host) and CD45.2 (donor)
436 expression by CD8⁺ cells, and detection of Ametrine signal within the CD8⁺CD45.2⁺T cell
437 population for the identification of adoptively transferred OT-I CD8⁺ T cells
438 (CD8⁺CD45.2⁺Ametrine⁺). *Right*, bar graph illustrating frequency of CD45.2⁺Ametrine⁺
439 OT-I T cells as a percentage of total CD8⁺ T cells. Data represent the mean \pm SEM (n=5).
440 **(e)** Distribution of OT-I⁺ T cells into various differentiation states at 7 dpi with *Lm*-OVA
441 (mean \pm SEM, n=5). T cell effector states are defined as: early effector cells (EEC),
442 KLRG1⁻CD127⁻; short lived effector cells (SLEC), KLRG1⁺CD127^{lo}; early memory
443 precursor cells (MPEC), KLRG1⁻CD127^{hi}. **, $p < 0.01$.
444
445

446 **Figure S5**



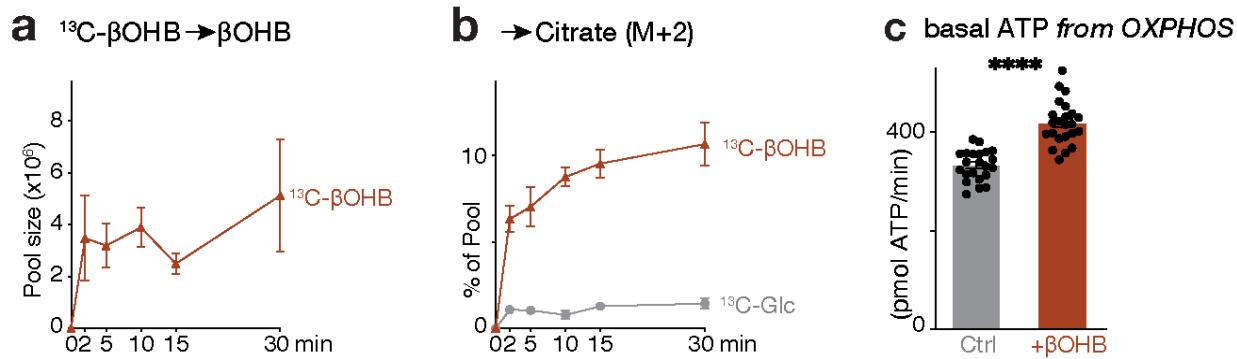
447

448 **Figure S5, related to Figure 1. Transcriptional analysis of *Lm*-OVA-specific**
449 **ketolysis-deficient CD8⁺ Teff cells. (a)** Heatmap depicting the top 25 differentially
450 expressed gene (DEG) transcripts ($p < 0.05$) altered in control (WT) and ketolysis-deficient
451 (DKO) OT-I CD8⁺ cells isolated from *Lm*-OVA-infected mice 7 dpi (n=3/group) (from
452 **Figure S4**). **(b)** Bar graphs representing top five pathways from KEGG and MSigDB
453 databases enriched in control (WT) versus ketolysis-deficient (DKO) OT-I CD8⁺ cells
454 isolated from *Lm*-OVA-infected mice 7 dpi (n=3/group). **(c)** Gene set enrichment analysis

455 (GSEA) of IFN response (MSigDB) and cell cycle (KEGG) genes comparing control (WT)
456 with ketolysis-deficient OT-I T cells responding to *Lm*-OVA (7 dpi, n=3 biological
457 replicates/group).

458

459 **Figure S6**



461 **Figure S6, related to Figure 2. Metabolic characterization of βOHB utilization in *in* vitro activated CD8^+ effector T cells.**

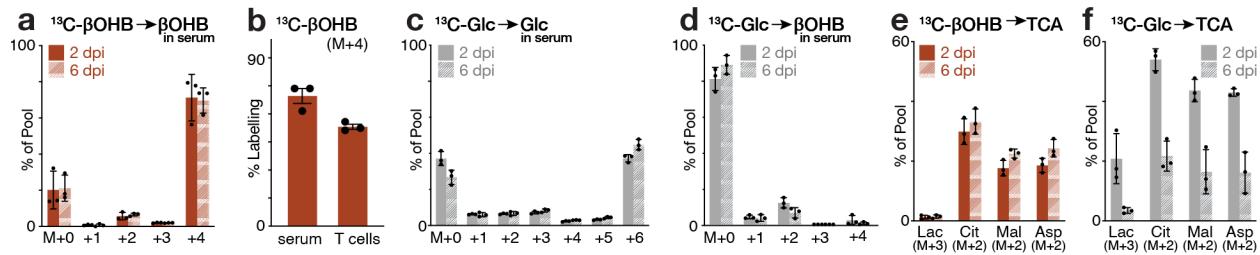
462

463 (a) Time course of βOHB pool sizes in activated CD8^+ T cells cultured with $[\text{U-}^{13}\text{C}_4]\beta\text{OHB}$.

464 (b) Relative contribution of $[\text{U-}^{13}\text{C}_4]\beta\text{OHB}$ or $[\text{U-}^{13}\text{C}_6]\text{glucose}$ to the $\text{M}+2$ pool of citrate in
465 activated CD8^+ T cells over time (mean \pm SEM, n=3). (c) Bioenergetic profile of *in vitro*
466 activated CD8^+ T cells cultured in the presence of 2mM βOHB . Bar graph represents
467 basal ATP production rate generated from OXPHOS. Data represents mean \pm SEM, n=22-
468 23/group ****, p < 0.0001

469

470 **Figure S7**



471

472 **Figure S7, related to Figure 2. Metabolic fate of $[U-^{13}\text{C}_4]\beta\text{OHB}$ and $[U-^{13}\text{C}_6]\text{glucose}$**

473 **during *in vivo* infusions during *Lm*-OVA infection.** Mice were adoptively transferred
474 with Thy1.1⁺OT-I T cells and infected with *Lm*-OVA (see **Fig. 1G**). At 2 or 6 dpi the mice
475 were infused with either $[U-^{13}\text{C}_4]\beta\text{OHB}$ or $[U-^{13}\text{C}_6]\text{glucose}$ for 2 h and serum and T cells
476 harvested for analysis. **(a)** Bar graph depicting MIDs of circulating βOHB after $[U-$
477 $^{13}\text{C}_4]\beta\text{OHB}$ infusions at 2 and 6 dpi; M+0 represents the endogenous unlabeled βOHB
478 pool and M+4 represents fully-labeled infused $[U-^{13}\text{C}_4]\beta\text{OHB}$. Data represent mean \pm
479 SEM for biological replicates (n=3). **(b)** Relative enrichment of $[U-^{13}\text{C}_4]\beta\text{OHB}$ of the total
480 βOHB pool in serum and the intracellular βOHB in isolated Thy1.1⁺OT-I T cells at 6 dpi
481 (mean \pm SEM, n=3). **(c-d)** MIDs of serum **(c)** glucose and **(d)** βOHB following $[U-$
482 $^{13}\text{C}_6]\text{glucose}$ infusions at 2 and 6 dpi. M+0 represents unlabeled metabolites, M+6
483 glucose in **(c)** represents enrichment of infused $[U-^{13}\text{C}_6]\text{glucose}$, and M+2 and M+4 in **(d)**
484 represents enrichment of ^{13}C -glucose-labeled βOHB due to ketogenesis. Data represent
485 mean \pm SEM for biological replicates (n=3). **(e-f)** Relative contribution of ^{13}C label into
486 lactate and TCA cycle derivatives citrate, malate and aspartate following **(e)** $[U-$
487 $^{13}\text{C}_4]\beta\text{OHB}$ and **(f)** $[U-^{13}\text{C}_6]\text{glucose}$ infusions at 2 and 6 dpi. MIDs for each metabolite were
488 normalized relative to serum enrichment of **(e)** M+4 βOHB and **(f)** M+6 glucose,

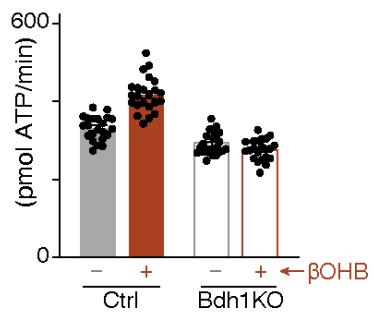
489 respectively. For all panels, the data represent the mean \pm SEM for 3 biological

490 replicates.

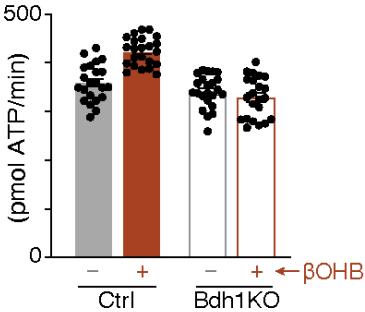
491

492 **Figure S8**

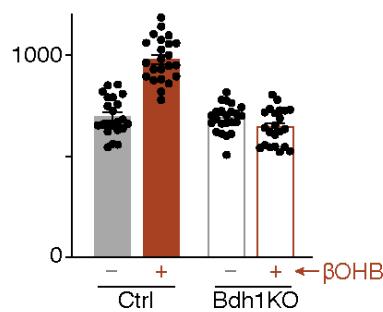
a basal ATP from OXPHOS



b basal ATP from glycolysis



max ATP from glycolysis



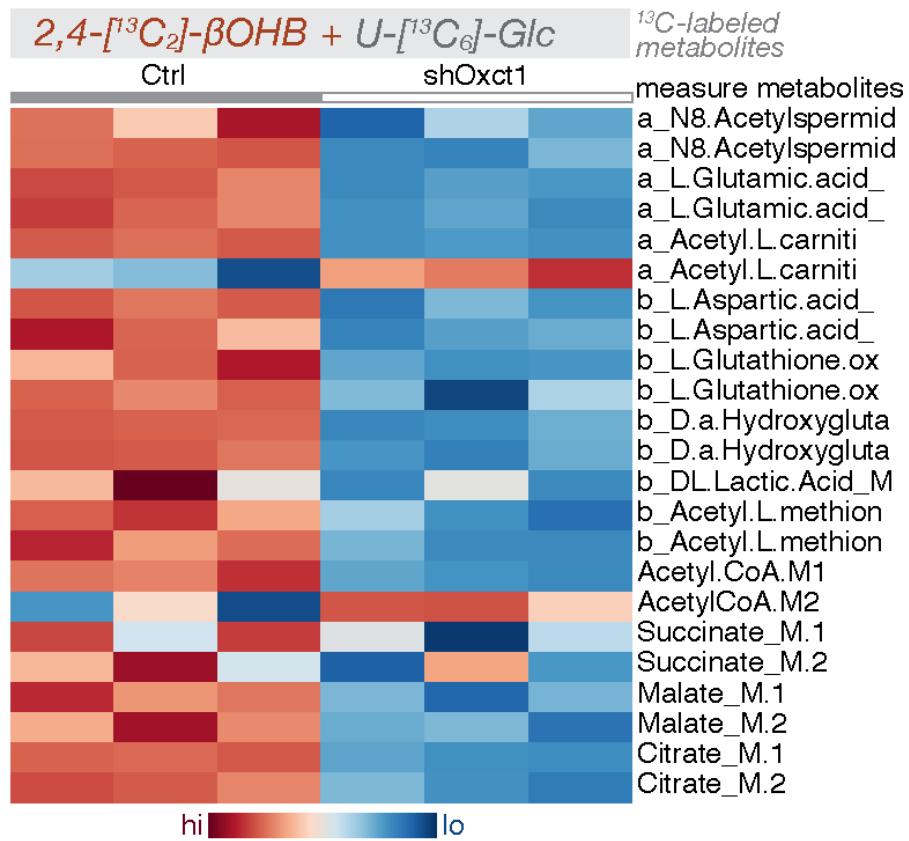
493

494 **Figure S8, related to Figure 3. β OHB increases the bioenergetic capacity of *in vitro*-**
495 **activated CD8⁺ Teff cells.** Bioenergetic profile of *in vitro*-activated control (Ctrl) and
496 Bdh1KO CD8⁺ T cells cultured in the presence (+) or absence (-) of 2mM β OHB. **(a)** Basal
497 ATP production rates from OXPHOS. **(b)** Basal (*left*) and maximal (*right*) ATP production
498 rates from glycolysis. Data represent the mean \pm SEM, n=22-24/group.

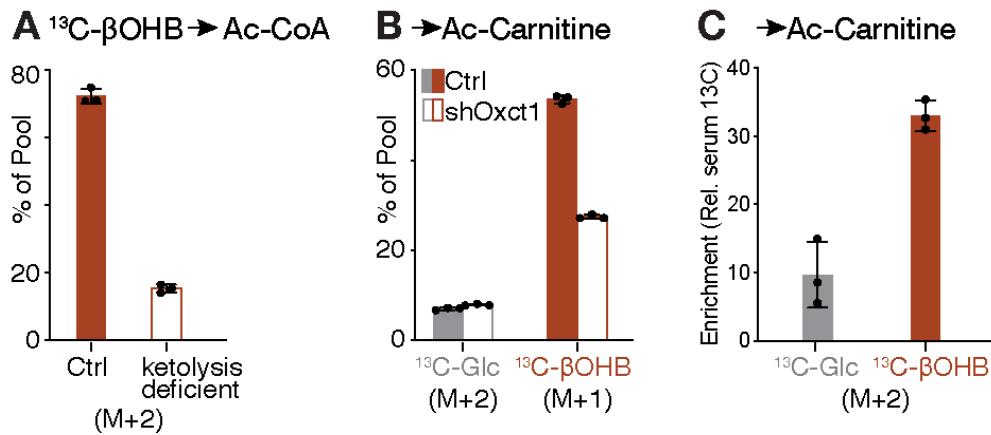
499

500

501 **Figure S9**



511 **Figure S10**



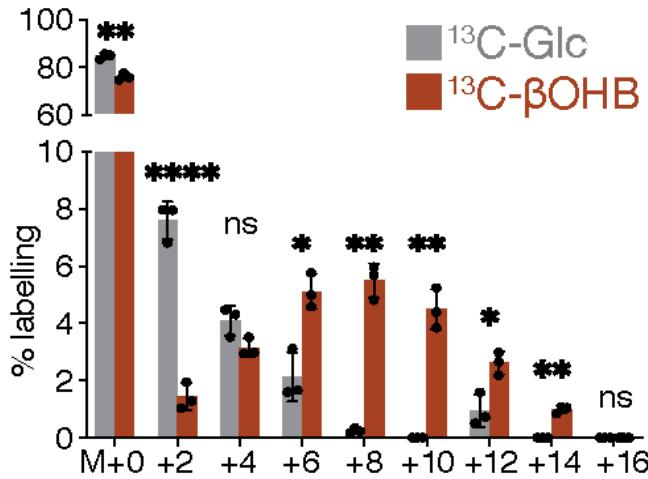
512

513 **Figure S10, related to Figure 3. βOHB -derived carbon contributes to acetyl-CoA**

514 **metabolism in activated CD8⁺ Teff cells. (a)** Metabolic production of acetyl-CoA from
515 $[\text{U-}^{13}\text{C}_4]\beta\text{OHB}$ in activated control (Ctrl) and ketolysis-deficient (*shOxct1;Bdh1KO*) CD8⁺
516 T cells. Shown is fractional enrichment of the acetyl group carbon (M+2) from ^{13}C - βOHB
517 into the acetyl-CoA pool *in vitro*-activated T cells (as in **Figure S4C**) after 2 h of culture.
518 Data represent the mean \pm SEM (n=3 replicates/group). **(b)** Relative ^{13}C contribution from
519 glucose (M+2) or βOHB (M+1) to the acetyl-carnitine pool in activated T cells. Control
520 (Ctrl, *shFF*) and *shOxct1*-expressing CD8⁺ T cells were cultured for 2 h with medium
521 containing 2 mM [2,4- $^{13}\text{C}_2]\beta\text{OHB}$ and 5 mM [U- $^{13}\text{C}_6]\text{glucose}$. Data represent the mean \pm
522 SEM (n=3/group). **(c)** Enrichment of ^{13}C carbon from $[\text{U-}^{13}\text{C}_6]\text{glucose}$ or $[\text{U-}^{13}\text{C}_4]\beta\text{OHB}$
523 into acetyl-carnitine (M+2) in Thy1.1⁺ OT-I T cells following 2 h infusion at 6 dpi with *Lm*-
524 OVA (as in **Figure 2j**). MIDs were normalized relative to serum enrichment of M+4 βOHB
525 and M+6 glucose, respectively. Data represents mean \pm SEM for 3 biological
526 replicates/group.

527 **Figure S11**

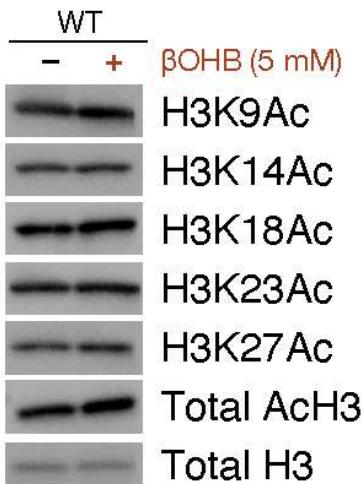
Palmitate labelling



528

529 **Figure S11, related to Figure 3. Contribution of glucose and β OHB to palmitate**
530 **synthesis in CD8 $^{+}$ Teff cells.** MID for [$\text{U-}^{13}\text{C}_6$]glucose- and [$\text{U-}^{13}\text{C}_4$] β OHB-derived carbon
531 into the intracellular palmitate pool for *in vitro*-activated CD8 $^{+}$ Teff cells after 24 h of
532 culture with each respective tracer. Data represent mean \pm SEM, n=3 replicates/group.
533 ns, not significant; *, $p < 0.05$; **, $p < 0.001$; ****, $p < 0.00001$.

534 **Figure S12**



535

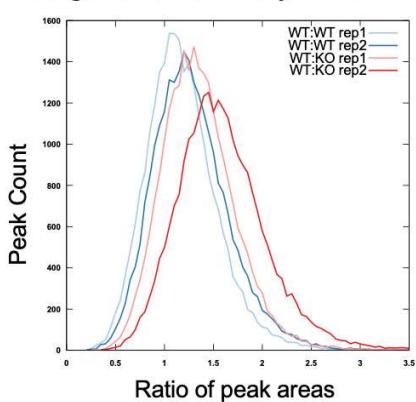
536 **Figure S12, related to Figure 4. Impact of exogenous β OHB on histone H3**

537 **acetylation in CD8⁺ Teff cells.** Immunoblot of total histone H3 acetylation (AcH3) and
538 acetylation at specific histone H3 residues (K9, K14, K18, K23, and K27) in isolated
539 histones from activated CD8⁺ T cells cultured in the presence (+) or absence (-) of 5 mM
540 β OHB for 24 h. Total histone H3 levels are shown as a loading control.

541

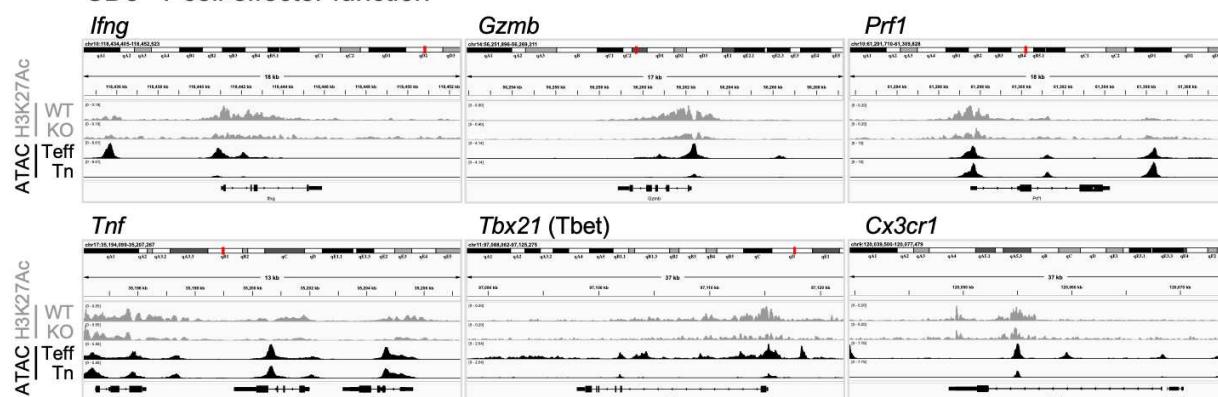
542 **Figure S13**

a Whole genome summary of H3K27Ac

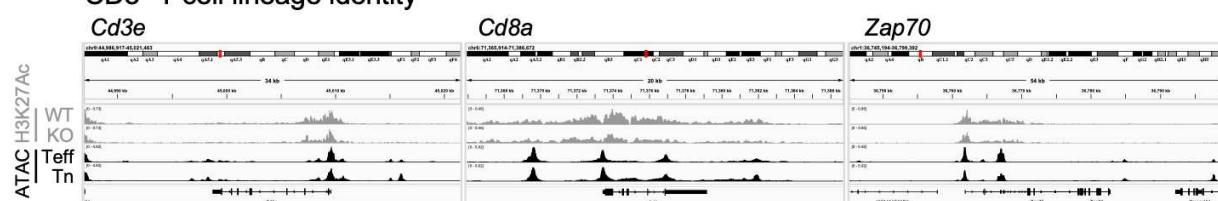


b H3K27Ac distribution at select loci (ChIP-seq)

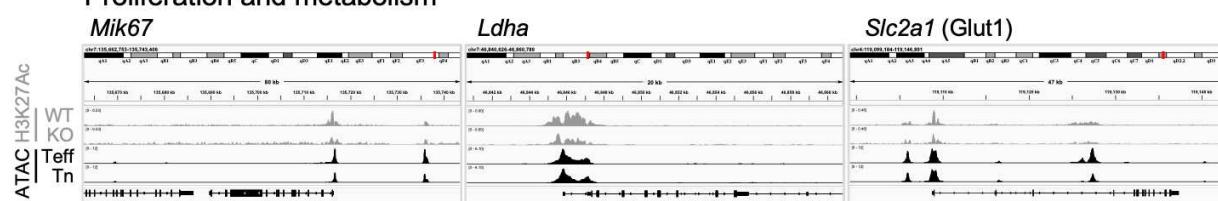
CD8⁺ T cell effector function



CD8⁺ T cell lineage identity



Proliferation and metabolism



543

544 **Figure S13, related to Figure 4. siQ-ChIP sequencing analysis of H3K27Ac**
 545 **enrichment at effector gene loci in CD8⁺ Teff cells. (a)** Histogram comparing genome-

546 wide enrichment of H3K27Ac siQ-ChIP signal (area under each peak) between control
547 (WT) and Bdh1KO (KO) replicates. **(b)** Representative genomic tracks (full genomic loci)
548 for H3K27Ac enrichment (grey) for genes associated with CD8⁺ effector T cell function,
549 CD8⁺ lineage identity, proliferation, and metabolism from control (WT) and Bdh1KO CD8⁺
550 T cells activated for 24 h with anti-CD3 and anti-CD28 antibodies. Chromatin
551 accessibility (ATC-seq) at corresponding genetic loci in naïve (Tn) and CD8⁺ effector
552 (Teff) cells from *Lm*-infected mice are shown in black (data from (27)). Data are
553 representative of duplicate samples.

554
555

556 **Table S4. List of primers used in this study**

Gene	Sequence	Amplicon Size (bp)	Reference
<i>Bdh1</i> genotyping	Fwd: TGCAGGAATCAGTGCTCTCCTAGCA	WT: 523 Flox: 697 KO: 275	(I)
	Rev: GGTGTCAGGGCTGAAGGATG		
	KO: ACAAGTGAATAAGTGGCAGAGGAATG		
Cre (transgene)	Fwd: CGATGCAACGAGTGATGAGGTTG	375	
	Rev: GCACGTTCACCGGCATCAAC		
Cre (Control)	Fwd: AACGATGAGGACCCGGTCTGTGAA	200	
	Rev: TGGACAGCTGGTTCACGATCATAGC		
OT-I (transgene)	Fwd: CAGCAGCAGGTGAGACAAAGT	300	
	Rev: GGCTTTATAATTAGCTTGGTCC		
OT-I (Control)	Fwd: CAAATGTTGCTTGTCTGGTG	200	
	Rev: GTCAGTCGAGTGCACAGTTT		
<i>Ifng</i> qPCR	Fwd: GATGCATTGAGTATTGCCAAGT	87	
	Rev: GTGGACCACTCGGATGAGCTC		
<i>Gzmb</i> qPCR	Fwd: TCAGGCTGCTGATCCTTGATCG	135	
	Rev: ATGAAGATCCTCCTGCTACTGC		

557

558 **Table S5. Key Resources Table**

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Hamster monoclonal anti-mouse CD3	eBioscience	Clone 145-2C11
Hamster monoclonal anti-mouse CD28	eBioscience	Clone 37.51
Rat monoclonal anti-mouse IFN-gamma	eBioscience	Clone AN-18
Rat monoclonal anti-mouse CD4	eBioscience	Clone GK1.5
Rat monoclonal anti-mouse IFN-gamma	eBioscience	Clone XMG1.2
Rat monoclonal anti-mouse CD8	eBioscience	Clone 53-6.7
Rat monoclonal anti-mouse TNF- α	eBioscience	Clone MP6-XT22
Rat monoclonal anti-mouse Granzyme B	eBioscience	Clone NGZB
Rat monoclonal anti-mouse CD44	eBioscience	Clone IM7
Hamster monoclonal anti-mouse CD69	eBioscience	Clone H1.2F3
Hamster monoclonal anti-mouse KLRG1	eBioscience	Clone 2F1
Anti-mouse CD127 (IL-7R)	BioLegend	Clone A7R34
Mouse monoclonal anti-mouse CD90.1 (Thy-1.1)	eBioscience	Clone HIS51
Mouse monoclonal anti-mouse CD45.1	BioLegend	Clone A20
Mouse monoclonal anti-mouse CD45.2	BioLegend	Clone 104
Rabbit polyclonal anti-BDH1	Protein Tech	15417-1-AP
Rabbit polyclonal anti-OXCT1	Protein Tech	12175-1-AP
Rabbit polyclonal anti-human/mouse β -actin	Cell Signaling	#4967
Rabbit polyclonal anti- α -Tubulin	Cell Signaling	#2146
Rabbit monoclonal anti-histone 3 (H3)	Cell Signaling	#4499, clone D1H2
Rabbit polyclonal anti-acetyl histone H3	Millipore	06-599
Rabbit monoclonal anti-acetyl histone H3 (Lys 9)	Cell Signaling	#9649, clone C5B11

Rabbit monoclonal anti-acetyl histone H3 (Lys 14)	Cell Signaling	#7627, clone D4B9
Rabbit monoclonal anti-acetyl histone H3 (Lys 18)	Cell Signaling	#13998, clone D8Z5H
Rabbit polyclonal anti-acetyl histone H3 (Lys 23)	Cell Signaling	#9674
Rabbit monoclonal anti-acetyl histone H3 (Lys 27)	Cell Signaling	#8173, clone D5E4
Rabbit polyclonal anti-acetyl histone H3 (Lys 27)	Active Motif	lot: 16119013
HRP-conjugated goat anti-rabbit IgG antibody	Cell Signaling	#7074
Bacterial and Virus Strains		
Attenuated (Δ actA) <i>Lm</i> -OVA	John Harty	(28)
Luciferase shRNA (LMPd-Amt backbone): AGCTCCCGTGAAATTGGAATCCTAGTGAAGCCACAG ATGTAGGATTCCAATTCAAGCGGGAGCC	Matthew Pipkin	(29)
Oxct1 shRNA1 (LMPd-Amt backbone): TGCTGTTGACAGTGAGCGCCGGAAGGGATGTCAGTA ATCAATAGTGAAGGCCACAGATGTATTGATTACTGAC ATCCTTCCGTTGCCTACTGCCTCGGA	This paper	custom
Chemicals, Peptides, and Recombinant Proteins		
D-glucose [$^{13}\text{C}_6$]	Cambridge Isotopes	CLM-1396
L-glutamine [$^{13}\text{C}_5$]	Cambridge Isotopes	CLM-1822
sodium acetate [$^{13}\text{C}_2$]	Cambridge Isotopes	CLM-440
sodium D-3-hydroxybutyrate [$^{13}\text{C}_4$]	Cambridge Isotopes	CLM-3853
sodium D-3-hydroxybutyrate [$2,4\text{-}^{13}\text{C}_2$]	Cambridge Isotopes	CLM-3706-1
citric acid [$^{13}\text{C}_6$]	Sigma Aldrich	606081
sodium lactate [$^{13}\text{C}_3$]	Cambridge Isotopes	CLM-10768
sodium pyruvate [$^{13}\text{C}_3$]	Cambridge Isotopes	CLM-2440
L-alanine [$^{13}\text{C}_3$]	Cambridge Isotopes	CLM-2184
IMDM GlutaMAX	Thermo Fisher	3198-022

Heat-inactivated FCS	Biochrom	S0115– Lot# 1289W
Penicillin-streptomycin	Millipore	A2213
2-mercaptoethanol	Gibco	#21985-023
Recombinant murine IL-2	Peprotech	212-12
Phorbol 12-myristate 13-acetate (PMA)	Sigma-Aldrich	P-8139
Ionomycin	Sigma-Aldrich	10634
Glucose ≥99,5 % D(+)	Roth	HN06.1
L-Glutamine	Biochrom AG	M11-004
Sodium Pyruvate	Gibco	#11360-070
Fixable Viability Dye eFluor 780	eBioscience	#65-0865-14
CellTrace Violet Cell Proliferation Kit	Thermo Fisher	C34557
BD GolgiStop	BD Biosciences	#51-2092KZ
Foxp3/transcription factor staining buffer set	eBioscience	#00-5523-00
Lyse/Fix Buffer, 5X	BD Biosciences	558049
Perm Buffer III	BD Biosciences	558050
16% Formaldehyde	Polysciences	18814-20
Hexadimethrine bromide (Polybrene)	Sigma-Aldrich	107689
OVA(257-264) SIINFEKL peptide	Bio-Synthesis Inc.	custom
SYBR green	Bio-Rad	#1725271
Critical Commercial Assays		
EasySep Mouse Naïve CD8+ T cell isolation kit	StemCell technologies	#19858
EasySep Mouse CD90.1 positive selection kit	StemCell technologies	#18958
Qiagen RNAeasy Kit	Qiagen	#74106
cOmplete, EDTA-free Protease Inhibitor Cocktail	Roche	11873580001
Seahorse XFe96 FluxPak	Agilent technologies	#102416-100
Histone Extraction Kit	Abcam	ab113476

High Capacity cDNA Reverse Transcriptase Kit	Life Technologies	#4368814
DNAse digestion kit	Qiagen	#79254
Autokit Total Ketone Bodies	Wako Fujifilm	415-73301
Experimental Models: Cell Lines		
293T cells	ATCC	CRL-3216
Experimental Models: Organisms/Strains		
C57BL/6J mice	Jackson Laboratories	JAX:000664
Tg(TcraTcrb)1100Mjb (OT-I) mice	Jackson Laboratories	JAX:003831
CD90.1 (Thy.1.1) mice	Jackson Laboratories	JAX:000406
B6.SJL- <i>Ptprc^a</i> <i>Pepc^b</i> /BoyJ (CD45.1) mice	Jackson Laboratories	JAX:002014
B6.Cg-Tg(Cd4-cre)1Cwi/BfluJ (CD4-Cre) mice	Jackson Laboratories	JAX:022071
<i>Bdh1</i> -floxed mice	Daniel Kelly	(1)
Software and Algorithms		
FlowJo 9.9.5	FlowJo LLC	www.flowjo.com
GraphPad Prism V9	GraphPad Software	www.graphpad.com
EI-Maven	Elucida Inc	https://elucidatainc.gitub.io/EIMaven/
Compound Discoverer V3.2	Thermo Scientific	https://www.thermoscifher.com/
Mass Hunter V10	Agilent Technologies	https://www.agilent.com
Integrative genomics viewer	UCSD/Broad Institute	www.igv.org
R V4.2.1	R Foundation for Statistical Computing	www.R-project.org

559

560

561 **Supplemental References**

562 1. J. L. Horton, M. T. Davidson, C. Kurishima, R. B. Vega, J. C. Powers, T. R.
563 Matsuura, C. Petucci, E. D. Lewandowski, P. A. Crawford, D. M. Muoio, F. A.
564 Recchia, D. P. Kelly, The failing heart utilizes 3-hydroxybutyrate as a metabolic
565 stress defense. *JCI Insight*. **4** (2019), doi:10.1172/JCI.INSIGHT.124079.

566 2. E. H. Ma, M. J. Verway, R. M. Johnson, D. G. Roy, M. Steadman, S. Hayes, K. S.
567 Williams, R. D. Sheldon, B. Samborska, P. A. Kosinski, H. Kim, T. Griss, B.
568 Faubert, S. A. Condotta, C. M. Krawczyk, R. J. DeBerardinis, K. Marsh, M. J.
569 Richer, V. Chubukov, T. P. Roddy, R. G. Jones, K. M. Stewart, M. J. Richer, V.
570 Chubukov, T. P. Roddy, R. G. Jones, K. Marsh, M. J. Richer, V. Chubukov, T. P.
571 Roddy, R. G. Jones, Metabolic Profiling Using Stable Isotope Tracing Reveals
572 Distinct Patterns of Glucose Utilization by Physiologically Activated CD8+ T
573 Cells. *Immunity*. **51**, 856-870.e5 (2019).

574 3. I. Kaymak, K. M. Luda, L. R. Duimstra, E. H. Ma, J. Longo, M. S. Dahabieh, B.
575 Faubert, B. M. Oswald, M. J. Watson, S. M. Kitchen-Goosen, L. M. DeCamp, S.
576 E. Compton, Z. Fu, R. J. DeBerardinis, K. S. Williams, R. D. Sheldon, R. G.
577 Jones, Carbon source availability drives nutrient utilization in CD8+ T cells. *Cell
578 Metab* (2022), doi:10.1016/J.CMET.2022.07.012.

579 4. D. G. Roy, J. Chen, V. Mamane, E. H. Ma, B. M. Muhire, R. D. Sheldon, T.
580 Shorstova, R. Koning, R. M. Johnson, E. Esaulova, K. S. Williams, S. Hayes, M.
581 Steadman, B. Samborska, A. Swain, A. Daigneault, V. Chubukov, T. P. Roddy,
582 W. Foulkes, J. A. Pospisilik, M. C. Bourgeois-Daigneault, M. N. Artyomov, M.
583 Witcher, C. M. Krawczyk, C. Larochelle, R. G. Jones, Methionine Metabolism

584 Shapes T Helper Cell Responses through Regulation of Epigenetic
585 Reprogramming. *Cell Metab.* **31**, 250-266.e9 (2020).

586 5. E. H. Ma, G. Bantug, T. Griss, S. Condotta, R. M. Johnson, B. Samborska, N.
587 Mainolfi, V. Suri, H. Guak, M. L. Balmer, M. J. Verway, T. C. Raissi, H. Tsui, G.
588 Boukhaled, S. Henriques da Costa, C. Frezza, C. M. Krawczyk, A. Friedman, M.
589 Manfredi, M. J. Richer, C. Hess, R. G. Jones, Serine Is an Essential Metabolite for
590 Effector T Cell Expansion. *Cell Metab.* **25**, 345–357 (2017).

591 6. J. Blagih, F. Coulombe, E. E. Vincent, F. Dupuy, G. Galicia-Vázquez, E.
592 Yurchenko, T. C. Raissi, G. J. W. VanderWindt, B. Viollet, E. L. Pearce, J.
593 Pelletier, C. A. Piccirillo, C. M. Krawczyk, M. Divangahi, R. G. Jones, G. Galicia-
594 Vazquez, E. Yurchenko, T. C. Raissi, G. J. van der Windt, B. Viollet, E. L. Pearce,
595 J. Pelletier, C. A. Piccirillo, C. M. Krawczyk, M. Divangahi, R. G. Jones, The
596 energy sensor AMPK regulates T cell metabolic adaptation and effector
597 responses in vivo. *Immunity.* **42**, 41–54 (2015).

598 7. R. D. Sheldon, E. H. Ma, L. M. DeCamp, K. S. Williams, R. G. Jones,
599 Interrogating in vivo T-cell metabolism in mice using stable isotope labeling
600 metabolomics and rapid cell sorting. *Nat Protoc.* **16**, 4494–4521 (2021).

601 8. S. A. Mookerjee, A. A. Gerencser, D. G. Nicholls, M. D. Brand, Quantifying
602 intracellular rates of glycolytic and oxidative ATP production and consumption
603 using extracellular flux measurements. *J Biol Chem.* **292**, 7189–7207 (2017).

604 9. E. G. Bligh, W. J. Dyer, A rapid method of total lipid extraction and purification.
605 *Can J Biochem Physiol.* **37**, 911–7 (1959).

606 10. E. H. Ma, M. J. Verway, R. M. Johnson, D. G. Roy, M. Steadman, S. Hayes, K. S.
607 Williams, R. D. Sheldon, B. Samborska, P. A. Kosinski, H. Kim, T. Griss, B.
608 Faubert, S. A. Condotta, C. M. Krawczyk, R. J. DeBerardinis, K. Marsh, M. J.
609 Richer, V. Chubukov, T. P. Roddy, R. G. Jones, K. M. Stewart, M. J. Richer, V.
610 Chubukov, T. P. Roddy, R. G. Jones, K. Marsh, M. J. Richer, V. Chubukov, T. P.
611 Roddy, R. G. Jones, Metabolic Profiling Using Stable Isotope Tracing Reveals
612 Distinct Patterns of Glucose Utilization by Physiologically Activated CD8+ T
613 Cells. *Immunity*. **51**, 856-870.e5 (2019).
614 11. T. Griss, E. E. Vincent, R. Egnatchik, J. Chen, E. H. Ma, B. Faubert, B. Viollet, R.
615 J. DeBerardinis, R. G. Jones, Metformin Antagonizes Cancer Cell Proliferation by
616 Suppressing Mitochondrial-Dependent Biosynthesis. *PLoS Biol.* **13**, e1002309
617 (2015).
618 12. S. Trefely, P. Ashwell, N. W. Snyder, FluxFix: automatic isotopologue
619 normalization for metabolic tracer analysis. *BMC Bioinformatics*. **17**, 485 (2016).
620 13. C. A. Fernandez, C. Des Rosiers, S. F. Previs, F. David, H. Brunengraber,
621 Correction of ¹³C mass isotopomer distributions for natural stable isotope
622 abundance. *J Mass Spectrom.* **31**, 255–62 (1996).
623 14. D. G. Roy, J. Chen, V. Mamane, E. H. Ma, B. M. Muhire, R. D. Sheldon, T.
624 Shorstova, R. Koning, R. M. Johnson, E. Esaulova, K. S. Williams, S. Hayes, M.
625 Steadman, B. Samborska, A. Swain, A. Daigneault, V. Chubukov, T. P. Roddy,
626 W. Foulkes, J. A. Pospisilik, M. C. Bourgeois-Daigneault, M. N. Artyomov, M.
627 Witcher, C. M. Krawczyk, C. Larochelle, R. G. Jones, Methionine Metabolism

628 Shapes T Helper Cell Responses through Regulation of Epigenetic
629 Reprogramming. *Cell Metab.* **31**, 250-266.e9 (2020).

630 15. W. Luo, M. S. Friedman, K. Shedden, K. D. Hankenson, P. J. Woolf, GAGE:
631 generally applicable gene set enrichment for pathway analysis. *BMC*
632 *Bioinformatics*. **10**, 161 (2009).

633 16. M. Martin, Cutadapt removes adapter sequences from high-throughput
634 sequencing reads. *EMBnet J.* **17**, 10–12 (2011).

635 17. A. Dobin, C. A. Davis, F. Schlesinger, J. Drenkow, C. Zaleski, S. Jha, P. Batut, M.
636 Chaisson, T. R. Gingeras, STAR: ultrafast universal RNA-seq aligner.
637 *Bioinformatics*. **29**, 15–21 (2013).

638 18. M. E. Ritchie, B. Phipson, D. Wu, Y. Hu, C. W. Law, W. Shi, G. K. Smyth, limma
639 powers differential expression analyses for RNA-sequencing and microarray
640 studies. *Nucleic Acids Res.* **43**, e47–e47 (2015).

641 19. M. I. Love, W. Huber, S. Anders, Moderated estimation of fold change and
642 dispersion for RNA-seq data with DESeq2. *Genome Biol.* **15**, 1–21 (2014).

643 20. S. Durinck, P. T. Spellman, E. Birney, W. Huber, Mapping identifiers for the
644 integration of genomic datasets with the R/Bioconductor package biomaRt.
645 *Nature Protocols 2009 4:8.* **4**, 1184–1191 (2009).

646 21. S. Durinck, Y. Moreau, A. Kasprzyk, S. Davis, B. de Moor, A. Brazma, W. Huber,
647 BioMart and Bioconductor: a powerful link between biological databases and
648 microarray data analysis. *Bioinformatics*. **21**, 3439–3440 (2005).

649 22. A. Subramanian, P. Tamayo, V. K. Mootha, S. Mukherjee, B. L. Ebert, M. A.
650 Gillette, A. Paulovich, S. L. Pomeroy, T. R. Golub, E. S. Lander, J. P. Mesirov,
651 Gene set enrichment analysis: A knowledge-based approach for interpreting
652 genome-wide expression profiles. *Proc Natl Acad Sci U S A.* **102**, 15545–15550
653 (2005).

654 23. T. Wu, E. Hu, S. Xu, M. Chen, P. Guo, Z. Dai, T. Feng, L. Zhou, W. Tang, L. Zhan,
655 X. Fu, S. Liu, X. Bo, G. Yu, clusterProfiler 4.0: A universal enrichment tool for
656 interpreting omics data. *The Innovation.* **2**, 100141 (2021).

657 24. B. M. Dickson, R. L. Tiedemann, A. A. Chomiak, E. M. Cornett, R. M. Vaughan, S.
658 B. Rothbart, A physical basis for quantitative ChIP-sequencing. *J Biol Chem.*
659 **295**, 15826–15837 (2020).

660 25. B. M. Dickson, A. Kupai, R. M. Vaughan, S. B. Rothbart, Theoretical and practical
661 refinements of sans spike-in quantitative ChIP-seq with application to p300/CBP
662 inhibition. *bioRxiv, under review*, doi:10.1101/2022.08.09.503331.

663 26. J. T. Robinson, H. Thorvaldsdóttir, W. Winckler, M. Guttman, E. S. Lander, G.
664 Getz, J. P. Mesirov, Integrative genomics viewer. *Nature Biotechnology* 2011
665 **29**:1. **29**, 24–26 (2011).

666 27. M. Philip, L. Fairchild, L. Sun, E. L. Horste, S. Camara, M. Shakiba, A. C. Scott,
667 A. Viale, P. Lauer, T. Merghoub, M. D. Hellmann, J. D. Wolchok, C. S. Leslie, A.
668 Schietinger, Chromatin states define tumour-specific T cell dysfunction and
669 reprogramming. *Nature.* **545**, 452–456 (2017).

670 28. A. R. Tvinnereim, S. E. Hamilton, J. T. Harty, CD8(+)T-cell response to secreted
671 and nonsecreted antigens delivered by recombinant *Listeria monocytogenes*
672 during secondary infection. *Infect Immun.* **70**, 153–162 (2002).

673 29. R. Chen, S. Bélanger, M. A. Frederick, B. Li, R. J. Johnston, N. Xiao, Y. C. Liu, S.
674 Sharma, B. Peters, A. Rao, S. Crotty, M. E. Pipkin, In vivo RNA interference
675 screens identify regulators of antiviral CD4(+) and CD8(+) T cell differentiation.
676 *Immunity.* **41**, 325–338 (2014).

677