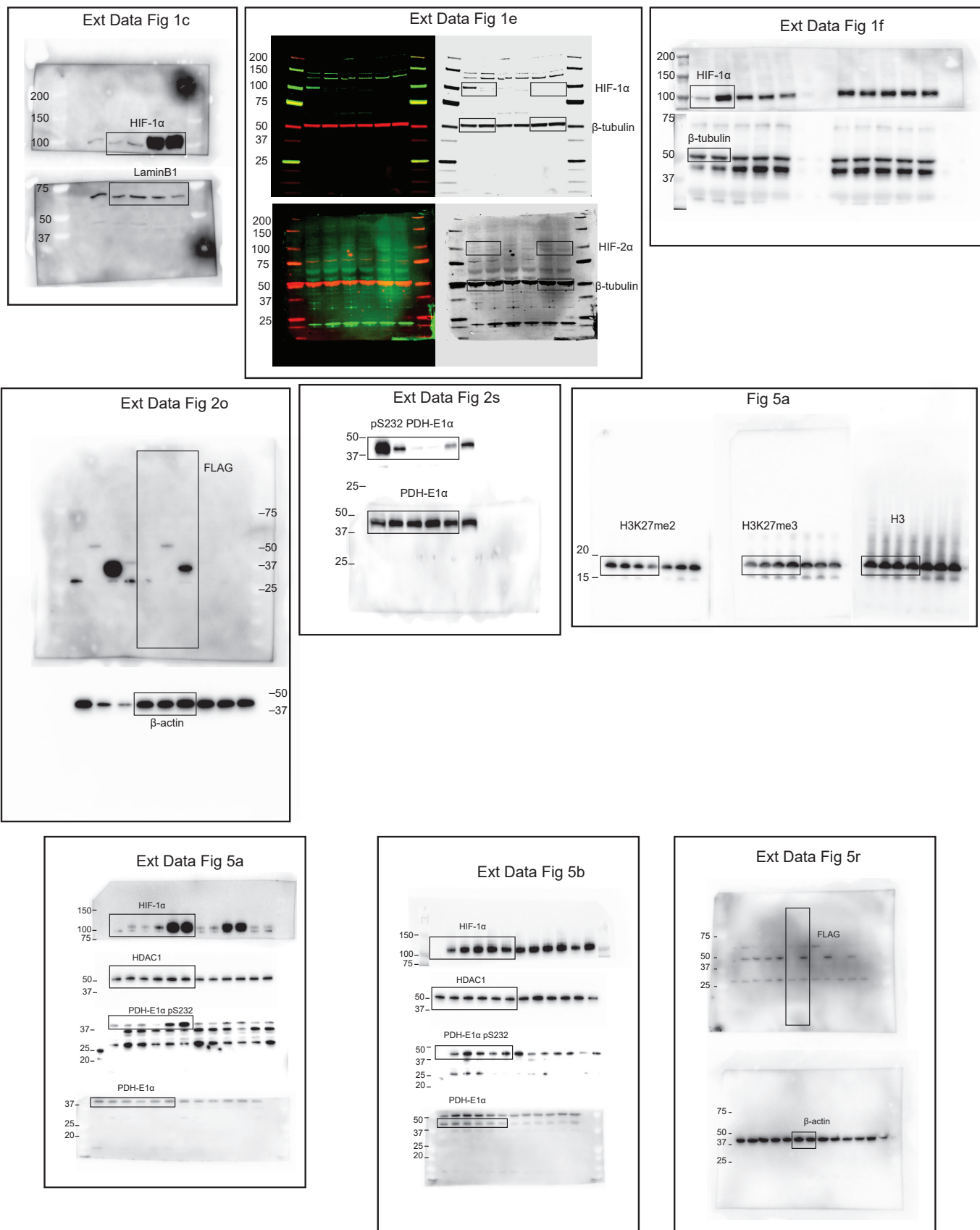
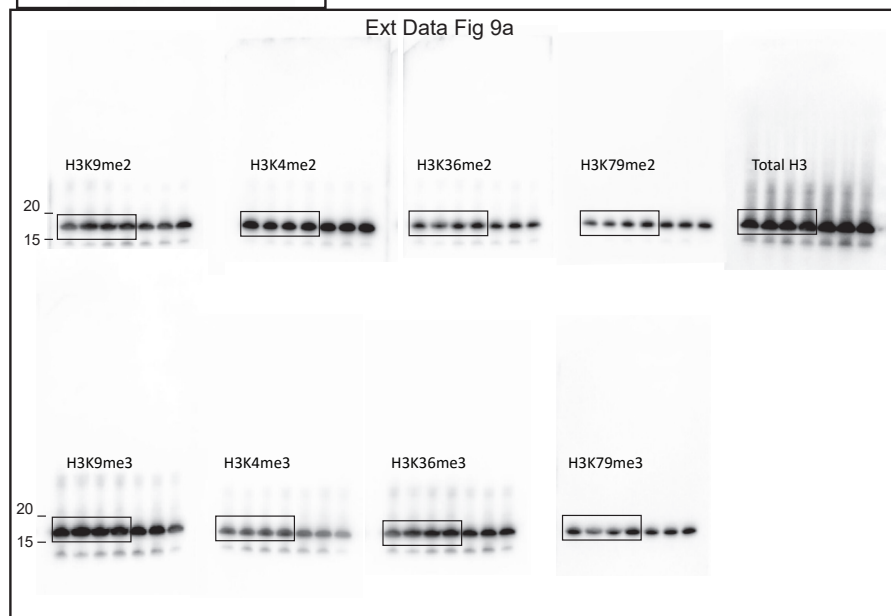
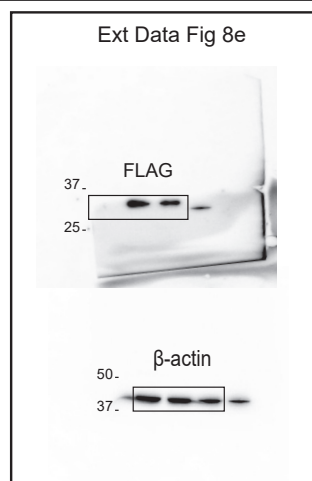
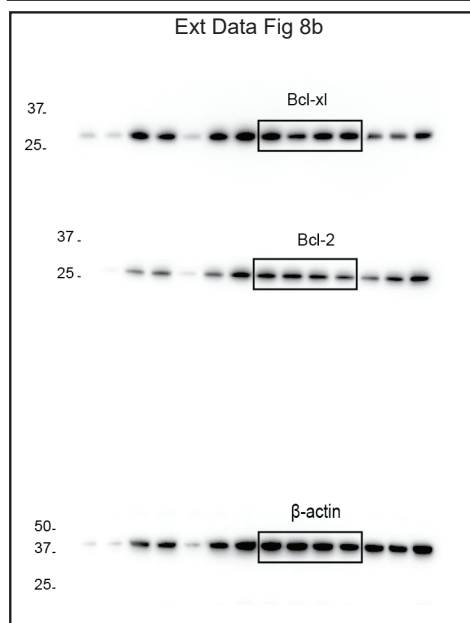
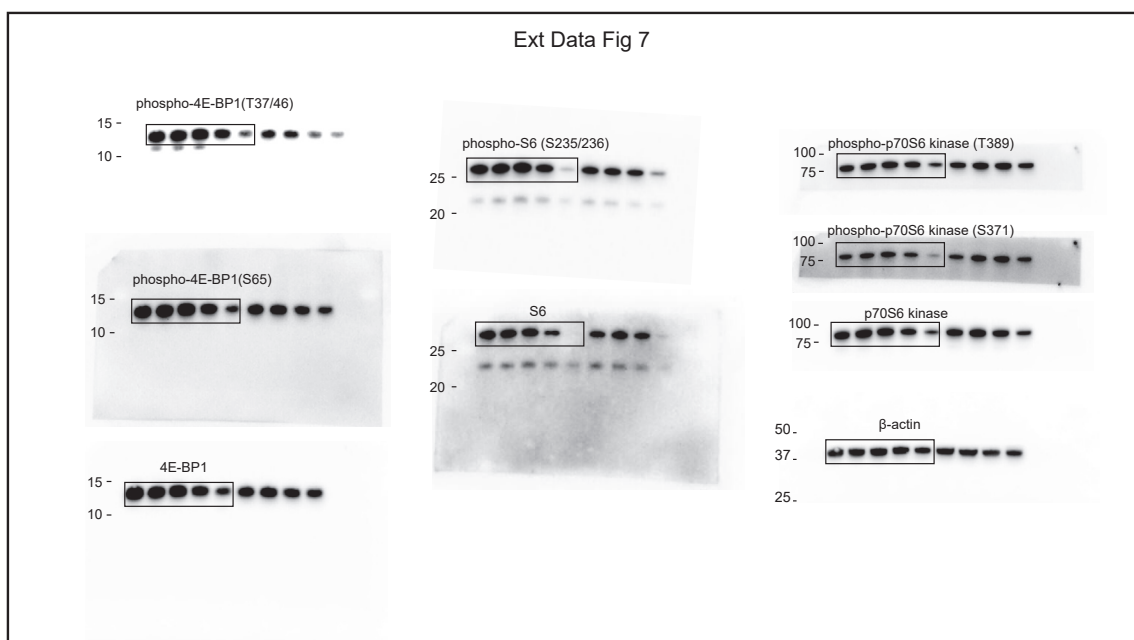


Supplementary Figure of Uncropped Gels with Size Marker Indications



Supplementary Figure of Uncropped Gels with Size Marker Indications



## **Methods**

### **Isolation and activation of CD8<sup>+</sup> T-lymphocytes**

CD8<sup>+</sup> T-lymphocytes were isolated from mouse spleens by positive selection. Incubation with MicroBeads conjugated to monoclonal anti-mouse CD8 $\alpha$  (Ly-2; isotype: rat IgG2a) antibody (Miltenyi, 130-049-401) was followed by magnetic bead isolation on a MACS column. Naïve CD8<sup>+</sup> T-lymphocytes were isolated from mouse spleens using the naïve CD8 $\alpha$ <sup>+</sup> T Cell Isolation Kit (Miltenyi, 130-096-543). Unless otherwise stated, CD8<sup>+</sup> T-lymphocytes were activated with plate-bound  $\alpha$ CD3 (5  $\mu$ g/ml) and soluble  $\alpha$ CD28 (1 $\mu$ g/ml) for 48 h. For activation of OT-I CD8<sup>+</sup> T-cells, total splenocytes from OT-I mice were cultured with SIINFEKL peptide for 48 h. All CD8<sup>+</sup> T-cells were cultured in RPMI-1640 medium containing 2 mM glutamine, 10% FBS, 25 mM HEPES, pH 7.2, 1% penicillin-streptomycin, 55  $\mu$ M  $\beta$ -mercaptoethanol and supplemented with or without 20 ng/ml recombinant murine IL-2 (Biolegend, 575404), unless otherwise stated.

### **Cell culture**

Following activation for 48 h, CD8<sup>+</sup> T-lymphocytes were cultured in either 21% or 1% oxygen conditions for various times. High glucose DMEM supplemented with 10% FBS and 0.8 mg/ml of G418, was used as standard growth medium for RCC4EV, RCC4VHL, 786-OEV and 786-OVHL cells. RCC4EV, RCC4VHL, 786-OEV and 786-OVHL cells were a gift from Prof Patrick Maxwell and authenticated by STR genotyping. MEFs, EL-4 and EG7-OVA cells were cultured in high glucose DMEM supplemented with 10% FBS, 1% penicillin/streptomycin. EG7-OVA cells were cultured with 0.4 mg/ml G481. EL-4 and EG7-OVA cells were obtained from the ATCC. All cell lines were free from mycoplasma as assessed by Mycoprobe (R&D Systems). For low oxygen experiments, cells were transferred into a Ruskinn Sci-tive hypoxia workstation for the indicated amount of time. S-2HG-octyl ester was purchased from Toronto Research Chemicals and DCA from Sigma.

### **Human CD8<sup>+</sup> T-lymphocyte isolation and culture**

Ethical approval was obtained from the East of England-Cambridge Central Research Ethics Committee (06/Q0108/281) and consent was obtained from all subjects. Peripheral blood mononuclear cells (PBMCs) from healthy donors were isolated from blood using discontinuous plasma-Percoll gradients. CD8<sup>+</sup> T-lymphocytes were then MACS purified from PBMCs by positive selection with anti-human CD8 microbeads (Miltenyi). Purification efficiency was confirmed by flow cytometry using fluorophore conjugated anti-CD8 $\alpha$  (Biolegend, HIT8a). CD8<sup>+</sup> T-lymphocytes were activated with CD3/CD28 Dynabeads (Thermo) and cultured in RPMI-1640 medium containing 2 mM glutamine, 10% FBS, 25 mM HEPES, pH 7.2, 1% penicillin-streptomycin and supplemented with 20 ng/ml recombinant human IL-2 (Biolegend, 589106). 2 days after activation, CD8<sup>+</sup> T-lymphocytes were cultured in either 5% or 1% oxygen conditions, or kept in 21% oxygen for a further 2 days at which point metabolites were extracted and measured.

### **Cell counting, volume and viability**

Cells were counted on an ADAM-MC automated cell counter (NanoEnTek) and viability was assessed by the exclusion of propidium iodide. Cell volume was determined using a Z2 Coulter counter (Beckman Coulter).

### **Flow cytometry and sorting**

Cells were stained and acquired on a Fortessa (BD Biosciences). The following fluorophores and fluorophore-conjugated antibodies were used: anti-CD62L (Biolegend, MEL-14), anti-CD44 (Biolegend, IM7), anti-CD8 $\alpha$  (Biolegend, 53-6.7), anti-CD45.1 (Biolegend, A20), anti-CD45.2 (Biolegend, 104), anti-CD127 (Biolegend, A7R34), anti-KLRG1 (Biolegend, 2F1/KLRG1), anti-CD19 (Biolegend, 6D5), anti-IFN $\gamma$  (Biolegend, XMG1.2), anti-TNF $\alpha$  (Biolegend, MP6-XT22), anti-Bcl-2 (Biolegend, BCL/10C4), anti-Eomes (eBioscience, Dan11mag), anti-PD-1 (Biolegend, 29F.1A12), anti-41BB (Biolegend, 17B5), anti-H3K27me3 (Cell Signalling Technologies, 5499 and 12158), CFSE (Life Technologies), LIVE/DEAD Violet (Life Technologies), PI-AnnexinV (Biolegend, 640928). For staining of nuclear-located markers Eomes and H3K27me3, the

Transcription Factor Staining Buffer Set (eBioscience) was used, according to the manufacturer's instructions. CD8<sup>+</sup> T-lymphocytes were treated for 7 days with the indicated dose of S-2HG-octyl ester. For staining of intracellular cytokines, OT-I cells were re-stimulated with SIINFEKL peptide for 4 h with GolgiStop solution (BD). For <sup>1</sup>H-NMR experiments, CD8<sup>+</sup> T-lymphocytes were sorted from total splenocytes by immunostaining with anti-mouse CD8-AF647 (Biolegend) on a MoFlo (Beckman Coulter). Cells were then activated and cultured as described above.

### Generation of *Vhl*<sup>-/-</sup> mouse embryonic fibroblasts

Mouse embryonic fibroblasts (MEFs) were isolated from E12.5-14.5 *Vhl*<sup>fl/fl</sup> embryos. MEFs were then immortalised by stable transfection with the SV40 large T antigen. Fifteen passages later, to perform acute deletions of *Vhl*, cells of each genotype were transiently (24 h) infected with 100 PFU/cell of adenovirus expressing either eGFP alone, or both Cre recombinase and eGFP (Vector Biolabs). Cell populations were then enriched for eGFP by sorting with on a MoFlo (Beckman Coulter) flow cytometry.

### Determination of deletion efficiency by qPCR

To quantify deletion efficiency, gDNA was isolated using a DNeasy Blood & Tissue Kit (Qiagen) followed by real-time PCR. Abundance of the target gene was normalized to *Actb* gDNA levels. The following primer and probe sequences (Integrated DNA Technologies) were used:

<i>Hif1α</i> forward	5' GGTGCTGGTGTCCAAAATGTAG 3'
<i>Hif1α</i> reverse	5' ATGGGTCTAGAGAGATAGCTCCACA 3'
<i>Hif1α</i> probe	5' [6-FAM] CCTGTTGGTTGCGCAGCAAGCATT [BHQ1a-Q] 3'
<i>Hif2α</i> forward	5' TCTATGAGTTGGCTCATGAGTTG 3'
<i>Hif2α</i> reverse	5' GTCCGAAGGAAGCTGATGG 3'
<i>Hif2α</i> probe	5' [6-FAM] CCACCTGGA/ZEN/CAAAGCCTCCATCAT [3IABkFQ] 3'
<i>Vhl</i> forward	5' GCTTGC GAATCCGAGGG 3'
<i>Vhl</i> reverse	5' TCCTCTGGACTGGCTGCC 3'
<i>Vhl</i> probe	5' [6-FAM] CCCGTTCCAATAATGCCCCGG [BHQ1a-Q] 3'
<i>Actb</i> forward	5' AGAGGGAAATCGTGCGTGA 3'
<i>Actb</i> reverse	5' CAATAGTGATGACCTGGCCGT 3'
<i>Actb</i> probe	5' [6-FAM] CACTGCCGCATCCTCTTCCTC [BHQ1a-Q] 3'

### Sequencing of *Idh1* and *Idh2*

gDNA was extracted from the following CD8<sup>+</sup> T-lymphocytes samples: freshly isolated, naïve *Hif1α*<sup>fl/fl</sup> and *Hif1α*<sup>fl/fl</sup>*dLck*<sup>cre</sup>; expanding *Hif1α*<sup>fl/fl</sup> and *Hif1α*<sup>fl/fl</sup>*dLck*<sup>cre</sup> after a total of 4 days in 21% oxygen; expanding *Hif1α*<sup>fl/fl</sup> and *Hif1α*<sup>fl/fl</sup>*dLck*<sup>cre</sup> after 2 days at 21% oxygen and a further 2 days at 1% oxygen (total of 4 days in culture). PCR was performed using the following primer pairs (5' to 3'), followed by purification of the product and Sanger sequencing:

*Idh1* forward, TGTAGATGCCCAAAGAACAAACA

*Idh1* reverse, AGGTTGCAGCCTGTCAGTTT

*Idh2* forward, AGCAAGGATAACAACTAGGCAGA

*Idh2* reverse, GGAAGCCTTGGCTTGAAGTGC

### qRT-PCR

RNA was isolated using the RNeasy kit (Qiagen). 0.25 µg-1 µg of RNA was used for cDNA synthesis with the First-Strand Synthesis kit (Invitrogen). All samples were run in technical triplicates using a StepOnePlus system (Applied Biosystems) with SYBR green and differential expression was calculated by the  $\Delta\Delta$ Ct method<sup>1</sup> using *Hprt* as the reference gene. Data were analysed by unsupervised hierarchical clustering and visualized by heat map. 500 µM S-2HG-octyl ester was used in experiments with S-2HG treatment, unless otherwise indicated in the figure. The following primers were used:

Gene Symbol	Primer Bank ID <sup>2-4</sup>	Forward Primer (5' to 3')	Reverse Primer (5' to 3')
<i>Cs</i>	13385942a1	GGACAATTTTCCAACCAATCTGC	TCGGTTCATTCCCTCTGCATA
<i>Aco2</i>	18079339a1	ATCGAGCGGGGAAAGACATAC	TGATGGTACAGCCACCTTAGG
<i>Idh1</i>	6754278a1	ATGCAAGGAGATGAAATGACACG	GCATCACGATTCTCTATGCCTAA
<i>Idh2</i>	1236984a1	GGAGAAGCCGGTAGTGGAGAT	GGTCTGGTCACGGTTTGGAA
<i>Idh3a</i>	18250284a1	TGGGTGTCCAAGGTCTCTC	CTCCCACTGAATAGGTGCTTTG
<i>Idh3b</i>	18700024a1	TGGAGAGGTCTCGGAACATCT	AGCCTTGAACACTTCCTTGAC
<i>Idh3g</i>	6680345a1	GGTGTGCAAAGGCAATGC	TATGCCGCCACCATACTTAG
<i>Ogdh</i>	15489120a1	AGGGCATATCAGATACGAGGG	CTGTGGATGAGATAATGTCAGCG
<i>Sucla2</i>	26349481a1	ACCTTTTCGCTGCATGAATAC	CCTGTGCCTTTATCACAACATCC
<i>Suclg2</i>	3766203a1	CCCCGAAGATGGCTGAACC	ACCTCCTTCAAACCGCTATTG
<i>Suclg1</i>	9845299a1	TGGGATACGACACGGGTCTTA	CAGAAGCCGTTGCTCCTGTT
<i>Sdha</i>	15030102a1	GGAACACTCCAAAAACAGACCT	CCACCACTGGGTATTGAGTAGAA
<i>Sdhb</i>	34328286a1	AATTTGCCATTTACCGATGGGA	AGCATCCAACACCATAGGTCC
<i>Sdhc</i>	13384690a1	GCTGCGTCTTGCTGAGACA	ATCTCCTCCTTAGCTGTGGTT
<i>Sdhd</i>	27229021a1	TGGTCAGACCCGCTTATGTG	GGTCCAGTGGAGAGATGCAG
<i>Fh1</i>	33859554a1	GAATGGCAAGCCAAAATTCCTT	CGTTCGCTAGCACCTCCAATCTT
<i>Mdh1</i>	31982178a1	TTCTGGACGGTGTCTGTATG	TTTCACATTGGCTTTCAGTAGGT
<i>Mdh2</i>	31982186a1	TTGGGCAACCCCTTCACTC	GCCTTTCACATTGCTCTGGTC
<i>Gls</i>	164607131c2	CTACAGGATTGCGAACATCTGAT	ACACCATCTGACGTTGTCTGA
<i>Gls2</i>	254826697c1	CGTCCGGTACTACCTCGGT	TGTCCCTCTGCAATAGTGTAGAA
<i>Glud1</i>	225543106c3	CTGTGGTCGATGTACCGTTTG	AGCTCCATAGTGAACCTCCGT
<i>Hprt</i>	7305155a1	TCAGTCAACGGGGGACATAAA	GGGGTGTACTGCTTAACCAG
<i>Got1</i>	6754034a1	GCGCTCCATCAGTCTTTG	ATTCATCTGTGCGGTACGCTC
<i>Got2</i>	6754036a1	GGACCTCCAGATCCCATCCT	GGTTTTCCGTTATCATCCCGGTA
<i>Glut1</i>	N/A	GGCATGTGCTTCCAGTATGT	ACGAGGAGCACCGTGAAGAT
<i>Pdk1</i>	N/A	GAAGCAGTTCCTGGACTTCG	CCAACCTTGCACCAGCTGTA
<i>Pgk1</i>	N/A	ATTCTGCTTGACAATGGAGC	AGGCATGGGAACACCATCA
<i>Mct4</i>	N/A	TCACGGGTTTCTCCTACGC	GCCAAAGCGGTTACACAC
<i>Ldha</i>	N/A	TGTCTCCAGCAAAGACTACTGT	GACTGTACTTGACAATGTTGGGA
<i>Hk2</i>	N/A	TGATCGCCTGCTTATTCACGG	AACCGCTAGAAATCTCCAGA
<i>Cox4i1</i>	N/A	ATTGGCAAGAGAGCCATTTCTAC	CACGCCGATCAGCGTAAGT
<i>Cox4i2</i>	N/A	CTGCCCGGAGTCTGGTAATG	CAGTCAACGTAGGGGGTCATC
<i>L2hgdh</i>	21703884a1	TAGTCATCGTTGGAGGTGGAA	TCCAGTCTGGTGAAGAGCTAAAT
<i>D2hgdh</i>	30725722a1	GGTCTGCAAGGGTCTCTG	ACCACGGAGTATGCTCTGTGA
<i>Ccr6</i>	6753318a1	CCTGGGCAACATTATGGTGGT	CAGAACGGTAGGGTGAGGACA
<i>Prdm1</i>	6680784a1	TTCTCTTGAAAAACGTGTGGG	GGAGCCGGAGCTAGACTTG
<i>Bcl6</i>	6753172a1	CCGGCACGCTAGTGATGTT	TGTCTTATGGGCTCTAAACTGCT
<i>Eomes</i>	26354683a1	GCGCATGTTTCCTTTCTTGAG	GGTCGGCCAGAACCACTTC
<i>Il2</i>	1504135a1	TGAGCAGGATGGAGAATTACAGG	GTCCAAGTTCATCTTCTAGGCAC
<i>Ifng</i>	33468859a1	ATGAACGCTACACACTGCATC	CCATCCTTTTGCCAGTTCCTC
<i>Tcf7</i>	6678245a1	AGCTTCTCCACTCTACGAACA	AATCCAGAGAGATCGGGGGTC
<i>Self<sup>5</sup></i>	Finaly et al	ACGGGCCCCAGTGTGAGTATGTG	TGAGAAATGCCAGCCCCGAGAA
<i>Kdm6a</i>	20073323a1	CGGGCGGACAAAAGAAGAAC	CATAGACTTGCATCAGATCCTCC
<i>Tet2</i>	22122441a1	AGAGAAGACAATCGAGAAGTCGG	CCTTCCGTACTCCAAACTCAT
<i>Bcl2</i>	6753168a1	ATGCCTTTGTGGAACATATGGC	GGTATGCACCCAGAGTGATGC
<i>Bcl-XL<sup>6</sup></i>	N/A	AACATCCCAGCTTCACATAACCCC	GCGACCCAGTTTACTCCATCC

**shRNA and overexpression**

For shRNA-mediated knockdown experiments, all shRNAs were cloned into pMKO.1GFP (pMKO.1 GFP was a gift from William Hahn, Addgene plasmid # 10676). shRNA-mediated knockdown of target genes was achieved by transduction with retrovirus expressing the following shRNAs from TRC:

Ldh1 shRNA#1: CGTCTCCCTGAAGTCTCTTAA, Ldh1 shRNA#2: GTTCCCAGTTAAGTCGTATAA, Mdh1 shRNA#1: CCCTGTCGTGATCAAGAATAA, Mdh1 shRNA#2: GCCCATCATTCTTGTGCTGTT, Mdh2 shRNA#1: CGGTGTGTACAACCCTAACAA, Mdh2 shRNA#2: CGGAATGCACTTACTTCTCTA, Tet2 shRNA#1: ATGCAGTATTTCCCGAATAAT, Tet2 shRNA#2: TCGTGCTTTGGCCAGATTAATA, Tet2 shRNA#3: GAGCGTTCCTCAGTATCATTT, Utx shRNA#1: CCGAGCAAACAGAAATAATTT, Utx shRNA#2: TGGACTTGCAGCACGAATTAA, Utx shRNA#3: GATTGCACATAGACTAAGAAA, L2hgdh shRNA#1 GCGTCATAGTAAGGTTGTGTT, L2hgdh shRNA#2: GATCCAGCAGGAGGATATAATA, L2hgdh shRNA#3: GTGTCATACACAGTGGTATTT.

scramble shRNA: CCGGCAACAAGATGAAGAGCACCAACTCGAGTTGGTGCTCTTCATCTTGTGTTTTTG. pMKO.1GFP vectors containing the shRNA of interest were transfected into Phoenix cells with pCL-Eco (pCL-Eco was a gift from Inder Verma (Addgene plasmid # 12371) ) using Lipofectamine 2000 (Thermo Fisher). Viral supernatants were collected 48 h later. Primary CD8<sup>+</sup> T-cells were transduced with viral supernatants using retronectin (Takara Clontech) according to the manufacturer's instructions. For measurement of 2-HG levels, GFP<sup>+</sup> cells were sorted 2 days later by flow cytometry, followed by culture in 1% oxygen for a further 48 h at which point metabolites were extracted. Metabolites were harvested from cells with shL2hgdh at day 8 after activation. Knockdown of target genes was confirmed by qRT-PCR. For expression of Bcl-2, Bcl-XL, L2hgdh, Pdk1 and Ldha enzymes, the vector SFG.wtCNb\_opt.IRES.eGFP (gift from Martin Pule (Addgene plasmid # 22492)) was used to generate an Empty Vector control, by replacing the insert with a multiple cloning site. Murine DNA sequences encoding C-terminal Flag tagged enzymes (L2hgdh, Pdk1 and Ldha) or N-terminal Flag tagged Bcl-2 or Bcl-XL were cloned into Empty Vector. Retrovirus encoding each enzyme was produced as for shRNA experiments and primary CD8<sup>+</sup> T-cells were transduced as before. For Pdk1 and Ldha overexpression experiments, GFP<sup>+</sup> cells were sorted 3 days later by flow cytometry and allowed to recover in 21% oxygen for 24 h, followed by culture in 1% oxygen for a further 48 h at which point metabolites were extracted. For L2hgdh expression experiments, cells were placed in 21% or 1% oxygen the day after transduction and GFP<sup>+</sup> cells were assessed by flow cytometry 7 days later with or without 300  $\mu$ M S-2HG-octyl ester treatment. Live, CD8<sup>+</sup>GFP<sup>+</sup> cells were gated.

**Glucose, lactate, VEGF, IL-2 and IFN- $\gamma$  measurements in media**

Glucose and lactate levels in culture medium were measured with a Dade-Behring Dimension RXL analyser. Changes in metabolite concentrations relative to fresh media were normalized to viable cell counts. Vegfa, IL-2 and IFN- $\gamma$  protein levels in media were determined using the following kits from MesoScale Discovery: K150BMC-2 for Vegfa, K15048D-2 for IFN- $\gamma$  and K152QQD-2 for IL-2. Values were normalized to viable cell counts. Cells were treated with 500  $\mu$ M S-2HG-octyl ester for 16-24h

***In vitro* cytotoxicity assay**

Total splenocytes were activated for 48 h with 1000 nM SIINFEKL and then expanded for a further 4 days in the presence of IL-2. OT-I-specific CD8<sup>+</sup> T-cells were then treated with or without 500  $\mu$ M S-2HG-octyl ester and then incubated with target (EG7-OVA, CFSE-low) and control (EL4, CFSE high) cells for 4 hours. Specific lysis was calculated by normalization of the loss in signal of the CFSE-low population relative to the CFSE-high population after co-culture for 4 hours with the vehicle or S-2HG-treated OT-I CD8<sup>+</sup> T-lymphocytes.

### **Metabolomics**

Metabolomics experiments were performed using GC/MS and LC/MS/MS platforms (Metabolon Inc.) for determination of intracellular metabolites. Samples were normalized using protein concentration measured by the Bradford assay and rescaled to set the median to 1. Missing values were imputed with the minimum value. Analyses performed with MetaboAnalyst<sup>7-9</sup> included unsupervised hierarchical clustering and principal component analysis.

### **<sup>1</sup>H-NMR Data Acquisition and Analysis**

<sup>1</sup>H nuclear magnetic resonance (<sup>1</sup>HNMR) spectroscopy was performed with solvent-suppression on a 600 MHz Bruker Avance NMR spectrometer with 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS) as an internal standard. Electronic RefeREnce To access *In vivo* Concentration (ERETIC) method<sup>7</sup> was used to determine the concentration of DSS in each sample. Processing of <sup>1</sup>HNMR spectra included both zero- and first-order phase corrections followed by baseline correction using Chenomx NMR Suite 7.6 (Chenomx Inc.). 2-hydroxyglutarate was identified, based on chemical shift assignment, also using the Chenomx NMR Suite 7.6. Spectral intensities were normalized to the internal standard in each sample.

### **Mass spectrometry**

Cells were counted to determine viable cell numbers. 2-3 million viable cells were harvested, washed in ice-cold PBS and extracted in ice-cold 80% methanol. After two-freeze thaw cycles, precipitated proteins were removed by centrifugation at 16,000g and kept for protein content determination by the Bradford assay. Supernatants were evaporated to dryness and samples were reconstituted in the appropriate buffer for each assay. For the quantification of 2HG, glutamate, fumarate, succinate and malate, cell extracts were dissolved in 0.1 % formic acid containing a known amount of deuterated 2HG (D<sub>3</sub>-2HG) as well as <sup>13</sup>C-labelled glutamate, malate, fumarate and succinate (all m+2), as internal standards. A calibration curve of stable isotope labelled internal standards was run with every batch of samples to allow for absolute quantification. 10 µl of each sample was injected onto a Sciex 6500 MS mounted with a Hypercarb column (Thermo), 100 mm x 2.1 mm, 3 µm particle size, held at 50°C, using an Agilent 1290 system. Mobile phase A consisted of 0.1% formic acid in water. Mobile phase B consisted of 0.1% formic acid in acetonitrile. The gradient profile, with a 0.4 ml/min flow rate, was as follows: 95% A for 1.0 min, 70% A for a further 2.5 min, 5% A for a further 1.5 min. The MS conditions included no splitting, HES ionization with a source temperature of 350°C and negative polarity. The precursor ions for 2HG, glutamate, succinate, fumarate, malate, D<sub>3</sub>-2HG, <sup>13</sup>C-glutamate, <sup>13</sup>C-succinate, <sup>13</sup>C-fumarate and <sup>13</sup>C-malate were: 147, 146, 117, 115, 133, 150, 148, 119, 117 and 135 m/z respectively. The product ions for 2HG, glutamate, succinate, fumarate, malate, D<sub>3</sub>-2HG, <sup>13</sup>C-glutamate, <sup>13</sup>C-succinate, <sup>13</sup>C-fumarate and <sup>13</sup>C-malate were: 129, 128, 73, 71, 115, 132, 130, 74, 72 and 117 m/z respectively. Typical retention times for 2HG, glutamate, succinate, fumarate, malate, D<sub>3</sub>-2HG, <sup>13</sup>C-glutamate, <sup>13</sup>C-succinate, <sup>13</sup>C-fumarate and <sup>13</sup>C-malate were 1.65, 0.63, 1.49, 2.91, 1.15, 1.67, 0.67, 1.49, 2.91 and 1.15 min respectively. For the enantioselective determination of 2HG, cell extracts were dissolved in H<sub>2</sub>O:MeOH (5:95 v/v) containing 0.3 % acetic acid and 0.1% ammonium hydroxide, as well as a known amount of D<sub>3</sub>-2HG. A calibration curve of stable isotope labelled internal standards was run with every batch of samples to allow for absolute quantification. 5 µl of each sample was injected onto a Sciex 6500 MS mounted with a Astec CHIROBIOTIC R column, 25 cm x 4.6 mm, 5 µm particle size, held at ambient temperature, using an Agilent 1290 system. The mobile phase consisted of H<sub>2</sub>O:MeOH (5:95 v/v) containing 0.3 % acetic acid and 0.1% ammonium hydroxide. The mobile phase was run isocratically at a flow rate of 1.2 ml /min for 9.6 min. The MS conditions were as above. Typical retention times were 3.71 and 4.33 min for S-2HG and R-2HG respectively. All <sup>13</sup>C tracer studies were performed using murine CD8<sup>+</sup> T-lymphocytes cultured in medium containing 10% dialysed FBS. RPMI-1640 medium free from glucose or glutamine was prepared so that each substrate pool was entirely labelled whilst the other not. The final concentrations of [U-<sup>13</sup>C<sub>6</sub>] glucose or [U-<sup>13</sup>C<sub>5</sub>] glutamine were 11 mM and 2 mM respectively. Medium was also supplemented with 25 mM HEPES pH 7.4, 1% penicillin-streptomycin and 55 µM β-mercaptoethanol. [U-<sup>13</sup>C<sub>6</sub>] glucose and [U-<sup>13</sup>C<sub>5</sub>] glutamine were purchased from Cambridge Isotope Labs. Steady-state labelling was achieved by culturing cells in the presence of tracer for 24 h. The multiple reaction monitoring (MRM) transitions monitored, corresponded to loss of water (-18 m/z).

The MRM transitions used for m+0, m+1, m+2, m+3, m+4 and m+5 2-HG were: 147-129, 148-130, 149-131, 150-132, 151-133 and 152-134 m/z respectively. Intracellular concentration of 2HG was determined by normalizing to cell volume.

For *in vivo* enantioselective 2HG measurements, samples containing a known amount of deuterated D<sub>3</sub>-2HG internal standard, were derivitised with diacetyl-L-tartaric anhydride following homogenization. Evaporated samples were dissolved in water. A calibration curve of stable isotope labelled internal standards was run with every batch of samples to allow for absolute quantification. 10 µl of each sample was injected onto a Sciex 6500 MS mounted with an Acquity UPLC HSS T3 column (Waters), 100 mm x 2.1 mm, 1.7 µm particle size, held at room temperature, using an Agilent 1290 system. Mobile phase A consisted of 125 mg/l ammonium formate pH 3.6. Mobile phase B consisted of acetonitrile. The gradient profile, with a 0.4 ml/min flow rate, was as follows: 97% A for 3.0 min, 30% A for a further 1 min and 100% A for a further 0.5 min. The MS conditions included no splitting, ESI ionization with a source temperature of 500°C and negative polarity. The precursor ions for derivitised-2HG, underivitised-2HG, derivitised-D<sub>3</sub>-2HG and underivitised-D<sub>3</sub>-2HG were: 362.9, 147.1, 366.9, and 150.1 respectively. The product ions were: derivitised-2HG (128.9 and 147.0), underivitised-2HG (129.1), derivitised-D<sub>3</sub>-2HG (150.0) and underivitised-D<sub>3</sub>-2HG (132.1). Typical retention times for S-2HG and R-2HG were 2.0 and 2.3 min respectively. Determination of 5hmC and 5mC levels in gDNA was performed as previously described<sup>10</sup>. Isolated murine CD8<sup>+</sup> T-lymphocytes were activated with plate bound αCD3 (5 µg/ml) and soluble αCD28 (1 µg/ml) antibodies for 48h. gDNA was isolated from cells before activation and at the indicated time points following activation. Cells were cultured with IL-2 (20 ng/ml) and in the presence or absence of 300 µM S-2HG-octyl-ester. Cells were passaged every 48 hours. gDNA was purified using the DNeasy Blood and Tissue Kit (Qiagen). 1 µg of gDNA was digested with 5 U of DNA Degradase Plus (Zymo Research) according to the manufacturer's instructions.

### **In vivo 2HG measurement**

Urine was collected from *Hif1α<sup>fl/fl</sup>* and *Hif1α<sup>fl/fl</sup> dLck<sup>cre</sup>* mice prior to sacrifice. Mice were sacrificed by cervical dislocation and spleens were immediately harvested and frozen in liquid nitrogen. Spleens were weighed and processed using a Precellys homogenizer. Following homogenization, samples were treated as above. Data are normalized to mass of tissue and converted to concentration (1g tissue = 1 ml water). Urine creatinine levels were measured by the kinetic Jaffe reaction using a Dade-Behring Dimension RXL analyser and used for normalization.

### **Immunoblotting**

Nuclear and cytosolic fractions were prepared from cells with the NE-PER kit (Thermo Scientific) and separated by SDS-PAGE. Proteins were transferred to PVDF membranes and then blocked in 5% milk prepared in phosphate-buffered saline (PBS) plus 0.05% Tween 20. Membranes were then incubated with primary antibodies overnight at 4 °C and horseradish peroxidase (HRP)-conjugated or Licore IRDye secondary antibodies for 1 h the next day. The following primary antibodies were used at a dilution of 1:1000: anti-Flag (Cell Signalling Technology, 2368), anti-HIF1α (Novus, NB100-449), anti-HIF2α (Novus, NB100-122), anti-PDH-E1α (Abcam, ab110330), anti-PDH-E1α pS232 (Merck Millipore, AP1063), anti-LaminB1 (Abcam, ab16048), anti-HDAC1 (Abcam, ab109411) and anti-β-tubulin (Abcam, ab21057). The following antibodies, all from Cell Signalling Technology, were used to assess mTOR signalling: anti-4E-BP1 (9644), anti-phospho-4E-BP1 S65 (9451), anti-phospho-4E-BP1 T37/46 (2885), anti-S6 (2217), anti-phospho-S6 S235/236 (4858), anti-p70S6 kinase (2708), anti-phospho-p70S6 kinase (9208), anti-phospho-p70S6 kinase T389 (9205). Histones were extracted from nuclear fractions by acid-extraction overnight at 4°C in 0.2 M HCl. The following antibodies, all from Cell Signalling Technology, were used to assess histone methylation status: anti-H3 (4499), anti-H3K4me2 (9725), anti-H3K4me3 (9751), anti-H3K9me2 (4658), anti-H3K9me3 (13969), anti-H3K27me2 (9728), anti-H3K27me3 (9733), anti-H3K36me2 (2901), anti-H3K36me3 (4909), anti-H3K79me2 (5427), anti-H3K79me3 (4360). Antibodies against Bcl-2 (D17C4) and Bcl-xl (54H6) were from Cell Signalling Technology. For histone immunoblots, CD8<sup>+</sup> T-lymphocytes were treated with the indicated dose of S-2HG-octyl ester for 7 days.



**hMeDIP/MeDIP-ePCR**

Freshly isolated naïve or activated CD8<sup>+</sup> T-lymphocytes treated with or without 500  $\mu$ M S-2HG-octyl ester for 7 days. Each profile shows the fold change over the non-binding control primer. Each dot represents an individual primer pair. Genomic DNA was purified using the DNeasy Blood & Tissue kit (Qiagen) according to the manufacturer's protocol and sonicated for 14 cycles of 30 secs on and 30 secs off on the high setting using the Bioruptor (Diagenode). DNA fragments were denatured by heating at 95 °C for 10 min, followed by snap chilling on ice. Antibodies were conjugated to protein G Dynabeads (Invitrogen) at 4 °C overnight. 2.5  $\mu$ g of denatured DNA was incubated with the conjugated antibodies for 5mC (MAb-006-100, Diagenode), 5hmC (#39769, Active Motif), rabbit IgG (sc-2027, Santa Cruz Biotechnology) and mouse IgG (sc-2025, Santa Cruz Biotechnology) at 4 °C overnight. Beads were collected and washed 5 times with IP buffer (100 mM sodium phosphate pH 7.0, 1.4 M NaCl, 0.5% Triton X-100) at room temperature. The immunoprecipitated DNA was eluted using digestion buffer (50 mM Tris-HCl pH 8.0, 10 mM EDTA, 0.5% SDS). After treatment with 0.8 mg/ml proteinase K at 55 °C for 1 h, the DNA was extracted by phenol-chloroform followed by ethanol precipitation and resuspended in 10 mM Tris-HCl pH 8.0. For qPCR, the samples were analysed using the BioMark HD System (Fluidigm) according to the manufacturer's protocol. See table below for primers.

**ChIP-PCR**

Freshly isolated naïve or activated CD8<sup>+</sup> T-lymphocytes treated with or without 500  $\mu$ M S-2HG-octyl ester for 7 days. Each profile shows the fold change over the non-binding control primer. Each dot represents an individual primer pair. Cells were fixed in 1% formaldehyde for 10 min at room temperature and quenched with 0.25 M glycine. After washing twice with ice-cold PBS, cells were resuspended in 250  $\mu$ l SDS lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris-HCl pH 8.1) at  $3.33 \times 10^7$  cells/ml. Samples were sonicated on a Bioruptor (Diagenode) at high power for 25 cycles, with 30s on and 30s off per cycle and centrifuged at 20,000g for 10 min at 4 °C, and the supernatant collected. DNA fragment sizes were checked by agarose gel electrophoresis to range from 200 to 500bp. 10  $\mu$ g of antibodies for H3K4Me3 (#305-34819, Active Motif), H3K27Me3 (GTX60816, GeneTex), RNA Pol II (sc-899, Santa Cruz Biotechnology), rabbit IgG (sc-2027, Santa Cruz Biotechnology) and mouse IgG (sc-2025, Santa Cruz Biotechnology) were used for each ChIP and conjugated to Protein A/G PLUS agarose beads (Santa Cruz Biotechnology) in ChIP dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl pH 8.1, 167 mM NaCl) overnight at 4 °C and washed three times with ChIP dilution buffer. Sheared chromatin was diluted 10-fold with ChIP dilution buffer and incubated with antibody/agarose bead mix overnight at 4 °C with  $5 \times 10^6$  cells used for each ChIP. The samples were then centrifuged at 200g for 2 min at 4 °C and the supernatants removed. The agarose beads were washed at 4 °C once with low salt immune complex wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl pH 8.1, 150 mM NaCl), once with high salt immune complex wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl pH 8.1, 500 mM NaCl), once with LiCl immune complex wash buffer (0.25 M LiCl, 1% NP40, 1% deoxycholate, 1 mM EDTA, 10 mM Tris-HCl pH 8.1) and twice with TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA). Protein-DNA complexes were eluted from the beads with elution buffer (1% SDS, 0.1 M NaHCO<sub>3</sub>) and reverse cross-linked with 0.2 M NaCl at 65 °C overnight. ChIP samples were treated with 0.2 mg/ml RNase A at 37 °C for 2 h and 0.8 mg/ml proteinase K at 55 °C for 3 h prior to phenol-chloroform extraction, ethanol precipitation and DNA resuspension in 10 mM Tris-HCl pH 8.0. For qPCR, samples were analysed using the BioMark HD System (Fluidigm) according to the manufacturer's protocol.

The following primer pairs were used:

Forward primers	Sequence	Reverse primers	Sequence
mSell-F1	CCTCACTCCTGACATCGTCC	mSell-R1	TAAGTGGCCACAGCTCATCA
mSell-F2	CCCCCAGTAAATAGCAATGGA	mSell-R2	TTTATGTGAGTAGCGCCCCC
mSell-F3	GGGGGCGCTACTCACATAAA	mSell-R3	AGCATTTACCTATGCTCCCA

mSell-F4	AGCTGTTTCTGGTGTGAGAA	mSell-R4	ACTCATCTGGCCTTACATGCT
mSell-F5	TTGTTCTGCTAGGTAAGGTGA	mSell-R5	GGACGTGAATCACTGCTTTCC
mSell-F6	GCTGCCAAAGCCTTCAATCG	mSell-R6	AGCCAATGGCAGGCTTGATT
mSell-F7	TAAGCTAGGGACTGGGAGAGAC	mSell-R7	GCTGGTGTTCACGGTAGCTT
mSell-F8	CTGTGATCCTCAGTGTCCCC	mSell-R8	TAAGCCCCACACTACACCAG
mSell-F9	CTGTGATCCTCAGTGTCCCC	mSell-R9	CTCTAAGCCCCACACTACACC
mSell-F10	TCCCGTAGGGTTCAATGGGT	mSell-R10	TGCTCCAGACATGAACTCCC
mSell-F11	CTGAGTGGCATTGGGAGTTC	mSell-R11	ACGATGCATTTGTGTCTCTGC
mSell-F12	TCCCGACTCCATGGATCAAAA	mSell-R12	CTCTCCCCCGTCCATTCCT
mSell-F13	TCTCTTGTTCCCAACACAGT	mSell-R13	GAAGTGCACCTTGTACCCGT
mSell-F14	TCAGTGGCTGCCGAAGTATT	mSell-R14	TTGCCATGTGATAGGCCACA
mSell-F15	TCTGCAGCCCACTTATTTTCA	mSell-R15	CCATGTCTTAAAACCAGCTACCG
mSell-F16	CTGGAAGAGAGACCATCCTTGAA	mSell-R16	GGTGACACCTCATCGTGGAT
mSell-F17	CCTGTAATCAATGCAGACAATGG	mSell-R17	GTGTTACCAGCTTTCCCT
mSell-F18	TCTAGAGGAGGGGAAAGCTGG	mSell-R18	AGCTGGCATTACAGACCAAG
mSell-F19	TCCTGCCAAAAGTGTCCCAA	mSell-R19	CAGCGAGGACCTCCCAAAAA
mSell-F20	ACGCGTTTCTACTTATCTTTGTGT	mSell-R20	GGATGTAATTTTTATGCGGTACAGA
mSell-F21	GTGCTTGCGTGCATCTGAAT	mSell-R21	GAGGGAGGTTTGGGTCAAGG
mSell-F22	TGCAACTCCTCCACTGTCATT	mSell-R22	CTCATGGCAGGTGTGACTGTA
mSell-F23	GCTCCAGTGTCTCCACATTCT	mSell-R23	TCCTTGGGCTTGGTGATTCT
mSell-F24	CTGTGGGCAGACGTGATCTAA	mSell-R24	GCTCAGGATTCACAGCGTAAG
mSell-F25	GCATCACTAAGTCTCCTGGTCC	mSell-R25	GGCTGAAACAATGACTGGGC
mSell-F26	TTGACGGAGCTGTTACACCC	mSell-R26	TGACCTGCTAACTGTAGTGTGT
mSell-F27	AGGCATGGTTATCTGAATGAGT	mSell-R27	TGGAGTCTAAGTAGTCTTGCTTGT
mSell-F28	AGAAGCAAGCAAGCATGTGA	mSell-R28	CATAGTTCCTTGGTGCTTAATCCA
mSell-F29	AGTGACTTCTCCAGATTGCTT	mSell-R29	AAGTGTGAAGAGGGTAAGACAGC
mSell-F30	AGTGACTTCTCCAGATTGCTT	mSell-R30	GAGAGGTTGGGGACCTAGAA
mSell-F31	TCTAGGTCCCCCAACCTCTC	mSell-R31	TTCTCGTTACCCCCCTAGT
mSell-F32	GGGGTGAACGAGGAAAGAGG	mSell-R32	GAAGTTCGGGAGCTTTCTGGA
mSell-F33	TCCACTAGGGGGTGAACGAG	mSell-R33	TGAACTTCGGGAGCTTTCTGG
mSell-F34	GTCTTCCAGAAAGTCCCGA	mSell-R34	AACTCCCACCATGGGGTA
mSell-F38	TATCTGAGCAGGGGGCATA	mSell-R38	ACCCCTTCTTAATCTGGGC
mSell-F39	GGCATAGCTTCTGGTCCACT	mSell-R39	CAATGTCACCAGTGGGGTTTG
mSell-F40	CACTGGTGACATTGGCACAT	mSell-R40	TATAGGCTGGGATGCTCACC
mSell-F41	GAGAGACTTGCAGAGAGACCC	mSell-R41	AAATGATAGCTTCTTGCCCCCT
mSell-F42	AGGGGGCAAGAAGCTATCATT	mSell-R42	CTGTGCCCTTCCAGCATTC
mSell-F43	CCTACGCATGCTGTCTCAAG	mSell-R43	TCACTCTGTGTCACCGCAA
mSell-F44	TTGATGGAATGCTGGAAGGGG	mSell-R44	GCCATAGGCTTTCTGCACATC
mSell-F45	CGCATGCTGTCTCAAGAAAATGA	mSell-R45	AGCCATAGGCTTTCTGCACAT
mSell-F46	CTTTGCGGTGACACAGAGTG	mSell-R46	TGTAGCCAGAAGCCAGGAAAG
mSell-F47	TTCTGGCTTCTGGCTACATC	mSell-R47	GCCTTTCACGCTGGGTCAT
mSell-F48	TCCTTCATGACCAGCGTGA	mSell-R48	GCTCTGTTTTAAATGCTCTTGGG

mSell-F49	CCAGCGTGAAAGGCCAAATGT	mSell-R49	TGTACCTACTTTTCGACTGAGC
mSell-F50	TGGGACTGAAACCTGGAAGG	mSell-R50	GCCCATCAGCCCACTATCAA
mSell-F51	TGAATTGAAGAAGTGCCAGGG	mSell-R51	CCACAACAGAGCAGTGTCCA
mSell-F52	TGGACACTGCTCTGTTGTGG	mSell-R52	TTTAAGAAAAGAACAATCACTGCCT
mSell-F53	AGTGATGTGCCCAAATCCTGA	mSell-R53	CGCTCTGGACTGTGGAAGTT
mSell-F54	AGGGGCTTTTGTGTCTTTCTTT	mSell-R54	TGAGCTGAAAGTCCAGGTGAG
mSell-F55	AAACTCAGTGAGCTGATGGCT	mSell-R55	TGTGCACAGTGACCTTGATGA
mSell-F56	CTTATGCCTGAAGGCCAGT	mSell-R56	GCCCTGGGAAAGCCTCAATA
mSell-F57	TGTTAATGTCTGCCTGACCCT	mSell-R57	TCCTGGGATAACTTCCTAAAGGC
mSell-F58	GGAAGTTATCCCAGGATTA AAAAGT	mSell-R58	TCTTCTCAA AATTCTTTGAAGTGT
mSell-F59	ATTCTGATCCAAGGCTGCCAA	mSell-R59	AGTGTCTAGGACCAGGCAC
mSell-F60	TGCTGCTGTTTTCCAGACTTC	mSell-R60	TCCCAGTTCATGGGCTTTTC
mSell-F61	GACATGGGTGGGAACCAACA	mSell-R61	GTTTGTGACAGGCGTCATCG
mSell-F62	AGTGTCTAGGCTCCTACGCT	mSell-R62	CCCTGTTC C C C C A C A T A C A C A
mSell-F63	TGGACAGACTGTGCCTCAAG	mSell-R63	TGCATAACGCACGCTACACT
mSell-F64	TGTGCAAAGAAGAGACTCTGG	mSell-R64	TTCCACACATTCTCCACGGC
mSell-F65	TGATGCAGGGTATTACGGGC	mSell-R65	AAGCAAAGCCAACACGACAC
mSell-F66	GGCCATTTCTTCCACGGTA	mSell-R66	TCCAAAGGCTC A C A C T G G A C
mSell-F67	GCTTCCAGTCCAAGTGTGCT	mSell-R67	TTGTCCCAACAACGAAAGATCCA
mSell-F68	TGGATCTTCGTTGTTGGGACA	mSell-R68	TGAAACTTCATGCTGACTTGGG
mSell-F69	TGGGAAGTTCCAAACAGAAAGC	mSell-R69	TGTAACCTGCTC A C C T T T G A C A
mSell-F70	GTCCTCTGAGCTTGCAGGATT	mSell-R70	GAAGAACAGGAGTACCTTCCCA
mSell-F71	GGGTATTTCTGGAGAATATGGAAGC	mSell-R71	AGGGGATCTCACCACAGGAT
mSell-F72	TGGCCACTTGGCATCTTACT	mSell-R72	ACCGCATCTCTGAGTGACTTC
mSell-F73	TGGGAGAACC G G A A A T G A G A G	mSell-R73	AGATTTGATGCGCCTTCCA
mSell-F74	GTCTGCTGCACACAATGGAA	mSell-R74	CTAAAGGGCAGTCCAACGGT
mSell-F75	AACATCGTGCTTGCTTTCTTGA	mSell-R75	GTGGAATATGCCTCCTACCCC
mSell-F76	AGGAGGCATATTCCACCACC	mSell-R76	ATGAAAGAACCAGAAGAAGTCAC
mSell-F77	AGCCATGACCTCCAAATATGCT	mSell-R77	TAGGGTGGCAGAGTTTCACAC
mSell-F78	GGGCTCCAGGCACAGAAATAG	mSell-R78	GGAGAGAGAAGCAGGGTTGAG
mSell-F79	ATATTCATCCCAGCCCCATT	mSell-R79	CACTGGACCACTTTCATGGAGA
mSell-F80	TGGATCTTTGTTGTGGCAAGC	mSell-R80	AACAGACTTCTAGGCAAGACTCC
mSell-F81	GTCTTTGAAGTCATCTGGCATT	mSell-R81	TCAACTAGGGTTCATCAAGGC
mSell-F82	CCTCTCCTTCAATGACACCCA	mSell-R82	GAACCTACCAACTGAAGCAATCA
mSell-F83	TCTCCAGTGTAAGCTCTCAGGA	mSell-R83	ACCCTGTCAGTCAAGAAAGGA
mSell-F84	TCCTTTCTTGACTGACAGGGTT	mSell-R84	TCAGTTCCATGATGTCAACGAT
mSell-F85	CTTCAGGGAGTAGGGGTGTC	mSell-R85	AGCCCATGGTGGTATAGGGTA
previous mSell-F37	TAAAGGGGGTGGTGCAAAGA	previous mSell-R37	AGTCAGGACCAAAGGCAGTTT
previous mSell-F38	CCCTGGCCAACTTCAGTTTT	previous mSell-R38	CTAGGACTAGACCAGGTGCC
previous mSell-F39	ACACCGTGCATCAGAGTACA	previous mSell-R39	CTACTCCAAACACCAGCGA
previous mSell-F40	AGTTACCCAGCTCAGATCGC	previous mSell-R40	CTGCCTATGGGACCACAGTT
mCD62L_PF2	TTCCAGAAAGCTCCCGAAGTT	mCD62L_PR2	GGACCCTGGTCTGACCATAGA

mCD62L_PF3	GGGGAAGAATCCAGTTGCCAT	mCD62L_PR3	ATCCCCAGTTTGAAGGAGG
mCD62L_bPF1	GGGGAAGAATCCAGTTGCCAT	mCD62L_bPR1	GGCTCAATCATTTGCCCCAA
mCD62L_PF4	TTGGCACATTCTTCCTGAGC	mCD62L_PR4	GGGTCTCTTGCAAGTCTCTC
mCD62L_bPF2	AGAGACTTGCAGAGAGACCCA	mCD62L_bPR2	TGATAGTCTCTTGCCCCCTT
mCD62L_PF5	AAGGGGGCAAGAAGCTATCA	mCD62L_PR5	ACACCAAGGGAGAAAATCAGCA
mCD62L_bPF3	CCCTACGCATGCTGTCTCAA	mCD62L_bPR3	TCACTCTGTGCACCCGAAA
mCD62L_PF7	GGGACTGAAACCTGGAAGGAA	mCD62L_PR7	ACAGATAAAGCCCATCAGCCC

### **Protein Quantification**

Protein quantification was performed using the DC-Protein Assay (BioRad) according to the manufacturer's instructions. Absorbance at 595 nm was measured and samples were quantified against a standard curve constructed using known concentrations of bovine serum albumin (BSA).

### ***In vivo* H3K27me3 quantification**

Spleens were harvested from 12 week old C57BL/6J male mice. Total splenocytes were stained with LIVE/DEAD violet, anti-CD8a, anti-CD62L, anti-CD44 and anti-H3K27me3 antibodies as described above, and acquired on a Fortessa (BD Biosciences). Live, CD8<sup>+</sup> cells were gated. H3K27me3 levels were quantified in the following CD8<sup>+</sup> populations: CD62L<sup>lo</sup>CD44<sup>hi</sup>, CD62L<sup>hi</sup>CD44<sup>hi</sup> and CD62L<sup>hi</sup>CD44<sup>lo</sup>.

### ***In vivo* persistence experiments**

Total splenocytes from OT-I CD45.1<sup>+</sup> mice were activated with 1000 nM SIINFEKL peptide in the presence of IL-2 and vehicle or 300  $\mu$ M S-2HG-octyl ester. After 48 h CD8<sup>+</sup> T-cells were purified by negative selection and cultured for 7 days in the presence of IL-2 and vehicle or 300  $\mu$ M S-2HG-octyl ester. 1 million CD45.1<sup>+</sup>CD8<sup>+</sup> cells were then injected intravenously into C57BL/6J wild type CD45.2.2 host mice. 30 days later, host mice were sacrificed to assess the persistence of transferred cells in the spleen. Absolute numbers of cells were determined with the use of counting beads (CountBright, Life Technologies).

### ***In vivo* recall experiments**

Total splenocytes from OT-I CD45.1.1 and CD45.1.2 mice were activated with 1000 nM SIINFEKL peptide in the presence of IL-2 and vehicle or 300  $\mu$ M S-2HG-octyl ester. After 48 h CD8<sup>+</sup> T-cells were purified by negative selection and cultured for 7 days in the presence of IL-2 and vehicle or 300  $\mu$ M S-2HG-octyl ester. Vehicle and S-2HG-treated cells were then mixed 1:1 and a total of 2 million cells (1 million per group) were injected intravenously into C57BL/6J wild type CD45.2.2 host mice. 30 days later, host mice were vaccinated i.p. with 1 million SIINFEKL-loaded bone marrow-derived dendritic cells. 7 days later, spleens, lymph nodes and half of the left lateral lobe of the liver were harvested from host mice and the presence of CD45.1<sup>+</sup>CD8<sup>+</sup> cells was determined by flow cytometry. Absolute numbers of cells were determined with the use of counting beads (CountBright, Life Technologies). Dendritic cells were prepared from bone marrow extracted from wild type C57BL/6J mice. Bone marrow-derived cells were cultured in non-TC treated petri dishes in RPMI-1640 medium containing 2 mM glutamine, 10% FBS, 25 mM HEPES, pH 7.2, 1% penicillin-streptomycin, 55  $\mu$ M  $\beta$ -mercaptoethanol supplemented 20ng/ml mGM-CSF (R&D Systems). After 8 days of culture, dendritic cells were activated with 1  $\mu$ g/ml LPS (Sigma) for 24 h. The maturation of dendritic cells was confirmed by flow cytometry using the following markers 24 h later: MHC class II (Biolegend, M5/114.15.2), CD11b (Biolegend, M1/70), CD11c (Biolegend, N418) and CD86 (Biolegend, GL-1). Mature dendritic cells were then loaded with 2  $\mu$ M SIINFEKL peptide at 37°C for 1 hour. After peptide loading, dendritic cells were detached with 3 mM EDTA in PBS for 5-10 min at 37°C and washed with PBS.

### **Homeostatic Proliferation**

Total splenocytes from OT-I CD45.1.1 and OT-I CD45.1.2 mice were activated with 1000 nM SIINFEKL peptide in the presence of IL-2 and vehicle or 300  $\mu$ M S-2HG-octyl ester. After 48 h CD8<sup>+</sup>

T-cells were purified by negative selection and cultured for a further 7 days in the presence of IL-2 and vehicle or 300  $\mu$ M S-2HG-octyl ester. Vehicle and S-2HG-treated cells were then mixed 1:1 and labelled with CFSE, followed by intravenous injection into sub-lethally irradiated (5 Gy total body irradiation) CD45.2.2 hosts. 7 days later, spleens were harvested and analysed by flow cytometry. Absolute numbers of cells were determined with the use of counting beads (CountBright, Life Technologies).

### **Adoptive cellular immunotherapy experiments**

For adoptive cell therapy experiments, OT-I CD8<sup>+</sup> T-lymphocytes were activated with 1000 nM SIINFEKL peptide in the presence of IL-2 and vehicle or 300  $\mu$ M S-2HG-octyl ester. After 48 h CD8<sup>+</sup> T-cells were purified by negative selection and cultured for a further 7 days in the presence of IL-2 and vehicle or 300  $\mu$ M S-2HG-octyl ester.  $0.7 \times 10^6$  -  $1.0 \times 10^6$  OT-I cells were transferred intravenously into tumour-bearing C57BL/6J lymphodepleted or lymphoreplete mice with 9-12 day established subcutaneous EG7-OVA tumours. Lymphodepletion was achieved with 5 Gy total body irradiation before adoptive transfer of OT-I CD8<sup>+</sup> T-lymphocytes. Tumour growth was monitored blindly by calliper measurements and volume was calculated by the modified ellipsoid formula ( $1/2(\text{Length} \times \text{Width}^2)$ )<sup>11</sup>. Mice were sacrificed when the maximal tumour size limit was reached.

### **Animal Models**

Mice were bred and housed in specific pathogen-free conditions in accordance with the ethical regulations of the UK Home Office and the University of Cambridge. Deletion of the following loxP-flanked alleles in CD8<sup>+</sup> T-lymphocytes was achieved via breeding with *dLck*<sup>cre</sup> mice<sup>12</sup>; *Hif1a*<sup>fl/fl</sup>, *Vh*<sup>fl/fl</sup><sup>13</sup> and *Epas1*<sup>fl/fl</sup><sup>14</sup>. All mice were backcrossed over ten generations to the C57BL/6J background. OT-I mice<sup>15</sup> containing transgenic inserts for mouse TCR-V $\alpha$ 2 and TCR-V $\beta$ 5 genes that recognise ovalbumin residues 257-264 (SIINFEKL) in the context of H-2K<sup>b</sup>, were crossed with CD45.1 mice. Both male and female mice were used in experiments and all mice were > 6 weeks of age. Blinding was introduced for all mouse experiments and the investigator was unaware of which mouse belonged to each group. Intravenous injection of T-lymphocytes was performed randomly. No statistical methods were used to pre-determine sample size.

### **Statistical analysis**

Statistical analyses were performed in GraphPad Prism 6 software. Pairwise comparisons were assessed using an unpaired two-tailed Student's t-test with Welch's correction when appropriate. Pairwise matched comparisons were assessed using a two-tailed paired t-test. Multiple comparisons were assessed with one-way ANOVA, including Bonferroni's correction for multiple testing. Grouped data were assessed by two-way ANOVA, including Bonferroni's correction, to adjust for multiple comparisons. Error bars are shown as s.d. unless otherwise indicated in figure legends and all experiments were performed at least twice. Sample sizes were chosen based on previous experience of *in vitro* and *in vivo* experiments.

### **Data Availability**

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

## Supplementary References

- 1 Schmittgen, T. D. & Livak, K. J. Analyzing real-time PCR data by the comparative C(T) method. *Nature protocols* **3**, 1101-1108 (2008).
- 2 Spandidos, A. *et al.* A comprehensive collection of experimentally validated primers for Polymerase Chain Reaction quantitation of murine transcript abundance. *BMC genomics* **9**, 633, doi:10.1186/1471-2164-9-633 (2008).
- 3 Spandidos, A., Wang, X., Wang, H. & Seed, B. PrimerBank: a resource of human and mouse PCR primer pairs for gene expression detection and quantification. *Nucleic acids research* **38**, D792-799, doi:10.1093/nar/gkp1005 (2010).
- 4 Wang, X., Spandidos, A., Wang, H. & Seed, B. PrimerBank: a PCR primer database for quantitative gene expression analysis, 2012 update. *Nucleic acids research* **40**, D1144-1149, doi:10.1093/nar/gkr1013 (2012).
- 5 Finlay, D. K. *et al.* PDK1 regulation of mTOR and hypoxia-inducible factor 1 integrate metabolism and migration of CD8+ T cells. *J Exp Med* **209**, 2441-2453, doi:10.1084/jem.20112607 (2012).
- 6 Medearis, S., Han, I. C., Huang, J. K., Yang, P. & Jaffe, G. J. The role of Bcl-xL in mouse RPE cell survival. *Invest Ophthalmol Vis Sci* **52**, 6545-6551, doi:10.1167/iovs.10-6772 (2011).
- 7 Xia, J., Mandal, R., Sinelnikov, I. V., Broadhurst, D. & Wishart, D. S. MetaboAnalyst 2.0--a comprehensive server for metabolomic data analysis. *Nucleic acids research* **40**, W127-133, doi:10.1093/nar/gks374 (2012).
- 8 Xia, J., Psychogios, N., Young, N. & Wishart, D. S. MetaboAnalyst: a web server for metabolomic data analysis and interpretation. *Nucleic acids research* **37**, W652-660, doi:10.1093/nar/gkp356 (2009).
- 9 Xia, J., Sinelnikov, I. V., Han, B. & Wishart, D. S. MetaboAnalyst 3.0--making metabolomics more meaningful. *Nucleic acids research* **43**, W251-257, doi:10.1093/nar/gkv380 (2015).
- 10 Bachman, M. *et al.* 5-Hydroxymethylcytosine is a predominantly stable DNA modification. *Nature chemistry* **6**, 1049-1055, doi:10.1038/nchem.2064 (2014).
- 11 Jensen, M. M., Jorgensen, J. T., Binderup, T. & Kjaer, A. Tumor volume in subcutaneous mouse xenografts measured by microCT is more accurate and reproducible than determined by 18F-FDG-microPET or external caliper. *BMC Med Imaging* **8**, 16, doi:10.1186/1471-2342-8-16 (2008).
- 12 Zhang, D. J. *et al.* Selective expression of the Cre recombinase in late-stage thymocytes using the distal promoter of the Lck gene. *Journal of immunology* **174**, 6725-6731 (2005).
- 13 Haase, V. H., Glickman, J. N., Socolovsky, M. & Jaenisch, R. Vascular tumors in livers with targeted inactivation of the von Hippel-Lindau tumor suppressor. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 1583-1588, doi:10.1073/pnas.98.4.1583 (2001).
- 14 Gruber, M. *et al.* Acute postnatal ablation of Hif-2alpha results in anemia. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 2301-2306, doi:10.1073/pnas.0608382104 (2007).
- 15 Hogquist, K. A. *et al.* T cell receptor antagonist peptides induce positive selection. *Cell* **76**, 17-27 (1994).