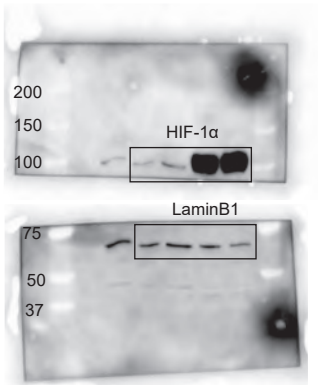
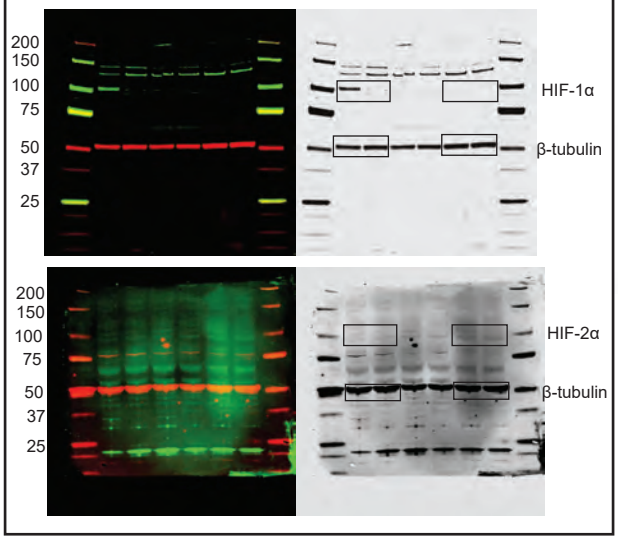
Supplementary Figure of Uncropped Gels with Size Marker Indications

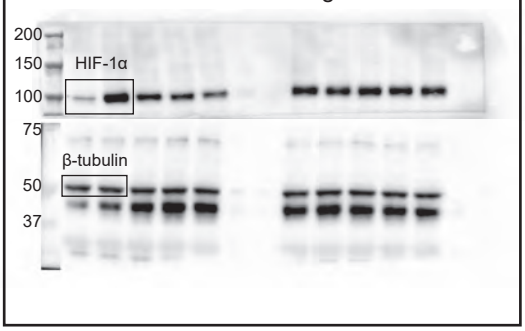
Ext Data Fig 1c



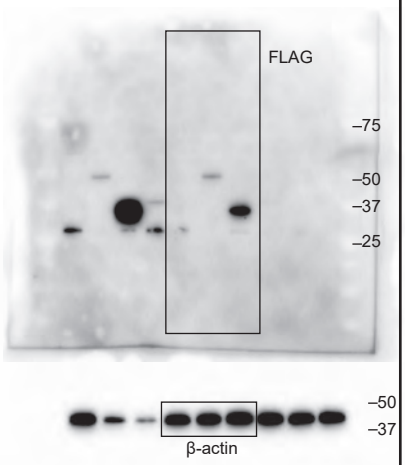
Ext Data Fig 1e



Ext Data Fig 1f



Ext Data Fig 2o



Ext Data Fig 2s

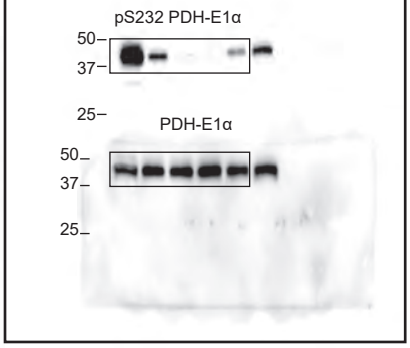
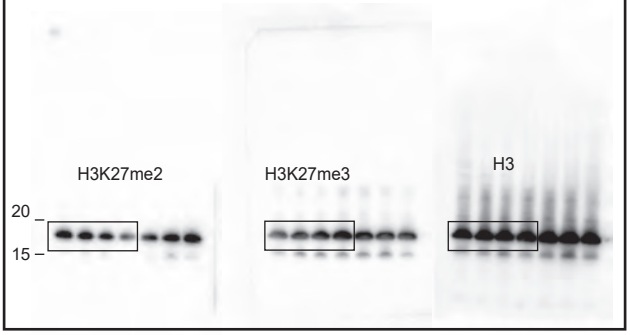


Fig 5a



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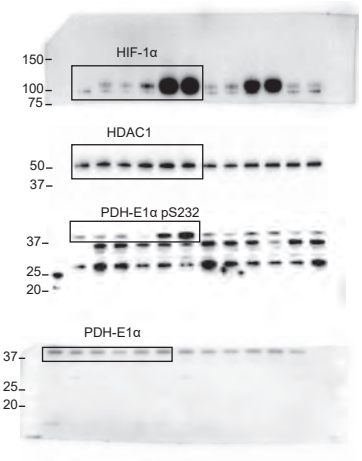
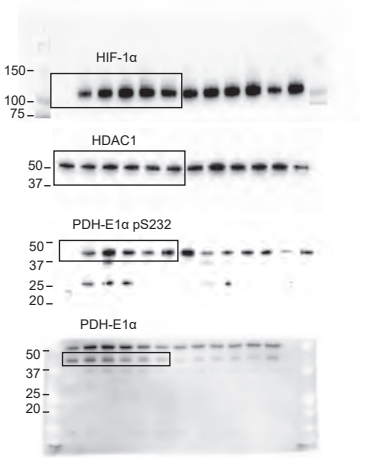
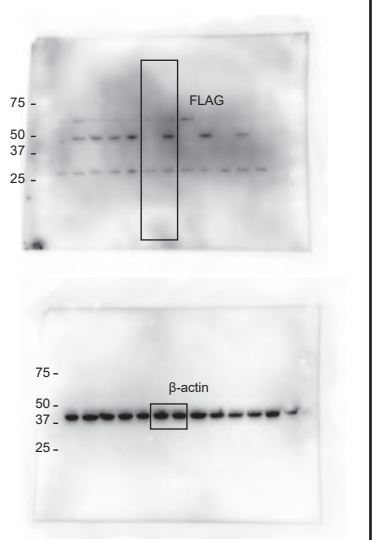


Ext Data Fig 5b

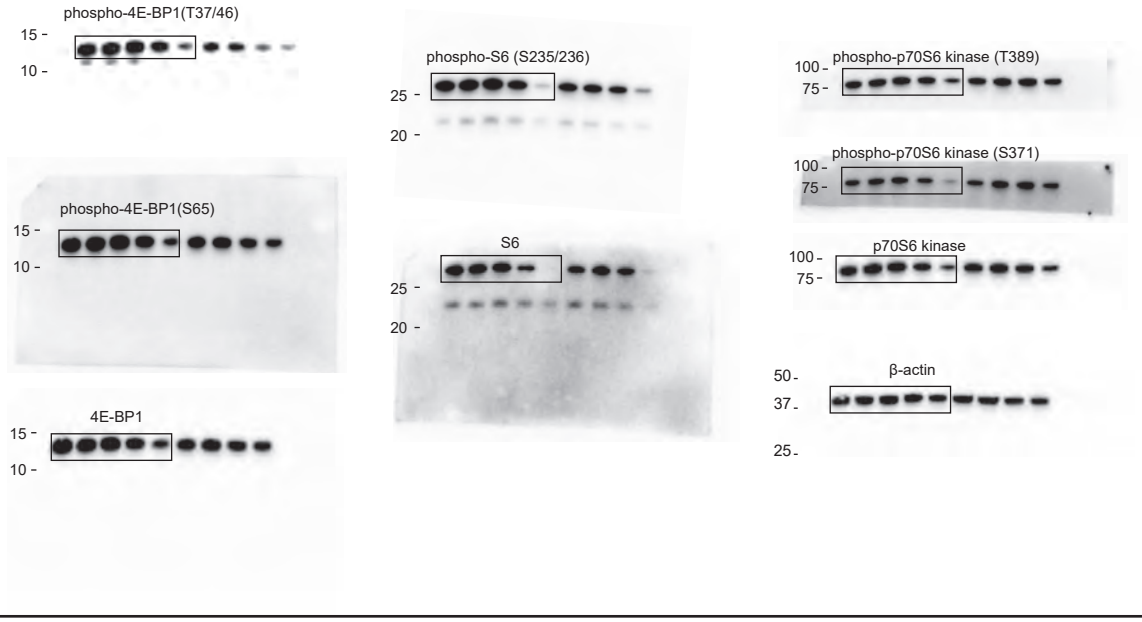


Ext Data Fig 5r



Supplementary Figure of Uncropped Gels with Size Marker Indications

Ext Data Fig 7



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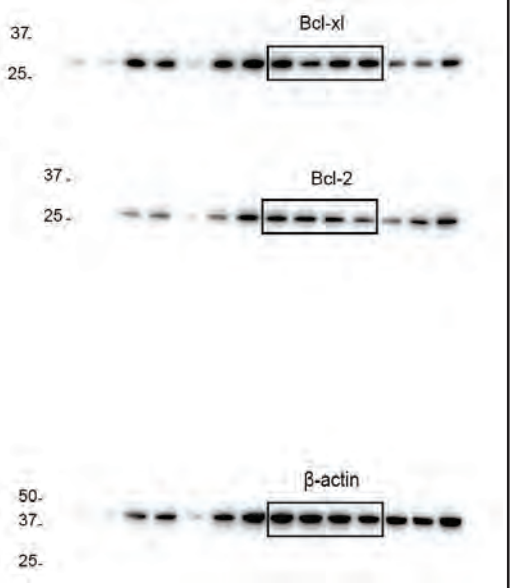
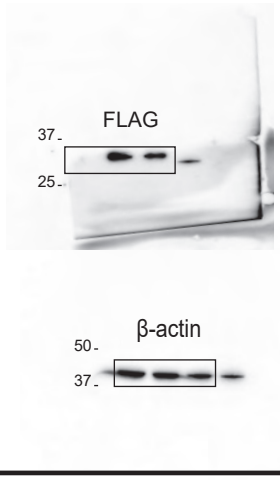
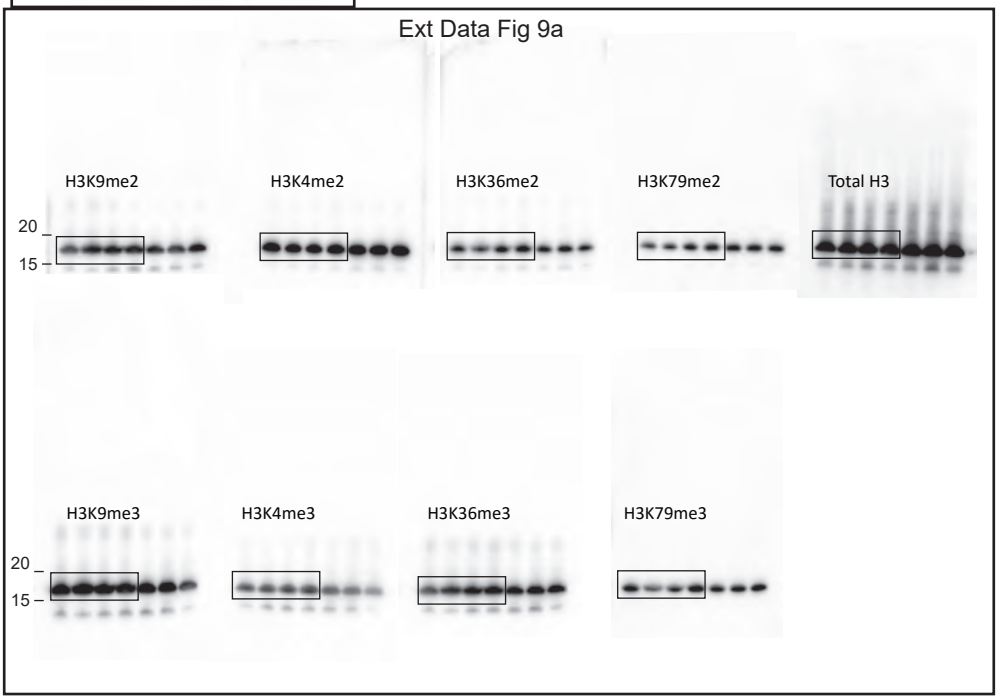


Ext Data Fig 8e



Ext Data Fig 9a



Methods

Isolation and activation of $CD8^+$ T-lymphocytes

$CD8^+$ T-lymphocytes were isolated from mouse spleens by positive selection. Incubation with MicroBeads conjugated to monoclonal anti-mouse CD8α (Ly-2; isotype: rat IgG2a) antibody (Miltenyi, 130-049-401) was followed by magnetic bead isolation on a MACS column. Naïve $CD8^+$ T-lymphocytes were isolated from mouse spleens using the naive $CD8a^+$ T Cell Isolation Kit (Miltenyi, 130-096-543). Unless otherwise stated, $CD8^+$ T-lymphocytes were activated with plate-bound αCD3 (5 μg/ml) and soluble αCD28 (1μg/ml) for 48 h. For activation of OT-I $CD8^+$ T-cells, total splenocytes from OT-I mice were cultured with SIINFEKL peptide for 48 h. All $CD8^+$ T-cells were cultured in RPMI-1640 medium containing 2 mM glutamine, 10% FBS, 25 mM HEPES, pH 7.2, 1% penicillin-streptomycin, 55 μM β-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, $CD8^+$ T-lymphocytes were cultured in either 21% or 1% oxygen conditions for various times. High glucose DMEM supplemented 10% FBS and 0.8 mg/ml of G418, was used as standard growth medium for RCC4EV, RCC4VHL, 786-OEV, 786-OVHL and EG7-OVA 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. 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 $CD8^+$ 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. $CD8^+$ 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-CD8a (Biolegend, HIT8a). $CD8^+$ 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, $CD8^+$ 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-CD8a (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-IFNγ (Biolegend, XMG1.2), anti-TNFα (Biolegend, MP6-XT22), anti-Bcl-2 (Biolegend, BCL/10C4), Eomes (eBioscience, Dan11mag), PD-1 (Biolegend, 29F.1A12), 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. $CD8^+$ 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 ^{1}H-NMR experiments, $CD8^+$ 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*$^{-/-}$ mouse embryonic fibroblasts

Mouse embryonic fibroblasts (MEFs) were isolated from E12.5-14.5 *Vhl*$^{fl/fl}$ 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:

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

Sequencing of *Idh1* and *Idh2*

gDNA was extracted from the following $CD8^+$ T-lymphocytes samples: freshly isolated, naïve *Hif1a*$^{fl/fl}$ and *Hif1a*$^{-/-}$; expanding *Hif1a*$^{fl/fl}$ and *Hif1a*$^{-/-}$ after a total of 4 days in 21% oxygen; expanding *Hif1a*$^{fl/fl}$ and *Hif1a*$^{-/-}$ 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, AGCAAGGATAACAAACTAGGCAGA, *Idh2* reverse, GGAAGCCTTGGCTTGAACTGC.

qRT–PCR

RNA was isolated using the RNeasy kit (Qiagen). 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 ΔΔCt method[1] using *Hprt* as the reference gene. Data were analysed unsupervised hierarchical clustering and visualized by heat map. 500 μM S-2HG was used in experiments with S-2HG treatment, unless otherwise indicated in the figure. The following primers were used:

Gene Symbol	Primer Bank ID[2-4]	Forward Primer (5' to 3')	Reverse Primer (5'to 3')
Cs	**13385942a1**	GGACAATTTTCCAACCAATCTGC	TCGGTTCATTCCCTCTGCATA
Aco2	**18079339a1**	ATCGAGCGGGGAAAGACATAC	TGATGGTACAGCCACCTTAGG
Idh1	**6754278a1**	ATGCAAGGAGATGAAATGACACG	GCATCACGATTCTCTATGCCTAA

Idh2	**1236984a1**	GGAGAAGCCGGTAGTGGAGAT	GGTCTGGTCACGGTTTGGAA
Idh3a	**18250284a1**	TGGGTGTCCAAGGTCTCTC	CTCCCACTGAATAGGTGCTTTG
Idh3b	**18700024a1**	TGGAGAGGTCTCGGAACATCT	AGCCTTGAACACTTCCTTGAC
Idh3g	**6680345a1**	GGTGCTGCAAAGGCAATGC	TATGCCGCCCACCATACTTAG
Ogdh	**15489120a1**	AGGGCATATCAGATACGAGGG	CTGTGGATGAGATAATGTCAGCG
Sucla2	**26349481a1**	ACCCTTTCGCTGCATGAATAC	CCTGTGCCTTTATCACAACATCC
Suclg2	**3766203a1**	CCCCGAAGATGGCTGAACC	ACCTCCTTTCAAACCGCTATTG
Suclg1	**9845299a1**	TGGGATACGACACGGGTCTTA	CAGAAGCCGTTGCTCCTGTT
Sdha	**15030102a1**	GGAACACTCCAAAAACAGACCT	CCACCACTGGGTATTGAGTAGAA
Sdhb	**34328286a1**	AATTTGCCATTTACCGATGGGA	AGCATCCAACACCATAGGTCC
Sdhc	**13384690a1**	GCTGCGTTCTTGCTGAGACA	ATCTCCTCCTTAGCTGTGGTT
Sdhd	**27229021a1**	TGGTCAGACCCGCTTATGTG	GGTCCAGTGGAGAGATGCAG
Fh1	**33859554a1**	GAATGGCAAGCCAAAATTCCTT	CGTTCCGTAGCACCTCCAATCTT
Mdh1	**31982178a1**	TTCTGGACGGTGTCCTGATG	TTTCACATTGGCTTTCAGTAGGT
Mdh2	**31982186a1**	TTGGGCAACCCCTTTCACTC	GCCTTTCACATTTGCTCTGGTC
Gls	**164607131c2**	CTACAGGATTGCGAACATCTGAT	ACACCATCTGACGTTGTCTGA
Gls2	**254826697c1**	CGTCCGGTACTACCTCGGT	TGTCCCTCTGCAATAGTGTAGAA
Glud1	**225543106c3**	CTGTGGTCGATGTACCGTTTG	AGCTCCATAGTGAACCTCCGT
Hprt	**7305155a1**	TCAGTCAACGGGGGACATAAA	GGGGCTGTACTGCTTAACCAG
Got1	**6754034a1**	GCGCCTCCATCAGTCTTTG	ATTCATCTGTGCGGTACGCTC
Got2	**6754036a1**	GGACCTCCAGATCCCATCCT	GGTTTTCCGTTATCATCCCGGTA
Glut1	N/A	GGGCATGTGCTTCCAGTATGT	ACGAGGAGCACCGTGAAGAT
Pdk1	N/A	GAAGCAGTTCCTGGACTTCG	CCAACTTTGCACCAGCTGTA
Pgk1	N/A	ATTCTGCTTGGACAATGGAGC	AGGCATGGGAACACCATCA
Mct4	N/A	TCACGGGTTTCTCCTACGC	GCCAAAGCGGTTCACACAC
Ldha	N/A	TGTCTCCAGCAAAGACTACTGT	GACTGTACTTGACAATGTTGGGA
Hk2	N/A	TGATCGCCTGCTTATTCACGG	AACCGCCTAGAAATCTCCAGA
Cox4i1	N/A	ATTGGCAAGAGAGCCATTTCTAC	CACGCCGATCAGCGTAAGT
Cox4i2	N/A	CTGCCCGGAGTCTGGTAATG	CAGTCAACGTAGGGGGTCATC
L2hgdh	**21703884a1**	TAGTCATCGTTGGAGGTGGAA	TCCAGTCTGGTGAAGAGCTAAAT
D2hgdh	**30725722a1**	GGTCTGCAAGGGTCCTCTG	ACCACGGAGTATGCTCTGTGA
Ccr6	**6753318a1**	CCTGGGCAACATTATGGTGGT	CAGAACGGTAGGGTGAGGACA
Prdm1	**6680784a1**	TTCTCTTGGAAAAACGTGTGGG	GGAGCCGGAGCTAGACTTG
Bcl6	**6753172a1**	CCGGCACGCTAGTGATGTT	TGTCTTATGGGCTCTAAACTGCT
Eomes	**26354683a1**	GCGCATGTTTCCTTTCTTGAG	GGTCGGCCAGAACCACTTC
Il2	**1504135a1**	TGAGCAGGATGGAGAATTACAGG	GTCCAAGTTCATCTTCTAGGCAC
Ifng	**33468859a1**	ATGAACGCTACACACTGCATC	CCATCCTTTTGCCAGTTCCTC
Tcf7	**6678245a1**	AGCTTTCTCCACTCTACGAACA	AATCCAGAGAGATCGGGGGTC
Sell[5]	**Finaly et al**	ACGGGCCCCAGTGTCAGTATGTG	TGAGAAATGCCAGCCCCGAGAA
Kdm6a	**20073323a1**	CGGGCGGACAAAAGAAGAAC	CATAGACTTGCATCAGATCCTCC
Tet2	**22122441a1**	AGAGAAGACAATCGAGAAGTCGG	CCTTCCGTACTCCCAAACTCAT
Bcl2	**6753168a1**	ATGCCTTTGTGGAACTATATGGC	GGTATGCACCCAGAGTGATGC
Bclxl[6]	N/A	AACATCCCAGCTTCACATAACCCC	GCGACCCCAGTTTACTCCATCC

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: TCGTGCTTTGGCCAGATTAAA, Tet2 shRNA#3: GAGCGTTCCTCAGTATCATTT, Utx shRNA#1: CCGAGCAAACAGAAATAATTT, Utx shRNA#2: TGGACTTGCAGCACGAATTAA, Utx shRNA#3: GATTGCACATAGACTAAGAAA, L2hgdh shRNA#1 GCGTCATAGTAAGGTTGTGTT, L2hgdh shRNA#2: GATCCAGCAGGAGGATATAAA, L2hgdh shRNA#3: GTGTCATACACAGTGGTATTT.

scramble shRNA: CCGGCAACAAGATGAAGAGCACCAACTCGAGTTGGTGCTCTTCATCTTGTTGTTTTTG.

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 $CD8^+$ T-cells were transduced with viral supernatants using rectronectin (Takara Clontech) according to the manufacturer's instructions. GFP^+ 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 shL2hdgh 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 $CD8^+$ T-cells were transduced as before. For Pdk1 and Ldha overexpression experiments, GFP^+ 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^+ cells were assessed by flow cytometry 7 days later with or without 300 μM S-2HG-octyl ester treatment. Live, $CD8^+GFP^+$ cells were gated.

Glucose, lactate, VEGF, IL-2 and IFN-γ 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. VEGF-A, IL-2 and IFN-γ protein levels in media were determined using the following kits from MesoScale Discovery: K150BMC-2 for VEGF-A, K15048D-2 for IFN-γ and K152QQD-2 for IL-2. Values were normalized to viable cell counts. Cells were treated with 500 μM S-2HG-octyl ester for 16-24h

***In vitro* cytotoxicity assay**

OT-I-specific $CD8^+$ T-cells were then treated with or without S-2HG-octyl ester and then incubated with target (EG7-OVA, CFSE-low) and control (EL4, CFSE high) cells. 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 $CD8^+$ 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[7-9] included unsupervised hierarchical clustering and principal component analysis.

^{1}H-NMR Data Acquisition and Analysis

^{1}H nuclear magnetic resonance (^{1}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[7] was used to determine the concentration of DSS in each sample. Processing of ^{1}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_3-2HG) as well as ^{13}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^{o}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^{o}C and negative polarity. The precursor ions for 2HG, glutamate, succinate, fumarate, malate, D_3-2HG, ^{13}C-glutamate, ^{13}C-succinate, ^{13}C-fumarate and ^{13}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_3-2HG, ^{13}C-glutamate, ^{13}C-succinate, ^{13}C-fumarate and ^{13}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_3-2HG, ^{13}C-glutamate, ^{13}C-succinate, ^{13}C-fumarate and ^{13}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_2O:MeOH (5:95 v/v) containing 0.3 % acetic acid and 0.1% ammonium hydroxide, as well as a known amount of D_3-2HG. 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_2O: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 ^{13}C tracer studies were performed using murine $CD8^{+}$ 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-$^{13}C_6$] glucose or [U-$^{13}C_5$] 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-$^{13}C_6$] glucose and [U-$^{13}C_5$] 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_3-2HG 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_3-2HG and underivitised-D_3-2HG 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_3-2HG (150.0) and underivitised-D_3-2HG (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 descirbed[10]. Isolated murine $CD8^+$ T-lymphocytes were activated with plate bound αCD3 (5 µg/ml and soluble αCD28 (and 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*$^{fl/fl}$ and *Hif1α*$^{fl/fl}$ *dlck*^{cre} 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 ^{0}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: FLAG (Cell Signalling Technology, 2368), HIF1α (Novus, NB100-449), HIF2α (Novus, NB100-122), PDH-E1α (Abcam, ab110330), PDH-E1α pS232 (Merck Millipore, AP1063) LaminB1 (Abcam, ab16048), HDAC1 (Abcam, ab109411) and β-tubulin (Abcam, ab21057). The following antibodies, all from Cell Signalling Technology, were used to assess mTOR signalling: 4E-BP1 (9644), phospho-4E-BP1 S65 (9451), phospho-4E-BP1 T37/46 (2885), S6 (2217), phospho-S6 S235/236 (4858), p70S6 kinase (2708), phospho-p70S6 kinase (9208), 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: H3 (4499), H3K4me2 (9725), H3K4me3 (9751), H3K9me2 (4658), H3K9me3 (13969), H3K27me2 (9728), H3K27me3 (9733), H3K36me2 (2901), H3K36me3 (4909), H3K79me2 (5427), H3K79me3 (4360). Antibodies against Bcl-2 (D17C4) and Bcl-xl (54H6) were from Cell Signalling Technology. For histone immunoblots, $CD8^+$ T-lymphocytes were treated with the indicated dose of S-2HG-octyl ester for 7 days.

hMeDIP/MeDIP-ePCR

Freshly isolated naïve or activated $CD8^+$ T-lymphocytes treated with or without 500 µM 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℃ for 10 min, followed by snap chilling on ice. Antibodies were conjugated to protein G dynabeads (Invitrogen) at 4℃ overnight. 2.5 ug 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℃ 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℃ 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 $CD8^+$ T-lymphocytes treated with or without 500 μM 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 re-suspended in 250μl SDS lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris-HCl pH 8.1) at 3.33×10^7 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℃, and the supernatant collected. DNA fragment sizes were checked by agarose gel electrophoresis to range from 200 to 500bp. 10μg 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℃ 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℃ with $5x10^6$ cells used for each ChIP. The samples were then centrifuged at 200g for 2 min at 4℃ and the supernatants removed. The agarose beads were washed at 4℃ 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 NaHCO3) and reverse cross-linked with 0.2 M NaCl at 65℃ overnight. ChIP samples were treated with 0.2 mg/ml RNase A at 37℃ for 2 h and 0.8 mg/ml proteinase K at 55℃ 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**	AGCATTTCACCTATGCTCCCA
mSell-F4	AGCTGTTTCCTGGTGTCAGAA	**mSell-R4**	ACTCATCTGGCCTTACATGCT
mSell-F5	TTGTTCCTGCTAGGTAAGGTGA	**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	TTCCGTAGGGTTCAATGGGT	**mSell-R10**	TGCTCCAGACATGAACTCCC

mSell-F11	CTGAGTGGCATTGGGAGTTC	**mSell-R11**	ACGATGCATTTGTGTTCCTGC
mSell-F12	TCCCGACTCCATGGATCAAA	**mSell-R12**	CTCTCCCCCGTCCATTCACT
mSell-F13	TCTCTTGTTCCCCACACAGT	**mSell-R13**	GAACTGCACCTTGTACCCGT
mSell-F14	TCAGTGGCTGCCGAAGTATT	**mSell-R14**	TTGCCATGTGATAGGCCACA
mSell-F15	TCTGCAGCCCACTTATTTTCA	**mSell-R15**	CCATGTCTTAAAACCAGCTACCG
mSell-F16	CTGGAAGAGAGACCATCCTTGAA	**mSell-R16**	GGTGACACCTCATCGTGGAT
mSell-F17	CCTGTAATCAATGCAGACAATGG	**mSell-R17**	GTGTTCACCAGCTTTCCCCT
mSell-F18	TCTAGAGGAGGGGAAAGCTGG	**mSell-R18**	AGCTGGCATTTACAGACCAAG
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**	TGACCTGCTAACTTGTAGTGTTGT
mSell-F27	AGGCATGGTTATCTGAATGAGT	**mSell-R27**	TGGAGTCTAAGTAGTCTTGCTTGT
mSell-F28	AGAAGCAAGCAAGCATGTGA	**mSell-R28**	CATAGTTCCTTGGTGCTTAATCCA
mSell-F29	AGTGACTTCCTCCAGATTGCTT	**mSell-R29**	AAGTGTGAAGAGGGTAAGACAGC
mSell-F30	AGTGACTTCCTCCAGATTGCTT	**mSell-R30**	GAGAGGTTGGGGGACCTAGAA
mSell-F31	TCTAGGTCCCCCAACCTCTC	**mSell-R31**	TTCCTCGTTCACCCCCTAGT
mSell-F32	GGGGTGAACGAGGAAAGAGG	**mSell-R32**	GAACTTCGGGAGCTTTCTGGA
mSell-F33	TCCACTAGGGGGTGAACGAG	**mSell-R33**	TGAACTTCGGGAGCTTTCTGG
mSell-F34	GTCTTCCAGAAAGCTCCCGA	**mSell-R34**	AACTCCCGACCATGGGGTA
mSell-F38	TATCCTGAGCAGGGGGCATA	**mSell-R38**	ACCCCTTTCCTAATCTGGGC
mSell-F39	GGCATAGCTTCTGGTCCACT	**mSell-R39**	CAATGTCACCAGTGGGGTTTG
mSell-F40	CACTGGTGACATTGGCACAT	**mSell-R40**	TATAGGCTGGGATGCTCACC
mSell-F41	GAGAGACTTGCAGAGAGACCC	**mSell-R41**	AAATGATAGCTTCTTGCCCCCT
mSell-F42	AGGGGGCAAGAAGCTATCATTT	**mSell-R42**	CTGTGCCCCTTCCAGCATTC
mSell-F43	CCTACGCATGCTGTCTCAAG	**mSell-R43**	TCACTCTGTGTCACCGCAAA
mSell-F44	TTGATGGAATGCTGGAAGGGG	**mSell-R44**	GCCATAGGCTTTCTGCACATC
mSell-F45	CGCATGCTGTCTCAAGAAAATGA	**mSell-R45**	AGCCATAGGCTTTCTGCACAT
mSell-F46	CTTTGCGGTGACACAGAGTG	**mSell-R46**	TGTAGCCAGAAGCCAGGAAAG
mSell-F47	TTCCTGGCTTCTGGCTACATC	**mSell-R47**	GCCTTTCACGCTGGGTCAT
mSell-F48	TCCTTCATGACCCAGCGTGA	**mSell-R48**	GCTCTTGTTTTTAATGCTCTTGGG
mSell-F49	CCAGCGTGAAAGGCAAATGT	**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	CTTATGCCTGAAGGCCCAGT	**mSell-R56**	GCCCTGGGAAAGCCTCAATA
mSell-F57	TGTTAATGTTCTGCCTGACCCT	**mSell-R57**	TCCTGGGATAACTTCCTAAAGGC
mSell-F58	GGAAGTTATCCCAGGATTAAAAAGT	**mSell-R58**	TCTTCCTCAAAATTCTTTGAAGTGT
mSell-F59	ATTCTGATCCAAGGCTGCCAA	**mSell-R59**	AGTGTTCTAGGACCAGGCAC
mSell-F60	TGCTGCTGTTTTCCAGACTTC	**mSell-R60**	TCCCAGTTCATGGGCTTTTC
mSell-F61	GACATGGGTGGGAACCAACA	**mSell-R61**	GTTTGTGACAGGCGTCATCG
mSell-F62	AGTGTCTAGGCTCCTACGCT	**mSell-R62**	CCCTGTTCCCCACATAACACA
mSell-F63	TGGACAGACTGTGCCTCAAG	**mSell-R63**	TGCATAACGCACGCTACACT
mSell-F64	TGTGCAAAGAAGAGACTCTGG	**mSell-R64**	TTCCACACATTCTCCACGGC
mSell-F65	TGATGCAGGGTATTACGGGC	**mSell-R65**	AAGCAAAGCCAACACGACAC
mSell-F66	GGCCATTTCTTCCCACGGTA	**mSell-R66**	TCCAAAGGCTCACACTGGAC
mSell-F67	GCTTCCAGTCCAAGTGTGCT	**mSell-R67**	TTGTCCCAACAACGAAGATCCA
mSell-F68	TGGATCTTCGTTGTTGGGACA	**mSell-R68**	TGAAACTTCATGCTGACTTGGG
mSell-F69	TGGGAAGTTCCAAACAGAAAGC	**mSell-R69**	TGTAACCTGCTCACCTTTGACA
mSell-F70	GTCCTCTGAGCTTGCAGGATT	**mSell-R70**	GAAGAACAGGAGTACCTTCCCA
mSell-F71	GGGTATTTCTGGAGAATATGGAAGC	**mSell-R71**	AGGGGATCTCACCACAGGAT
mSell-F72	TGGCCACTTGGCATCTTACT	**mSell-R72**	ACCGCATCTCTGAGTGACTTC
mSell-F73	TGGGAGAACCGGAAATGAGAG	**mSell-R73**	AGATTTGATGCGCCTTTCCA
mSell-F74	GTCTGCTGCACACAATGGAA	**mSell-R74**	CTAAAGGGCAGTCCAACGGT
mSell-F75	AACATCGTGCTTGCTTTCTTGA	**mSell-R75**	GTGGAATATGCCTCCTACCCC
mSell-F76	AGGAGGCATATTCCACCACC	**mSell-R76**	ATGAAAGAACCCAGAAGAAGTCAC
mSell-F77	AGCCATGACCTCCAAATATGCT	**mSell-R77**	TAGGGTGGCAGAGTTTCACAC
mSell-F78	GGGCTCCAGGCACAGAAATAG	**mSell-R78**	GGAGAGAGAAGCAGGGTTGAG
mSell-F79	ATATTCATCCCCAGCCCCATT	**mSell-R79**	CACTGGACCACTTTCATGGAGA
mSell-F80	TGGATCTTTGTTGTGGCAAGC	**mSell-R80**	AACAGACTTCTAGGCAAGACTCC
mSell-F81	GTCTTTGAAGTCATCTGGCATTT	**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**	CTACTCCAAACACCCAGCGA
previous mSell-F40	AGTTACCCAGCTCAGATCGC	**previous mSell-R40**	CTGCCTATGGGACCACAGTT
mCD62L_PF2	TTCCAGAAAGCTCCCGAAGTT	**mCD62L_PR2**	GGACCCTGGTCGTACCATAGA
mCD62L_PF3	GGGGAAGAATCCAGTTGCCAT	**mCD62L_PR3**	ATCCCCAGTTTGGAAGGAGG
mCD62L_bPF1	GGGGAAGAATCCAGTTGCCAT	**mCD62L_bPR1**	GGCTCAATCATTTGCCCCAA
mCD62L_PF4	TTGGCACATTCTTCCTGAGC	**mCD62L_PR4**	GGGTCTCTCTGCAAGTCTCTC
mCD62L_bPF2	AGAGACTTGCAGAGAGACCCA	**mCD62L_bPR2**	TGATAGCTTCTTGCCCCCTT
mCD62L_PF5	AAGGGGGCAAGAAGCTATCA	**mCD62L_PR5**	ACACCAAGGGAGAAAATCAGCA
mCD62L_bPF3	CCCTACGCATGCTGTCTCAA	**mCD62L_bPR3**	TCACTCTGTGTCACCGCAAA
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+ cells were gated. H3K27me3 levels were quantified in the following $CD8^+$ populations: $CD62L^{Low}CD44^{High}$, $CD62L^{High}CD44^{High}$ and $CD62L^{High}CD44^{Low}$.

***In vivo* persistence experiments**

Total splenocytes from OT-I $CD45.1^+$ mice were activated with 1000 nM SIINFEKL peptide in the presence of IL-2 and vehicle or 300 μM S-2HG-octyl ester. After 48 h $CD8^+$ T-cells were purified by negative selection and cultured for 7 days in the presence of IL-2 and vehicle or 300 μM S-2HG-octyl ester. 1 million $CD45.1^+CD8^+$ 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 μM S-2HG-octyl ester. After 48 h $CD8^+$ T-cells were purified by negative selection and cultured for 7 days in the presence of IL-2 and vehicle or 300 μM 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 $CD45.1^+CD8^+$ 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 μM β-mercaptoethanol supplemented 20ng/ml mGM-CSF (R&D Systems). After 8 days of culture, dendritic cells were activated with 1 μg/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 μM SIINFEKL peptide at 37^0C for 1 hour. After peptide loading, dendritic cells were detached with 3 mM EDTA in PBS for 5-10 min at 37^0C and washed with PBS.

Homeostatic Proliferation

Total splenocytes from OT-I CD45.1/CD45.1 and OT-I CD45.1/CD45.2 mice were activated with 1000 nM SIINFEKL peptide in the presence of IL-2 and vehicle or 300 μM S-2HG-octyl ester. After 48 h $CD8^+$ T-cells were purified by negative selection and cultured for a further 7 days in the presence of IL-2 and vehicle or 300 μM 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 CD45.2/CD45.2 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 $CD8^+$ T-lymphocytes were activated with 1000 nM SIINFEKL peptide in the presence of IL-2 and vehicle or 300 μM S-2HG-octyl ester. After 48 h $CD8^+$ T-cells were purified by negative selection and cultured for a further 7 days in the presence of IL-2 and vehicle or 300 μM S-2HG-octyl ester. 0.7×10^6 - 1.0×10^6 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 $CD8^+$ T-lymphocytes. Tumour growth was monitored blindly by calliper measurements and volume was calculated by the modified ellipsoid formula $(1/2(\text{Length} \times \text{Width}^2))$[11]. The maximal tumour measurements permitted was a mean length and width up to 12 mm and mice were sacrificed when the maximal 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 $CD8^+$ T-lymphocytes was achieved via breeding with dLck mice[12]: *Hif1a*$^{fl/fl}$, *Vh*$^{fl/fl}$[13] and *EpasI*$^{fl/fl}$ [14]. All mice were backcrossed over ten generations to the C57BL/6J background. OT-1 mice[15] containing transgenic inserts for mouse TCR-Vα2 and TCR-Vβ5 genes that recognise ovalbumin residues 257-264 (SIINFEKL) in the context of H-$2K^b$, 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. as 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.

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