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SUPPLEMENTARY INFORMATION

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Entry of glucose- and glutamine-derived carbons into the citric acid cycle supports early steps of HIV-1 infection in CD4 T cells

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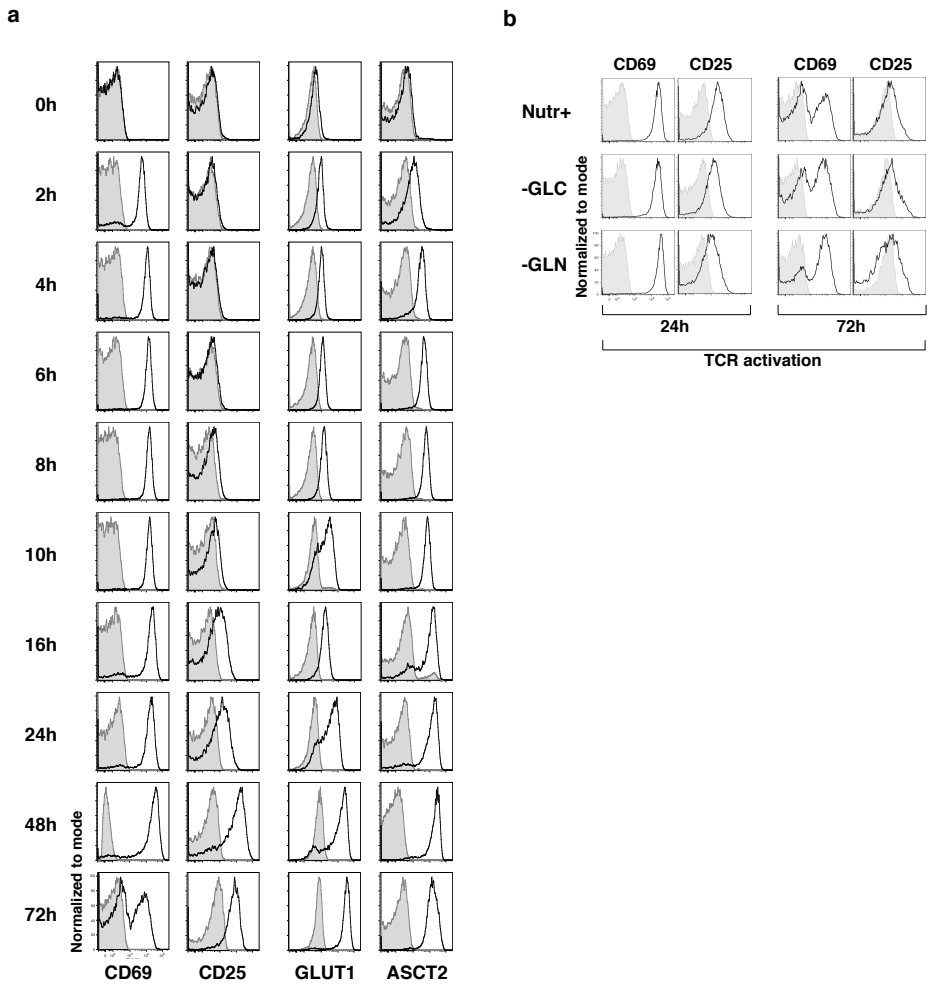
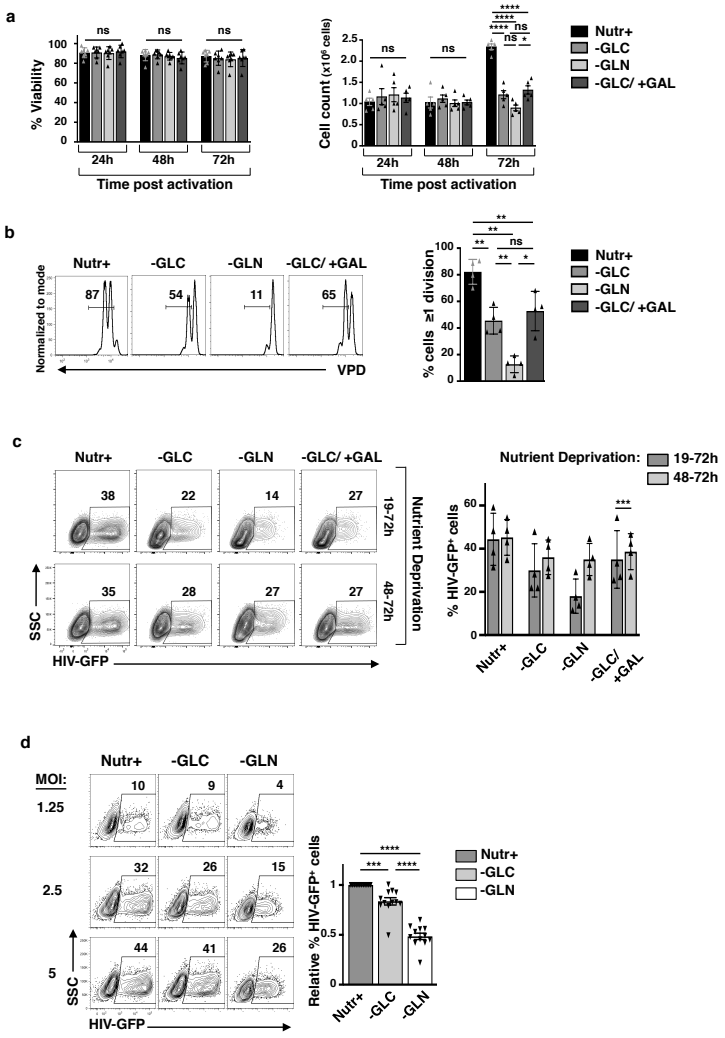
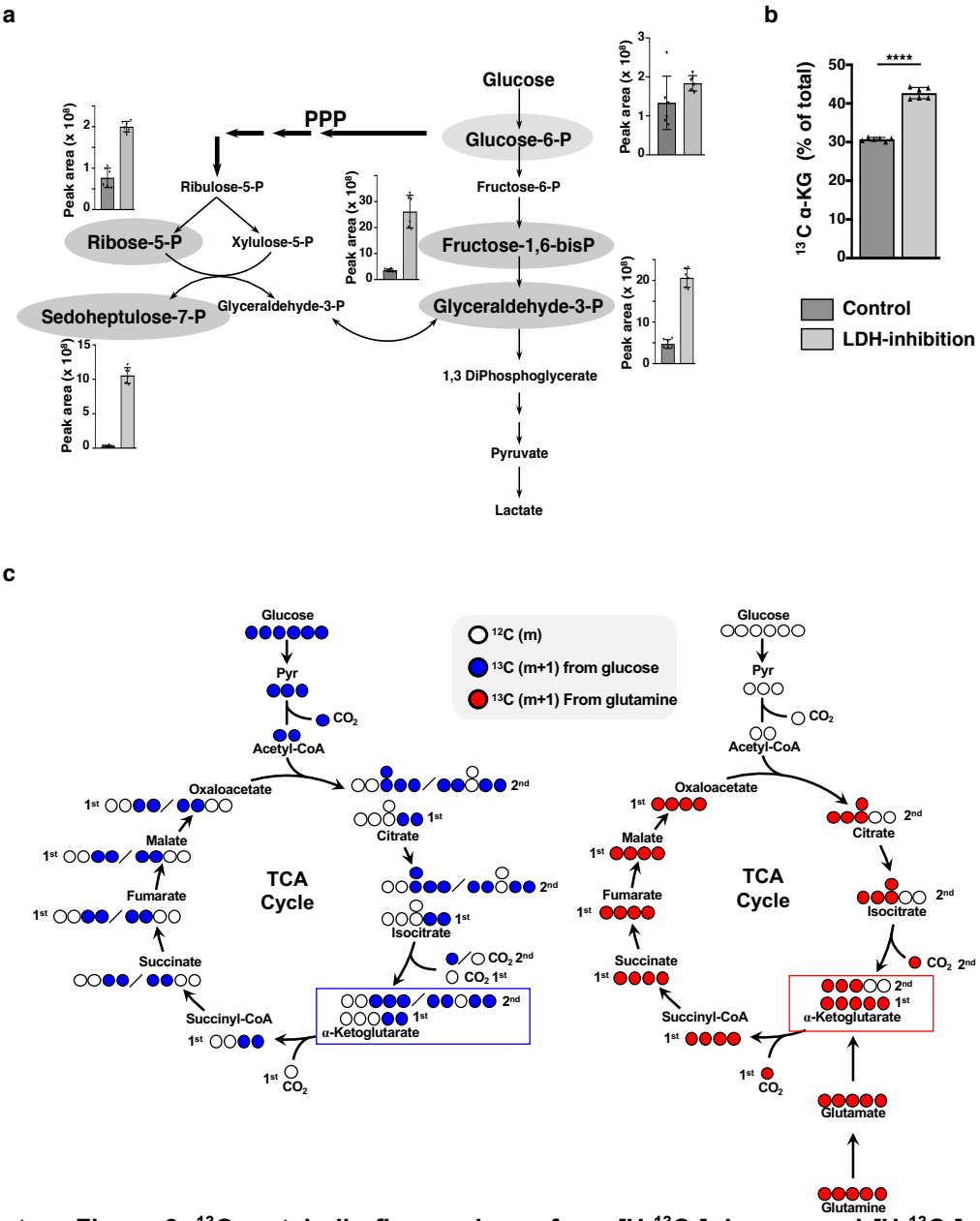


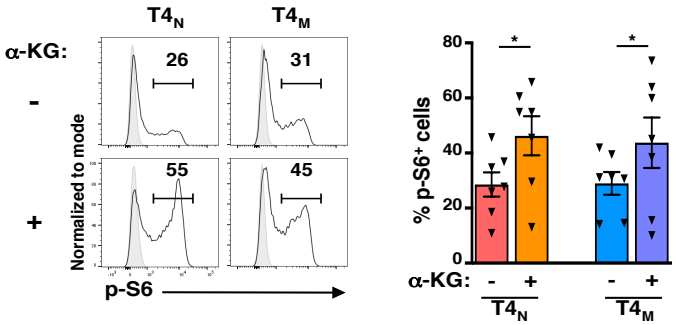
Supplementary Figure 1. GLUT1 and ASCT2 nutrient transporters are rapidly upregulated following TCR stimulation. (**a**) Human CD4 T cells were stimulated with αCD3/αCD28 mAbs and surface expression of early activation markers (CD25 and CD69) as well as the GLUT1 glucose and ASCT2 glutamine transporters were monitored at 2, 4, 6, 8, 10, 18, 24, 48 and 72 hours post stimulation. Representative histograms are presented. Control immunofluorescence is shown in grey histograms and specific staining is shown in black line histograms (representative of n=2 biologically independent samples). (**b**) Expression of CD25 and CD69 activation markers were assessed at 24 and 72h post activation in nutrient-replete conditions (Nutr+) as well as following deprivation of glucose (-GLC) or glutamine (-GLN) at 19h (representative of n=4 biologically independent samples).



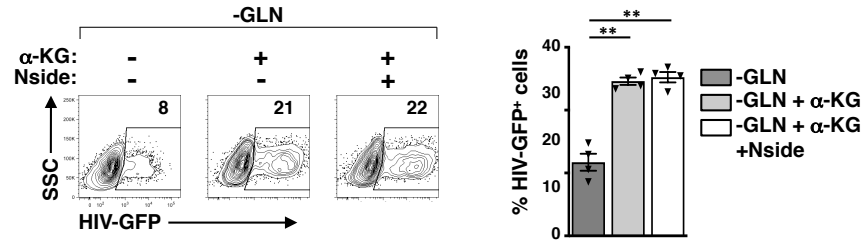
Supplementary Figure 2. Impact of nutrient deprivation on CD4 T cell survival, proliferation and HIV-1 gene expression. (**a**) CD4 T cells (1x10^6/well) were activated for 19h with coated αCD3/αCD28 mAbs and then transferred to either complete (Nutr+), glucose-deprived (-GLC), glutamine-deprived media (-GLN) or glucose-deprived/galactose-supplemented media (-GLC/+GAL). Viability (monitored by viability dye analysis; top; n=7 individual donors, 1-way ANOVA test) and absolute cell counts (bottom panel; n=5 biologically independent samples, 1-way ANOVA test) were monitored at 24 and 48h. (**b**) CD4 T cells were labeled with VPD and then activated in the conditions described above. Representative histograms showing proliferation profiles at 72h are presented together with a quantification of the percentages of dividing cells ± SEM (n=4 biologically independent samples, 2-tailed t-test). (**c**) CD4 T cells were activated as above for 19h and exposed to nutrient deprivation conditions at either 19h or at 48h. In all conditions, cells were infected with single round HIV-1 virion harboring GFP at 24h and GFP reporter expression was evaluated at 72h. Representative dot plots showing percentages of infected cells (top) and histograms showing quantification of means ± SEM are presented (n=4 biologically independent samples; 2-tailed t-test; ***, p=0.0004). (**d**) CD4 T cells were activated as above and infected at the indicated MOIs. Infection was monitored 48h later as a function of GFP expression. Dot plots showing the percentage of infected cells are presented at different MOIs (left) as well as a quantification of the means ± SEM of GFP$^+$ cells relative to control conditions, set at 1 (right; n=4 biologically independent samples performed in technical triplicates; 1-way ANOVA test). *, p<0.05; **, p<0.01; ***p<0.005; ****p<0.0001. All precise p values are indicated in Supplementary Table 3.



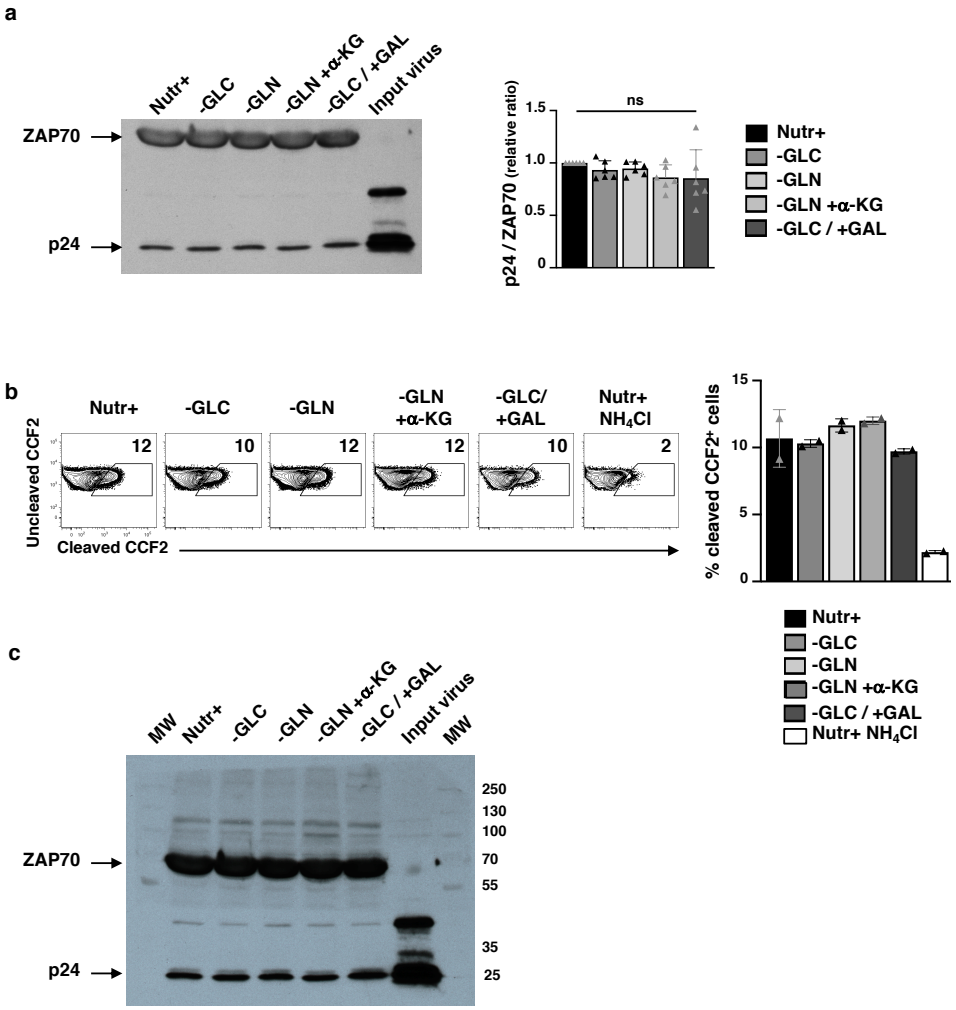
Supplementary Figure 3. ^{13}C metabolic flux analyses from [U-$^{13}C_6$]glucose and [U-$^{13}C_5$]glutamine into pentose phosphate pathway and TCA cycle intermediates. (**a**) The presence of pentose phosphate pathway intermediates following TCR stimulation of CD4 T cells under control conditions or following LDH inhibition (LDHi) with the competitive pyruvate analogue oxamate was monitored by HPLC-MS (mean ±SEM, n=2 biologically independent samples performed in technical triplicates). (**b**) The percentage of α-KG derived from ^{13}C glucose carbons in T cells activated in the absence vs presence of oxamate was monitored by HPLC-MS (mean ±SEM, n=2 biologically independent samples performed in technical triplicates; $p<0.0001$, unpaired t-test, 2-tailed). (**c**) A simplified representation of the distribution of ^{13}C carbon atoms derived from glucose (blue dots) and glutamine (red dots) in TCA cycle intermediates. The isotopologues of α-ketoglutarate derived from the 1st and 2nd rounds of the cycle are shown in boxes and isotopologues in other intermediates are indicated.



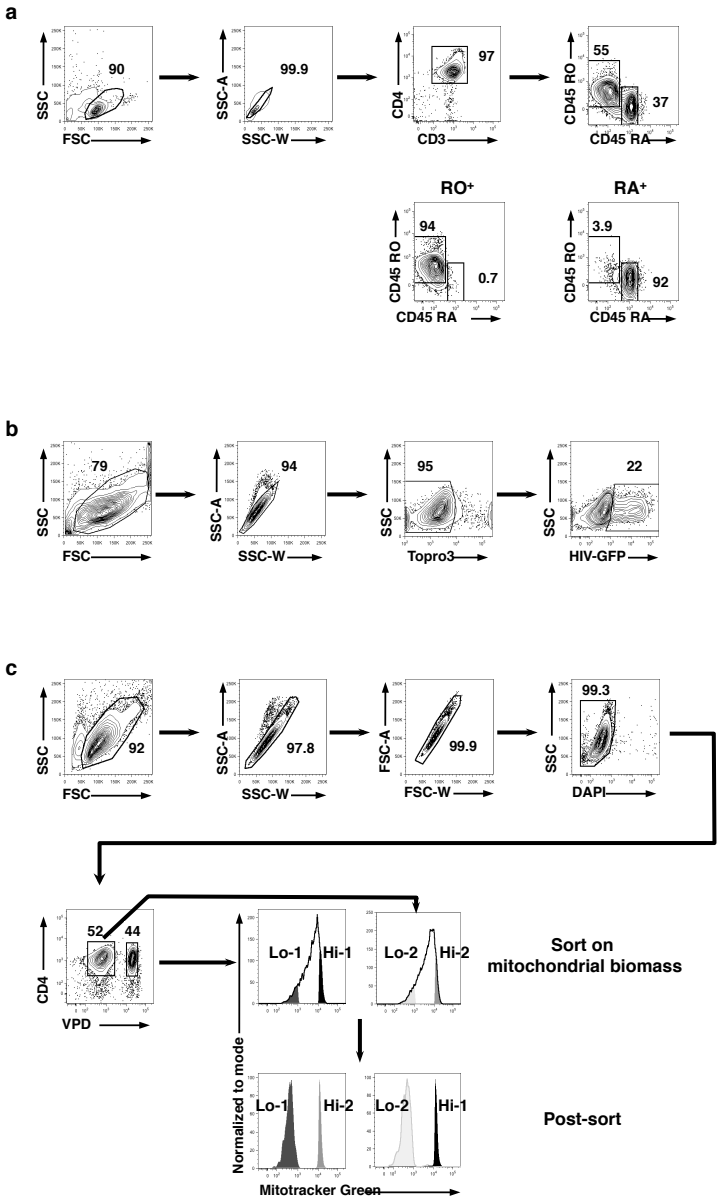
Supplementary Figure 4. α-KG increases mTOR signaling in activated CD4 T cells under conditions of glutamine deprivation. Naïve ($T4_N$) and memory ($T4_M$) CD4 T cells were TCR-stimulated in glutamine deprivation conditions in the absence or presence of cell permeable α-KG (dimethyl ketoglutarate, 3.5mM). S6 phosphorylation (p-S6) was monitored and representative plots are presented with isotype controls (grey histograms) and specific staining (black line, left). The mean percentages of $p\text{-}S6^+$ cells ± SEM are presented (right; n=7 biologically independent samples; 2-tailed t-test; *, p=0.0119 for $T4_N$ and p=0.0466 for $T4_M$. Precise p values are indicated in Supplementary Table 3.



Supplementary Figure 5. The impact of α-KG on HIV-1 infection in glutamine-deprived CD4 T cells is not altered by exogenous nucleosides. CD4 T cells activated in glutamine-deprived conditions were cultured in the absence or presence of α-KG and exogenous nucleosides (Nside; 30 μM). Cells were infected with single round HIV-1 virions and reporter expression was assessed 48h later. Representative histograms are shown (left panels) and quantification ± SEM of HIV infection is presented (right; n=4 biologically independent samples; 2-tailed t-test). **p=0.0041 for -GLN vs. -GLN+α-KG and p=0.0016 for -GLN vs. -GLN+α-KG+Nside.



Supplementary Figure 6. **Fusion of HIV-1 virions to CD4 T cells is not modulated by nutrient conditions.** (**a**) Fusion was assessed as a function of cellular uptake of Gag p24. CD4 T cells were activated for 19h, transferred to the indicated nutrient conditions for 5h (24h post activation) and then exposed to virus particles for 2h at 37°C. Cell lysates were subject to immunoblot and probed with anti-p24 and anti-ZAP-70 mAbs. A representative blot is presented (left, of n=6 biologically independent samples) and the level of p24 input virus is shown. The level of p24 relative to ZAP-70 in control conditions (Nutr+) was arbitrarily set to 1 and ratios in the indicated positions are presented (n=6 biologically independent samples, 1-way ANOVA test; ns, non-significant p>0.05). (**b**) Fusion was assessed using virus containing β-lactamase (BlaM)-Vpr chimeric protein. CD4 T cells were activated as above prior to a 2h incubation with virions containing BlaM-Vpr at 37°C. Cells were then loaded with CCF2-AM to monitor fusion; cells harboring BlaM-Vpr exhibit blue fluorescence due to cleavage of CCF2. Incubation with ammonium chloride (NH_4CL) was used as a negative control, inhibiting fusion. Percent fusion is presented (n=2 independent experiments from n=1 biologically independent sample). (**c**) The uncropped western blot shown in panel a is presented with full molecular weight markers. Precise p values are presented in Supplementary Table 3.



Supplementary Figure 7. Gating strategy for flow cytometry analysis of CD4 T cells. (**a**) Gating strategies showing FSC/SSC profiles, followed by FSC-A/SSC-W profiles for live freshly isolated CD4-selected T cells. For evaluation of naïve and memory CD4 T cells, CD45RA/CD45RO profiles are presented. (**b**) Representative gating strategies for TCR-stimulated CD4 T cells are presented showing sequential FSC/SSC, SSC-A/SSC-W and SSC/Topro3 profiles followed by HIV-GFP evaluation are presented. The percentages of cells in each gate are indicated. (**c**) Gating strategies for FACS-sorting of VDP-unlabeled and labeled CD4 T cells as a function of Mitotracker Green staining. Sequential FSC/SSC, SSC-A/SSC-W, FSC-A/FSC-W, and SSC/DAPI profiles are presented followed by sorting of CD4/VDP cells on the basis of Mitotracker Green profiles and evaluation of sorted cells.

List of Antibodies/Reagents

Protein	Ref.	Supplier
α-CD3 purified (clone OKT3)	317302	BioLegend
α-CD28 purified (clone 9.3)		Dr. Carl June
α-hCD3-APC-AF750 (clone UCHT1)	A66329	Beckman Coulter
α-hCD4-PE (clone 13B8.2)	A07751	Beckman Coulter
α-hCD4-BV786 (clone SK3)	563881	Becton Dickinson
α-hCD45RA-FITC (clone ALB-11)	A07786	Beckman Coulter
α-hCD45RO-PC7 (clone UCHL1)	B13648	Beckman Coulter
α-hCD25-APC (clone B1.49.9)	B09684	Beckman Coulter
α-hCD69-PC7 (clone TPI.55.3)	A80710	Beckman Coulter
α-hCD8-purified (clone OKT8)	BE0004-2	BioXcell
α-hCD8-APCeF780 (clone RPA-T8)	47-0088	eBiosciences
α-hCD45RA purified (clone 5H9)	556625	Becton Dickinson
α-hCD45RO purified (clone UCHL1)	555491	Becton Dickinson
α-P-RPS6 (Ser 235/236) (clone 91B2)	2211	Cell Signaling Technology
α-ZAP70 (clone 2F3.2)		Dr A. Weiss
α-HIV p24 (clone 183H125C)	NIH3537	NIH AIDS Reagent Program
GLUT1 RBD-GFP	GLUT1_G100	Metafora Biosystems
ASCT2 RBD-rFc	ASCT2.RBD	Metafora Biosystems
MitoTracker Green	M7514	Molecular Probes/ThermoFisher
MitoSox Red	M36008	Molecular Probes/ThermoFisher
Gene Blazer In Vivo Detection kit	12578134	Molecular Probes/ThermoFisher

Supplementary Table 1. List of antibodies/ reagents. Clones, references and suppliers are indicated.

Sequence	Primers
R/U5	Fwd 5'-GGCTAACTAGGGAACCCACTG-3'
	Rev 5'-CTGCTAGAGATTTTCCACACTGAC-3'
LTR/Gag	Fwd 5'-TGTGTGCCCGTCTGTTGTGT-3'
	Rev 5'-GAGTCCTGCGTCGAGAGAGC-3'
2LTRc	Fwd 5'-GCCTCAATAAAGCTTGCCTTG-3'
	Rev 5'-TCCCAGGCTCAGATCTGGTCTAAC-3'
β2m	Fwd 5'-TGCTGTCTCCATGTTTGATGTATCT-3'
	Rev 5'-TCTCTGCTCCCCACCTCTAAGT-3'

Supplementary Table 2. List of Primers.

Supplementary Table 3. **Statistical analyses of data presented in all figures**.

Figure 1

Panel	test	n			p value
1d	2-tailed t-test	5	$T4_N$ vs $T4_M$	**	0.0010
1f	2-tailed t-test	5	$T4_N$ Nutr+ vs $T4_N$ -GLC	*	0.0130
			$T4_N$ Nutr+ vs $T4_N$ -GLN	***	0.0003
			$T4_N$ -GLC vs $T4_N$ -GLN	***	0.0004
			$T4_M$ Nutr+ vs $T4_M$ -GLC	**	0.0011
			$T4_M$ Nutr+ vs $T4_M$ -GLN	****	<0.0001
			$T4_M$ -GLC vs $T4_M$ -GLN	***	0.0002

Figure 2

Panel	test	n			p value
2a	2-tailed t-test	13	$T4_N$ Nutr+ vs $T4_N$ -GLC	****	<0.0001
			$T4_N$ Nutr+ vs $T4_N$ -GLN	****	<0.0001
			$T4_N$ -GLC vs $T4_N$ -GLN	****	<0.0001
			$T4_M$ Nutr+ vs $T4_M$ -GLC	****	<0.0001
			$T4_M$ Nutr+ vs $T4_M$ -GLN	****	<0.0001
			$T4_M$ -GLC vs $T4_M$ -GLN		
2b	2-tailed t-test	3	Nutr+ vs Nutr NSide	ns	0.3802
			-GLC vs -GLC NSide	ns	0.5681
			-GLN vs –GLN NSide	**	0.0062
2c	2-tailed t-test	4	Nutr+ vs Nutr NSide	ns	0.1366
			-GLC vs -GLC NSide	ns	0.6402
			-GLN vs -GLN NSide	ns	0.2028

Figure 3

Panel	test	n			p value
3a	2-tailed t-test	8	$T4_N$ Nutr+ vs $T4_N$ -GLC	*	0.0142
			$T4_N$ Nutr+ vs $T4_N$ -GLN	****	<0.0001
			$T4_N$ -GLC vs $T4_N$ -GLN	****	<0.0001
			$T4_M$ Nutr+ vs $T4_M$ -GLC	ns	0.1337
			$T4_M$ Nutr+ vs $T4_M$ -GLN	****	<0.0001
			$T4_M$ -GLC vs $T4_M$ -GLN	****	<0.0001
3b GLUT1	2-tailed t-test	7	$T4_N$ Nutr+ vs $T4_N$ -GLC	**	0.0011
			$T4_N$ Nutr+ vs $T4_N$ -GLN	**	0.0022
			$T4_N$ -GLC vs $T4_N$ -GLN	***	0.0004
			$T4_M$ Nutr+ vs $T4_M$ -GLC	ns	0.1857
			$T4_M$ Nutr+ vs $T4_M$ -GLN	****	<0.0001
			$T4_M$ -GLC vs $T4_M$ -GLN	***	0.0002
3b ASCT2	2-tailed t-test	7	$T4_N$ Nutr+ vs $T4_N$ -GLC	***	0.0008
			$T4_N$ Nutr+ vs $T4_N$ -GLN	**	0.0043
			$T4_N$ -GLC vs $T4_N$ -GLN	**	0.0060
			$T4_M$ Nutr+ vs $T4_M$ -GLC	ns	0.1027
			$T4_M$ Nutr+ vs $T4_M$ -GLN	ns	0.3138
			$T4_M$ -GLC vs $T4_M$ -GLN	ns	0.1971

3c OCR	2-tailed t-test	5	NS vs Nutr+	*	0.0125
		4	NS vs -GLC	*	0.0257
		4	NS vs -GLN	*	0.0469
		8	Nutr+ vs -GLC	*	0.0100
		8	Nutr+ vs -GLN	**	0.0067
		8	-GLC vs -GLN	**	0.0026
3c ECAR	2-tailed t-test	6	NS vs Nutr+	**	0.0051
		5	NS vs -GLC	*	0.0351
		5	NS vs -GLN	**	0.0048
		9	Nutr+ vs -GLC	**	0.0038
		9	Nutr+ vs -GLN	*	0.0332
		9	-GLC vs -GLN	****	<0.0001
3d	2-tailed t-test	6	NS vs Nutr+	*	0.0243
		5	NS vs -GLC	*	0.0197
		5	NS vs -GLN	*	0.0104
		9	Nutr+ vs -GLC	****	<0.0001
		9	Nutr+ vs -GLN	*	0.0106
		9	-GLC vs -GLN	****	<0.0001

Figure 4

Panel	**test**	**n**			**p value**
4c	2-tailed t-test	4	TCR vs Oxamate	**	0.0067
		4	TCR vs GSKi	*	0.0347
		4	Oxamate vs GSKi	ns	0.6651
4d	1w ANOVA - Tukey	6	TCR vs Oxamate	**	0.0029
			TCR vs Lactate	*	0.0401
			TCR vs TCR Pyr	ns	0.1378
			Oxamate vs Oxamate Pyr	*	0.0149
			Lactate vs Lactate Pyr	*	0.0197
4e RU5	2-tailed t-test	3	T6 TCR vs Oxamate	ns	0.7864
			T6 TCR vs Lactate	ns	0.4168
			T24 TCR vs Oxamate	**	0.0062
			T24 TCR vs Lactate	ns	0.1200
4e LTR Gag	2-tailed t-test	3	T24 TCR vs Oxamate	*	0.0454
			T24 TCR vs Lactate	*	0.0216
4e 2LTRc	2-tailed t-test	3	T24 TCR vs Oxamate	*	0.0251
			T24 TCR vs Lactate	*	0.0243

Figure 5

Panel	**test**	**n**			**p value**
5a	2-tailed t-test	4	-GLC no drug vs oligomycin	***	0.0008
		3	-GLC no drug vs AntA	**	0.0089
		4	-GLN no drug vs oligomycin	ns	0.5807
		3	-GLN no drug vs AntA	ns	0.3965
5c	2-tailed t-test	5	$T4_N$ -GLN vs $T4_N$ -GLN aKG	**	0.0059
			$T4_M$ -GLN vs $T4_M$ -GLN aKG	*	0.0135

5d RU5	2-tailed t-test	6	T6 Nutr+ vs -GLC	ns	0.6754
			T6 Nutr+ vs -GLN	ns	0.4935
			T6 Nutr+ vs -GLN DMK	ns	0.9338
			T6 -GLN vs -GLN + aKG	ns	0.5999
			T24 Nutr+ vs -GLC	**	0.0081
			T24 Nutr+ vs -GLN	**	0.0022
			T24 -GLN vs -GLN + aKG	**	0.0025
5d LTR Gag	2-tailed t-test	6	T6 Nutr+ vs -GLC	ns	0.2768
			T6 Nutr+ vs -GLN	ns	0.3478
			T6 Nutr+ vs -GLN DMK	ns	0.4967
			T6 -GLN vs -GLN + aKG	ns	0.7673
			T24 Nutr+ vs -GLC	**	0.0062
			T24 Nutr+ vs -GLN	**	0.0092
			T24 -GLC vs –GLN	*	0.0394
			T24 -GLN vs -GLN + aKG	*	0.0130
5d 2LTRc	2-tailed t-test	6	T24 -GLN vs -GLN + aKG	*	0.0192

Figure 6

Panel	**test**	**n**			**p value**
6a OCR/ECAR	2-tailed t-test	7	$T4_N$ -GLN vs $T4_N$ -GLN inj aKG	***	0.0003
			$T4_N$ -GLN vs $T4_N$ -GLN aKG	***	0.0010
			$T4_M$ -GLN vs $T4_M$ -GLN inj aKG	***	0.0002
			$T4_M$ -GLN vs $T4_M$ -GLN aKG	***	0.0002
6a ATP	2-tailed t-test	7	$T4_N$ -GLN vs $T4_N$ -GLN inj aKG	***	0.0002
			$T4_N$ -GLN vs $T4_N$ -GLN aKG	**	0.0016
			$T4_M$ -GLN vs $T4_M$ -GLN inj aKG	****	<0.0001
			$T4_M$ -GLN vs $T4_M$ -GLN aKG	**	0.0013
6b OCR/ECAR	2-tailed t-test	11	TCR vs Oxamate	****	<0.0001
			TCR vs Lactate	**	0.0014
			Oxamate vs Lactate	**	0.0073
6b ATP	2-tailed t-test	11	TCR vs Oxamate	**	0.0010
			TCR vs Lactate	**	0.0054
			Oxamate vs Lactate	ns	0.6066
6c	2-tailed t-test	4	TCR vs Oxamate	*	0.0222
6d Mitogreen	2-tailed t-test	18	TCR neg vs TCR HIV	****	<0.0001
			TCR neg vs Oxamate neg	****	<0.0001
			Oxamate neg vs Oxamate HIV	****	<0.0001
			TCR HIV vs Oxamate HIV	****	<0.0001
6d Mitosox	2-tailed t-test	15	TCR neg vs TCR HIV	***	0.0008
			TCR neg vs Oxamate neg	***	0.0008
			Oxamate neg vs Oxamate HIV	***	0.0007
			TCR HIV vs Oxamate HIV	***	0.0005
6e	2-tailed t-test	5	TCR neg vs TCR HIV	****	<0.0001
			Oxamate neg vs Oxamate HIV	****	<0.0001

Figure 7

Panel	test	n			p value
7a	2-tailed t-test	4	Mito Low vs Mito High	**	0.0015
7b infection	2-tailed t-test	6	Mito Low vs Mito High	*	0.0450
7b MFI	2-tailed t-test	6	Mito Low vs Mito High	**	0.0016

Suppl Figure 2

Panel	test	n			p value
S2a viability	1w-ANOVA Tukey	7	T24 Nutr+ vs -GLC	ns	0.9834
			T24 Nutr+ vs -GLN	ns	0.9774
			T24 Nutr+ vs GAL	ns	0.6811
			T48 Nutr+ vs -GLC	ns	0.9930
			T48 Nutr+ vs -GLN	ns	0.9727
			T48 Nutr+ vs GAL	ns	0.7095
			T72 Nutr+ vs -GLC	ns	0.6779
			T72 Nutr+ vs -GLN	ns	0.5232
			T72 Nutr+ vs GAL	ns	0.8374
S2a cell count	1w-ANOVA Tukey	5	T24 Nutr+ vs -GLC	ns	0.7654
			T24 Nutr+ vs -GLN	ns	0.5547
			T24 Nutr+ vs GAL	ns	0.8741
			T48 Nutr+ vs -GLC	ns	0.8657
			T48 Nutr+ vs -GLN	ns	0.9986
			T48 Nutr+ vs GAL	ns	0.9999
			T72 Nutr+ vs -GLC	****	<0.0001
			T72 Nutr+ vs -GLN	****	<0.0001
			T72 Nutr+ vs GAL	****	<0.0001
			T72 -GLC vs -GLN	ns	0.0906
			T72 -GLC vs -GAL	ns	0.7842
			T72 -GLN vs GAL	*	0.0178
S2b	2-tailed t-test	4	Nutr+ vs -GLC	**	0.0016
			Nutr+ vs -GLN	**	0.0016
			Nutr+ vs GAL	**	0.0049
			-GLC vs -GLN	**	0.0088
			-GLC vs -GAL	ns	0.2202
			-GLN vs -GAL	*	0.0228
S2c	2-tailed t-test	4	Nutr+ 19h vs Nutr+ 48h	ns	0.7069
			-GLC 19h vs -GLC 48h	ns	0.1837
			-GLN 19h vs -GLN 48h	***	0.0004
			GAL 19h vs GAL 48h	ns	0.4675
S2d	1w-ANOVA Tukey	12	Nutr+ vs -GLC	***	0.0010
			Nutr+ vs -GLN	****	<0.0001
			-GLC vs -GLN	****	<0.0001

Suppl Figure 3

Panel	test	n			p value
S3b	2-tailed t-test	6	CTL vs LDH inhibition	****	<0.0001

Suppl Figure 4

Panel	test	n			p value
S4	2-tailed t-test	7	$T4_N$ -GLN vs $T4_N$ -GLN aKG	*	0.0119
			$T4_M$ -GLN vs $T4_M$ -GLN aKG	*	0.0466

Suppl Figure 5

Panel	test	n			p value
S5	2-tailed t-test	12	-GLN vs -GLN aKG	****	<0.0001
			-GLN vs -GLN aKG NSide	****	<0.0001

Suppl Figure 6

Panel	test	n			p value
S6a p24	1w-ANOVA Tukey	6	Nutr+ vs -GLC	ns	0.8327
			Nutr+ vs -GLN	ns	0.9177
			Nutr+ vs –GLN a-KG	ns	0.2397
			Nutr+ vs -GAL	ns	0.1980