






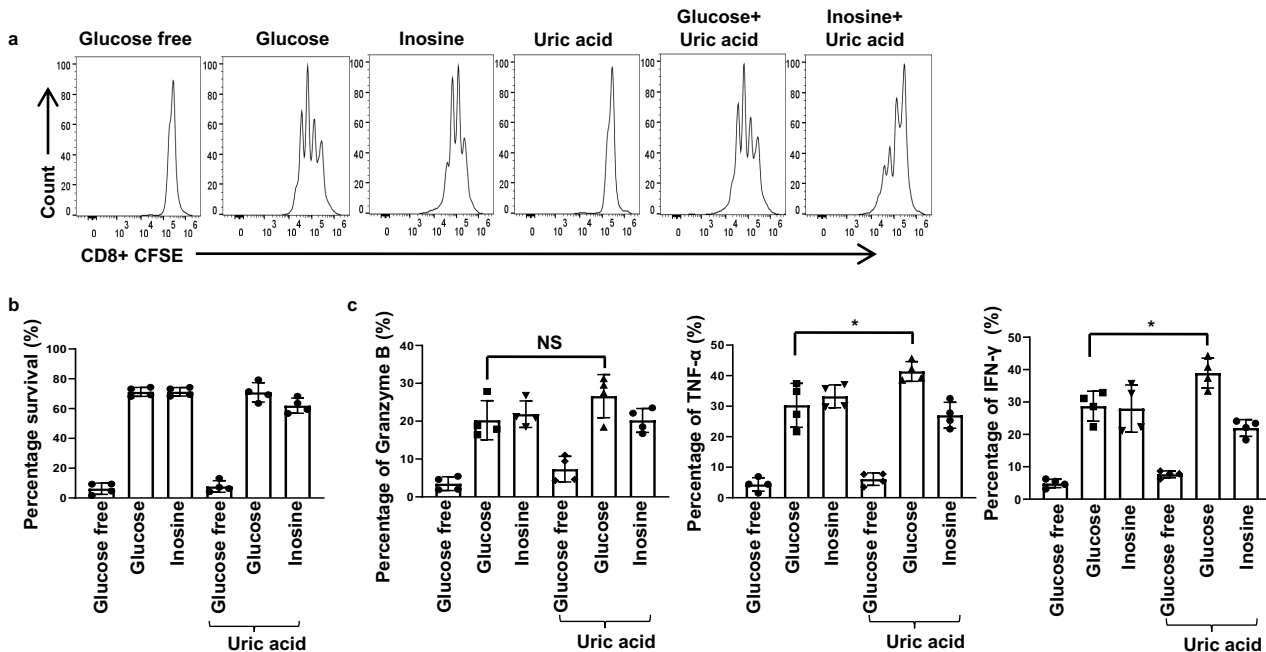


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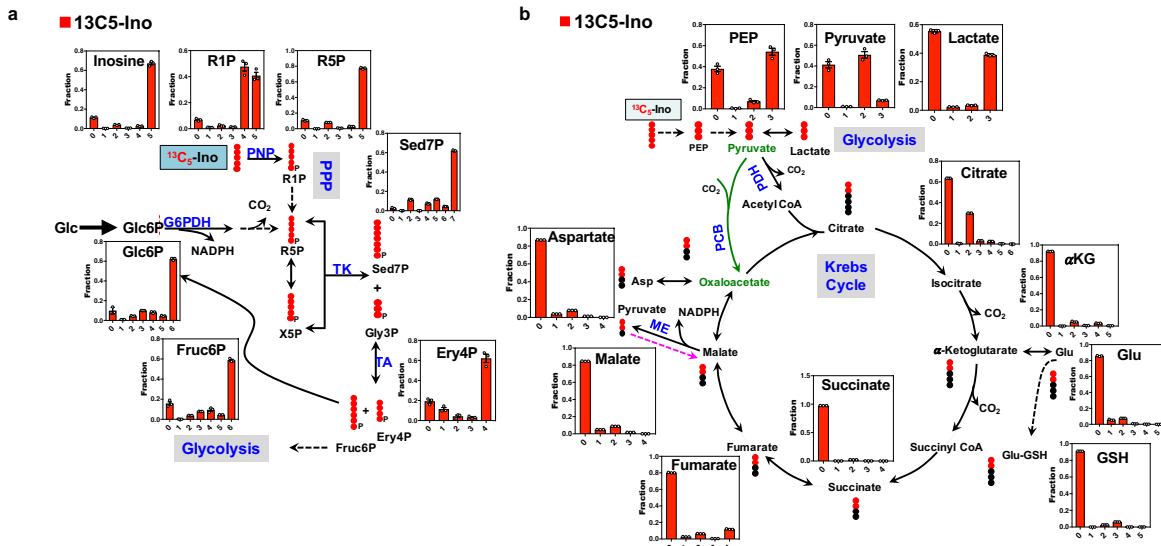
Inosine is an alternative carbon source for CD8⁺-T-cell function under glucose restriction

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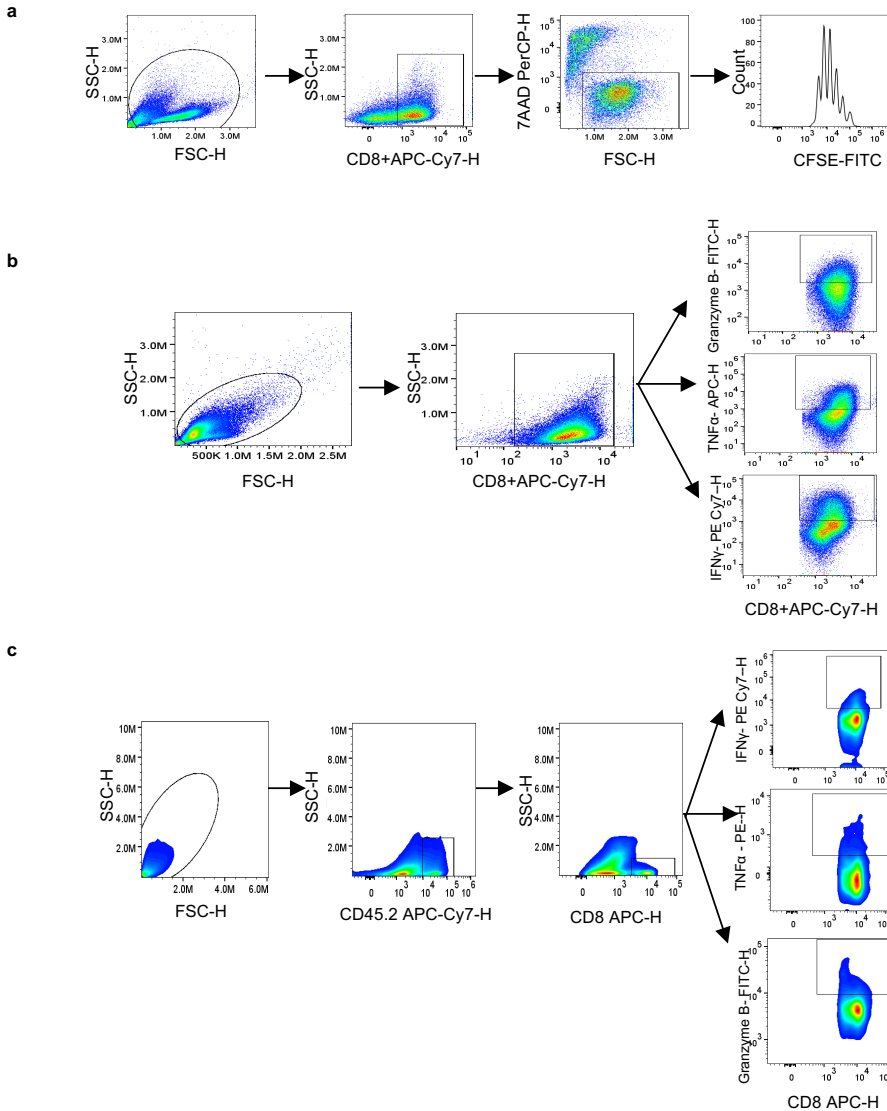
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Supplementary Figure 1. Uric acid is not the downstream effector metabolite of inosine. (a-c) Naive CD8⁺ T cells from C57BL/6 mice were activated by plate bound anti-CD3 and anti-CD28 antibodies and differentiated in the indicated conditional media in the presence and absence of uric acid (1mM) for 4 days. (a) Cell proliferation (CFSE), (b) cell survival (7AAD), and (c) indicated intracellular effector molecules were determined by FACS. Data are presented as mean \pm SD (n=4). (c) NS, $P=0.1477$; *, $P=0.0307$ and 0.0205 for Granzyme B, TNF- α , and IFN- γ for Glucose versus Glucose+Uric acid by unpaired two-tailed t-test. Data are representative of three independent experiments. Sample size (n) represents biologically independent samples (b-c).



Supplementary Figure 2. Incorporation of the ribose subunit of inosine into the central carbon metabolism in the presence of glucose in human T effector cells. Active human T cells were incubated in media containing both $^{13}\text{C}_5\text{-Inosine}$ and $[6,6\text{-D}_2\text{-Glucose}]$ for 24 h, extracted as described in methods, and analyzed for the incorporation of $^{13}\text{C}_5\text{-Ino}$ in the presence of glucose into PPP metabolites (a) and glycolytic/Krebs cycle metabolites (b) by IC-UHR-FTMS. Data are presented as mean \pm SEM (n=3). All symbols and abbreviations are as described in **Figure 2**. Numbers in the X-axis represent those of ^{13}C atoms in given metabolites.



Supplementary Figure 3. Gating strategy for analysis of (a) CD8+ T cell proliferation (b) intracellular cytokines and effector molecule expression (c) tumor infiltrated effector T cells and intracellular cytokines.

Supplementary Table 1

Antibody	Cat#/Vendor
Mouse anti-CD3 mAb	BE0001-1, BioXcell
Mouse anti-CD28 mAb	BE0015-1, BioXcell
Anti mouse CD8-APC-Cy7	100714, Biolegend
Anti human/mouse Granzyme B-FITC	515403, Biolegend
Anti mouse TNF- α - APC	17-7321-82, eBioscience
Anti mouse IFN- γ - PE-Cy7	25-7319-82, eBioscience
Anti-human CD8 α Antibody - APC-Cy7	300926, Biolegend
Anti-human TNF- α -PE	502909, Biolegend
Anti-human IFN- γ - PE-Cy7	506518, Biolegend
Anti mouse CD45.2 - PerCP-Cyanine5.5	45-0454-82, eBioscience
InVivoMAb anti m PD-L1	BE0101, BioXcell
InVivoMAb anti m PD-1	BE0146, BioXcell
Anti-human CD3 (OKT-3)	BE0001, BioXcell
Anti-human/monkey CD28.2	BE0291, BioXcell
anti-PNP	sc-271890, Santa Cruz
anti-actin	sc-47778, Santa Cruz
Reagents	Cat#/Vendor
Recombinant human IL-2	200-02, Peprotech
Recombinant murine IL-2	212-12, Peprotech
Cell Stimulation Cocktail (plus protein transport inhibitors) (500X)	00-4975-93, eBioscience
carboxyfluorescein diacetate succinimidyl ester(CFSE)	Invitrogen
7-amino-actinomycin D(7AAD)	420404, Biolegend
D-(+)-Glucose	G7021, Sigma-Aldrich
Inosine	I4125, Sigma-Aldrich
Adenosine	164040050, ACROS
D-(+)-Glucose	G7021, Sigma-Aldrich
RPMI 1640 Medium, No Glucose	11-879-020, Gibco
Retronectin	T100, Takara/clontech
Human gp100	RP20344, GenScript
Forodesine Hydrochloride	HY-16209, MedChem Express
¹³ C ₆ -Glucose	CLM-1396, Cambridge Isotope Lab
¹³ C ₆ -Inosine	NUC-072, OMCIRON
D-GLUCOSE (6,6-D2, 99%)	DLM-349, Cambridge Isotope Lab
¹⁴ C ₂ -L-glutamine	ART0115, American Radiolabeled Chemicals
[9,10- ³ H]-palmitic acid	MT 845, Moravek
[2,8- ³ H]-inosine	MT 678, Moravek
[2,8- ³ H]-adenosine	MT 793, Moravek
Etomoxir	11969, Caymen
Methotrexate	13960100, Caymen
Calcein AM	C3099, Invitrogen
Sodium pyruvate solution	S8636, Sigma-Aldrich
Uric Acid	69-93-2, Alfa Aesar
hypoxathine	H9636, Sigma-Aldrich
ribose-1-phosphate	83866, Sigma-Aldrich
perchloric acid	311413, Sigma-Aldrich
sucrose	S7930, Sigma-Aldrich
1-Bromododecane	106912500, ACROS
CD45R(B220) microbeads, mouse	5150309030, Miltenyi Biotec
Naive CD8+ T cell isolation kit,mouse	130-096-543, Miltenyi Biotec
MojoSort™ Mouse CD8 Naive T Cell Isolation Kit	480044, Biolegend
MojoSort™ Human CD3 T Cell Isolation Kit	480021, Biolegend

Supplementary Table 2

Data sets	Asterisks	P value (13C5-Iso versus 13C5-Iso+Foro)
13C2-Citrate	***	8.18755E-05
13C3-Citrate	***	2.96234E-06
13C4-Citrate	***	2.15878E-06
13C5-Citrate	***	4.70221E-06
13C6-Citrate	***	6.49864E-06
13C2- α KG	**	0.00431901
13C3- α KG	**	0.001316994
13C4- α KG	***	0.000277638
13C5- α KG	***	0.000550666
13C1-Glu	***	6.64732E-06
13C2-Glu	***	7.0498E-11
13C3-Glu	***	8.341E-12
13C4-Glu	***	3.41648E-10
13C5-Glu	***	1.6913E-09
13C1-GSH	***	1.63113E-05
13C2-GSH	***	1.71179E-10
13C3-GSH	***	4.47E-13
13C4-GSH	***	4.553E-12
13C5-GSH	***	2.52106E-10
13C1-Succinate	*	0.018325258
13C2-Succinate	***	0.000546566
13C3-Succinate	***	0.00069771
13C4-Succinate	**	0.00218597
13C1-Fumarate	***	4.94266E-05
13C2-Fumarate	***	9.47052E-08
13C3-Fumarate	***	8.385E-12
13C4-Fumarate	***	3.1535E-09
13C1-Malate	***	2.37424E-05
13C2-Malate	***	1.97296E-07
13C3-Malate	***	6.0447E-08
13C4-Malate	***	2.33909E-08
13C1-Aspartate	***	6.72424E-08
13C2-Aspartate	***	7.3491E-11
13C3-Aspartate	***	9.183E-12
13C4-Aspartate	***	1.32059E-10