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Supplemental information

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to regulate their homeostasis and function**

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This PDF file includes Supplemental Figures 1-6 and Tables 1-3.

Figure S1

A

Pathway Name	Match Status	p	-log(p)	Impact
Alanine, aspartate and glutamate metabolism	9/24	9.7215E-6	11.541	0.7289
Purine metabolism	14/68	7.6799E-5	9.4743	0.304
Pyrimidine metabolism	10/41	2.0564E-4	8.4894	0.22992
Phenylalanine, tyrosine and tryptophan biosynthesis	3/4	0.0010784	6.8323	1.0
D-Glutamine and D-glutamate metabolism	3/5	0.0025663	5.9653	1.0
Arginine and proline metabolism	8/44	0.0067465	4.9987	0.23187
Taurine and hypotaurine metabolism	3/8	0.012405	4.3896	0.42857
Phenylalanine metabolism	3/11	0.031594	3.4548	0.64815
Aminoacyl-tRNA biosynthesis	9/69	0.034417	3.3692	0.0
Nicotinate and nicotinamide metabolism	3/13	0.049733	3.0011	0.20833
Butanoate metabolism	4/22	0.052807	2.9411	0.02899
Pantothenate and CoA biosynthesis	3/15	0.0719	2.6325	0.02041
Glycolysis or Gluconeogenesis	4/26	0.088274	2.4273	0.13904
Amino sugar and nucleotide sugar metabolism	5/37	0.09311	2.374	0.23554
Cysteine and methionine metabolism	4/27	0.098579	2.3169	0.22557
Nitrogen metabolism	2/9	0.11559	2.1577	0.0
Vitamin B6 metabolism	2/9	0.11559	2.1577	0.07843
Citrate cycle (TCA cycle)	3/20	0.14224	1.9502	0.09365
Fructose and mannose metabolism	3/21	0.15837	1.8428	0.12754
Valine, leucine and isoleucine biosynthesis	2/11	0.16221	1.8189	0.0

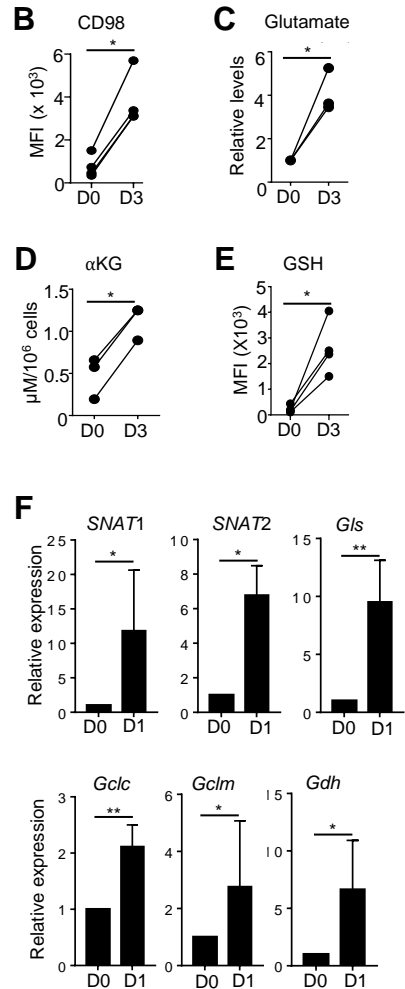


Figure S1. NKT cells increase glutaminolysis upon activation. Related to Figure 1. (A) Freshly sorted NKT and CD4 T cells from C57BL/6 mice were subjected to metabolomic analysis through LC-MS/MS. The table shows a summary of the most upregulated metabolic pathways in NKT cells compared to CD4 T cells (n=3). (B-E) Sorted NKT cells were stimulated without (D0) or with (D3) αGalCer (100 ng/mL). Graphs show the levels of (B) CD98 expression (n=3), (C) glutamate (n=3), (D) αKG (n=3), and (E) GSH (n=3) in resting and activated NKT cells. D0 sample values were set at 1 to calculate the relative levels of glutamate. (F) Graphs show expression of the indicated genes in freshly sorted NKT cells (D0) and NKT cells stimulated with αGalCer (D1) (n=3). All data are representative of or combined from at least three independent experiments. Data are shown as mean ± SEM. *p<0.05, **p<0.01.

Figure S2. Glutamine metabolism is essential for NKT cell survival and proliferation. Related to Figure 2. (A) Sorted splenic NKT cells from C57BL/6 mice were stimulated with α GalCer (100 ng/mL) for 3 days in the indicated culture conditions. Data show mitochondrial mass and membrane potential measured by MitoTracker and TMRM, respectively. (n=3). (B and C) Sorted NKT cells were activated in the presence or absence of CB839 (250 nM). NKT cells were analyzed for (B) CD25 expression (n=3) and (C) mitochondrial mass and membrane potential (n=3). (D) CD4 T cells were activated with α CD3 and α CD28 for 3 days in the presence or absence of indicated concentrations of CB839. Graphs show cell survival (left panel) and proliferation (right panel) along with the histogram layout (n=4). (E) Graph shows ATP levels in activated NKT cells (n=4). (F) Thymic NKT cells from WT and GLS1 KO mice were analyzed for expression of CD24 to identify Stage 0 (CD24⁺) cells as well as CD44 and NK1.1 to identify stage 1 (CD44⁻NK1.1⁻), stage 2 (CD44⁺NK1.1⁻), and stage 3 (CD44⁺NK1.1⁺) cells (n=3). (G) Representative plots comparing NKT1, NKT2, and NKT17 subsets gated using PLZF and ROR γ t expression in the spleens of WT and GLS1 KO mice (n=3). (H) Graphs show NKT cell numbers in thymi (left panel) and spleens (right panel) of WT and GLS1 KO mice (n=5). (I) Graphs show cell survival (left panel) and proliferation (right panel) of CD4 T cells activated in the presence or absence of BSO (n=3). (J) Graphs show mitochondrial mass and membrane potential in EGCG (10 μ M) treated NKT cells. (n=3). (K and L) Graphs show cell survival (left panel), proliferation (right panel) (K) and mitochondrial mass and membrane potential (L) of CD4 T cells activated in the presence or absence of EGCG (n=3). All relative levels were calculated using the average control value as a reference point. All data are representative of or combined from at least three independent experiments. Data are shown as mean \pm SEM. *p<0.05, **p<0.01.

Figure S3

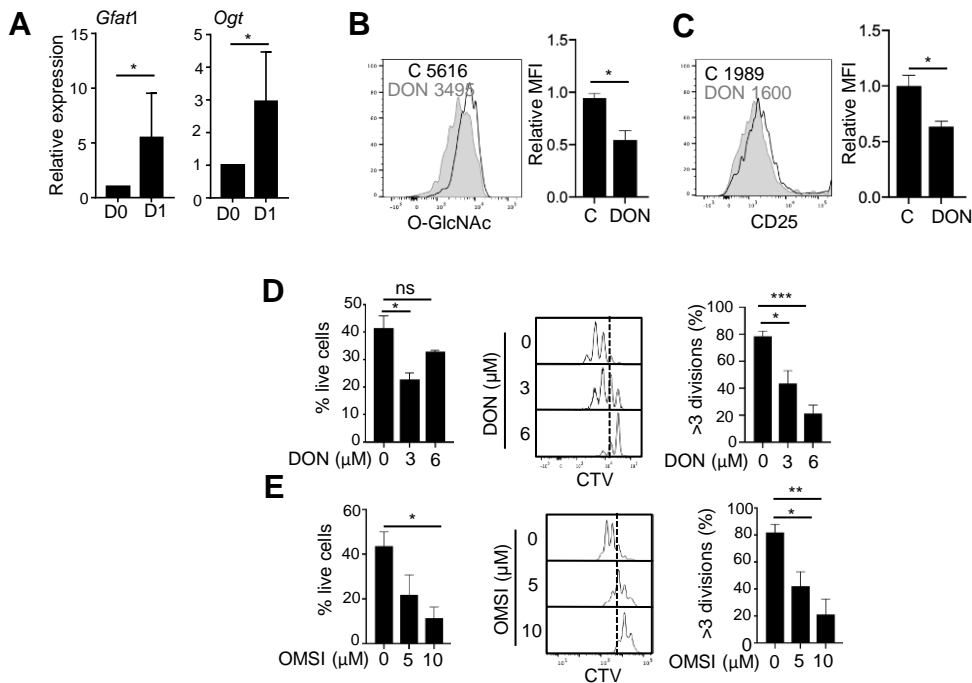


Figure S3. Glutamine metabolism is essential for NKT cell survival and proliferation. Related to Figure 2. (A) Graphs show relative expression of the indicated genes in resting (D0) and stimulated (D1) NKT cells (n=4). (B and C) Sorted splenic NKT cells were activated with and without DON (6 μ M). On day 3, NKT cells were stained for (B) O-GlcNAc levels (n=3) and (C) CD25 (n=3). (D and E) Graphs show cell survival (left panel) and proliferation (right panel) along with the histogram layout in CD4 T cells activated in the presence or absence of DON (D) or OMSI (E) (n=3). All relative levels were calculated using the average control value as a reference point. All data are representative of or combined from at least three independent experiments. Data are shown as mean \pm SEM. *p<0.05, **p<0.01.

Figure S4

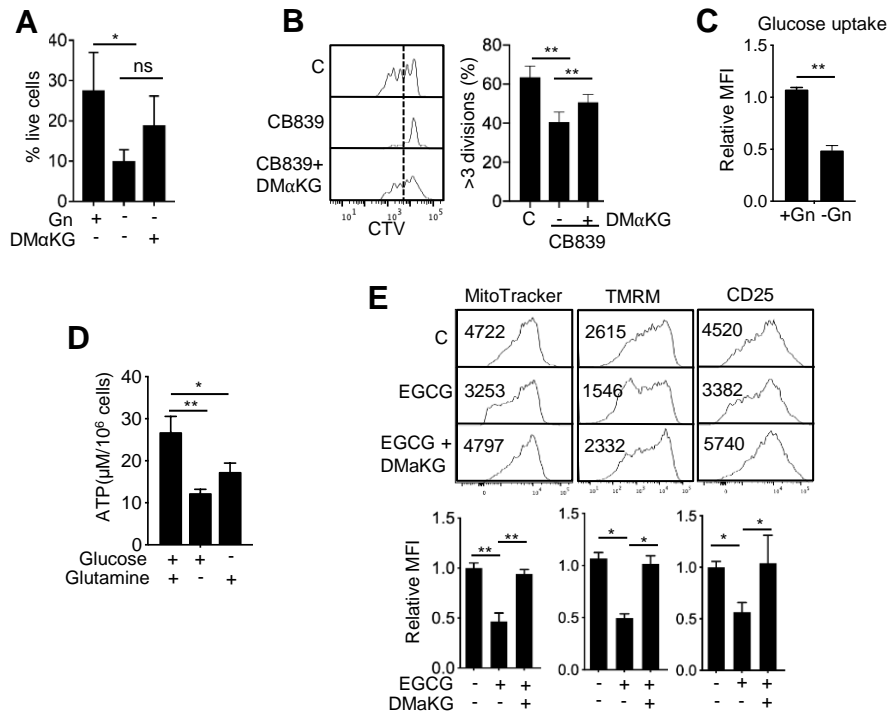


Figure S4. NKT cells exhibit a glutamine-addicted phenotype. Related to Figure 4. Sorted splenic NKT cells were stimulated with α GalCer (100 ng/mL) under the indicated culture conditions. (A and B) NKT cells were stimulated with and without glutamine (Gn) (2 mM) or CB839 (250 mM) in the presence or absence of DM α KG (1.5 mM). (A) Graph shows NKT cell survival in the absence of glutamine. (B) Representative histograms and summary graph show proliferation as measured by CellTrace Violet (CTV) after CB839 treatment (n=3). (C) Graph shows relative glucose uptake in NKT cells grown under glutamine-deprived conditions as measured by the mean fluorescent intensity (MFI) of 2-NBDG (n=3). (D) Graph shows ATP production in NKT cells activated under the specified culture conditions (n=3). NKT cells were stimulated with and without EGCG (20 μ M) in the presence or absence of DM α KG. (E) Histograms and graphs show mitochondrial mass, membrane potential, and CD25 expression in NKT cells stimulated at the indicated conditions. All relative levels were calculated using the average control value as a reference point. All data are representative of or combined from at least three independent experiments. Data are shown as mean \pm SEM. *p<0.05, **p<0.01. ns: not significant.

Figure S5

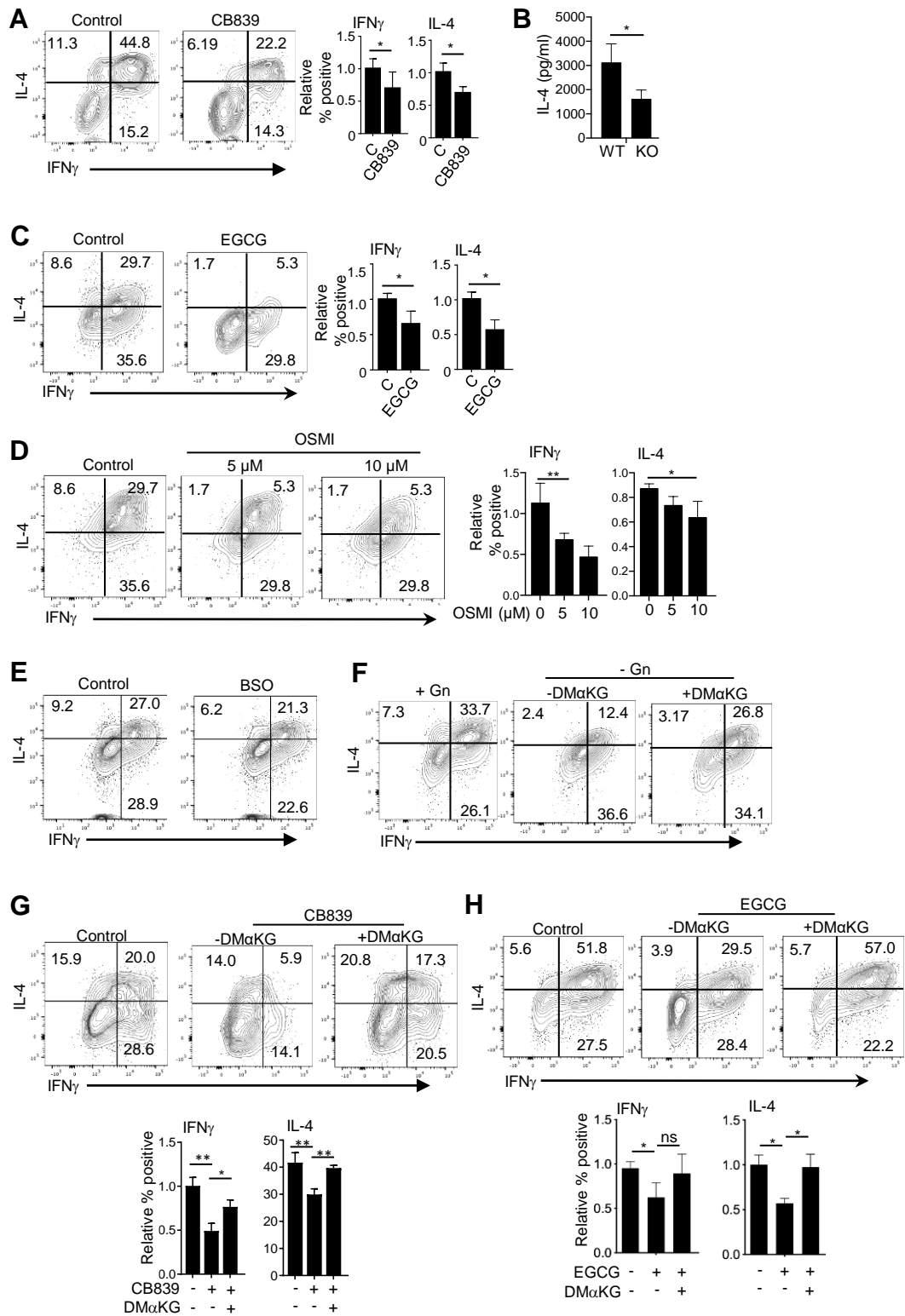


Figure S5. IFN γ and IL-4 production in NKT cells rely on distinct branches of glutamine metabolism. Related to Figure 5. Sorted splenic NKT cells were stimulated with α GalCer (100 ng/mL). At day 3 post-activation, cells were re-stimulated with PMA/Ionomycin and Monensin for 4 h followed by intracellular cytokine staining. (A) Cytokine expression in NKT cells activated with and without CB839 (250 nM) (n=3). (B) Graph shows the level of IL-4 secreted into the media by WT and GLS1 KO NKT cells as measured by ELISA (n=3). (C-E) Cytokine expression in NKT cells activated with and without EGCG (20 μ M) (C), OSMI (5 μ M and 10 μ M) (n=3) (D), and BSO (250 μ M) (E) (n=3). (F-H) NKT cells were stimulated with and without glutamine (Gn) (2 mM) (F) or CB839 (250 nM) (G) or EGCG (20 μ M) (H) in the presence or absence of DM α KG (1.5 mM). Representative dot plots and summary graphs show the cytokine expression in NKT cells treated as noted (n=3). All relative levels were calculated using the average control value as a reference point. All data are representative of or combined from at least three independent experiments. Data are shown as mean \pm SEM. *p<0.05, **p<0.01. ns: not significant.

Figure S6

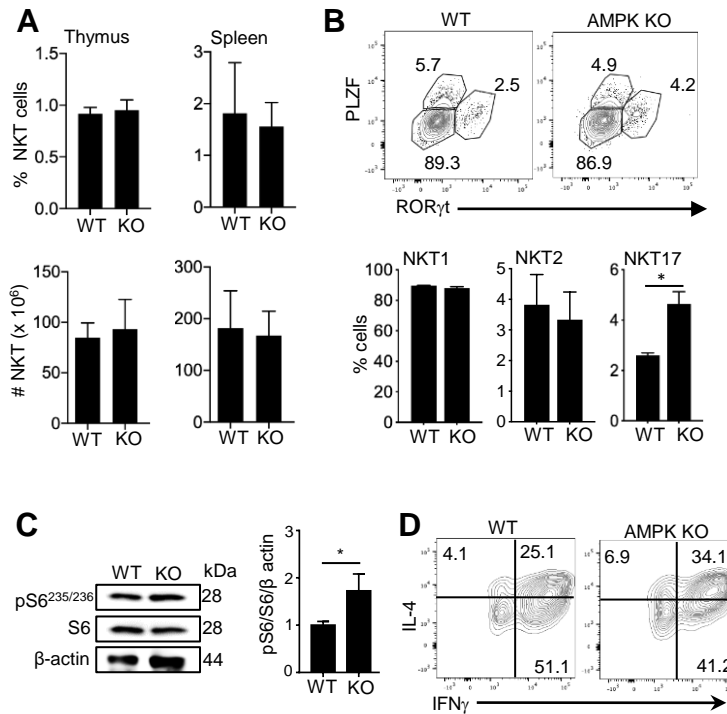


Figure S6. mTORC1-AMPK signaling regulates both glucose and glutamine metabolism in NKT cells. Related to Figure 7. (A) Total cells from the thymi and spleens of WT and AMPK KO mice were counted. Cells were stained for TCR- β and CD1d-tetramer antibodies and analyzed by flow cytometry for NKT cell frequencies. Graphs show percentages (upper panels) and numbers (bottom panels) of NKT cells in the thymi and spleens of WT and AMPK KO mice ($n=3$). (B) Representative plots and summary graphs show NKT1, NKT2, and NKT17 subsets gated using PLZF and ROR γ t expression in the spleens of WT and AMPK KO mice ($n=3$). (C) Representative images show western blot analysis in NKT cells activated with α Galcer (100 ng/ml) for 5 h. The graph on the right shows the relative levels of S6 phosphorylation. The ratio was first calculated using the density of S6 over that of β -actin (S6/ β -actin) by densitometry followed by the normalization of pS6^{235/236} over S6/ β -actin values (pS6/S6/ β -actin) ($n=3$). (D) Representative dot plots show cytokine expression in WT and AMPK KO NKT cells activated as shown in figure 7L ($n=3$). All data are representative of or combined from at least three independent experiments. Data are shown as mean \pm SEM. * $p<0.05$.

Table S1. Related to Figure 1. Intracellular metabolite levels in unstimulated (D0) and stimulated (D3) NKT cells.

Compound	D0 1	D0 2	D0 3	D3 1	D3 2	D3 3
beta-Nicotinamide adenine dinucleotide	0.27	0.19	0.33	2.67	1.67	3.79
Taurine	0.49	0.31	0.65	2.64	1.35	3.24
Uridine 5'-diphosphogalactose	0.54	0.31	0.58	2.18	1.42	3.29
Uridine 5-diphosphoglucose	0.54	0.31	0.58	2.18	1.42	3.29
2-Deoxycytidine	0.54	0.33	0.69	1.31	2.70	2.50
Citramalic acid	0.48	0.47	0.97	1.92	1.03	2.12
2-Methyl-1-butanol	0.70	0.26	0.88	1.12	2.04	1.60
trans-Aconitic acid	0.71	0.31	0.94	1.06	1.80	1.41
Arabinose-5-phosphate	0.66	0.33	0.90	1.27	1.10	1.45
L-Malic acid	0.77	0.35	0.96	1.04	1.20	1.48
Adenosine 5-monophosphate	1.77	1.37	1.98	0.42	0.38	0.63
3-Hydroxy-DL-kynurenine	1.81	0.95	2.73	1.05	0.24	0.52
3-Hydroxyanthranilic acid	0.45	0.22	0.94	4.08	1.06	2.79
Riboflavin	0.80	0.40	1.06	0.94	1.47	1.23
Deoxyadenosine 5-triphosphate	3.62	1.05	4.25	0.66	0.95	0.36
2-Isopropylmalic acid	0.81	0.79	0.94	1.06	2.71	2.26
4-Hydroxy-L-glutamic acid	0.90	0.44	0.83	1.10	1.94	3.11
L-Glutamic acid	0.81	0.39	0.805	0.98	1.02	1.26
D-Glucosamine 6-phosphate	3.14	1.16	1.47	0.45	0.49	0.84
N-Carbamoyl-DL-aspartic acid	0.29	0.06	0.20	1.71	13.17	24.14
2-Deoxyadenosine 5-monophosphate	0.63	0.90	1.06	0.94	1.59	2.31
D-erythro-Dihydrosphingosine	0.36	0.67	1.01	2.28	4.66	0.99
D-Fructose 6-phosphate	0.98	0.56	1.02	1.27	0.86	1.56
Pyridoxal hydrochloride	5.62	0.43	7.47	0.29	1.46	0.54
Epicatechin	1.30	0.29	0.48	0.70	1.68	1.59
2-Deoxyguanosine 5-diphosphate	0.49	0.73	1.10	1.04	0.96	2.00
Adenosine 5-diphosphate	0.49	0.73	1.10	1.04	0.96	2.00
N-Acetyl-alpha-D-glucosamine 1-phosphate	0.53	0.57	1.24	1.69	0.76	1.40
O-Phosphorylethanolamine	1.33	0.83	1.70	0.77	0.65	1.17
Nicotinic acid mononucleotide	1.80	0.86	3.83	0.96	0.22	1.04
gamma-Glu-Cys	0.92	0.57	1.08	3.02	0.48	3.82
2-4-Quinolinediol	1.04	0.49	0.96	2.20	0.96	1.15
Dihydroxyacetone phosphate	0.81	0.74	1.19	2.04	0.81	3.51
Ketovaleric acid	0.12	0.42	1.38	0.62	1.60	1.65
o-Phospho-L-Serine	0.50	0.76	0.74	1.89	1.24	6.69
trans-trans Muconic acid	1.03	0.05	1.01	1.96	0.93	0.99
D-+-Galactosamine	1.91	1.01	0.99	0.92	0.42	1.13
L-Cystine	1.61	0.84	2.15	0.92	0.96	1.04
Quinic acid	0.85	0.42	1.15	2.48	0.46	4.72
2-Phosphoglyceric acid	0.94	0.50	1.17	1.04	0.96	1.62
5-Deoxy-5-(methylthio)adenosine	1.34	0.33	0.66	1.69	0.55	2.03
5-Methoxytryptamine	0.38	0.87	0.71	1.13	1.79	7.75
Cytidine	1.92	0.79	1.13	0.40	1.14	0.87
Vanillic acid	1.34	0.66	0.55	3.21	0.12	3.56
alpha-D-Glucose-1-phosphate	0.89	0.46	1.11	1.38	0.72	1.33
D-Glucose 6-phosphate	1.88	0.87	2.02	0.97	0.96	1.03

Uridine 5-monophosphate	1.32	0.79	1.79	1.00	0.76	1.00
Adipic acid	1.04	0.72	0.96	1.28	0.79	1.31
Indoline-2-carboxylate	0.73	1.57	3.97	0.74	0.59	1.26
Thiamine	1.05	0.46	1.03	0.97	0.95	1.35
2-Deoxyguanosine	1.62	0.44	1.86	0.17	0.20	1.56
2-Deoxy-D-ribose	0.60	0.24	1.28	1.12	1.19	0.88
Nicotinic acid	0.96	0.99	0.72	2.17	1.01	14.47
Pyridoxamine	2.93	0.80	1.03	0.97	0.24	1.17
2-Deoxyuridine 5-triphosphate	2.68	0.25	1.73	1.47	0.11	0.53
Oxamic acid	1.30	0.29	0.70	3.02	1.81	0.24
Cytidine 5-diphosphate	2.48	0.17	2.61	1.37	0.63	0.55
L-Canavanine	0.78	11.36	1.51	1.22	0.63	0.31
3-2-Hydroxyethylindole	0.93	0.92	1.07	11.13	0.40	2.46
Hypoxanthine	1.47	0.56	1.91	0.60	1.14	0.86
Guanosine 3,5-cyclic monophosphate	1.40	0.60	0.02	3.09	0.21	1.49
D-Maltose	1.05	0.54	0.95	1.84	0.40	1.87
Cytidine 5-triphosphate	1.20	0.90	0.80	0.89	1.82	1.10
D-Gluconic acid	0.90	0.47	1.21	1.07	0.93	1.27
Galactonic acid	0.90	0.48	1.21	1.07	0.93	1.27
D-Fructose 1,6-biphosphate	1.04	0.24	1.10	0.73	0.96	3.77
Inosine 5-triphosphate	0.29	1.43	5.77	0.57	1.57	0.16
Folinic acid	1.13	0.55	0.99	1.01	13.29	0.94
alpha-D(+)Mannose 1-phosphate	1.23	0.77	0.61	1.74	0.44	1.81
Uridine 5-diphosphate	0.09	0.47	2.17	1.53	0.46	5.23
4-Quinolol	1.19	0.55	3.34	1.23	0.46	0.81
Trehalose	2.49	0.98	1.02	1.47	0.62	0.84
2-Deoxyguanosine 5-monophosphate	0.90	0.90	1.10	15.96	0.72	1.11
trans-4-Hydroxy-L-proline	0.96	0.49	1.04	1.08	0.81	1.13
Succinic semialdehyde	0.24	0.78	1.22	2.02	0.13	1.99
2-Deoxy-D-glucose 6-phosphate	0.72	0.58	1.30	1.19	0.81	1.26
3-Indoleacetic acid	0.90	0.87	1.10	1.59	0.23	5.75
3-Hydroxyphenylacetic acid	0.68	0.45	1.38	1.33	0.78	1.22
Pyridoxal 5 phosphate	1.04	0.96	1.55	0.80	0.63	1.38
5-Hydroxy-3-indoleacetic acid	1.27	0.82	0.73	0.26	1.18	9.51
DL-Glyceraldehyde 3-phosphate	0.49	1.17	5.08	0.80	1.16	0.84
Allantoin	0.93	0.28	1.20	0.91	1.17	1.07
Taurocholic acid	0.94	0.36	1.15	0.89	1.06	1.15
O-Succinyl-L-homoserine	1.26	0.50	1.29	1.07	0.07	0.93
L-Proline	0.82	0.44	1.22	1.02	0.98	1.07
2-3-Dihydroxyisovalerate	0.71	0.56	1.50	2.64	1.29	0.53
4-Aminobenzoic acid	0.06	0.47	1.67	0.89	1.49	1.11
L-Kynurenine	0.79	0.21	14.51	0.58	1.21	1.63
Uridine	1.27	0.59	1.84	0.77	1.10	0.90
Inosine 5-diphosphate	0.96	0.87	1.06	0.55	1.04	4.39
N-Formyl-L-Tyrosine	0.32	0.91	1.09	0.17	1.10	3.66
Citric acid	1.06	2.22	0.70	0.94	1.16	0.73
L-Hydroxyglutaric acid	0.92	0.38	1.19	0.84	1.14	1.08
Mevalonic acid	0.92	0.38	1.19	0.84	1.14	1.08
4-Methyl-2-oxovaleric acid	0.90	0.56	1.10	1.17	0.70	1.16

Itaconic acid	0.90	0.56	1.10	1.17	0.70	1.16
2-Ketobutyrate	0.81	0.35	1.28	1.50	0.56	1.19
N-Acetylneuraminic acid	0.89	0.45	1.23	0.84	1.14	1.11
L-Homocystine	0.80	0.57	5.47	1.20	1.28	0.69
Cytidine-5-monophosphate	1.17	0.36	1.54	0.97	0.10	1.03
D-pantothenic acid	0.94	0.37	1.24	0.79	1.31	1.06
Argininosuccinic acid	1.34	0.49	1.60	1.33	0.67	0.57
Flavin adenine dinucleotide	2.29	0.59	1.30	0.45	0.70	9.53
2-Deoxyribose 5-phosphate	0.76	0.58	1.34	0.67	1.24	1.39
Creatine phosphate	0.62	0.67	1.33	1.60	0.10	2.26
L-Carnitine	1.11	0.87	1.39	1.44	0.35	0.89
Adenylosuccinic acid	0.05	1.00	1.02	1.13	0.61	1.00
L-Homocysteine	1.24	0.76	0.29	1.55	0.33	1.24
Cysteine	1.37	0.28	8.36	3.83	0.38	0.63
Guanine	1.77	0.28	0.93	1.07	2.40	0.67
NADH	1.50	0.64	0.28	1.66	0.31	1.36
Lactic acid	0.97	0.49	1.27	1.03	0.93	1.18
Adenine	0.52	0.41	7.38	1.20	0.80	2.22
Guanosine 5-diphosphate	1.13	0.60	2.40	0.87	1.58	0.68
±-Mevalonolactone	0.56	1.02	4.51	2.25	0.71	0.98
Xanthosine	1.04	0.45	1.30	0.88	1.40	0.96
Uridine 5-triphosphate	1.03	0.41	4.64	2.37	0.97	0.46
Ketoisovaleric acid	1.51	0.23	1.06	2.30	0.94	0.52
Cellobiose	0.91	0.65	1.37	1.13	0.18	1.09
Adenosine 5-triphosphate	1.85	0.15	2.46	0.14	0.05	2.71
2-Deoxycytidine 5-diphosphate	0.89	0.61	1.61	1.11	0.50	2.36
Isopentyl acetate	0.87	0.54	1.21	1.14	0.68	1.13
Lipoamide	0.80	0.73	1.44	1.20	0.35	2.24
Homocitrate	0.99	0.29	1.07	0.63	1.06	1.01
L-Arabinose	0.98	0.52	1.23	1.02	0.93	1.05
NADPH	0.62	0.79	2.90	1.15	3.83	0.85
Pyridoxine	1.00	0.39	1.31	0.76	1.33	1.00
L-Threonine	0.91	0.53	1.30	1.09	0.81	1.13
2-2-Dimethyl Succinic acid	0.89	0.52	1.19	1.14	0.65	1.11
N-acetylaspartate	1.15	0.27	1.14	0.93	0.98	1.02
L-Aspartic Acid	0.85	0.41	1.11	1	1	1.34
L-Arabitol	0.94	0.51	1.19	1.06	0.71	1.14
Xylitol	0.94	0.51	1.19	1.06	0.71	1.14
gamma-Aminobutyric acid	0.83	1.17	2.48	0.11	0.30	6.70
Creatine	1.00	0.49	1.23	1.00	0.86	1.11
Xanthine	1.02	0.37	1.33	0.72	1.38	0.98
DL-2-Amino adipic acid	0.43	1.30	1.21	0.79	0.58	2.19
CoA	1.15	0.85	3.62	0.52	0.75	3.09
2-3-Pyridinedicarboxylic acid	0.95	0.46	1.15	1.10	0.65	1.05
Deoxythymidine 5-triphosphate	1.05	0.95	2.01	0.26	0.21	5.36
Isopentenyl pyrophosphate	2.47	0.32	1.07	2.00	0.08	0.93
beta-Nicotinamide mononucleotide	1.27	1.02	0.98	1.78	0.49	0.54
Succinic acid	1.00	0.43	1.33	0.79	1.23	1.00
DL-Isocitric acid	1.09	1.73	0.43	0.91	1.20	0.75

S-5-Adenosyl-L-homocysteine	1.64	0.87	0.69	0.38	1.13	2.27
L-Histidine	0.98	0.49	1.25	1.02	0.77	1.15
Salicylic acid	1.06	0.65	1.18	1.06	0.71	0.94
S-2-Aminoethyl-L-cysteine	0.72	1.27	4.48	7.47	0.49	0.73
L-Serine	0.95	0.53	1.28	1.05	0.71	1.21
Deoxyguanosine 5-triphosphate	0.86	0.07	1.14	1.21	0.02	1.26
Melibiose	1.32	0.45	1.04	1.59	0.57	0.96
N-Carbamyl-L-glutamic acid	0.27	0.88	1.18	1.30	0.24	1.12
D-Mannose	0.98	0.54	1.21	1.02	0.77	1.09
L-Sorbose	0.98	0.54	1.21	1.02	0.77	1.09
N-Acetyl D-galactosamine	1.25	0.36	1.25	0.58	1.03	0.97
Guanosine	0.88	0.35	2.07	1.15	1.12	0.62
Inosine	1.00	0.63	1.24	1.00	1.05	0.95
L-Citrulline	1.13	0.41	1.20	0.92	0.99	1.01
L-Tryptophan	1.02	0.44	1.27	0.74	1.20	0.98
Uracil	1.05	0.38	1.30	0.76	1.22	0.95
Adenosine	0.68	1.64	0.52	1.86	0.03	1.32
2-3-Dihydroxybenzoic acid	1.60	0.86	0.46	1.14	0.47	1.57
myo-Inositol	0.99	0.53	1.24	1.01	0.81	1.06
4-Hydroxyphenyl-pyruvic acid	0.64	0.97	1.05	1.17	0.28	1.03
Pyruvic acid	0.7	0.26	0.88	1.12	2.03	1.59
Mevalonic acid 5-phosphate	0.93	0.44	1.27	1.07	0.61	1.11
6-Hydroxynicotinic acid	0.46	0.62	4.97	3.11	0.93	1.07
L-Dihydroorotic acid	1.08	0.72	3.86	0.92	0.79	4.82
m-Hydroxybenzoic acid	0.71	0.62	2.31	1.32	0.80	1.20
L-Homoserine	1.13	0.38	1.83	2.08	0.87	0.67
Deoxycytidine 5-triphosphate	0.76	1.24	0.53	1.40	0.07	1.28
2-Deoxyadenosine 5-diphosphate	0.30	0.77	2.80	2.85	0.28	1.23
Maleic acid	0.97	0.54	1.26	1.03	0.71	1.14
L-Phenylalanine	1.04	0.41	1.35	0.71	1.26	0.96
3-Dehydroshikimic acid	1.59	0.10	1.06	0.94	1.40	0.16
Malonic acid	0.87	0.61	1.88	1.08	0.92	1.16
Adenosine 3-5-cyclic monophosphate	0.93	0.28	1.47	0.08	1.07	1.80
2-Deoxyinosine	2.34	0.78	0.84	1.79	0.73	1.16
L-Tyrosine	0.99	0.42	1.35	0.79	1.09	1.01
2-Deoxyadenosine	4.09	0.33	0.67	1.33	0.38	3.98
4-Guanidobutyric acid	0.88	0.20	2.22	1.12	0.80	1.14
o-Hydroxy hippuric acid	1.24	0.39	1.14	0.28	1.47	0.86
Glyceric acid	0.96	0.39	1.18	0.27	1.04	1.08
Uric acid	1.05	0.63	1.60	1.79	0.33	0.95
Prephenic acid	0.79	0.72	1.21	1.21	0.37	1.24
Thymine	0.86	0.70	4.79	4.96	1.14	0.79
4-Pyridoxic acid	1.11	0.43	1.36	0.79	1.30	0.89
Trehalose 6-phosphate	1.06	1.23	0.44	1.39	0.94	0.48
L-Isoleucine	1.03	0.42	1.39	0.88	1.07	0.97
L-Leucine	1.03	0.42	1.39	0.88	1.07	0.97
Glucoheptonic acid	0.95	0.53	1.38	1.05	0.72	1.15
4-Hydroxybenzoic acid	3.76	0.21	1.17	0.83	0.27	4.41
Chorismic acid	1.63	0.14	1.10	1.32	0.53	0.90

Cytosine	1.72	0.83	1.17	0.62	0.05	3.26
L-Methionine	1.03	0.41	1.35	0.74	1.13	0.97
3-Methylglutaric acid	1.41	0.88	1.06	1.77	0.58	0.94
2-Deoxyuridine	1.18	0.01	1.27	0.82	1.21	0.45
Creatinine	1.05	0.50	1.52	0.94	0.95	1.18
Pyruvic acid	0.7	0.26	0.88	1.12	2.03	1.59
cis-Aconitic acid	0.7	0.31	0.93	1.07	1.79	1.4
Phosphoenolpyruvic acid	0.83	0.37	1.17	1.69	1.285	1.96
N-Acetyl-D-glucosamine 6-phosphate	0.65	0.32	0.95	1.18	1.05	1.76
alpha-Ketoglutaric acid	0.74	0.72	0.96	1.29	0.99	1.31
L-asparagine	1	0.5	1.28	1	1.11	1.1

Table S2: Related to Figure 1. Metabolite levels in media from unstimulated (D0) and stimulated (D3) NKT cells.

Compound	D0 1	D0 2	D0 3	D3 1	D3 2	D3 3
2-Deoxyadenosine 5-monophosphate	0.02	0.01	0.01	1.98	2.71	2.45
Guanine	0.05	0.05	0.04	3.84	1.95	3.13
Uridine 5-monophosphate	0.09	0.06	0.02	1.91	2.13	2.14
Dihydroxyacetone phosphate	0.10	0.15	0.06	1.85	3.89	2.82
2-Deoxyribose 5-phosphate	0.21	0.03	0.11	2.38	1.79	2.36
2-Methyl-1-butanol	0.09	0.12	0.13	1.87	2.01	2.02
Pyruvic acid	0.09	0.12	0.13	1.87	2.01	2.02
2-Deoxycytidine	0.17	0.24	0.25	1.78	1.75	1.93
2-2-Dimethyl Succinic acid	0.23	0.34	0.35	1.65	1.86	1.88
cis-Aconitic acid	0.41	0.56	0.64	1.37	1.36	1.54
trans-Aconitic acid	0.41	0.56	0.64	1.37	1.36	1.54
O-Phosphorylethanolamine	0.81	0.63	0.56	1.19	2.01	1.69
2-Deoxyuridine	0.45	0.62	0.73	1.27	1.42	1.46
Arabinose-5-phosphate	0.70	0.92	0.98	1.21	1.02	1.13
D-Mannose	1.07	1.32	1.49	0.87	0.90	0.93
L-Sorbose	1.07	1.32	1.49	0.87	0.90	0.93
L-Phenylalanine	1.06	1.40	1.58	0.86	0.89	0.94
myo-Inositol	1.14	1.28	1.47	0.82	0.83	0.86
Pyridoxine	1.07	1.41	1.66	0.82	0.86	0.93
Riboflavin	1.10	1.43	1.67	0.84	0.88	0.90
L-Proline	1.10	1.34	1.53	0.77	0.90	0.81
L-Methionine	1.09	1.42	1.71	0.79	0.85	0.91
Uracil	1.10	1.45	1.71	0.81	0.84	0.90
Xanthosine	1.15	1.48	1.59	0.85	0.80	0.84
2-3-Pyridinedicarboxylic acid	1.12	1.61	1.53	0.81	0.88	0.76
L-Threonine	1.15	1.22	1.51	0.54	0.81	0.85
5-Hydroxy-3-indoleacetic acid	1.19	1.34	1.66	0.81	0.69	0.78
Glucoheptonic acid	1.75	1.25	1.13	0.60	0.87	0.76
L-Tryptophan	1.22	1.41	1.62	0.74	0.74	0.78
Mevalonic acid 5-phosphate	1.24	1.32	1.51	0.70	0.76	0.68
trans-4-Hydroxy-L-proline	2.12	1.23	1.38	0.57	0.77	0.71
3-Dehydroshikimic acid	1.22	1.53	2.23	0.60	0.78	0.71
4-Aminobenzoic acid	1.30	1.66	1.85	0.62	0.62	0.70
L-Serine	1.47	1.31	1.84	0.56	0.56	0.69
L-Glutamine	1.59	1.28	1.73	0.51	0.72	0.52
2-Ketobutyrate	1.25	1.78	2.29	0.60	0.63	0.75
L-Histidine	1.34	1.50	2.17	0.57	0.60	0.66
Uridine	1.49	1.72	2.00	0.43	0.48	0.51
Cytidine	1.44	2.28	2.19	0.52	0.51	0.56
Taurine	1.75	1.40	1.68	0.26	0.35	0.60
Pyridoxal hydrochloride	1.71	2.14	2.57	0.27	0.29	0.23
Hypoxanthine	1.92	2.67	3.18	0.06	0.07	0.08
4-Hydroxyphenyl-pyruvic acid	0.07	0.05	0.05	8.53	6.57	1.93
o-Hydroxy hippuric acid	1.00	1.32	1.51	0.82	0.87	1.00
Trehalose 6-phosphate	3.48	1.49	1.48	0.23	0.52	0.24
3-Methylglutaric acid	1.27	1.02	1.36	0.92	0.95	0.98

Allantoin	1.16	0.98	1.07	1.02	0.70	0.68
Glyceric acid	1.00	1.04	1.58	0.68	1.00	0.37
Inosine	1.08	1.24	1.68	0.85	0.87	0.92
Inosine 5-diphosphate	0.28	0.42	1.11	2.10	0.89	1.42
2-Isopropylmalic acid	0.88	1.39	1.53	0.50	0.53	1.12
Lipoamide	1.01	1.07	1.06	0.79	0.99	0.92
3-Hydroxyphenylacetic acid	1.03	1.81	1.34	0.69	0.90	0.97
Succinic acid	1.02	1.31	1.49	0.89	0.92	0.98
Citramalic acid	0.99	1.24	1.34	0.88	0.93	1.01
L-Arginine	1.37	1.48	0.97	0.96	0.79	1.03
Xanthine	1.00	1.32	1.50	0.88	0.92	1.00
L-Isoleucine	1.03	1.37	1.52	0.95	0.95	0.97
L-Leucine	1.03	1.37	1.52	0.95	0.95	0.97
Creatine	1.03	1.31	1.56	0.92	0.97	0.96
L-Tyrosine	1.00	1.30	1.52	0.88	0.94	1.00
N-acetylaspartylglutamate	1.09	1.07	2.54	0.37	0.26	0.93
Taurocholic acid	0.98	1.36	1.60	0.85	0.91	1.02
L-Hydroxyglutaric acid	1.00	1.31	1.49	0.93	0.93	1.00
Mevalonic acid	1.00	1.31	1.49	0.93	0.93	1.00
D-pantothenic acid	0.99	1.30	1.48	0.90	0.94	1.01
4-Hydroxybenzoic acid	1.04	7.10	7.65	0.54	0.66	0.96
Orotic acid	0.24	0.63	0.31	17.90	1.37	20.56
L-Citrulline	1.31	0.91	1.59	0.51	0.91	1.09
4-Pyridoxic acid	0.98	1.40	1.70	0.88	0.90	1.02
D-Fructose 1,6-biphosphate	1.13	1.68	0.99	1.01	0.52	0.96
N-Acetylglutamic acid	0.36	0.48	1.17	0.83	1.30	1.41
Guanosine 3,5-cyclic monophosphate	1.33	0.86	1.97	0.52	0.63	1.14
3-2-Hydroxyethylindole	1.01	1.76	3.38	0.81	0.38	0.99
Adenosine	7.01	0.88	4.56	0.20	1.08	0.92
Nicotinic acid mononucleotide	1.15	4.48	1.58	0.85	0.27	0.17
2-3-Dihydroxyisovalerate	2.10	0.86	1.58	1.14	0.79	0.60
Epicatechin	1.12	1.16	0.90	0.42	1.10	0.31
Thymine	0.95	1.62	1.56	0.96	0.95	1.04
DL-Glyceraldehyde 3-phosphate	1.97	0.77	1.30	0.55	0.17	1.23
L-Arabinose	0.99	1.04	1.44	0.82	0.88	1.01
Ketovaleric acid	0.73	0.82	1.08	0.97	1.03	1.18
L-Arabitol	0.76	1.16	0.72	1.41	1.53	0.84
Xylitol	0.76	1.16	0.72	1.41	1.53	0.84
Maleic acid	1.11	0.89	1.21	0.67	0.65	1.11
Homocitrate	0.92	1.50	1.98	0.87	0.87	1.08
N-acetylaspartate	0.93	1.28	1.35	0.90	0.98	1.02
Remove	4.07	0.83	1.89	0.67	0.26	1.17
S-5-Adenosyl-L-homocysteine	1.32	0.49	0.68	0.41	8.00	4.99
Vanillic acid	0.64	1.37	2.49	0.08	0.29	1.36
Pyridoxamine	1.07	0.70	0.66	0.93	1.19	2.26
4-Quinolol	0.89	0.26	1.11	1.68	0.85	1.16
2-Deoxyuridine 5-triphosphate	0.78	2.95	1.77	0.69	1.22	0.61
2-Deoxyadenosine 5-diphosphate	4.37	0.87	2.06	1.04	0.96	0.43
Creatinine	2.70	0.62	2.63	0.76	0.92	1.08

trans-trans Muconic acid	0.57	4.20	2.26	0.99	1.01	0.14
L-Homoserine	2.65	1.66	0.76	1.07	0.54	0.93
Inosine 5-triphosphate	0.33	0.09	1.32	1.08	1.47	0.92
Adenine	11.73	1.78	2.10	0.18	0.20	0.22
2-Deoxyguanosine 5-monophosphate	0.53	2.46	1.47	1.47	0.11	0.11
DL-2-Aminoadipic acid	0.21	1.00	0.55	3.66	1.02	1.00
N-Formyl-L-Tyrosine	2.06	0.54	3.88	0.20	1.02	0.98
Shikimic acid	0.89	0.78	1.04	1.80	0.96	1.10
3-Indoleacetic acid	1.18	0.47	0.15	1.13	0.87	1.13
Pyridoxal 5 phosphate	2.15	2.92	0.41	1.07	0.93	0.36
4-Methyl-2-oxovaleric acid	0.93	1.18	1.32	0.95	0.99	1.01
Itaconic acid	0.93	1.18	1.32	0.95	0.99	1.01
Malonic acid	0.73	0.91	1.03	0.97	1.04	1.04
Cytosine	1.28	0.72	2.75	0.15	1.38	0.64
2-Deoxy-D-glucose 6-phosphate	1.09	8.46	2.16	0.91	0.84	0.65
2-Deoxycytidine 5-monophosphate	0.07	0.48	0.70	2.90	16.83	1.30
Glutathione red	0.93	1.27	1.07	1.29	0.49	0.20
Chorismic acid	1.10	0.90	0.21	3.04	1.79	0.40
Melibiose	1.05	0.95	1.77	0.77	0.72	1.21
Galactonic acid	0.91	1.18	1.32	0.98	0.95	1.02
o-Phospho-L-Serine	2.19	1.06	13.91	0.55	0.37	0.94
alpha-D-Glucose-1-phosphate	0.84	1.27	1.23	0.82	0.99	1.01
3-Hydroxy-DL-kynurenine	2.18	0.94	1.06	0.22	0.68	1.51
Ketoisovaleric acid	1.18	1.00	4.60	0.83	0.81	1.00
Oxamic acid	0.38	1.64	2.15	0.55	1.45	0.14
Isopentenyl pyrophosphate	1.20	0.67	0.80	3.59	0.41	1.90
2-Deoxyguanosine	0.69	1.98	1.43	0.80	1.09	0.91
D-Glucosamine 6-phosphate	0.59	1.23	0.77	0.60	1.42	2.50
D-Fructose 6-phosphate	0.84	1.14	1.32	0.81	0.99	1.01
Cellobiose	0.54	1.08	0.96	1.04	0.89	1.22
L-Kynurenine	0.74	0.95	1.05	0.96	1.05	1.04
4-Guanidobutyric acid	1.17	1.00	0.31	0.79	1.00	2.86
Glyoxylic acid	0.43	1.61	0.64	1.25	0.75	4.42
beta-Nicotinamide mononucleotide	1.75	0.26	5.57	1.46	0.53	0.54
Isopentyl acetate	0.89	1.13	1.28	1.03	0.95	0.97
N-Carbamyl-L-glutamic acid	0.86	1.01	1.33	0.99	0.04	1.09
Salicylic acid	0.73	0.90	1.10	1.06	0.95	1.05
D-+-Galactosamine	1.06	0.94	8.36	0.48	0.78	1.44
Argininosuccinic acid	6.30	0.99	0.88	1.01	0.59	1.01
DL-Isocitric acid	1.28	0.71	0.30	1.16	1.08	0.92
4-Hydroxy-L-glutamic acid	0.62	1.13	5.32	1.12	0.88	0.48
D-Maltose	2.25	0.40	1.47	1.13	0.45	0.87
D-Gluconic acid	0.89	1.15	1.25	0.96	1.01	0.99
beta-Nicotinamide adenine dinucleotide	1.43	0.05	0.03	0.57	110.05	1.55
Citric acid	1.28	0.72	0.28	1.16	1.07	0.93
Uridine 5'-diphosphogalactose	0.03	0.08	3.32	1.58	16.98	0.42
Uridine 5-diphosphoglucose	0.03	0.08	3.32	1.58	16.98	0.42
2-4-Quinolinediol	5.78	0.33	1.35	0.65	0.56	1.40
Folinic acid	0.85	2.39	0.72	39.63	1.15	0.54

Cytidine 5-triphosphate	0.85	0.90	2.91	0.27	1.10	1.30
Thymidine	0.36	1.08	9.49	0.92	0.66	1.64
2-Deoxycytidine 5-diphosphate	1.09	0.91	0.28	0.29	1.16	2.55
Ribonic acid gamma lactone	1.25	0.44	3.03	0.75	0.87	1.13
2-Deoxyadenosine	1.35	0.52	2.72	0.65	16.24	0.52
Deoxycytidine 5-triphosphate	1.88	0.13	1.56	1.23	0.16	0.77
2-Deoxy-D-ribose	0.92	0.70	1.26	1.08	1.39	0.87
Nicotinic acid	2.03	0.12	0.15	0.12	1.85	2.05
Succinic semialdehyde	0.31	0.78	1.38	1.22	2.04	0.35
Thymidine 5-diphosphate	0.87	2.21	1.13	0.47	0.51	2.03
m-Hydroxybenzoic acid	0.59	1.12	1.65	0.77	0.94	1.06
L-Carnitine	0.25	1.33	1.62	0.59	4.13	0.67
D-erythro-Dihydrosphingosine	1.82	0.61	0.45	2.05	0.45	1.39
Deoxyguanosine 5-triphosphate	1.53	1.41	0.19	1.29	0.31	0.71
Uridine 5-triphosphate	0.64	1.42	0.99	0.07	1.27	1.01
L-Dihydroorotic acid	0.42	1.18	1.80	0.85	1.15	0.71
L-Homocysteine	0.04	1.92	0.71	1.26	0.74	1.65
Guanosine 5-triphosphate	0.65	0.83	5.95	11.47	1.17	0.80
alpha-D(+)Mannose 1-phosphate	1.24	0.73	0.92	0.78	1.08	1.35
Cytidine 5-diphosphate	1.54	0.36	0.68	1.32	0.49	1.45
Deoxyadenosine 5-triphosphate	0.36	2.27	0.17	2.43	0.13	1.64
gamma-Glu-Cys	0.94	1.06	1.13	1.71	0.46	0.26
±-Mevalonolactone	0.85	1.00	1.54	0.93	1.00	1.14
Adipic acid	0.63	1.78	1.03	0.95	0.99	1.01
alpha-Ketoglutaric acid	0.63	1.78	1.03	0.95	0.99	1.01
L-Malic acid	0.81	1.08	1.20	0.91	0.98	1.02
Thiamine	0.39	1.29	1.66	0.98	1.02	0.81
Trehalose	0.71	1.05	1.85	0.94	1.26	0.95
N-Acetylneuraminic acid	0.82	0.95	1.36	0.74	1.05	1.08
NADH	1.26	0.47	0.77	1.23	0.10	1.92
2-Deoxyguanosine 5-diphosphate	0.48	0.80	1.66	0.50	1.80	1.20
Adenosine 5-diphosphate	0.48	0.80	1.66	0.50	1.80	1.20
CoA	1.41	0.28	3.63	2.97	0.59	0.40
L-Homocystine	0.97	1.31	0.40	1.03	0.92	1.03
S-2-Aminoethyl-L-cysteine	1.27	0.73	26.74	0.37	0.18	17.24
gamma-Aminobutyric acid	0.35	0.54	1.89	1.49	0.46	1.46
Creatine phosphate	0.53	1.35	1.10	0.53	2.07	0.90
Adenosine 5-triphosphate	1.53	1.41	0.19	1.29	0.66	0.71
L-Gluthathione (oxidized)	0.40	1.01	1.72	1.90	0.99	0.65
Uric acid	0.50	1.43	1.13	0.87	0.78	1.20
L-Cystathionine	1.48	0.19	0.80	0.33	1.20	1.27
5-Methoxytryptamine	1.83	0.83	0.60	1.17	1.61	0.73
L-Glutamic acid	0.77	1.01	1.09	0.91	0.99	1.02
Prephenic acid	0.67	1.69	0.72	1.22	0.82	1.18
L-Canavanine	0.10	5.12	0.06	3.87	0.30	1.70
N-Carbamoyl-DL-aspartic acid	0.79	0.32	1.99	1.25	0.94	1.06
2-3-Dihydroxybenzoic acid	1.10	1.20	0.14	0.90	1.36	0.04
AICAR	0.39	4.13	1.54	0.46	4.79	0.35
Adenylosuccinic acid	7.60	1.03	0.07	0.40	0.97	8.10

Guanosine	0.23	0.19	4.44	0.25	2.55	1.75
N-Acetyl D-galactosamine	0.82	0.98	1.24	1.02	0.94	1.11
Uridine 5-diphosphate	0.76	0.99	1.38	1.01	1.92	0.27
2-Deoxyinosine	1.18	0.33	1.69	2.25	0.24	0.82
NADPH	-	0.14	2.39	0.39	1.07	1.00
Cytidine-5-monophosphate	0.47	2.69	0.69	2.09	0.40	1.31
Indoline-2-carboxylate	0.32	2.27	0.72	1.02	0.98	1.33
Phenylpyruvic acid	0.03	0.01	0.01	1.97	2.32	0.85
Lactic acid	1.06	1.09	1.26	0.92	0.94	0.52
N-Acetyl-D-glucosamine 6-phosphate	1.01	1.18	1.30	0.86	0.99	0.28
L-Aspartic Acid	1.11	1.13	1.43	0.83	0.84	0.82
L-asparagine	1.42	1.24	1.76	0.62	0.69	0.60
L-Glutamine	1.59	1.28	1.73	0.51	0.72	0.69
L-Cystine	1.88	1.45	1.80	0.31	0.55	0.72

Table S3: Details of the oligonucleotides used in this paper (Related to STAR Methods).

S. No.	Mouse Gene	Forward primer sequence	Reverse primer sequence
1	<i>β-Actin</i>	AGCCATGTACGTAGCCATC	CTCTCAGCTGTGGTGGTG
2	<i>Gclc</i>	GGCTCTCTGCACCATCACTT	GTTAGAGTACCGAAGCGGGG
3	<i>Gclm</i>	AGGAGCTTCGGGACTGTATCC	GTTGGGACATGGTGCATTCCAAAA
4	<i>Ogt</i>	TTCGGGAATCACCCCTACTTCA	TACCATCATCCGGGCTCAA
5	<i>Gfat1</i>	TAAGGAGATCCAGCGGTGTC	GTT CAGCTGTCTCGCCTGATTGA
6	<i>SNAT1</i>	CCTCACAAGTACCAGAGCAC	GGCCAGCTCAAATAACGATGA
7	<i>SNAT2</i>	GATGAACGTGTCCAAGATTTT	CTACTGGGACCGTGAGGGTGAC
8	<i>Gls</i>	GGGTAATCACTTTTGTACGA	GACTTCACCCTTTGATCACC
9	<i>Gdh</i>	CCCAACTTCTTCAAGATGGTG	AGAGGCTCAACACATGGTTGC