**Supplementary Material, Siska et al.**

Kynurenine induces T cell fat catabolism and has limited suppressive effects in vivo

Supplemental table S1: Key resource table

| Reagent or Resource	Source	Identifiers
Antibodies		
CD4 Monoclonal Antibody (GK1.5), Alexa Fluor 488	Thermo Fisher Scientific	Cat# 53-0041-82, RRID:AB_469893
CD4 Monoclonal Antibody (GK1.5), APC	Thermo Fisher Scientific	Cat# 17-0041-82, RRID:AB_469320
Rat Anti-CD8a Monoclonal Antibody, PE-Cy7 Conjugated, Clone 53-6.7	BD Biosciences	Cat# 552877, RRID:AB_394506
Rat Anti-CD4 Monoclonal Antibody, Pacific Blue Conjugated, Clone RM4-5	BD Biosciences	Cat# 558107, RRID:AB_397030
Mouse Anti-CD8 Monoclonal Antibody, Allophycocyanin Conjugated, Clone RPA-T8	BD Biosciences	Cat# 555369, RRID:AB_398595
FOXP3 Monoclonal Antibody (FJK-16s), APC	Thermo Fisher Scientific	Cat# 17-5773-80, RRID: AB_469456
Pacific Blue(TM) anti-human CD4 antibody	BioLegend	Cat# 300521, RRID:AB_493098
Rat Anti-IFN-gamma Monoclonal Antibody, Alexa Fluor 647 Conjugated, Clone XMG1.2	BD Biosciences	Cat# 557735, RRID:AB_396843
Rat Anti-CD44 Monoclonal Antibody, Phycoerythrin Conjugated, Clone IM7	BD Biosciences	Cat# 553134, RRID:AB_394649
APC-Cy7 Rat Anti-Mouse CD62L antibody	BD Biosciences	Cat# 560514, RRID:AB_10611861
Rat Anti-CD25 Monoclonal Antibody, Allophycocyanin Conjugated, Clone PC61	BD Biosciences	Cat# 557192, RRID:AB_398623
Mouse Anti-CD25 Monoclonal Antibody, PE-Cy7 Conjugated, Clone M-A251	BD Biosciences	Cat# 557741, RRID:AB_396847
7-AAD Staining Solution 2mL antibody	BD Biosciences	Cat# 559925, RRID:AB_2869266
Annexin V - Apoptosis stain antibody	BD Biosciences	Cat# 556419, RRID:AB_2665412
PerCP anti-human CD4 antibody	BioLegend	Cat# 344624, RRID:AB_2563326
FOXP3 Monoclonal Antibody (PCH101), APC	Thermo Fisher Scientific	Cat# 17-4776-42, RRID:AB_1603280
LEAF™ Purified anti-human CD3 antibody	BioLegend	Cat# 317304, RRID:AB_571925
Syrian Hamster Anti-CD28 Monoclonal Antibody, Unconjugated, Clone 37.51	BD Biosciences	Cat# 553294, RRID:AB_394763
Purified NA/LE Hamster Anti-Mouse CD3e Clone 145-2C11 antibody	BD Biosciences	Cat# 553057, RRID:AB_394590
Dynabeads™ Human T-Activator CD3/CD28	Thermo Fisher Scientific	Cat# 11131D
Dynabeads™ Mouse T-Activator CD3/CD28	Thermo Fisher Scientific	Cat# 11456D
Chemicals, Peptides, and Recombinant Proteins		
LIVE/DEAD Fixable Aqua Dead Cell Stain Kit	Thermo Fisher Scientific	Cat# L34966
CellTrace CFSE Cell Proliferation Kit	Thermo Fisher Scientific	Cat# C34554
L-Kynurenine	Cayman Chemicals	Cat# 11305, CAS# 2922-83-0
L-Kynurenine	Sigma Aldrich	Cat# K8625, CAS# 2922-83-0
D-Kynurenine	Cayman Chemicals	Cat# 28254, CAS# 13441-51-5
D-Kynurenine	Sigma Aldrich	Cat# K2380, CAS# 13441-51-5
Kynurenic acid	Sigma Aldrich	Cat# K3375, CAS# 492-27-3
Cell-Tak	BD Biosciences	Cat# 354240
D-Glucose (U-13C6, 99%)	Cambridge Isotope Laboratories	Cat# CLM-1396-PK
L-Glutamine (13C5, 99%)	Cambridge Isotope Laboratories	Cat# CLM-1822-H-PK
Palmitic acid (U-13C16, 98%)	Cambridge Isotope Laboratories	Cat# CLM-409-PK
Bovine Serum Albumin solution, 30% in saline, fatty acid free, aseptically filled	Sigma Aldrich	Cat# A9205, CAS# 9048-46-8
D-(+)-Glucose	Cayman Chemicals	Cat# 23733, CAS# 50-99-7
L-Glutamine	Cayman Chemicals	Cat# 23716, CAS# 56-85-9

Sodium Palmitate	Sigma Aldrich	Cat# P9767, CAS# 408-35-5
Sodium Oleate	Sigma Aldrich	Cat# O7501, CAS# 143-19-1
1 Kb Plus DNA Ladder	Invitrogen	Cat# 10787018
Recombinant Mouse TGF-beta 1	R&D systems	Cat# 7666-MB-005
Human TGF-beta 1	Peprotech	Cat# 100-21
Recombinant Mouse IL-2, carrier-free	Biolegend	Cat# 575406
Interleukin-2, human	Roche	Cat# 11011456001
Dextran sulphate sodium 36-50 kDa	MP Biomedicals	Cat# 160110
Ionomycin	Sigma-Aldrich	Cat# 56092-82-1
Phorbol 12-myristate 13-acetate (PMA)	Sigma-Aldrich	Cat# 16561-29-8
BD GolgiStop	BD Biosciences	Cat# 54724
Critical Commercial Assays		
L-Kynurenine ELISA kit	ImmuSmol	Cat# BA-E-2200
Seahorse XF Cell Mito Stress Test Kit	Agilent	Cat# 103015-100
Seahorse XF Glycolysis Stress Test Kit	Agilent	Cat# 103020-100
CD4+CD25+ Regulatory T Cell Isolation Kit, mouse	Miltenyi Biotec	Cat# 130-091-041
CD90.2 MicroBeads, mouse (for APC)	Miltenyi Biotec	Cat# 130-049-101
CD8a (Ly-2) MicroBeads, mouse	Miltenyi Biotec	Cat# 130-049-401
CD4 MicroBeads, human antibody	Miltenyi Biotec	Cat# 130-045-101, RRID:AB_2889919
CD8 MicroBeads, human antibody	Miltenyi Biotec	Cat# 130-045-201, RRID:AB_2889920
BD Cytofix/Cytoperm™ Kit	Thermo Fisher Scientific	Cat# BDB554714
RNeasy Mini Kit	Qiagen	Cat# 74104
TaqMan™ Universal PCR Master Mix	Thermo Fisher Scientific	Cat# 4304437
Experimental Models: Cell Lines		
U-87 MG, human brain tumour (likely glioblastoma), male	American Type Culture Collection	Cat# CRL-1772; RRID:CVCL_0188
B16.F10 mouse melanoma, male	American Type Culture Collection	Cat# CVCL_0159 RRID:CVCL_0159
Ae17 mouse mesothelioma, sOVA, female	(34)	RRID:CVCL_LJ85
Experimental Models: Organisms/Strains		
B6.129S7-$Rag1^{tm1Mom}$/J (B6/Rag1$^{-/-}$)	The Jackson Laboratory	RRID:IMSR_JAX:002216
C57BL/6J	The Jackson Laboratory	RRID:IMSR_JAX:000664
BALB/cJ	The Jackson Laboratory	RRID:IMSR_JAX:000651
AhR$^{-/-}$	(21)	
B6.PL-Thy1a/CyJ (Thy1.1, CD90.1)	The Jackson Laboratory	RRID:IMSR_JAX:000406
Oligonucleotides		
Cyp1a1	Thermo Fisher Scientific	Cat# Mm00487218_m1
TiPARP	Thermo Fisher Scientific	Cat# Mm00724822_m1
18S rRNA	Thermo Fisher Scientific	Cat# Hs03003631_g1
Software and Algorithms		
Wave 2.6.1	Agilent	www.agilent.com
Other		
RPMI 1640 Medium	Thermo Fisher Scientific	Cat# 11875085
Foetal Bovine Serum, certified, United States	Thermo Fisher Scientific	Cat# 16000044
RPMI 1640 medium, no glucose	Thermo Fisher Scientific	Cat# 11879020
Foetal Bovine Serum, dialyzed, US origin	Thermo Fisher Scientific	Cat# 26400044
Seahorse XF Media & Calibrant	Agilent	Cat# 102353-100

Supplemental Figure S1. Kynurenine effects on T cell function are attributable to cell death.
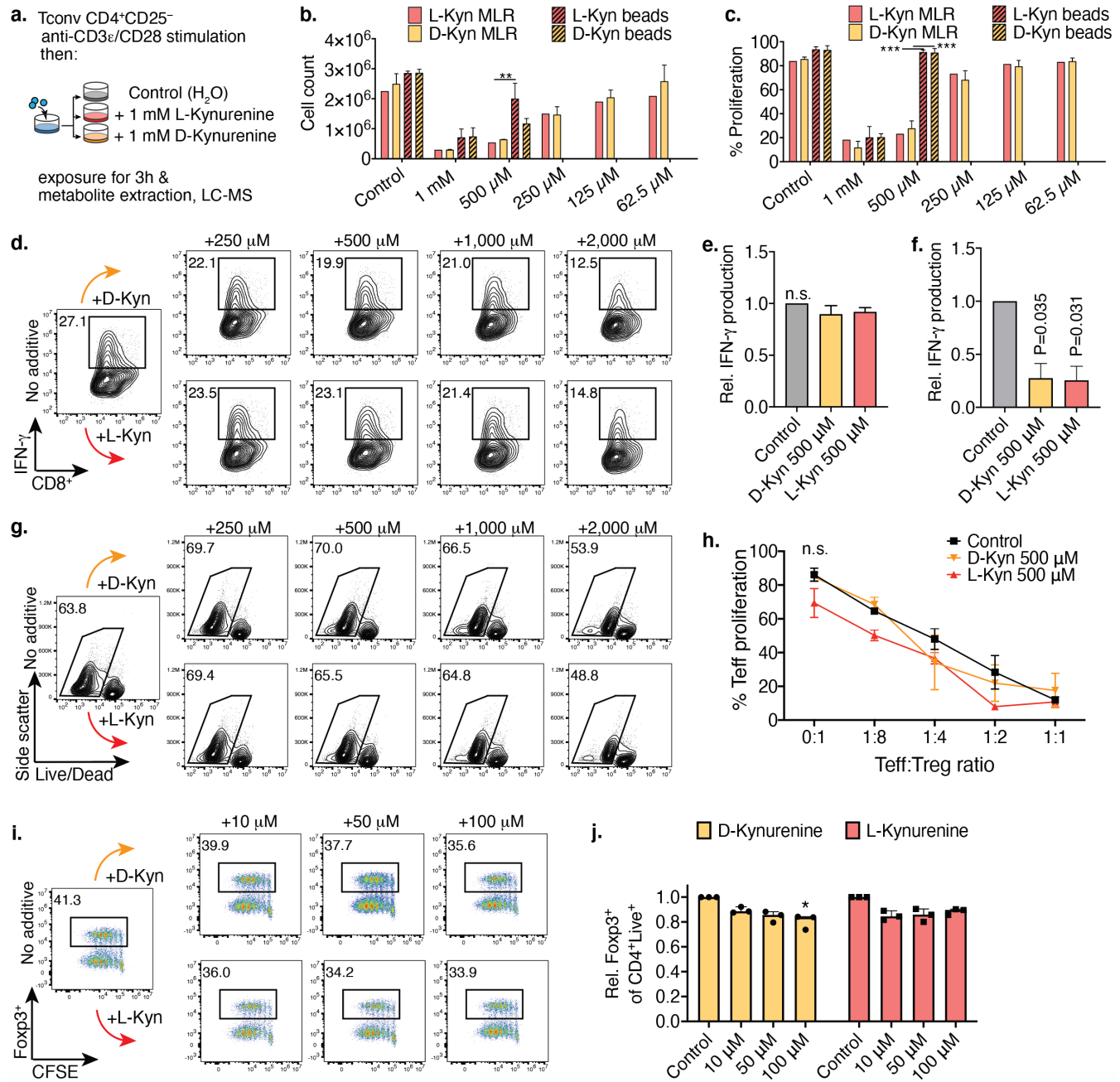
(**a**) Experimental design to Figure 1B, C as well as Figure 4 & Figure 5. (**b, c**) Human CD4 T cells in mixed leukocyte reaction (MLR) or with CD3/CD28 bead stimulation, proliferation assessed by cell counting and CFSE-dilution, respectively. Data derived from three (all conditions except D-Kyn beads) or two (D-Kyn beads) independent experiments. Two-way ANOVA comparing MLR with corresponding bead stimulated condition. (**d-g**) Interferon (IFN)-γ production in murine CD8$^+$ stimulated with CD3ε/CD28 mAb-coated beads overnight and with PMA/ionomycin for five hours with or without L-/D-kynurenine at the indicated doses (**d, e, g**) and human T cells stimulated with CD3/CD28 mAb-coated beads for three days (**f**). (**d, g**) shows representative flow cytometry of (**d**) IFN-γ production and (**G**) incremental cell injury with higher L- and D-kynurenine doses, (**e, f**) cumulative IFN-γ production by murine (**e**) and human (**f**) T cells at 500 μM kynurenine (3/group, Student t-test). (**h**) CD4$^+$CD25$^-$ T cells were CFSE-labelled and co-stimulated with anti-CD3ε and irradiated antigen presenting cells and co-cultured with Treg at the indicated ratios. After three days, Treg and Tconv cell viability was assessed using Aqua Life/Dead and effector T cell proliferation measured by CFSE dilution (3/group, area under the curve, Student t-test). (**i, j**) Murine Tconv cells were CFSE-labelled and co-stimulated and polarized to induce Foxp3 by adding IL-2 and TGF-β. After four days, Foxp3 expression in CD4$^+$ T cells was assessed. D-/L-kynurenine did not increase Foxp3 expression. (I) Representatives and (J) quantitative data normalized to control (Kruskal Wallis test, 3/group). *, ** and *** indicate p<0.05, p<0.01 and p<0.001, respectively. Data shown as median with IQR or range (**b, c, j**) or mean with SEM (**e, f, h**).

**Supplemental Figure S2. Brain kynurenine levels and colitis histology scoring.**
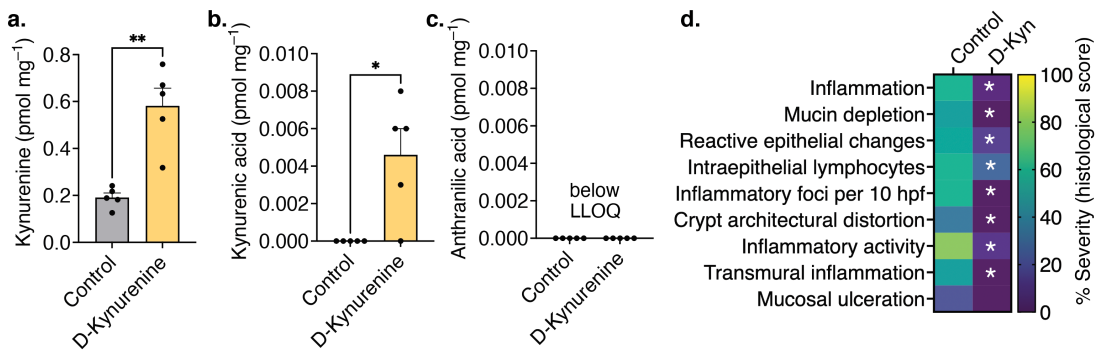
(**a-c**) C57BL/6J mice were fed D-kynurenine enriched (300 mg/kg/d) or control chow for 10 days (5/group), and brain tissue was snap frozen. Metabolites were extracted and measured by LC-MS/MS. As expected, D-kynurenine (**a**) and kynurenic acid (**b**) were increased under D-kynurenine diet. (**c**) Anthranilic acid was not detected above the lower limit of quantification (LLOQ). (**d**) Heatmap of blinded histological scoring of colitis data in Figure 2F, G. Data were normalized to a percent scale (Student t-test, 5/group). * and ** indicates $p<0.05$ and $p<0.01$, respectively.

**Supplemental Figure S3. AhR genotype and phenotype of donor mice.**
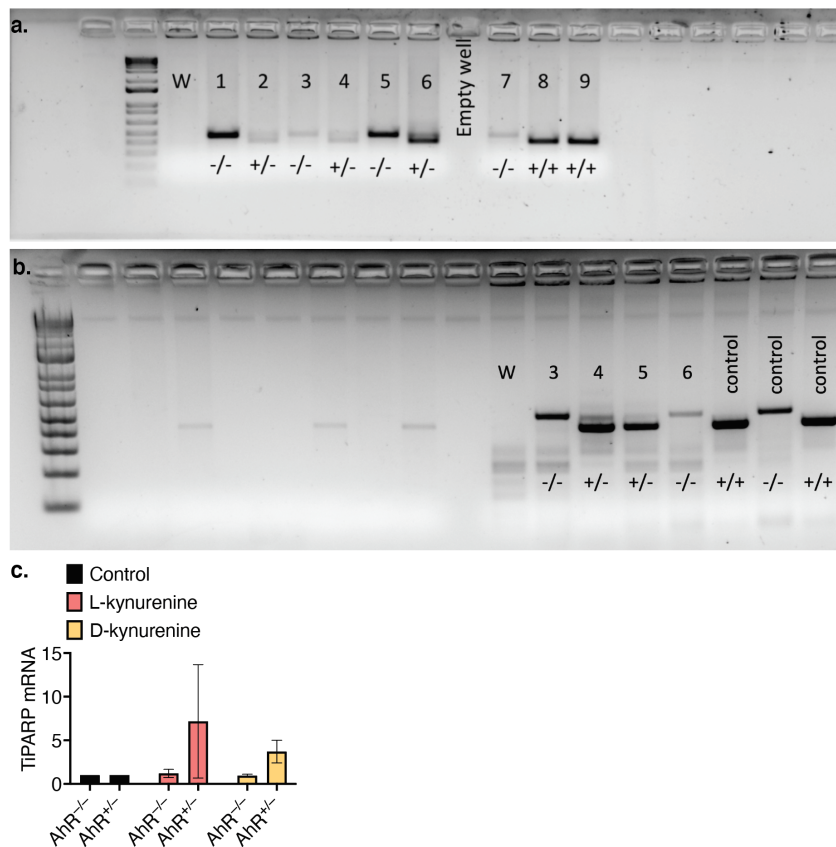
(**a, b**) Agarose gels with AhR PCR products from RNA extracted from tail tips of spleen-donor mice sent from the University of Florida to confirm AhR genotype in Figure 3 (a) and Figure S3 (b) All data were derived from littermates. (**c**) AhR-dependent gene expression through qPCR. Murine CD4$^+$CD25$^-$ Tconv from AhR$^{-/-}$ and AhR$^{+/-}$ littermate T cells were co-stimulated with CD3ε/CD28 mAb-coated beads and exposed to 1 mM L- or D-kynurenine overnight. TiPARP mRNA gene expression was induced through kynurenine if AhR is present. Data derived from two independent experiments and normalized to the control condition without kynurenine. Error bars indicate SEM.

**Supplemental Figure S4. Bioenergetic measurements in kynurenine exposed T cells.**
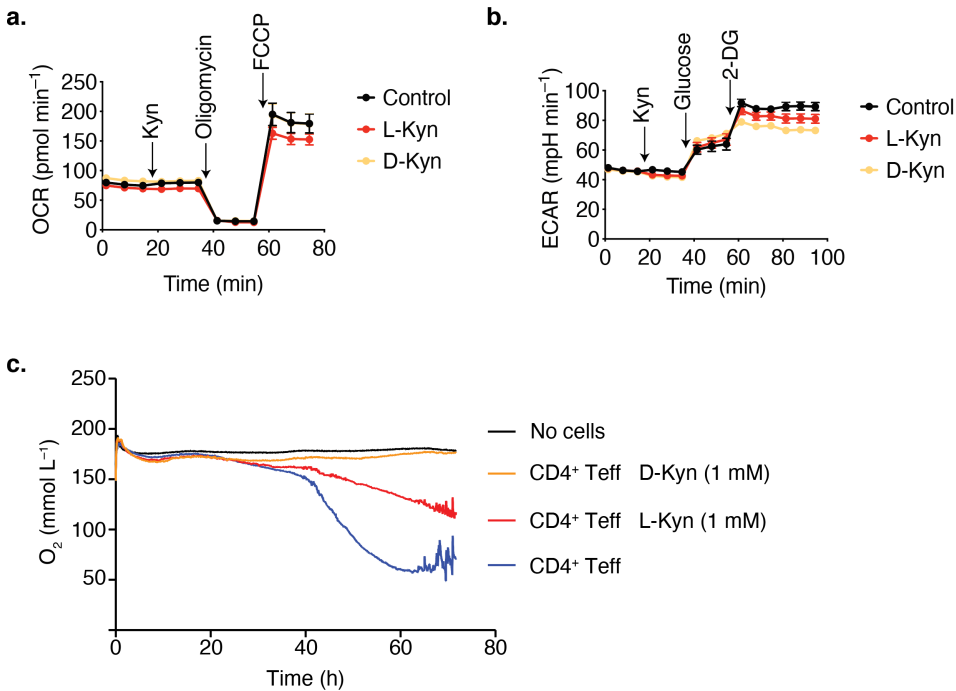
(**a, b**) Bioenergetic Seahorse assays examining overnight stimulated Tconv for (**a**) mitochondrial function (corrected for antimycin/rotenone) and (**b**) glycolytic function. Tconv cells were stimulated with anti-CD3ε/CD28 beads and 25 U IL-2 × mL^{-1} for 16 hours and the subjected to Seahorse, with L- or D-kynurenine injected into the chamber during the experiment to a final concentration of 1 mM. Data representative for two independent experiments. (**c**) Cell culture oxygen consumption experiments were performed with SDR SensorDish Reader using stimulated CD4$^+$ T cells ± 1 mM D-Kyn or L-Kyn. Abbreviations: 2-DG, 2-Deoxy-D-glycose; FCCP, cyanide-4-[trifluoromethoxy]phenylhydrazone; Kyn, kynurenine; OCR, oxygen consumption rate; ECAR, extracellular acidification rate.

**Supplemental Figure S5. ATP and citrate isocitrate tracing, amino acid and nucleotide ion counts.**
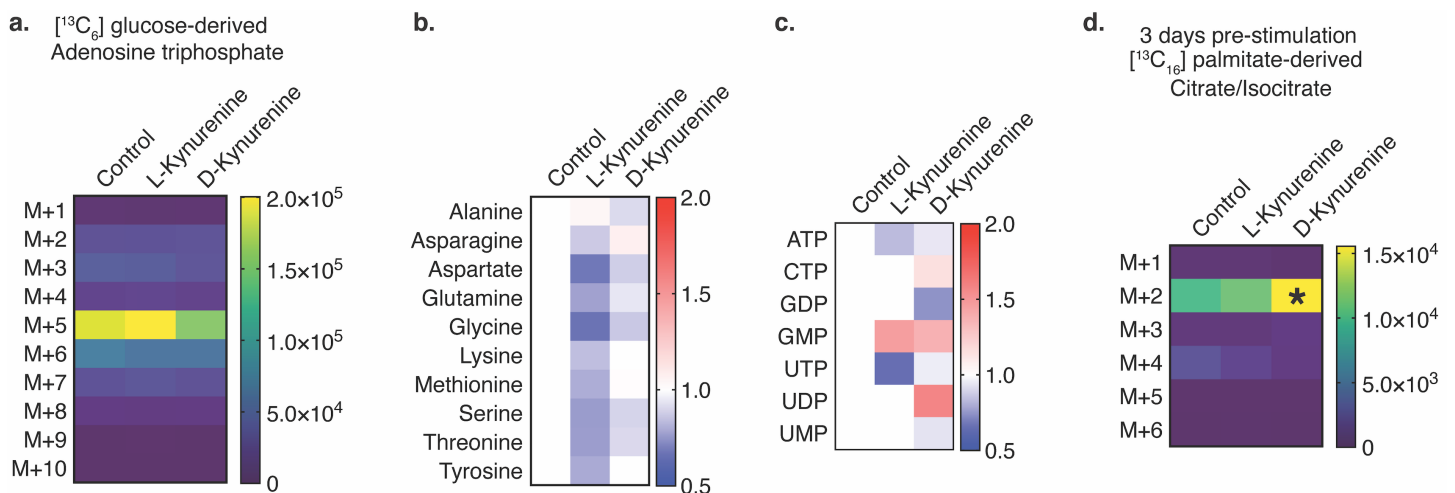
(**a-d**) Tconv T cells were co-stimulated overnight (a-c) or three days (b) and cultured with [^{13}C$_6$] D-glucose (**a-c**) or [^{13}C$_{16}$] palmitate (**b**) for three hours ±1 mM L-/D-kynurenine or water control. Metabolites were extracted and analysed for derivative analysis (**a, d**) or as pooled total ion counts of all isotopologues (**b, c**). (**a, d**) Derivative analysis data for glucose derived ATP (**a**) and palmitate derived citrate/isocitrate (**d**) were displayed as heatmaps showing total ion counts, with M+1-10 indicating the number of ^{13}C-atoms per isotopologue. (**b, c**) Amino acid (**b**) and nucleotide (**c**) relative total ion count normalized to the control condition. (**a-d**) Data pooled from three independent experiments (two-way ANOVA with Benjamini, Krieger and Yekutieli FDR correction, a-d). * indicates $p < 0.05$ to control.

Supplemental Figure S6. Human T cell rescue by serum, albumin, lipids or higher cell number.
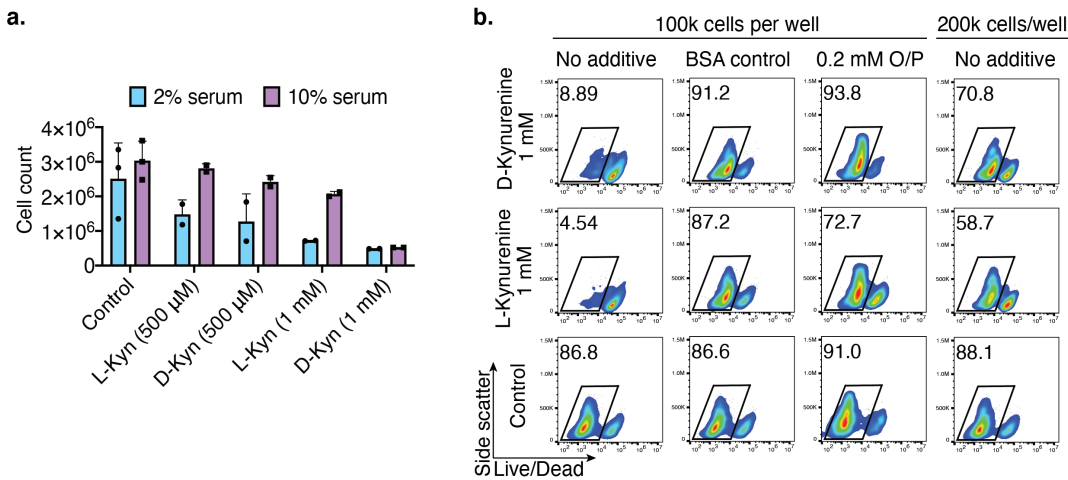
(**a**) Human CD4$^+$ T cells were stimulated with or without D-Kyn or L-Kyn at the indicated concentrations in media containing 2% or 10% serum. Data pooled from three independent experiments. (**b**) Human CD4$^+$ T cells were co-stimulated ±1 mM L- or D-kynurenine or water control for four days, and cell viability assessed using Aqua Life/Dead staining. Similar to the murine experiments, increasing lipids, but also just lipid free albumin or the amount of T cells per well diminished the apoptosis-inducing effect of kynurenine. Data representative of two independent experiments. Error bars indicate SEM.

Supplemental Figure S7. Additional tryptophan metabolites in human cancer tissue and kynurenine fed mice.
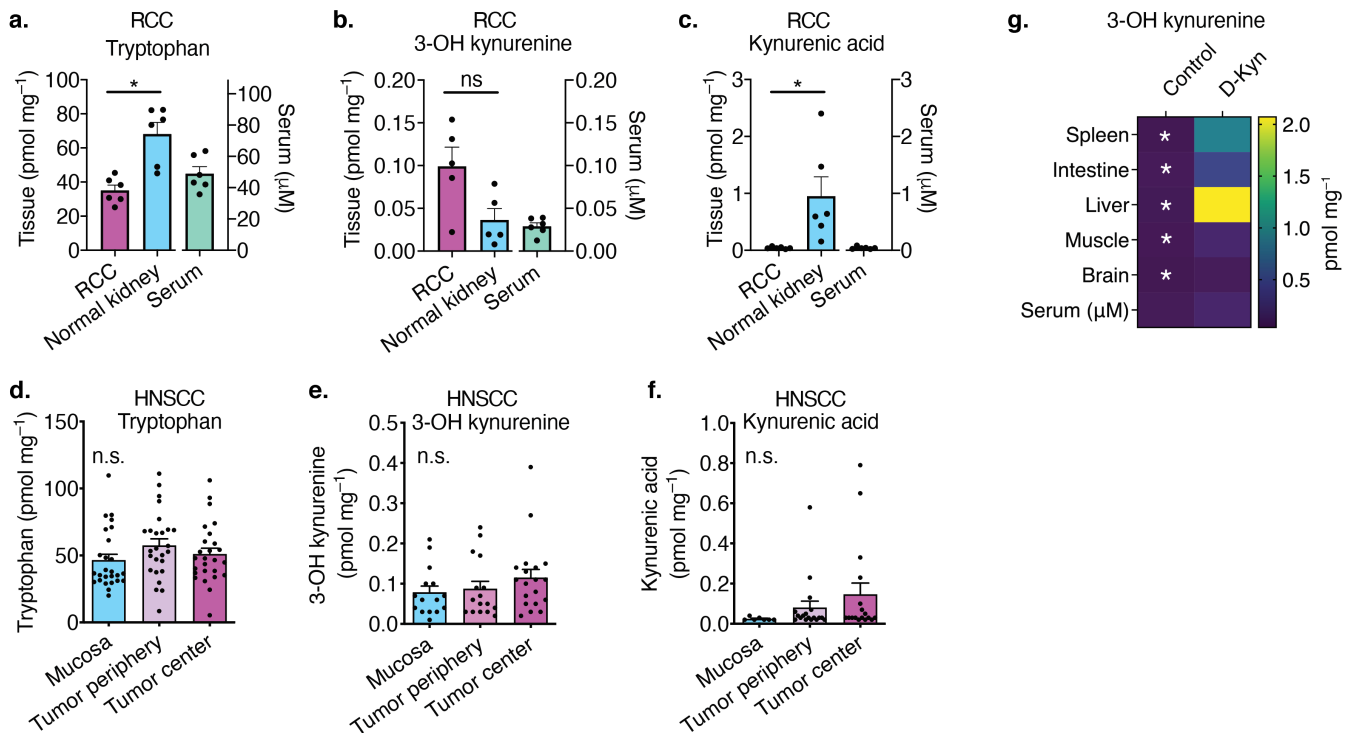
(**a-f**) Human renal clear cell carcinoma (a-c) and head and neck squamous cell carcinoma (d-f) were frozen in liquid nitrogen, and metabolites were extracted and quantified by LC-MS/MS. Levels of tryptophan (**a, d**), 3-OH kynurenine (**b, e**) and kynurenic acid (**c, f**) were determined in serum, healthy tissue and tumours. Each dot represents an individual patient. (**g**) C57BL/6J mice were fed D-kynurenine enriched (300 mg/kg/d) or control chow for 10 days (5/group). Serum was obtained from living mice, tissue obtained and snap frozen after euthanasia in rapid succession. Metabolites were extracted and measured by LC-MS/MS. 3-OH kynurenine concentrations are shown as heatmaps. (**a-c, g**) Student t-test; (**d-f**) One-way ANOVA. * indicates p<0.05. Error bars indicate SEM.