Science Advances
AAAS

Supplementary Materials for

Immunomodulatory actions of a kynurenine-derived endogenous electrophile

Mara Carreño *et al.*

Corresponding author: Dario A. Vitturi, dav28@pitt.edu

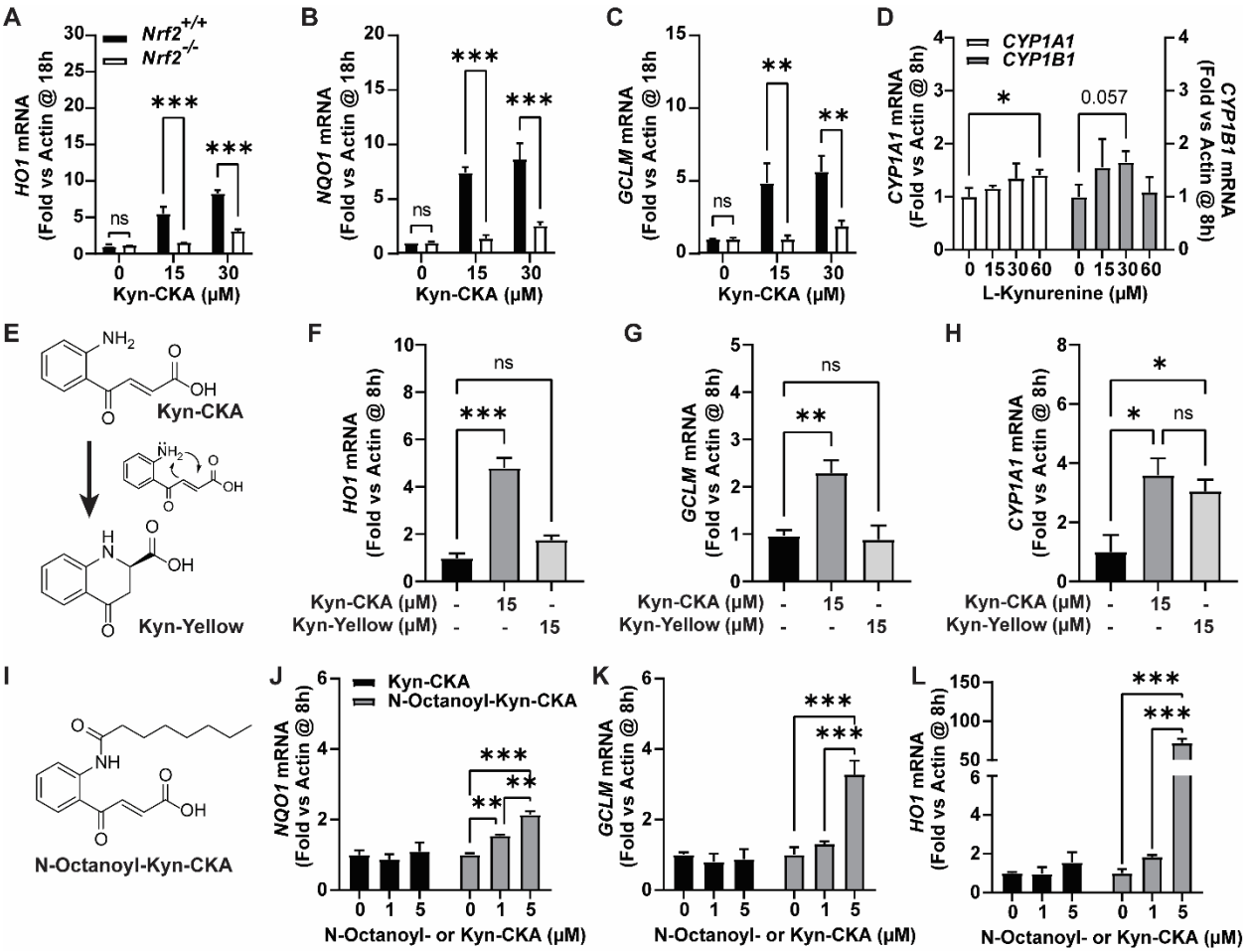
Sci. Adv. **8**, eabm9138 (2022)
DOI: 10.1126/sciadv.abm9138

The PDF file includes:

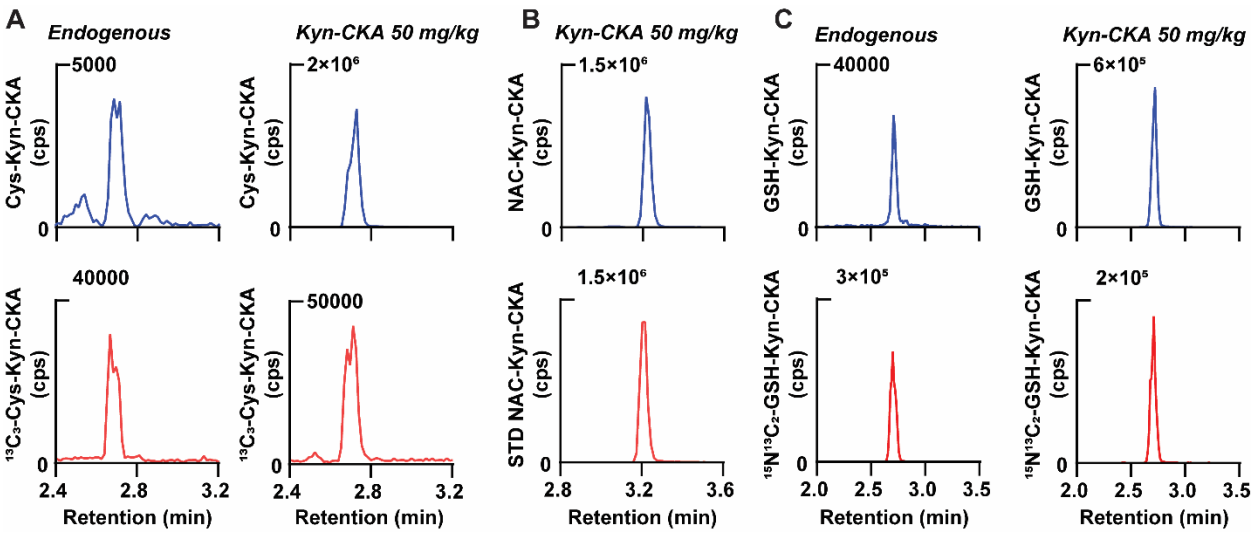
Figs. S1 to S6
Tables S1 to S3

Other Supplementary Material for this manuscript includes the following:

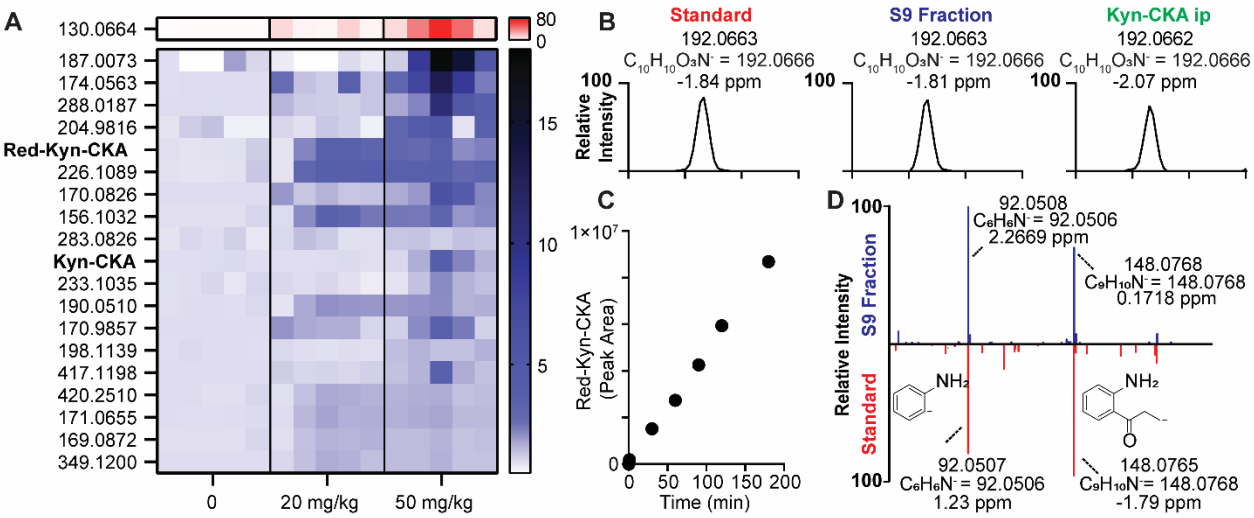
Movies S1 to S10



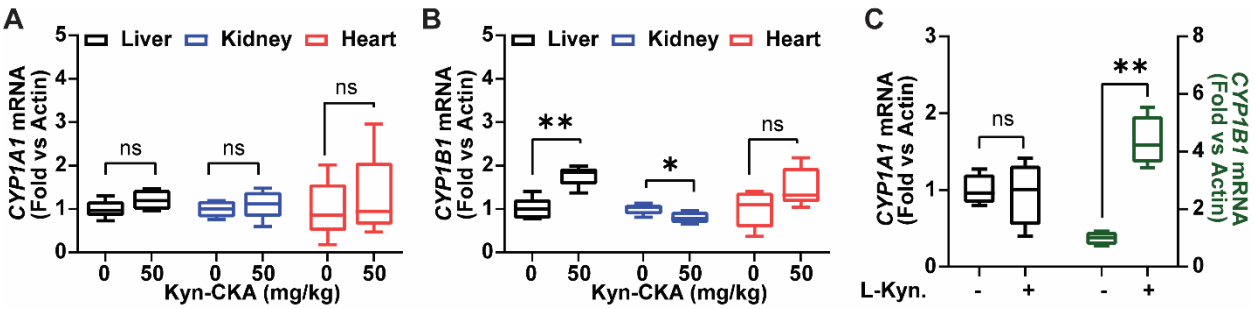
Supplementary Fig. 1. Kyn-CKA engages Nrf2- and AhR-dependent signaling via distinct mechanisms. (A-C) Nrf2 deficiency in mouse embryonic fibroblasts (MEF) results in significant attenuation of *HO1*, *NQO1* and *GCLM* expression following Kyn-CKA treatment (n=3). To ensure clarity, the data was normalized to 0 μM Kyn-CKA treatment within each genotype. Basal *HO1* and *NQO1* expression levels are 80-90% lower and basal *GCLM* expression is 30% lower in *Nrf2*$^{-/-}$ versus *Nrf2*$^{+/+}$ cells. (D) Activation of AhR-dependent genes by 8 h L-Kynurenine treatment in AML-12 (n=3). (E) Kyn-Yellow formation via Kyn-CKA cyclization secondary to intramolecular nucleophilic attack by the aromatic amine on the α,β-unsaturated carbonyl moiety. (F-H) Nrf2- and AhR-dependent gene expression 8 h post Kyn-CKA or Kyn-Yellow in AML-12 (n=3). (I) Cell permeable N-Octanoyl-Kyn-CKA structure. (J-L) Nrf2-dependent gene expression 8 h post Kyn-CKA or N-Octanoyl-Kyn-CKA in AML-12 (n=3). * $p < 0.05$, ** $p < 0.01$, ***$p < 0.0001$ by two- (A-C, I-K) or one-way (D, F-H) ANOVA and Tuckey's test.



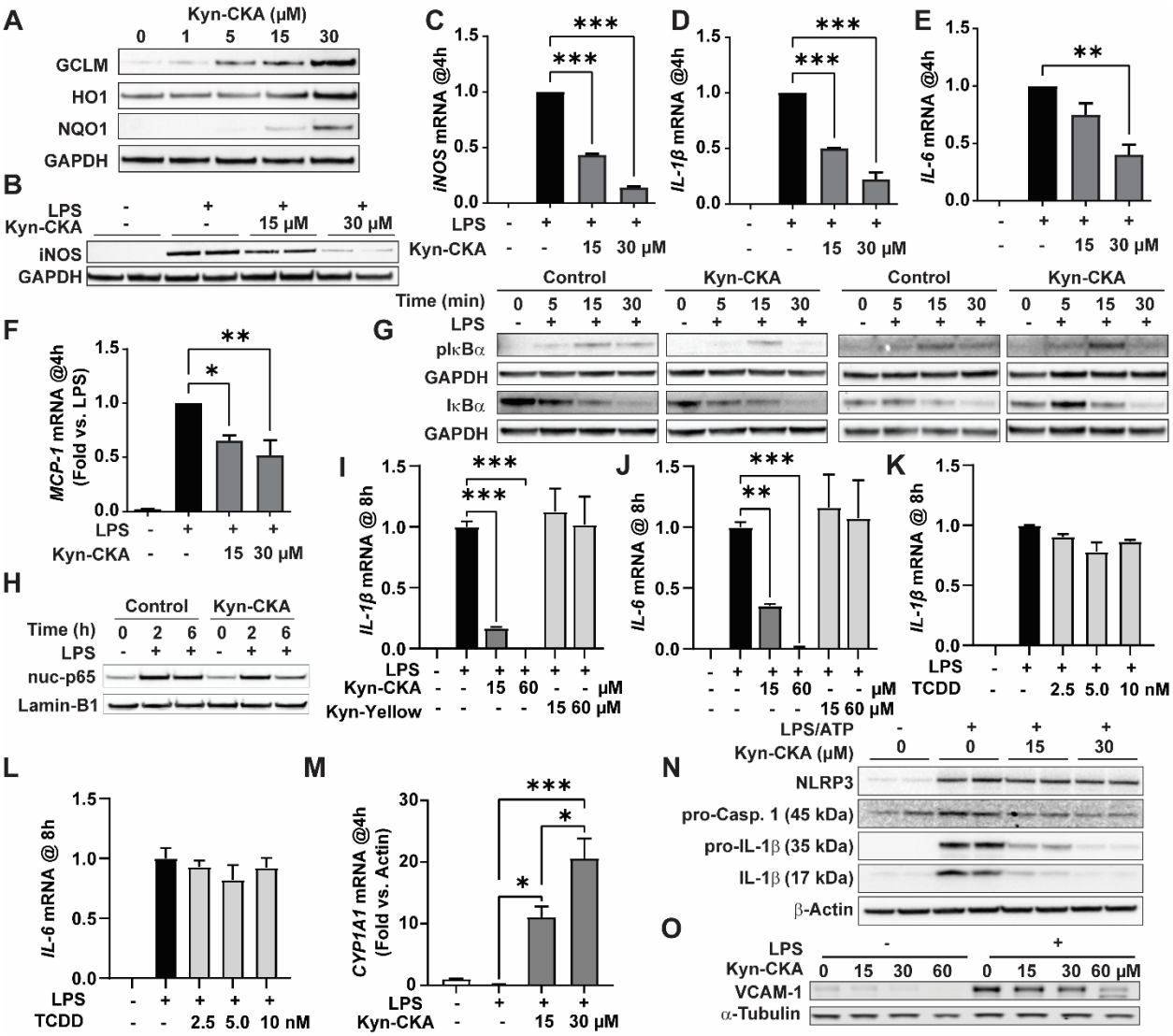
Supplementary Fig. 2. Kyn-CKA metabolites are present *in vivo*. (**A**) Representative LC-MS/MS traces showing urinary Cys-Kyn-CKA (MRM 311/120) in the absence and 8 h following intraperitoneal Kyn-CKA co-eluting with an isotopically labeled standard (MRM 314/123). (B) Representative LC-MS/MS trace of urine NAC-Kyn-CKA (MRM 353/162) 8 h post Kyn-CKA co-eluting with a synthetic standard. (C) Representative LC-MS/MS traces of hepatic GSH-Kyn-CKA adducts in the absence and presence of Kyn-CKA supplementation at 8 h. Abscissas are detector intensities expressed in counts per second (cps).



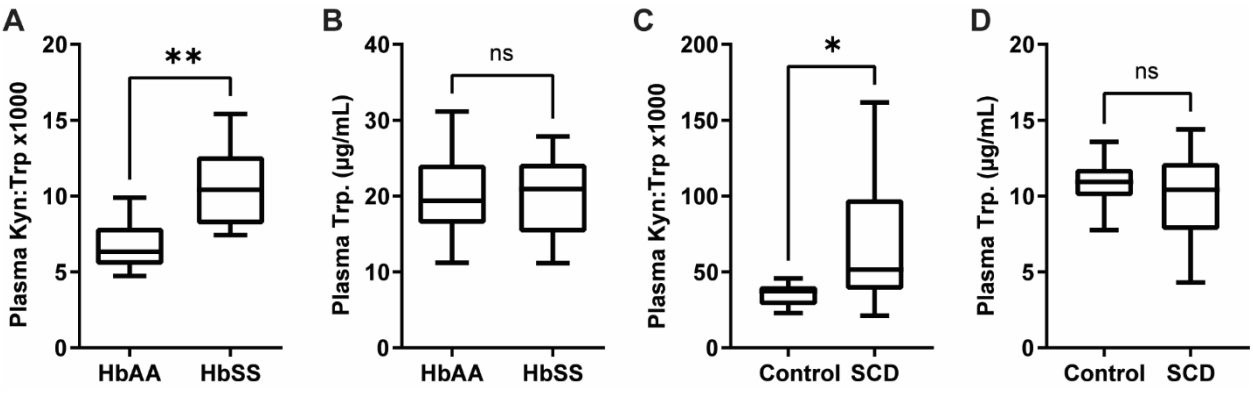
Supplementary Fig. 3. Candidate metabolites modulated by Kyn-CKA administration. (**A**) Heatmap showing the 20 top features detected by untargeted LC-HRMS to be increased in mouse plasma following Kyn-CKA treatment using the free XCMS suite. (B) Confirmation of molecular composition (at the 2 ppm level), using high-resolution mass spectrometry, for synthetic Red-Kyn-CKA, the product of S9 fraction reduction of Kyn-CKA and Red-Kyn-CKA detected in the plasma from Kyn-CKA treated mice at 8 h. (C) Kinetic trace showing the enzymatic conversion of Kyn-CKA (200 µM) to Red-Kyn-CKA by a rat liver S9 fraction (1 mg) in the presence of NADPH (1 mM) at 37ºC, pH 7.4. (D) Structural confirmation of S9-derived products by MS^2 using synthetic Red-Kyn-CKA.



Supplementary Fig. 4. Kyn-CKA induces AhR-dependent genes *in vivo*. (**A-B**) AhR-dependent gene expression in mice 8 h post Kyn-CKA (50 mg/kg, n=5 per dose). (C) AhR-dependent gene expression in mouse liver 12 h post second L-Kyn dose (50 mg/kg ip, 2 doses, 24 h apart, n=4). * $p < 0.05$, ** $p < 0.01$ by t-test.



Supplementary Fig. 5. Kyn-CKA induces Nrf2-dependent proteins and inhibits NF-κB and NLRP3 inflammasome engagement in macrophages (**A**) Dose-dependent induction of Nrf2 target proteins by Kyn-CKA at 24 h in BMDM. (B) Kyn-CKA inhibits LPS-induced (1 μg/mL) iNOS protein at 8 h, and (C) *iNOS* and (D-F) pro-inflammatory cytokine RNA expression at 4 h in BMDM (n=3). (G) Additional replicates for total and phospho-IκBα levels following LPS in the presence of Kyn-CKA (0 or 30 μM) in J774a.1 cells. (H) Additional replicate for nuclear P65 in LPS-treated J774a1 versus time and Kyn-CKA (0, 30 μM). Each lane are three combined independent wells. (I,J) Kyn-Yellow and (K,L) TCDD have no effect on LPS (1 μg/mL) induced gene expression in J774a.1 cells (n=3). (M) Dose-dependent induction of *CYP1A1* expression in BMDM 4 h post LPS (1 μg/mL, n=3). (N) Kyn-CKA inhibits pro-caspase-1 and pro-IL-1β expression, and pro-IL-1β processing in BMDM treated with LPS (100 ng/mL) and ATP (2 mM) for 8 h. (O) VCAM-1 expression in HPMVEC 16 h post LPS (100 ng/mL) and Kyn-CKA. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$ by one-way ANOVA and Tukey's test.



Supplementary Fig. 6. Kynurenine synthesis is upregulated in murine and human SCD (**A**) Plasma kynurenine:tryptophan ratios and (B) tryptophan levels in HbAA (n = 13) and HbSS (n = 8) Townes mice. ** $p < 0.001$ by t-test. (C) Plasma kynurenine:tryptophan ratios and (D) tryptophan levels in control (n = 12) and SCD (n = 10) human donors. * $p < 0.05$ by t-test.

Supplementary Table 1 - Antibodies:

Antibody	Catalog number	Company	RRID
NLRP3	15101	Cell Signaling	AB_2722591
Pro-IL-1β	12242	Cell Signaling	AB_2715503
IL-1β	12242	Cell Signaling	AB_2715503
iNOS	13120	Cell Signaling	AB_2687529
VCAM-1	ab134047	Abcam	AB_2721053
HO1	ADI-SPA-895	Enzo Life Sciences	AB_10618757
NQO1	ab34173	Abcam	AB_2251526
GCLM	14241-1-AP	Proteintech	AB_2107832
GAPDH	2118	Cell Signaling	AB_561053
p65	8242	Cell Signaling	AB_10859369
IKBα	4812	Cell Signaling	AB_10694416
Phospho-IKBα	2859	Cell Signaling	AB_561111
Lamin-B1	12586	Cell Signaling	AB_2650517
α-Rabbit (secondary)	7074	Cell Signaling	AB_2099233
α-Mouse (secondary)	7076	Cell Signaling	AB_330924

Supplementary Table 2 - Primers used for RT-PCR:

Primer	Catalog number	Company
IL1B	Mm00434228	ThermoFisher
IL6	Mm00446190	ThermoFisher
MCP1 (Ccl2)	Mm00441242	ThermoFisher
HO1 (Hmox1)	Mm00516005	ThermoFisher
NQO1	Mm01253561	ThermoFisher
GCLM	Mm01324400	ThermoFisher
iNOS (Nos2)	Mm00440502	ThermoFisher
KIM-1 (Havcr1)	12001950	BioRad
CYP1A1	Mm00487218	ThermoFisher
CYP1B1	Mm00487229	ThermoFisher
Actin	4351315	Applied Biosystems

Supplementary Table 3 - Transitions for MRM analysis:

Metabolite	Q1	Q3	DP	EP	CE	CXP
Kynurenine	207.1	190.0	-65	-8	-22	-10
N-Formyl-kynurenine	235.1	190.0	-65	-8	-22	-10
Kyn-CKA	190.0	128.0	-65	-5	-15	-8
Kyn-CKA	190.0	144.0	-65	-8	-22	-12
Kyn-CKA	190.0	146.0	-65	-8	-15	-10
GSH-Kyn-CKA	497.0	306.0	-55	-10	-20	-8
Red-Kyn-CKA	192.1	148.1	-55	-7	-15	-6
Red-Kyn-CKA	192.1	92.0	-55	-3	-27	-12
Cys-Kyn-CKA	311.1	120.0	-70	-5	-30	-10
Cys-Kyn-CKA	311.1	190.0	-70	-5	-25	-10
NAC-Kyn-CKA	369.0	162.0	-70	-8	-20	-7
$^{15}N^{13}C_2$-GSH-Kyn-CKA	500.1	309.1	-70	-5	-20	-10
$^{13}C_3$-Cys-Kyn-CKA	314.1	123.0	-70	-5	-30	-10
$^{13}C_3$-Cys-Kyn-CKA	314.1	190.0	-70	-5	-25	-10
Trp-d_3	206.1	116.0	-60	-8	-22	-8

DP: declustering potential, EP: entrance potential, CE: collision energy, CXP: collision cell exit potential