

SUPPLEMENTARY INFORMATION

The role of the tryptophan-nicotinamide pathway in a model of severe malnutrition induced liver dysfunction

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9 **SUPPLEMENTARY FILES:**

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11 **Supplementary Figure 1**

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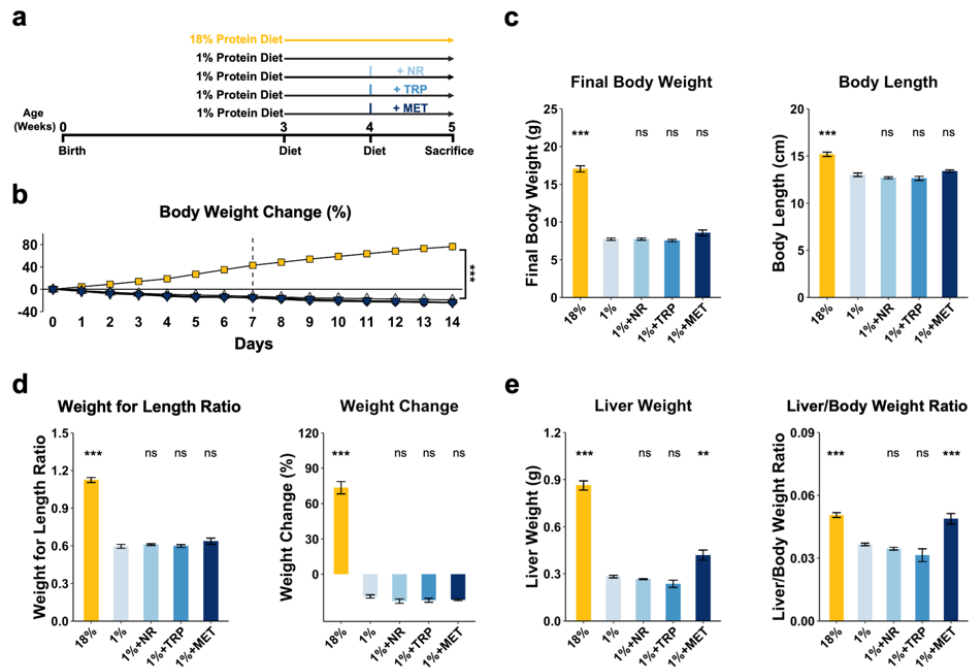
14 **Supplementary Table 1**

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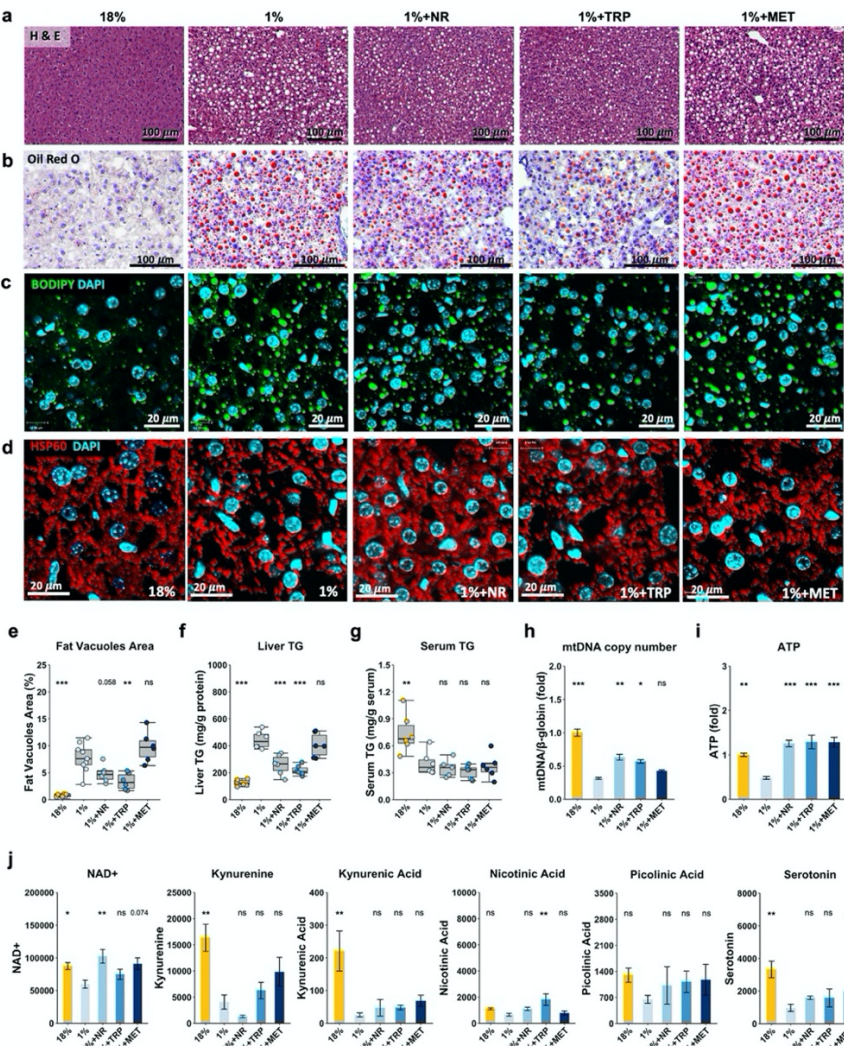
17 **Supplementary Table 4**

Supplementary Figure 1



Supplementary Fig. 1 Feeding a 1% protein diet with or without NR, TRP, and MET supplementation on basic animal characteristics. **a** Experiment design of NR, TRP and MET supplementation. **b** Body weight change throughout experiment (n=15 for 18% and 1%; n=6 for all other groups). **c** Final body weight and body length assessed at day 14 (n=12 for 18%; n=10 for 1%; n=6 for all other groups). **d** Weight for length ratio and weight change assessed at day 14 (n=12 for 18%; n=10 for 1%; n=6 for all other groups). **e** Liver weight, liver weight/body weight ratio (n=12 for 18%; n=10 for 1%; n=6 for all other groups). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns as not significant, one-way ANOVA followed by Tukey's post hoc test. Data are shown as the mean $\pm$ S.E.M.

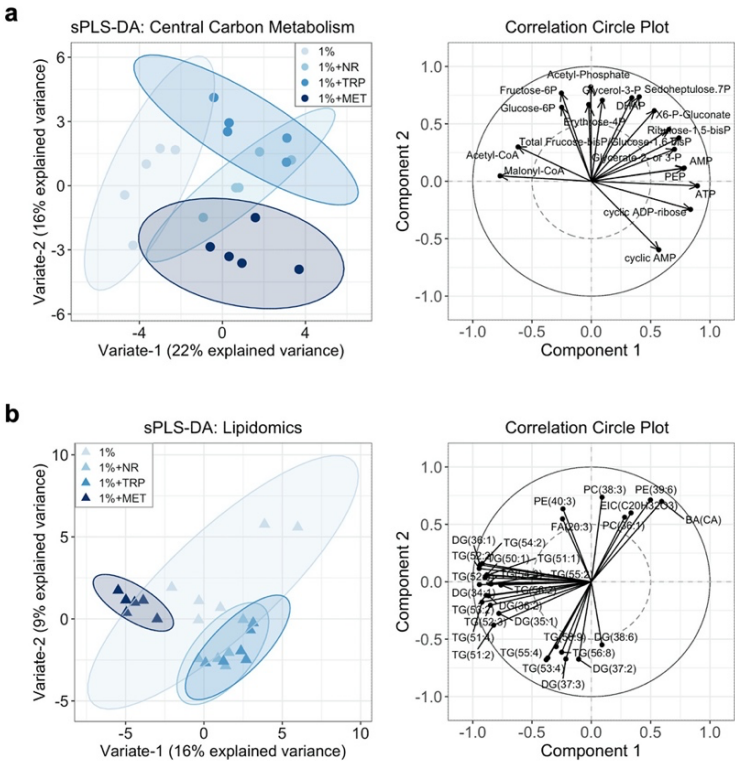
29 **Supplementary Figure 2**



30

31 **Supplementary Fig. 2** Liver histology, mitochondrial characteristics, and TRP-NAM pathway
32 metabolites of NR, TRP, and MET supplementation. **a** Representative hematoxylin and eosin
33 staining images of the liver (20X magnification). Cytoplasm was stained in red, and nucleus was
34 stained in purple. **b** Representative oil red o stain staining images of the liver (20X magnification).
35 Fat droplet was stained in red, and nucleus was stained in purple. **c** Representative
36 immunofluorescence images of the liver (40X magnification). BODIPY was used to stain fat
37 droplet in green, and DAPI was used to counter stain nucleus in blue. **d** Representative
38 immunofluorescence images of mitochondrial (60X magnification). HSP60 was used to stain
39 mitochondrial in red, and DAPI was used to counter stain nucleus in blue. **e** Quantification of fat
40 vacuoles area (n=9 for 18% and 1%; n=6 for all other groups). **f** Liver TG concentrations (n=6). **g**
41 Serum TG concentrations (n=6). **h** mtDNA copy number (n=6). **i** ATP levels (n=11 for 18% and
42 1%; n=5 for 1%+NR, 1%+TRP, and 1%+MET). **j** Hepatic NAD⁺ levels and TRP-NAM pathway
43 metabolites (n=6). * p < 0.05, ** p < 0.01, *** p < 0.001, ns as not significant, one-way ANOVA
44 followed by Tukey's post hoc test. Data are shown as the mean $\pm$ S.E.M. Scale bars are as
45 indicated.

46 **Supplementary Figure 3**



47

48 **Supplementary Fig. 3** Hepatic metabolic and lipidomic profiles under different treatment of TRP-
49 NAM pathway modulators. **a** sPLS-DA and correlation circle plots of hepatic central carbon
50 metabolism showing separation of different treatments (n=5). **b** sPLS-DA and correlation circle
51 plots of hepatic lipidomics showing separation of different treatments (n=6).

52 **Supplementary Table 1**

53 **Supplementary Table 1** Hepatic central carbon metabolism profiles under different treatments
54 (n=5 for all groups except n=7 for 1%+NAM).

	18%	1%	1%+NAM	1%+NR	1%+TRP	1%+MET
AMP	2.92±0.54	1.66±0.09	2.74±0.41	2.32±0.22	2.28±0.17	2.07±0.18
ADP	2.32±0.45	1.46±0.11	1.66±0.20	1.45±0.27	1.39±0.19	1.71±0.31
ATP	1.34±0.14	0.74±0.10	1.93±0.36	1.67±0.17	1.72±0.38	1.71±0.25
GMP	3.27±1.24	0.35±0.11	0.86±0.17	0.82±0.28	0.76±0.25	1.21±0.29
GDP	0.31±0.02	0.27±0.03	0.35±0.04	0.24±0.02	0.29±0.02	0.38±0.06
GTP	0.036±0.006	0.034±0.009	0.082±0.025	0.052±0.010	0.062±0.013	0.062±0.016
UMP	6.50±1.74	0.73±0.19	1.64±0.31	1.54±0.46	1.12±0.34	1.88±0.33
UDP	0.033±0.005	0.020±0.002	0.030±0.004	0.026±0.004	0.024±0.006	0.024±0.005
UTP	0.58±0.12	0.26±0.05	1.36±0.33	0.76±0.23	1.03±0.35	0.62±0.16
cyclic-ADP-ribose	1.22±0.27	0.53±0.11	1.25±0.33	1.23±0.12	0.97±0.19	1.27±0.25
cyclic-AMP	0.015±0.002	0.010±0.001	0.018±0.002	0.012±0.001	0.014±0.002	0.023±0.003
6-P-Gluconate	3.85±0.88	2.38±0.89	3.73±0.65	5.05±1.22	6.33±0.68	2.64±1.24
Acetylglucosamine-1P	17.21±1.78	5.96±0.79	8.24±1.05	6.66±0.59	8.61±1.16	6.77±0.50
Acetyl-Phosphate	12.01±1.277	15.37±4.28	13.88±2.46	13.44±2.29	17.75±1.17	6.98±1.11
ADP-Glucose	0.004±0.001	0.001±0.001	0.005±0.001	0.003±0.001	0.003±0.001	0.001±0.000
2,3-bisP-Glycerate	0.048±0.019	0.033±0.003	0.121±0.038	0.034±0.014	0.088±0.027	0.078±0.032
DHAP	1.21±0.13	1.58±0.36	2.57±0.45	1.69±0.32	2.87±0.35	1.41±0.26
Total Fructose-bisP/Glucose-1,6-bisP	0.56±0.10	0.62±0.19	1.08±0.11	1.09±0.17	1.28±0.14	0.89±0.13
Fructose-6P	54.43±6.12	70.20±14.78	56.35±9.24	52.69±9.89	69.16±11.84	28.96±2.94
Glucosamine-6P	3.22±0.36	2.76±0.41	2.31±0.42	4.25±1.03	3.79±1.28	1.88±0.57
Glycerate-2/3-P	11.23±6.28	5.90±2.25	11.19±2.44	11.47±2.42	16.14±1.70	12.48±1.80
Glycerol-3-P	9.73±1.25	12.18±3.24	14.91±3.98	22.96±6.33	41.01±13.60	6.66±1.19
PEP	3.32±0.50	3.31±0.97	5.99±1.12	6.52±1.18	7.68±0.59	7.25±1.10
Phosphocreatine	0.021±0.012	0.010±0.003	0.020±0.006	0.010±0.003	0.022±0.009	0.018±0.010
Ribulose-1,5-bisP	0.16±0.05	0.07±0.01	0.20±0.05	0.15±0.03	0.17±0.02	0.10±0.03
Ribulose-5P	32.84±5.50	24.54±5.54	35.74±3.75	30.81±2.87	33.52±4.50	22.95±2.74
Sedoheptulose-7P	3.89±0.72	8.52±2.71	11.18±2.91	11.18±2.62	15.63±1.60	4.36±1.93
UDP-Glucose	0.138±0.041	0.013±0.003	0.560±0.171	0.167±0.148	0.192±0.111	0.063±0.040
Acetyl-CoA	0.003±0.000	0.004±0.000	0.003±0.000	0.003±0.000	0.003±0.000	0.003±0.000
Hs-CoA	0.035±0.014	0.010±0.001	0.084±0.023	0.023±0.013	0.081±0.035	0.021±0.007
Malonyl-CoA	0.005±0.001	0.010±0.001	0.004±0.000	0.003±0.000	0.004±0.001	0.003±0.001
Succinyl-CoA	0.209±0.125	0.023±0.004	0.145±0.049	0.059±0.021	0.099±0.030	0.090±0.052
Erythrose-4P	0.260±0.030	0.494±0.064	0.771±0.167	0.461±0.025	0.614±0.037	0.404±0.038
Glucose	4800±434	2611±628	2580±437	2775±658	3171±220	2211±358
Glucose-6P	23.13±5.99	55.89±15.89	52.61±8.96	36.22±11.84	54.96±13.60	6.90±1.46
Glyceraldehyde-3P	2.20±0.15	5.38±0.74	8.90±1.62	5.82±1.01	7.30±1.15	5.55±0.60
Mannose-6P	14.17±0.68	34.35±11.16	27.54±3.07	21.03±6.66	34.70±13.02	11.55±4.05
Ribose-5P	9.81±1.19	10.93±1.72	16.05±2.00	14.71±3.07	14.48±1.99	8.79±2.39
α-Hydroxyglutaric acid	42.00±16.04	4.14±1.08	6.06±0.93	5.97±0.96	8.00±0.64	4.48±1.24
α-Ketoglutaric acid	9.36±3.43	3.57±1.14	3.30±1.40	3.56±1.35	2.57±0.57	4.21±0.96
Citric acid	0.58±0.17	33.21±12.72	34.55±14.69	14.55±14.13	44.46±20.43	21.74±10.54
Fumaric acid	985.0±108.3	1005.0±217.2	796.9±171.3	931.1±173.0	727.6±107.6	672.6±132.9
Glycolic acid	39.78±3.08	41.27±6.93	110.38±47.27	69.96±21.83	69.31±11.18	29.45±0.92
Isocitric acid	1.52±0.19	2.78±1.30	2.71±0.86	2.15±0.59	3.49±1.31	1.52±0.36
Lactic acid	9340±1005	4437±1043	7080±1412	6103±1272	6603±918	5818±706
Malic acid	1236.8±29.2	1106.8±261.4	1311.7±303.8	1154.6±236.3	1397.8±161.1	994.7±189.3
Pyruvic acid	90.05±12.46	51.59±7.02	67.19±9.69	77.38±9.79	63.52±8.16	65.76±7.51
Succinic acid	21.62±5.00	13.30±5.56	17.30±10.61	5.37±1.14	9.44±1.01	5.00±1.03

55 Data are shown as the mean ± S.E.M.

Supplementary Table 2

Supplementary Table 2 Hepatic lipidomic profiles of total lipid for each class (n=6).

		18%	1%	1%+NAM	1%+NR	1%+TRP	1%+MET
Totals for each class	Glycerides (Gly)	256.5±181.6*	558.3±235.7	597.2±84.9	485.5±248.4	529.4±78.7	724.0±94.6
	Phospholipids (PLs)	638.2±169.5**	268.0±175.7	249.5±85.1	200.4±135.7	244.6±77.3	156.5±63.1
	Sphingolipids (SLs)	26.1±5.5**	44.7±13.8	44.2±5.2	41.2±20.8	41.3±3.2	42.4±7.6
	Sterols	13.8±4.7**	111.0±64.7	81.3±21.6	89.3±56.0	167.8±71.8	64.3±39.9
Ratio	Gly/PLs	0.5±0.6	4.2±4.7	2.7±1.2	2.8±1.3	2.4±0.8	6.1±4.7
	Gly/SLs	9.1±4.0	12.2±2.9	13.5±0.9	11.8±1.0	12.8±1.1	17.2±1.0*
	Gly/Sterols	23.2±26.0	7.9±6.5	7.7±2.0	6.1±2.3	4.2±3.3	18.1±14.9

Gly: Glycerides. PLs: Phospholipids. SLs: Sphingolipids. *p < 0.05, **p < 0.01, ***p < 0.001, ns as not significant, versus the 1% group. Data are shown as the mean ± S.E.M.

Supplementary Table 3

Supplementary Table 3 List of antibodies used for western blot and immunofluorescent (related to Methods)

Antibody	Company	Species
SIRT1	Cell Signaling	Rabbit
PGC-1 α	Abcam	Rabbit
LC3B	Sigma	Rabbit
β -actin	Sigma	Mouse
Complex I	Abcam	Rabbit
Complex IV	Abcam	Mouse
Complex V	Abcam	Rabbit
p65	Abcam	Rabbit
Ac-p65	Abcam	Rabbit
TOM20	Santa Cruz	Rabbit
BODIPY	Cell Signaling	Rabbit
HSP60	Cell Signaling	Rabbit
DAPI	Cell Signaling	Rabbit
Anti-Rabbit	Invitrogen	
Anti-Mouse	Invitrogen	
Goat anti-rabbit IgG	Thermos Fisher Scientific	

SIRT1: NAD-dependent deacetylase sirtuin-1. PGC-1 α : the peroxisome proliferator-activated receptor-gamma coactivator-1alpha. LC3B: autophagy marker light chain 3B. β -actin: beta-actin (human gene and protein abbreviation ACTB/ACTB). p65: nuclear factor NF-kappa-B p65. Ac-p65: acetyl-NF-kappa-B p65. TOM20: the mitochondrial preprotein translocases of the outer membrane 20. BODIPY: fluorinated boron-dipyrromethene. HSP60: heat shock protein 60. DAPI: 4',6-diamidino-2-phenylindole, dihydrochloride. Goat anti-rabbit IgG: goat anti-rabbit Immunoglobulin G.

Supplementary Table 4

Supplementary Table 4 List of primers used for qPCR (related to Methods)

Gene		Forward and Reverse primer (5' to 3')	Size
mtDNA	F	CCCAGCTACTACCATCATTCAAGT	117
	R	GATGGTTTGGGAGATTGGTTGATGT	
β-globin	F	AAGGTGAACGCCGATGAAGT	81
	R	ATCAAAGTACCGCTGGGTCC	
Acaa2	F	CCTGCTACGAGGTGTGTTCA	76
	R	GAAGTCCTTGAGAAGGCCCC	
Acadl	F	TGCACACATACAGACGGTGC	130
	R	CATGGAAGCAGAACCGGAGT	
Acadm	F	TGACAAAAGCGGGGAGTACC	144
	R	CCATACGCCAACTCTTCGGT	
Hadha	F	AGTGGAAAGCGTGACTCCAG	115
	R	GTAGTGCATGCCGATCACCT	
Acaca	F	CGATCTATCCGTCGGTGGTC	118
	R	GGTCTGCCATCTTAATGTATTCTGC	
Fasn	F	TGCACCTCACAGGCATCAAT	104
	R	GTCCCACTTGATGTGAGGGG	
Rpl13a	F	TCCCTCCACCCTATGACAAG	136
	R	GTCACCTGCCTGGTACTTCC	

mtDNA: mitochondrial DNA. β-globin: beta globin. Acaa2: acetyl-CoA acyltransferase 2. Acadl: acyl-CoA dehydrogenase long chain. Acadm: acyl-CoA dehydrogenase medium-chain. Hadha: hydroxyacyl-CoA dehydrogenase trifunctional multienzyme complex subunit alpha. Acaca: acetyl-CoA carboxylase alpha. Fasn: fatty acid synthase. Rpl13a: ribosomal protein 13a (reference gene).