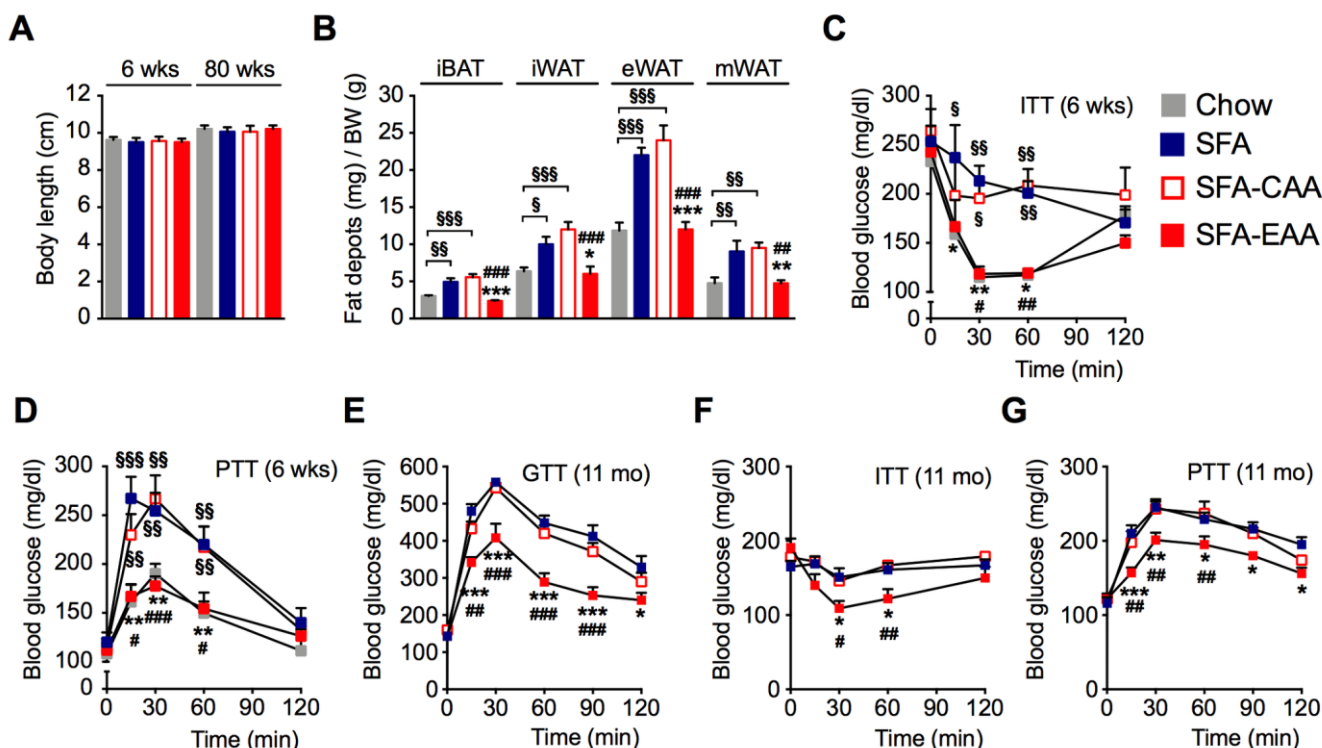


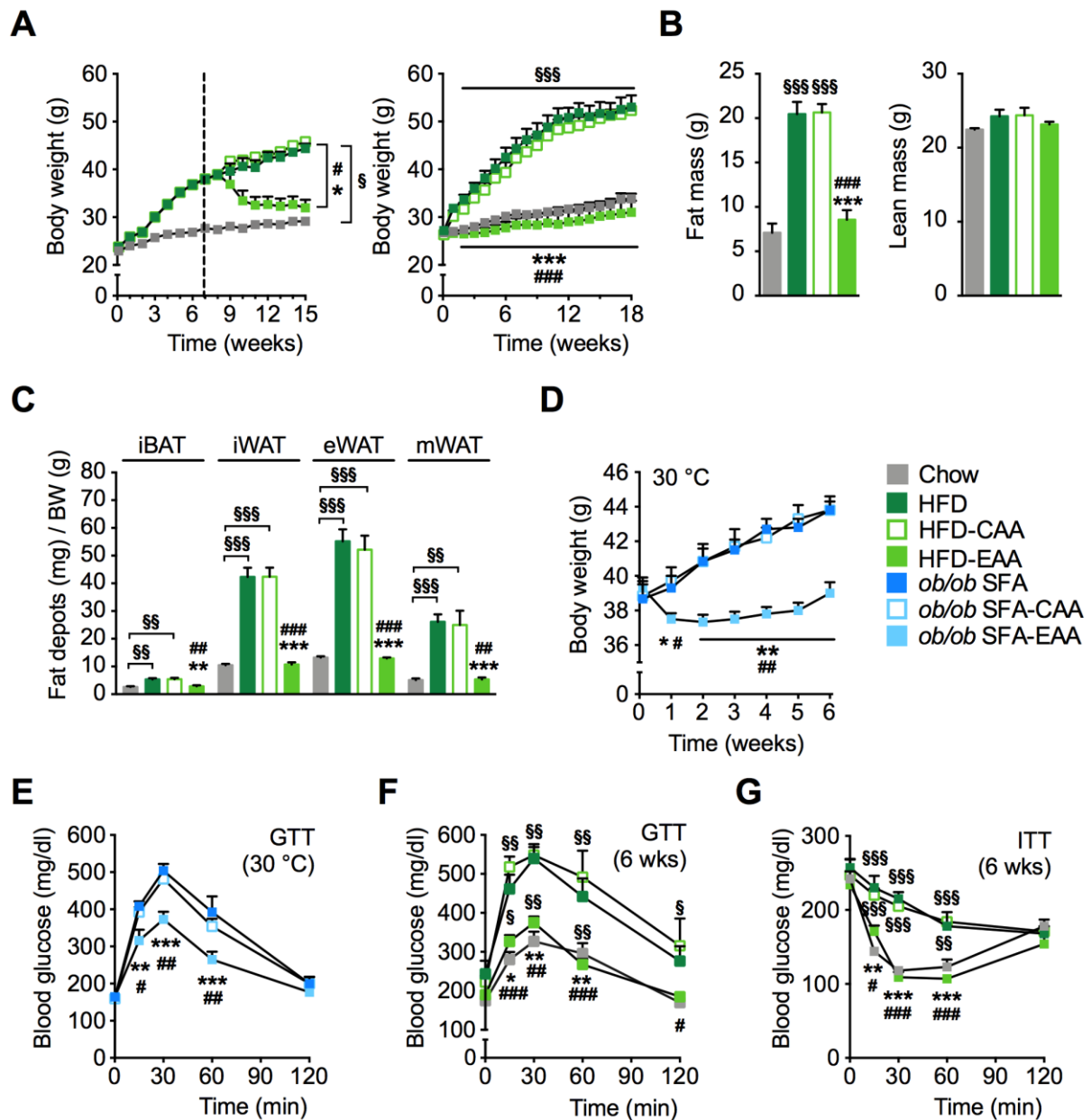
Online Supplemental Materials

Manipulation of dietary amino acids prevents and reverses obesity in mice through multiple mechanisms that modulate energy homeostasis

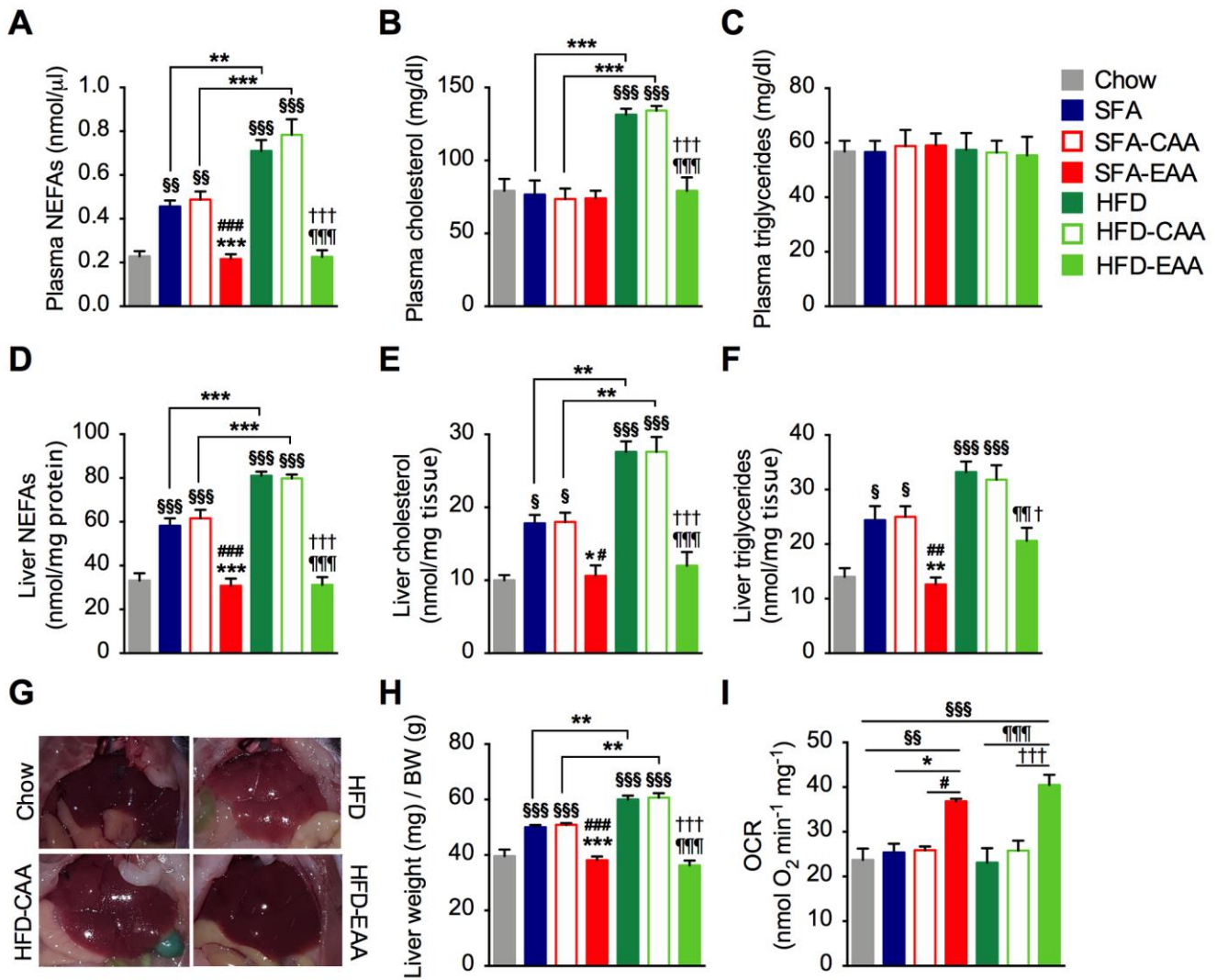
Chiara Ruocco, Maurizio Ragni, Fabio Rossi, Pierluigi Carullo, Veronica Ghini, Fabiana Piscitelli, Adele Cutignano, Emiliano Manzo, Rafael Maciel Ioris, Franck Bontems, Laura Tedesco, Carolina Greco, Annachiara Pino, Ilenia Severi, Dianxin Liu, Ryan P. Ceddia, Luisa Ponzoni, Leonardo Tenori, Lisa Rizzetto, Matthias Scholz, Kieran Tuohy, Francesco Bifari, Vincenzo Di Marzo, Claudio Luchinat, Michele O. Carruba, Saverio Cinti, Ilaria Decimo, Gianluigi Condorelli, Roberto Coppari, Sheila Collins, Alessandra Valerio, and Enzo Nisoli



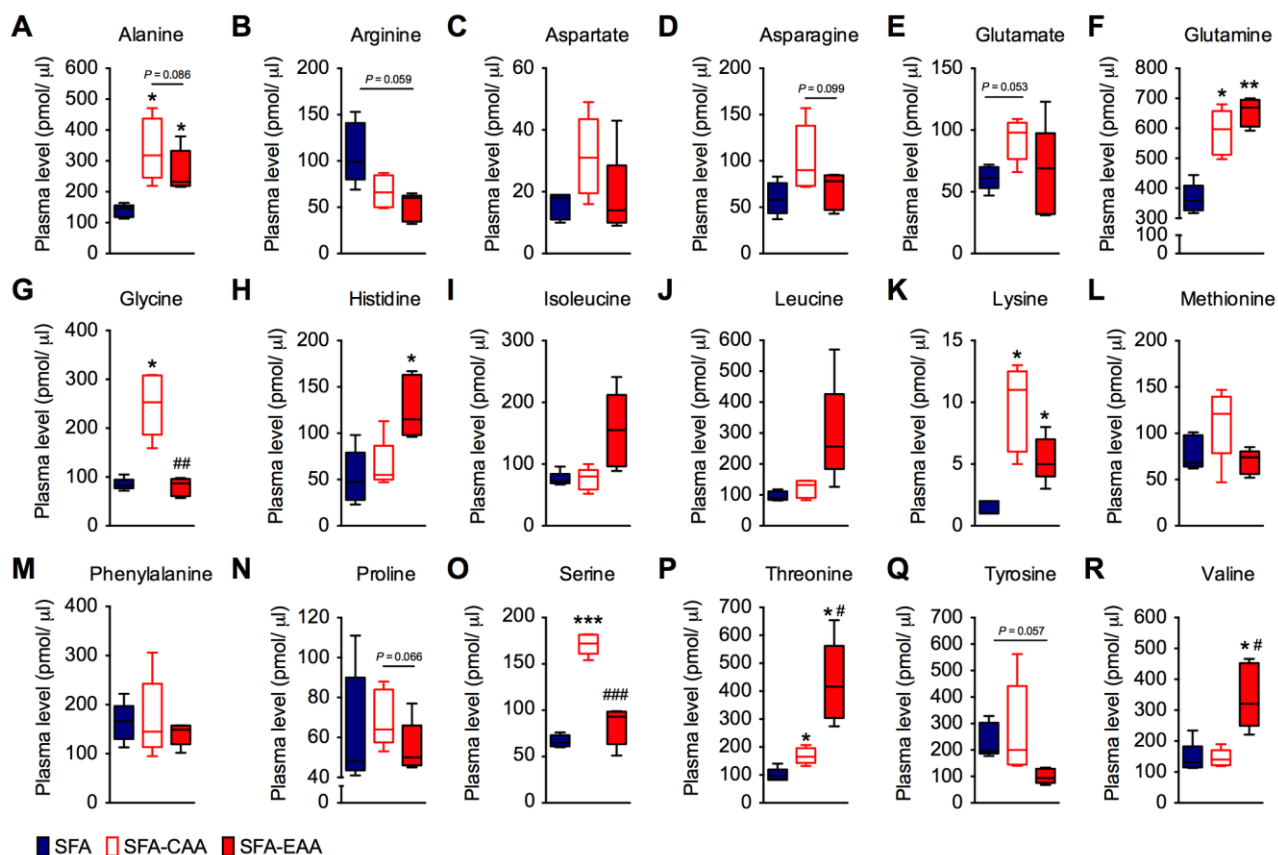
Supplementary Figure 1 — EAA unlike CAA substitution reduces fat accumulation maintaining the healthy effect on glucose homeostasis also after a long period of treatment. **A**: Body length as nasal-to-anal distance ($n = 8$ mice per group) of mice fed with different dietary regimens for 6 and 80 weeks. **B**: Weight of different fat pads expressed as mg over whole-body weight. **C-G**: Insulin tolerance tests (ITT) (**C** and **F**), pyruvate tolerance tests (PTT) (**D** and **G**), and glucose tolerance tests (**E**) in mice fed with chow, SFA, SFA-CAA, and SFA-EAA diets for 6 weeks or 11 months. iBAT, interscapular brown fat; iWAT, inguinal fat; eWAT, epididymal fat; mWAT, mesenteric fat. All data are presented as mean $\pm$ SEM. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ vs. SFA diet; $\#P < 0.05$, $\#\#P < 0.01$, and $\#\#\#P < 0.001$ vs. SFA-CAA diet; $\$P < 0.05$, $\$\$P < 0.01$, and $\$\$\$P < 0.001$ vs. chow diet.



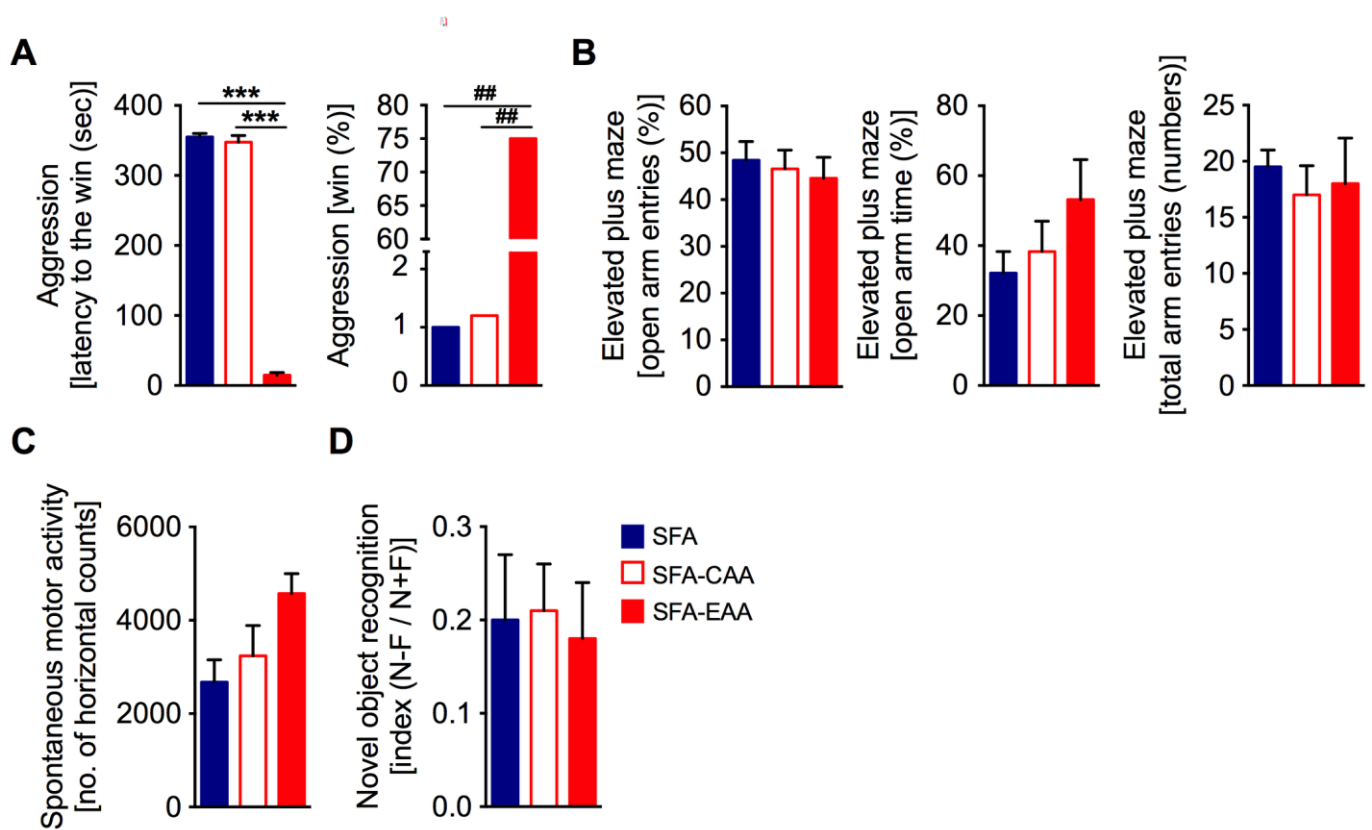
Supplementary Figure 2 — EAA-substituted diet prevents and reverses obesity and glucose imbalance induced either by consumption of HFD diet or in *ob/ob* mice. **A**: (Left) Body weight of mice fed with chow, HFD, HFD-EAA, and HFD-CAA diet at room temperature ($n = 7-10$ mice per group). After nine weeks (dashed line), mice fed with HFD were switched to either HFD-EAA diet (HFD > HFD-EAA) or HFD-CAA diet (HFD > SFA-CAA) ($n = 5$ mice per group). (Right) Body weight of mice fed with chow, HFD, HFD-EAA, and HFD-CAA diet for 18 weeks ($n = 5$ mice per group). **B**: Body composition: fat mass (left) and lean mass (right). **C**: Weight of different fat pads expressed as mg over whole-body weight. Mice in **B** and **C** were fed with HFD, HFD-EAA, and HFD-CAA for six weeks ($n = 5-6$ mice per group). **D** and **E**: Body weight (**D**) and glucose tolerance tests (GTT) (**E**) in *ob/ob* mice, housed at thermoneutrality (30 °C) and fed with SFA, SFA-EAA, and SFA-CAA for six weeks ($n = 6$ mice per group). **F** and **G**: Glucose homeostasis in HFD groups. GTT (**F**) and insulin tolerance tests (ITT) in mice fed with chow, HFD, HFD-EAA, and HFD-CAA diets for six weeks ($n = 5$ mice per group). All data are presented as mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ vs. SFA or HFD diet; # $P < 0.05$, ## $P < 0.01$, and ### $P < 0.001$ vs. SFA-CAA diet and HFD-CAA; § $P < 0.05$, §§ $P < 0.01$, and §§§ $P < 0.001$ vs. chow diet.



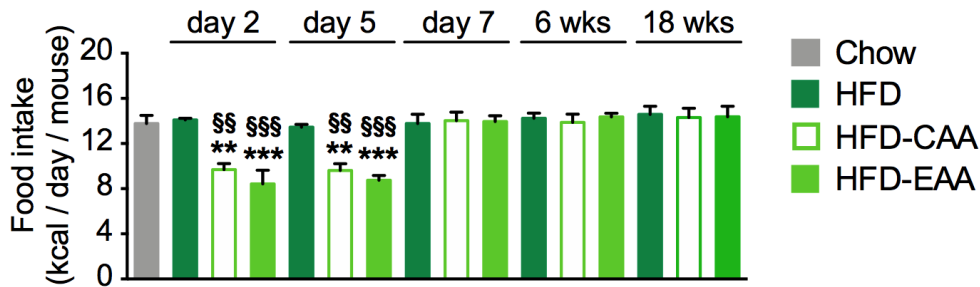
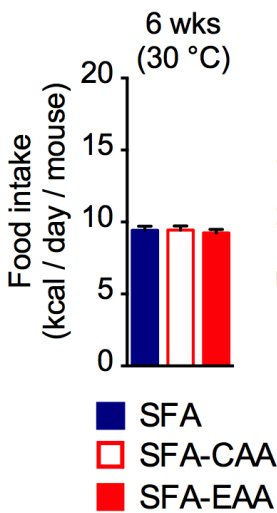
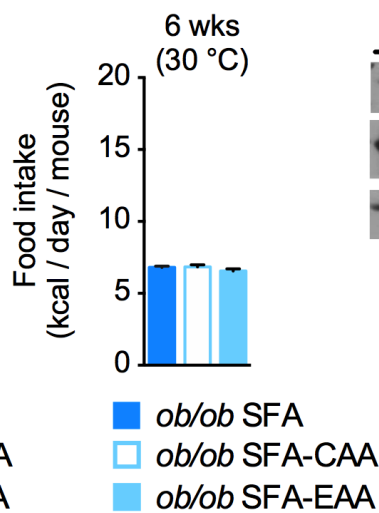
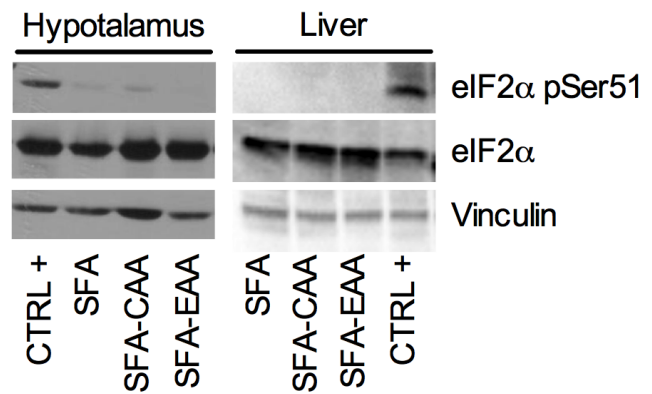
Supplementary Figure 3 — Effects on lipid homeostasis. A-C: Plasma concentrations of nonesterified fatty acids (NEFAs) (nmol/μl) (A), cholesterol (mg/dl) (B), and triglycerides (mg/dl) (C). D-F: Hepatic content of NEFAs (nmol/mg protein) (D), total cholesterol (nmol/mg tissue) (E), and triglycerides (nmol/mg tissue) (F). G: Representative macroscopic pictures of the liver. H: Liver weight (mg/BW). I: Oxygen consumption rates (OCR) were normalized to mitochondrial protein content. Mitochondria were isolated from liver of mice fed with different dietary regimens for 6 weeks at room temperature. Data are presented as means ± SEM ($n = 5-8$ mice per group). * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ vs. SFA-fed mice; $^{\$}P < 0.05$, $^{\$\$}P < 0.01$, and $^{\$ \$ \$}P < 0.001$ vs. chow diet-fed mice; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. SFA-CAA-fed mice; $^{\P}P < 0.01$ and $^{\P \P \P}P < 0.001$ vs. HFD-fed mice; $^{\dagger}P < 0.05$ and $^{\dagger \dagger \dagger}P < 0.001$ vs. HFD-CAA-fed mice.



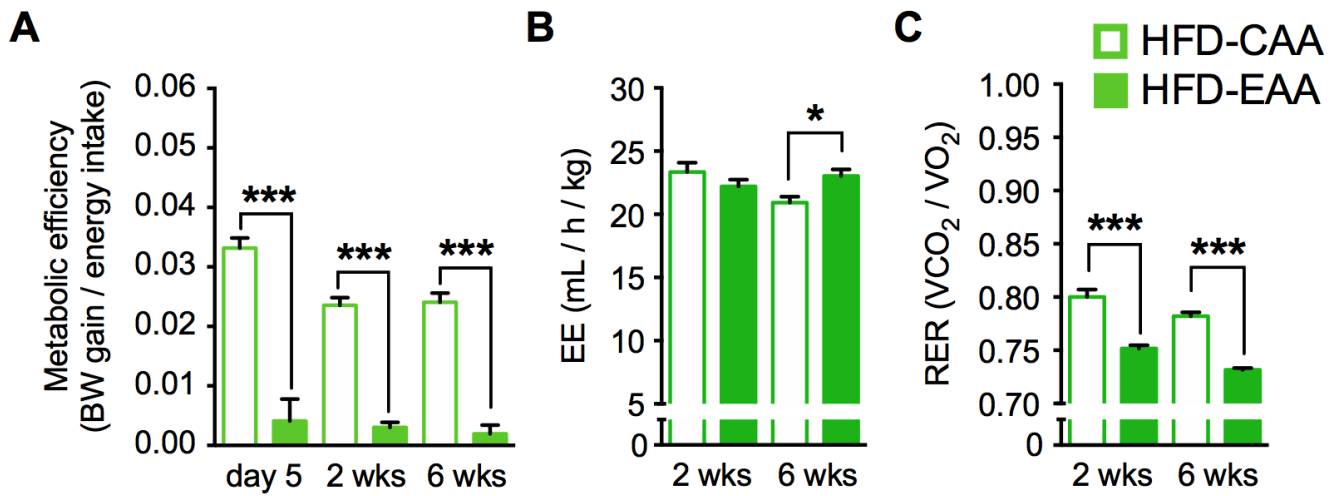
Supplementary Figure 4 — Plasma amino acid profile. A-R: Amino acid concentrations in plasma samples of mice fed with SFA, SFA-EAA, and SFA-CAA diet for six weeks at room temperature. Box plots, with center hinge representing the mean, and the upper and lower hinges representing the first and third quartiles; whiskers represent 1.5 times the interquartile range. All data are presented as mean $\pm$ SEM ($n = 5$ mice per group). * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ vs. SFA diet; # $P < 0.05$, ## $P < 0.01$, and ### $P < 0.01$ vs. SFA-CAA.



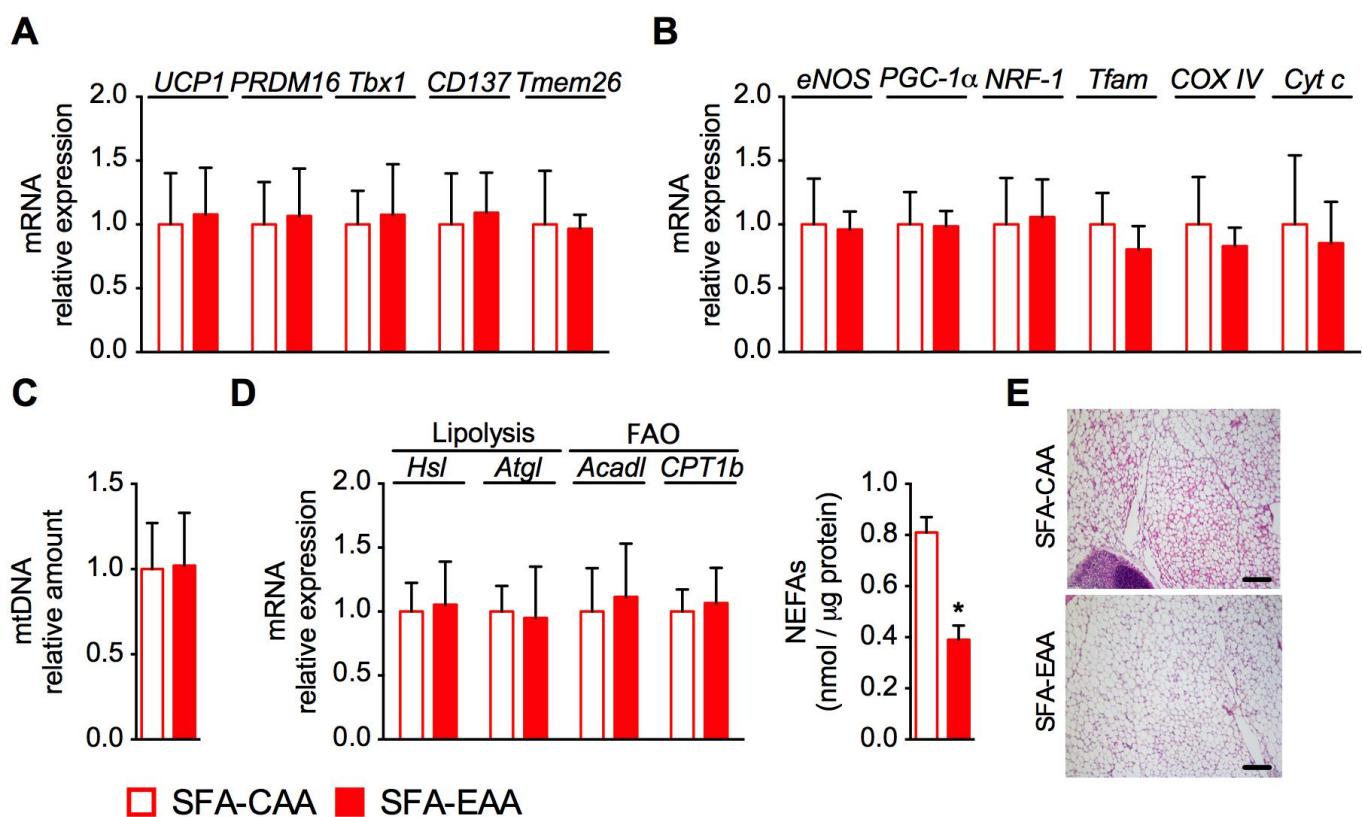
Supplementary Figure 5 — SFA-EAA diet consumption preserved energetic behavior. Mice fed with SFA, SFA-CAA, and SFA-EAA for 20 months at room temperature. **A:** Energetic behavior, as demonstrated by the percentage of winning in the tube test for social dominance, was preserved in mice fed with SFA-EAA compared to SFA and SFA-CAA diets. **B:** Anxiety-like behaviour, **C:** Motor activity and **D:** Episodic memory were similar in all dietary groups. Mice were subjected to familiarization (T1) and novel object recognition (T2). The novel object recognition task was performed using a delay time of 120 min. The performance was evaluated by calculating a discrimination index ($N-F / N+F$), where N = time spent exploring the new object during T2, F = time spent exploring the familiar object during T2. Mean $\pm$ SEM are reported ($n = 5$ mice per group). *** $P < 0.001$ vs. SFA- and SFA-CAA-fed mice (Student t-test) and ## $P < 0.01$ vs. SFA- and SFA-CAA-fed mice (Chi-square test).

A**B****C****D**

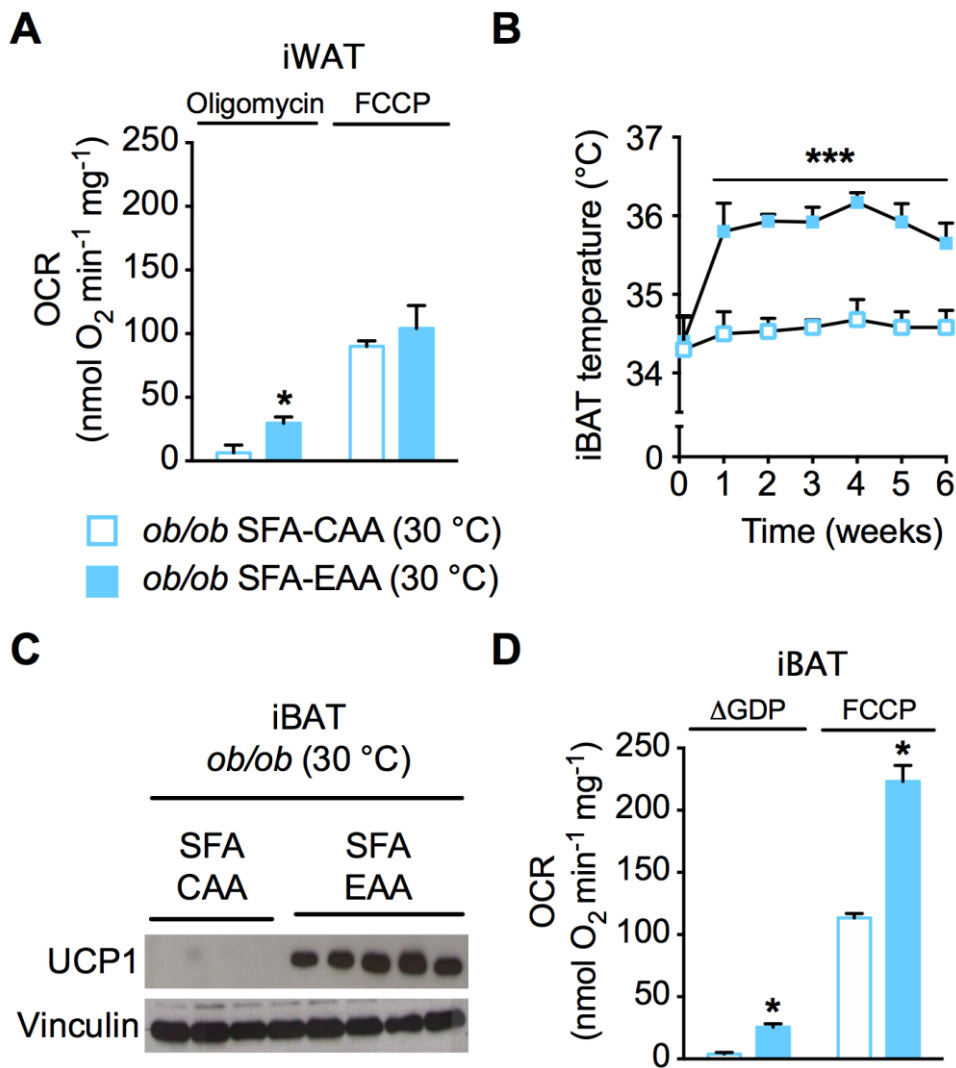
Supplementary Figure 6 — Both EAA and CAA substitution does not affect food intake and stress response pathway linked to amino acid balance in both SFA and HFD diet. *A-D*: Food intake was measured twice a week in different models of obesity ($n = 5-10$ mice per group). *E*: Western blot analysis of (Ser51) phosphorylation of α subunit of eukaryotic translation initiation factor 2 (eIF2)—a marker of kinase general control nonderepressible 2 (GCN2) kinase activity, the critical regulator of cellular responses under amino acid deficiency sensor—in hypothalamus and liver of mice fed with SFA-CAA and SFA-EAA diet for six weeks at room temperature. One experiment representative of three reproducible ones is shown. CTRL+, internal positive control (*i.e.*, amino acid starved HeLa cells). All data are presented as mean $\pm$ SEM.



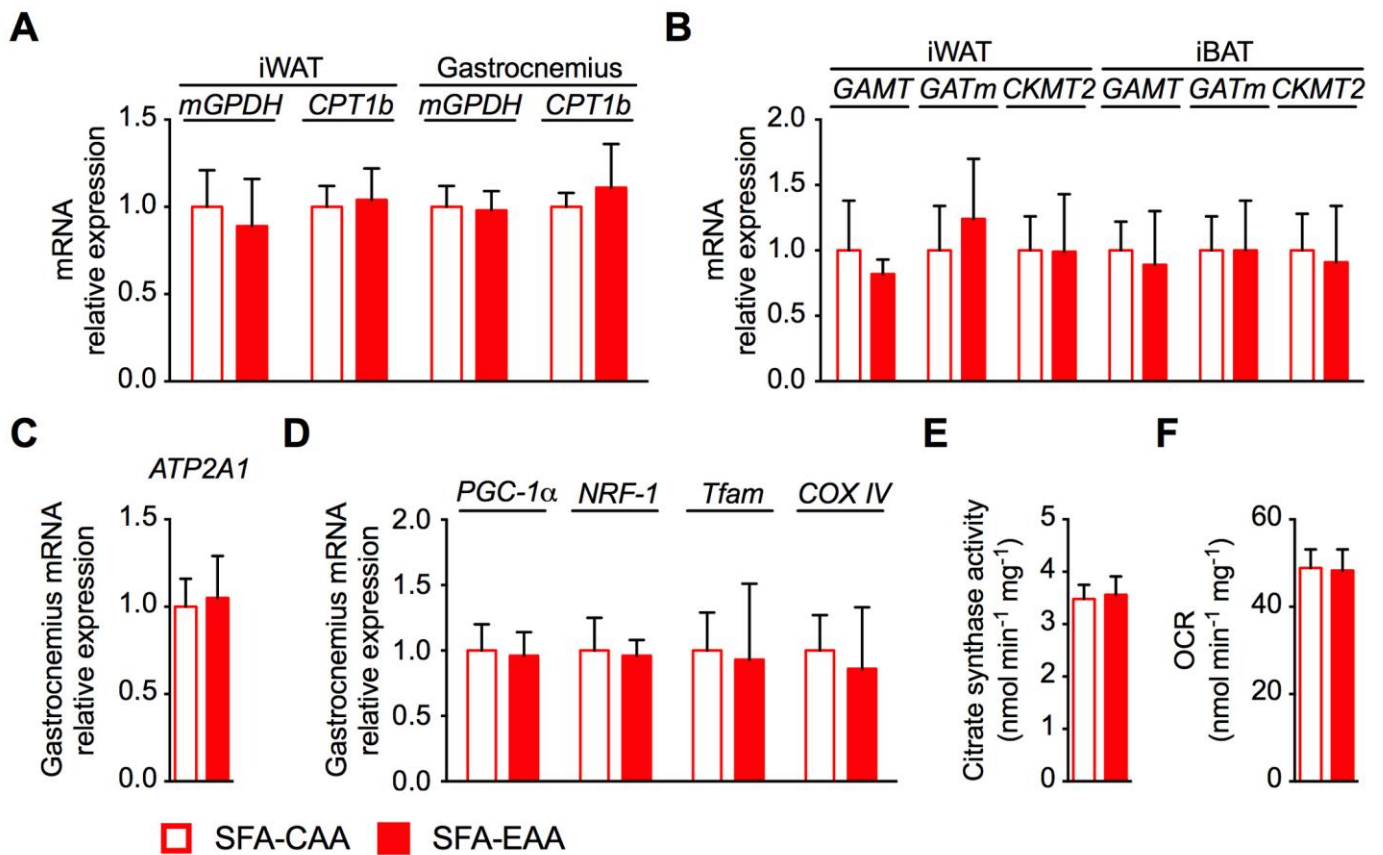
Supplementary Figure 7 — Energy metabolism in mice on HFD diets. *A*: Metabolic efficiency was calculated as the ratio between the body weight gain and the energy intake (*i.e.*, total food consumed during five days or 2 weeks or 6 weeks). *B* and *C*: Energy expenditure (EE) (*B*) and respiratory exchange ratio (RER) (*C*) during one 24 h cycle. Mice in *B* and *C* were fed with HFD-CAA and HFD-EAA diets in two separate experiments for 2 or 6 weeks. All data are presented as mean $\pm$ SEM. * $P < 0.05$, and *** $P < 0.001$ vs. HFD-CAA diet.



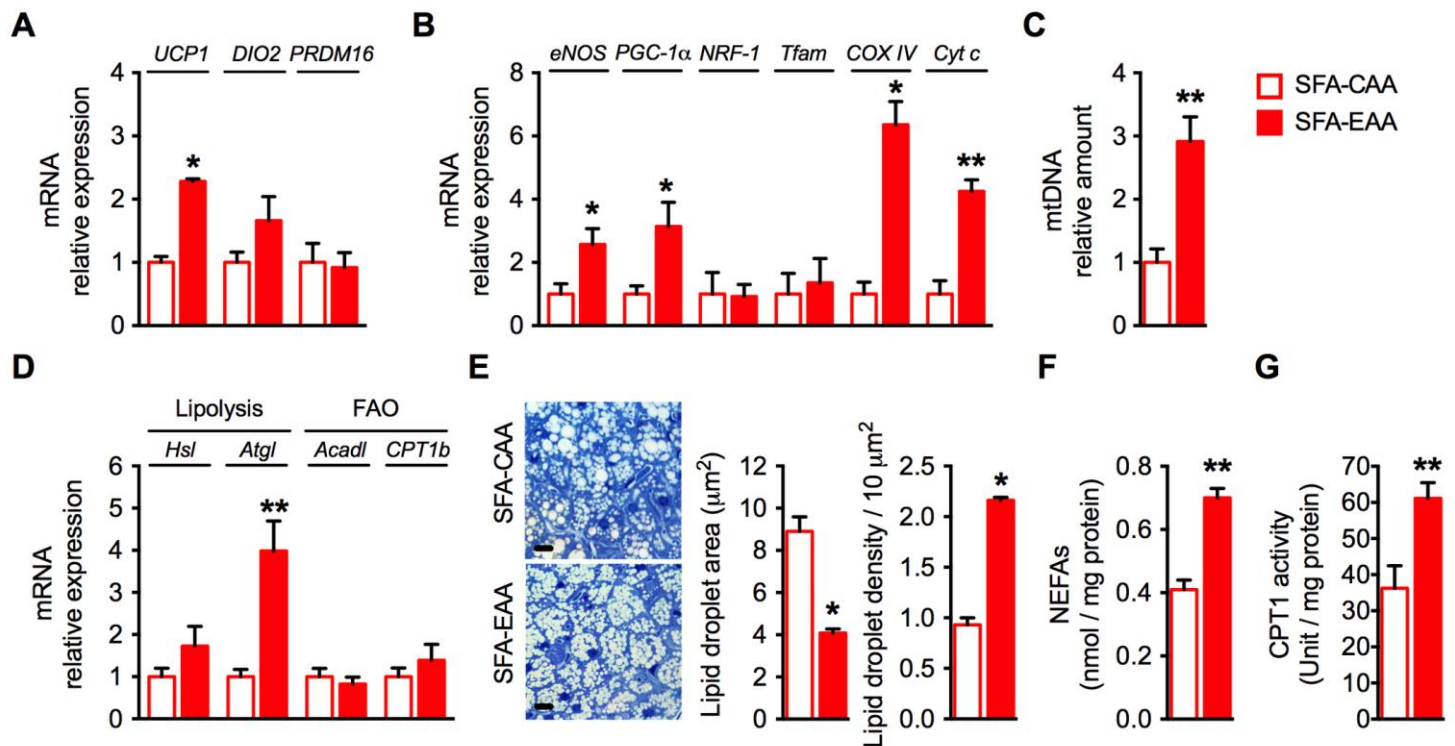
Supplementary Figure 8 — SFA-EAA diet does not promote browning of iWAT. **A**: Relative mRNA levels of *UCP1* and browning genes. **B**: Relative mRNA levels of *eNOS* and mitochondrial biogenesis genes. **C**: Mitochondrial DNA amount. **D**: Relative mRNA levels of lipolysis and fatty acid oxidation genes (left) and non-esterified fatty acid (NEFA) levels (right). The experiments were repeated at least three times. **E**: Hematoxylin and eosin staining of semithin sections (3 μm) of iWAT. Scale bar, 50 μm. All data are presented as mean ± SEM ($n = 5$ mice per group). * $P < 0.05$ vs. SFA-CAA diet.



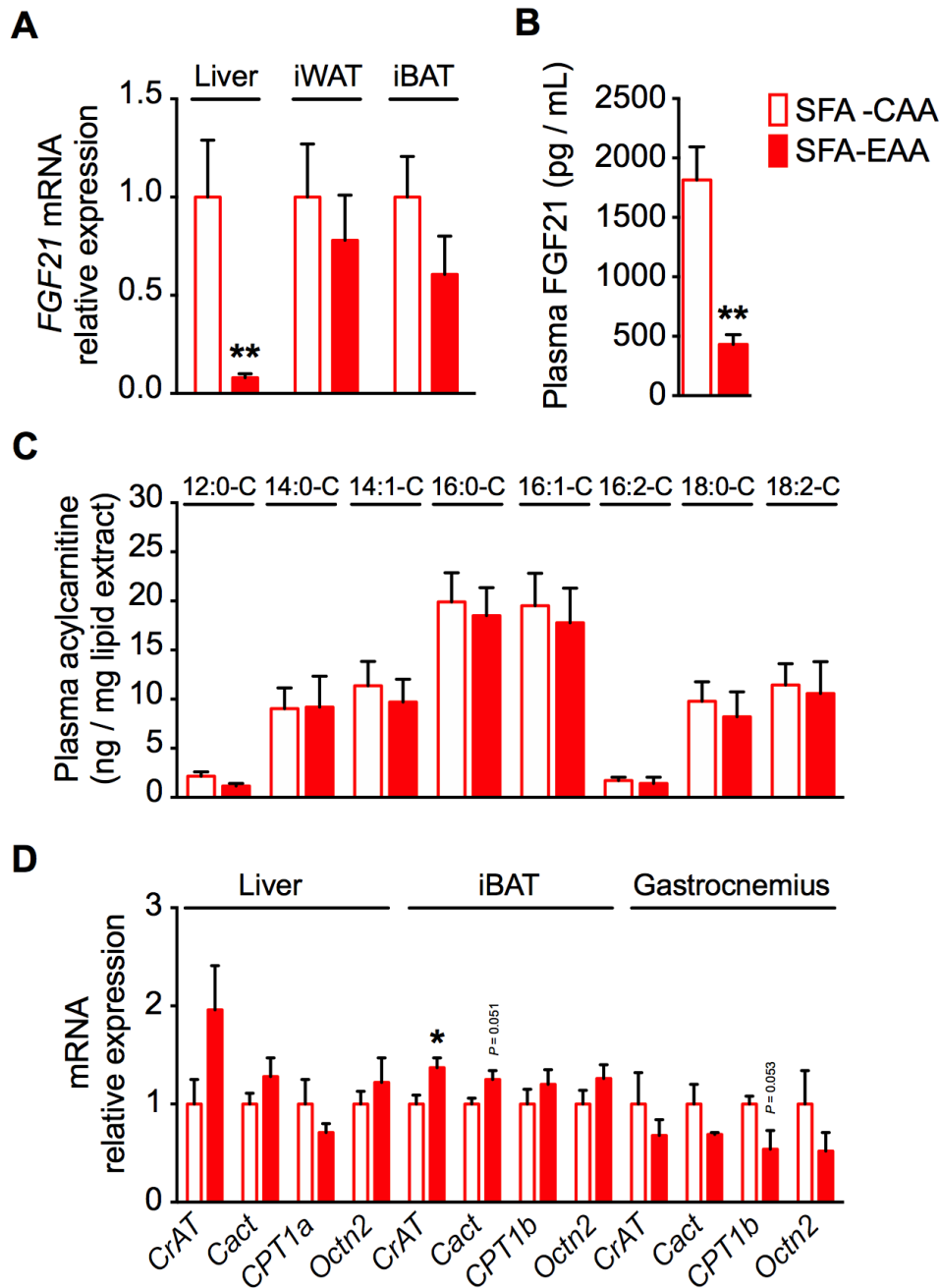
Supplementary Figure 9 — SFA-EAA diet confirms its thermogenic effects on iWAT and iBAT of *ob/ob* mice. **A:** Uncoupled (*i.e.*, with oligomycin) and maximal (*i.e.*, with FCCP) oxygen consumption rates (OCR) in mitochondria isolated from iWAT; respiration was normalized to mitochondrial protein content ($n = 5$ mice per group). **B:** iBAT temperature measurement with Flir camera ($n = 6$ mice per group). **C:** Western blot analysis of UCP1 and vinculin protein expression in iBAT. One experiment representative of three reproducible ones ($n = 5$ mice per group). **D:** UCP-1-dependent (*i.e.*, Δ GDP) and maximal (*i.e.*, with FCCP) OCR in iBAT mitochondria ($n = 6$ mice per group); respiration was normalized to mitochondrial protein content. *ob/ob* mice in **A-D** were fed with SFA-CAA and SFA-EAA diet for six weeks at thermoneutrality. All data are presented as mean $\pm$ SEM. * $P < 0.05$ and *** $P < 0.001$ vs. SFA-CAA diet.



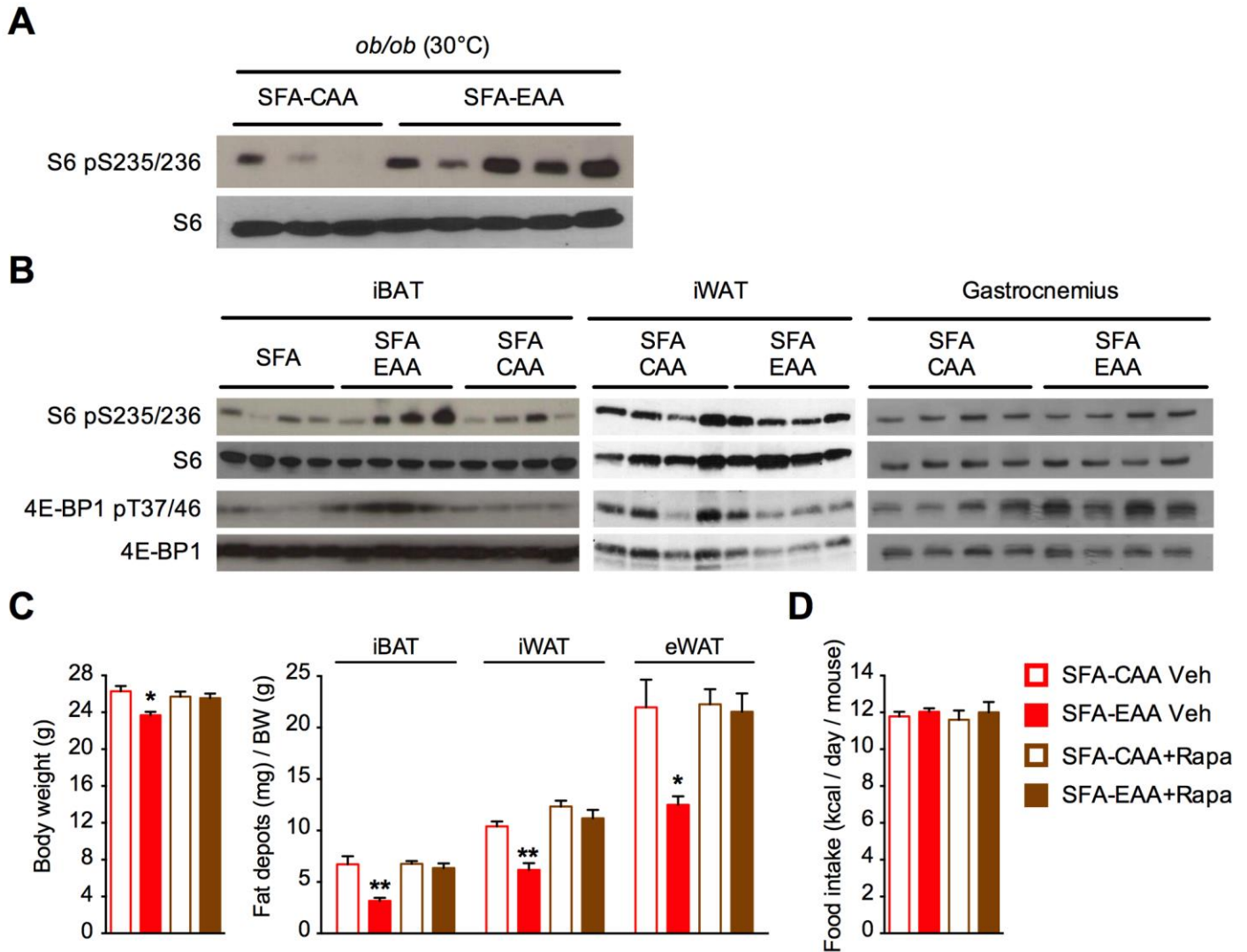
Supplementary Figure 10 — SFA-EAA diet does not promote futile cycle activation or mitochondrial biogenesis stimulation in adipose and muscle. A-C: Relative mRNA levels of futile cycles genes, including glycerol phosphate shuttle in iWAT and gastrocnemius (A), creatine-driven substrate cycle in iWAT and iBAT (B), and calcium release and reuptake cycle in gastrocnemius (ATPase Sarcoplasmic/Endoplasmic Reticulum Calcium Transporting 1 - ATP2A1) (C) of mice fed with SFA-CAA and SFA-EAA diet for six weeks at room temperature. D-F: Mitochondrial biogenesis and function in gastrocnemius muscle of mice fed with SFA-CAA and SFA-EAA diet for six weeks at room temperature. Relative mRNA levels of mitochondrial biogenesis genes (D), citrate synthase activity (normalized to protein content) (E), and oxygen consumption rates (OCR) (normalized to mitochondrial protein content) (F). All data are presented as mean $\pm$ SEM ($n = 5-6$ mice per group).



Supplementary Figure 11 — SFA-EAA diet increases expression of genes involved in mitochondrial biogenesis, thermogenesis, lipolysis, and fatty acid oxidation in iBAT of mice fed with SFA-CAA and SFA-EAA diet for six weeks at room temperature. **A**: Relative mRNA levels of thermogenic genes. **B**: Relative mRNA levels of mitochondrial biogenesis genes. **C**: Mitochondrial DNA (mtDNA) amount. **D**: Relative mRNA levels of lipolysis and fatty acid oxidation (FAO). The experiments were repeated at least three times. **E**: Lipid droplets in iBAT slices (toluidine blue staining) and lipid droplet area and density. Scale bar, 25 μm . **F**: Non-esterified free fatty acid (NEFA) levels. **G**: Carnitine palmitoyltransferase I (CPT1) activity in iBAT mitochondria. All data are presented as mean $\pm$ SEM ($n = 5-6$ mice per group). * $P < 0.05$ and ** $P < 0.01$ vs. SFA-CAA diet.



Supplementary Figure 12 — SFA-EAA diet does not induce production of thermogenic FGF21 and long-chain acylcarnitines. *A*: Relative mRNA levels of *Fgf21* gene in different tissues of mice fed with SFA-CAA and SFA-EAA diet for six weeks at room temperature. *B*: Plasma FGF21 concentrations in mice fed with SFA-CAA and SFA-EAA diet for six weeks at room temperature. *C*: Serum long-chain acylcarnitine levels of mice fed with SFA-CAA and SFA-EAA diet for six weeks at room temperature analyzed using tandem mass spectrometry. *D*: Relative mRNA levels of genes implicated in acylcarnitine synthesis in different tissues of mice fed with SFA-CAA and SFA-EAA diet for six weeks at room temperature. The experiments were repeated at least three times. CrAT, carnitine acetyltransferase; Cact, carnitine-acylcarnitine translocase; CPT1a, carnitine palmitoyltransferase 1a; CPT1b, carnitine palmitoyltransferase 1b; Octn2, organic cation transporter type 2. All data are presented as mean $\pm$ SEM ($n = 5$ mice per group). * $P < 0.05$ and ** $P < 0.01$ vs. SFA-CAA diet.



Supplementary Figure 13 — mTORC1 signaling contributes to iBAT thermogenesis induced by SFA-EAA diet under different experimental settings. **A:** Western blot analysis of (Ser 235/236) phosphorylated S6 and S6 protein levels in iBAT of *ob/ob* mice fed with SFA-CAA and SFA-EAA diet for six weeks at thermoneutrality. One immunoblot experiment representative of three reproducible ones ($n = 3-5$ mice per group). **B:** Western blot analysis of mTORC1 pathway in iBAT, iWAT, and muscle of mice fed with SFA, SFA-CAA, and SFA-EAA diet for six weeks at room temperature ($n = 4$ mice per group). **C:** Body weight (left) and fat depots (expressed as mg over whole-body weight) (right). **D:** Food intake (expressed as kcal per mouse per day). Mice in **C** and **D** were fed with SFA-CAA and SFA-EAA diet for six weeks at room temperature, with or without Rapa (i.p. 2.5 mg/kg body weight) delivered in 200 μ l, 5 days per week for six weeks, starting with diets ($n = 5-6$ mice per group). All data are presented as mean $\pm$ SEM. * $P < 0.05$ and ** $P < 0.01$ vs. Veh-treated mice fed with SFA-CAA diet.

Supplementary Table 1 — Diet compositions

Dietary interventions										
% kcal from	SFA	SFA-EAA		SFA-CAA		HFD	HFD-EAA		HFD-CAA	
Protein	20	20		20		20	20		20	
Carbohydrate	70	70		70		20	20		20	
Fat	10	10		10		60	60		60	
kcal/g	3.8	3.8		3.8		5.2	5.2		5.2	
Amino acid sources										
Amino acids (g)	SFA	SFA-EAA		SFA-CAA		HFD	HFD-EAA		HFD-CAA	
	casein ^a	casein ^b	EAAm ^c	casein ^b	CAAm ^d	casein ^a	casein ^b	EAAm ^c	casein ^b	CAAm ^d
Leucine	18	1.17	58.6	1.17	16.8	18	1.17	58.6	1.17	16.8
Valine	10.6	0.69	29.22	0.69	9.91	10.6	0.69	29.22	0.69	9.91
Lysine	15	0.98	30.34	0.98	14.03	15	0.98	30.34	0.98	14.03
Isoleucine	8.6	0.56	29.22	0.56	8.04	8.6	0.56	29.22	0.56	8.04
Threonine	8.2	0.53	16.48	0.53	7.67	8.2	0.53	16.48	0.53	7.67
Histidine	5.2	0.34	7.12	0.34	4.86	5.2	0.34	7.12	0.34	4.86
Cysteine /cystine ^e	3.4	0.09	7.12	0.09	1.31	3.4	0.09	7.12	0.09	1.31
Phenylalanine	9.6	0.62	4.68	0.62	8.98	9.6	0.62	4.68	0.62	8.98
Methionine	5.8	0.38	2.44	0.38	5.42	5.8	0.38	2.44	0.38	5.42
Tyrosine	10.4	0.68	1.31	0.68	9.72	10.4	0.68	1.31	0.68	9.72
Tryptophan	2.4	0.17	0.94	0.17	2.24	2.4	0.17	0.94	0.17	2.24
Alanine	5.8	0.4	-	0.4	5.42	5.8	0.4	-	0.4	5.42
Arginine	6.8	0.44	-	0.44	6.36	6.8	0.44	-	0.44	6.36
Aspartic acid	13.8	0.9	-	0.9	12.9	13.8	0.9	-	0.9	12.9
Glutamic acid	43.4	2.82	-	2.82	40.58	43.4	2.82	-	2.82	40.58
Glycine	3.4	0.22	-	0.22	3.18	3.4	0.22	-	0.22	3.18
Proline	20.2	1.31	-	1.31	18.89	20.2	1.31	-	1.31	18.89
Serine	11.4	0.74	-	0.74	10.66	11.4	0.74	-	0.74	10.66
Caloric amino acid intake ^f										
			Δ caloric intake		Δ caloric intake			Δ caloric intake	HFD- CAA	Δ caloric intake
kcal/day	SFA	SFA-EAA		SFA-CAA		HFD	HFD-EAA			
Leucine	1.041	3.425	2.384	1.042	0.001	1.027	3.326	2.299	1.029	0.003
Valine	0.585	1.709	1.125	0.620	0.035	0.577	1.660	1.084	0.612	0.036
Lysine	0.864	1.785	0.921	0.871	0.007	0.852	1.733	0.882	0.860	0.009
Isoleucine	0.497	1.704	1.207	0.498	0.001	0.490	1.655	1.165	0.492	0.002
Threonine	0.474	0.973	0.500	0.481	0.008	0.467	0.945	0.478	0.476	0.009
Histidine	0.296	0.419	0.123	0.296	0.000	0.292	0.407	0.115	0.292	0.000
Cysteine /cystine ^e	0.192	0.405	0.213	0.078	-0.114	0.189	0.393	0.204	0.077	-0.113
Phenylalanine	0.563	0.307	-0.256	0.560	-0.003	0.555	0.299	-0.257	0.553	-0.002
Methionine	0.348	0.152	-0.196	0.329	-0.019	0.343	0.148	-0.195	0.325	-0.018
Tyrosine	0.591	0.110	-0.481	0.606	0.015	0.582	0.106	-0.476	0.598	0.016
Tryptophan	0.147	0.067	-0.080	0.141	-0.006	0.145	0.065	-0.080	0.140	-0.006
Alanine	0.329	-	-	0.343	0.013	0.325	-	-	0.338	0.014
Arginine	0.386	-	-	0.389	0.003	0.380	-	-	0.384	0.004
Aspartic acid	0.781	-	-	0.807	0.026	0.770	-	-	0.797	0.027
Glutamic acid	2.205	-	-	2.499	0.294	2.174	-	-	2.469	0.294
Glycine	0.192	-	-	0.201	0.009	0.189	-	-	0.199	0.010
Proline	1.147	-	-	1.180	0.033	1.131	-	-	1.165	0.035
Serine	0.647	-	-	0.667	0.020	0.638	-	-	0.659	0.021

^aSingle amino acid amount in 200 g of casein (composition provided by Research Diets); ^bsingle amino acid amount in 13 g of casein; ^csingle amino acid amount of EAAM [the specific amino acid formula is stoichiometrically similar to a mixture we previously found to promote mitochondrial biogenesis in cardiac and skeletal muscle when administered via drinking water (32)]; and ^dCAAM, purified amino acid mixture designed on the amino acid profile of casein. The amount of EAA and CAA mixture in the substituted diets was calculated as 93.5 % casein content (187 g out 200 g); ^ereported amount is equal to the cysteine/cystine ratio typically present in 200 g casein (*i.e.*, 1.4 g) added with further 3.0 g cystine; ^fcaloric amino acid intakes (kcal/day) were determined on average kcal/day consumed by mice fed with SFA (14.44 ± 0.61 kcal/day), SFA-EAA (14.29 ± 0.45 kcal/day), SFA-CAA (14.54 ± 0.61 kcal/day), HFD (14.24 ± 0.45 kcal/day), HFD-EAA (13.88 ± 0.73 kcal/day), and HFD-CAA (14.36 ± 0.33 kcal/day). The optimal amino acid amounts in the diets were assessed by preliminary experiments, in which different percentages of casein were substituted with the EAA mixture.

Supplementary Table 2 — Antibodies and assay kits

REAGENT	SOURCE	CAT. #	SAMPLE TYPE
Assay kits			
Leptin (mouse) ELISA Kit	Enzo Life Sciences	ADI-900-019A	Plasma
Mouse Adiponectin ELISA Kit	Abcam	ab108785	Plasma
Mouse/Rat FGF-21 Quantikine ELISA Kit	R&D Systems	MF2100	Plasma, iWAT, iBAT, liver
TSH (Rodent) ELISA Kit	Abnova	KA2336	Plasma
Free Fatty Acid Quantification Colorimetric/Fluorometric Kit	BioVision	K612-100	Plasma, iWAT, iBAT, liver
Mouse Ultrasensitive Insulin ELISA kit	Alpco	80-INSMSU-E01	Plasma
Mouse IGF-1 ELISA Kit	Thermo Fisher Scientific	EMIGF1	Plasma
Cholesterol Assay Kit	Abcam	ab65390	Plasma, Liver
Triglyceride Assay Kit	Abcam	ab65336	Plasma, Liver
Antibodies			
anti-p-S6 (Ser235/236)	Cell Signaling	2211	iWAT, iBAT, gastrocnemius, primary brown adipocytes
anti-S6	Cell Signaling	2217	iWAT, iBAT, gastrocnemius, primary brown adipocytes
anti-p-4EBP1 (Thr37/46)	Cell Signaling	2855	iWAT, iBAT, gastrocnemius
anti-4EBP1	Cell Signaling	9644	iWAT, iBAT, gastrocnemius
anti p-eIF2 α	Cell Signaling	3398	Hypothalamus, Liver
anti eIF2 α	Cell Signaling	5324	Hypothalamus, Liver
anti-COX IV	Cell Signaling	4844	iBAT
anti-Cyt c	Cell Signaling	4280	iBAT
anti-UCP1	Abcam	ab23841	iWAT, iBAT, primary brown adipocytes
anti-GAPDH	Cell Signaling	2118	iBAT, primary brown adipocytes
anti-HSP60	Cell Signaling	4870	iWAT
anti-vinculin	Sigma Aldrich	V9131	iBAT

Supplementary Table 3 — Mass spectrometry parameters

Amino acid	RT (min)	m/z	Calibration curve	r
Alanine	2.86	260.1	$y = 13573.47x$	0.99
Glycine	2.51	246.1	$y = 11700.40x$	0.99
Isoleucine	5.88	302.1	$y = 11676.90x$	0.97
Phenylalanine	6.53	336.1	$y = 6553.14x$	0.96
Valine	4.06	288.1	$y = 16128.62x$	0.97
Threonine	2.74	290.1	$y = 8556.89x$	0.99
Serine	2.46	276.1	$y = 8505.54x$	0.99
Tyrosine	3.62	352.1	$y = 2392.91x$	0.97
Glutamine	2.44	317.1	$y = 3891.44x$	0.93
Arginine- $^{13}\text{C}_6$	2.33	351.2	$y = 476.85x$	0.98
Lysine- D_4	3.81	321.1	$y = 1637.19x$	0.98
Proline	2.98	286.1	$y = 10992.47x$	0.98
Aspartic acid	2.57	304.1	$y = 4061.32x$	0.92
Glutamic acid	2.65	318.1	$y = 3228.65x$	0.97
Histidine	1.91	326.1	$y = 220.13x$	0.98
Methionine	3.89	320.1	$y = 5644.93x$	0.96
Asparagine	2.29	303.1	$y = 3236.46x$	0.99
Leucine	6.18	302.1	$y = 13907.29x$	0.98
Tryptophan	6.57	375.1	—	

Supplementary Table 4 — Levels of free amino acids in iBAT of mice fed with SFA-CAA or SFA-EAA diet (iBAT aminograms)

	SFA-CAA		SFA-EAA		Fold change	<i>P</i>
	pmol/mg iBAT	%	pmol/mg iBAT	%		
Leucine	15.11 ± 0.65	1.3	29.77 ± 1.12	2.5	1.97	0.03
Lysine	68.61 ± 1.06	6.0	23.84 ± 1.51	1.9	0.35	0.04
Isoleucine	15.97 ± 0.92	1.4	34.90 ± 2.06	3.0	2.19	0.04
Valine	10.85 ± 1.73	0.9	31.18 ± 0.78	2.6	2.87	0.03
Threonine	21.84 ± 1.81	1.9	157.88 ± 2.81	13.0	7.23	0.02
Cysteine/cystine	-	-	-	-	-	-
Histidine	-	-	-	-	-	-
Phenylalanine	23.99 ± 1.78	2.1	22.25 ± 1.23	1.8	0.93	0.95
Methionine	-	-	-	-	-	-
Tyrosine	31.10 ± 3.72	2.7	29.22 ± 2.20	2.4	0.94	0.98
Tryptophan	-	-	-	-	-	-
Alanine	285.14 ± 36.52	24.9	260.51 ± 52.36	21.4	0.91	0.97
Arginine	37.56 ± 1.42	3.3	70.73 ± 2.12	5.8	1.88	0.04
Aspartic acid	20.73 ± 2.34	1.8	19.22 ± 3.73	1.6	0.93	0.62
Glutamic acid	371.08 ± 25.40	32.4	343.99 ± 26.84	28.3	0.93	0.92
Glycine	124.19 ± 18.95	10.8	91.57 ± 13.56	7.5	0.74	0.04
Proline	4.85 ± 1.75	0.4	87.79 ± 1.43	7.2	18.12	0.03
Serine	114.27 ± 9.64	10.0	13.13 ± 1.22	1.1	0.11	0.03

Amino acid levels in iBAT were measured with UPLC-MS/MS (in technical triplicates) after night consumption of SFA-CAA or SFA-EAA diets (6 weeks of treatment) (*n* = 5 mice per group). Increased amino acids in SFA-EAA- vs SFA-CAA-fed mice (in red); decreased amino acids in SFA-EAA- vs SFA-CAA-fed mice (in blue); undetected amino acid (in grey). The amino acid percentages (w/V) (grey shadow) were used to prepare EAA and CAA mixtures for the *in vitro* experiments. Values are expressed as mean ± SEM.

Supplementary Table 5 — Primers for qRT-PCR

Gene	Primer Sense (5'-3')	Primer Antisense (5'-3')	PCR Product (bp)	T _a (°C)
<i>Acadl</i>	TGGGGACTTGCTCTCAACA	GGCCTGTGCAATTGGAGTA	62	58
<i>Atgl</i>	CAGCACATTTATCCCGGTGTAC	AAATGCCGCCATCCACATAG	76	61
<i>ATP2A1</i>	TGCTGCTTTGCTCGTGTGGA	CGGCCTTCTTCAGAGCAGGG	124	63
<i>Cact</i>	GGTGGCTGTCCAGACAAACT	TCCGTTTAAGAACCTCCTGG	122	59
<i>CD137</i>	CGTGCAGAACTCCTGTGATAAC	GTCCACCTATGCTGGAGAAGG	103	60
<i>CKMT2</i>	GCATGGTGGCTGGTGATGAG	AAACTGCCCCTGAGTAATCTTG	146	61
<i>COX IV</i>	TGGGACTATGACAAGAATGAGTGG	TTAGCATGGACCATTGGATACGG	113	63
<i>CPT1a</i>	CGTTACTTCAAGGTCTGGCTCTAC	GCTGCGGCTCTGAGGTGTC	103	62
<i>CPT1b</i>	TGCCAGCCACAATTCACC	GTCATCACATAGTTAGTTGCC	167	58
<i>CrAT</i>	GTTTCACCTGGGCCGCACTG	TCAGTGTAGGCTCGGTGGGC	146	65
<i>Cyt c</i>	ATAGGGGCATGTCACCTCAAAC	GTGGTTAGCCATGACCTGAAAG	172	61
<i>DIO2</i>	GTCCGCAAATGACCCCTTT	CCCACCCACTCTCTGACTTTC	65	59
<i>eNOS</i>	CCTTCCGCTACCAGCCAGA	CAGAGATCTTCACTGCATTGGCTA	105	61
<i>FGF21</i>	CTGCTGCTGGCTGTCTTCC	GTCTTGCTCGTCATCTGTGTAG	124	60
<i>GAMT</i>	GCACCTACCCTGCCTGACGG	CCCCGGTCTTCAGCAAGCG	130	64
<i>GAPDH</i>	AACTTTGGCATTGTGGAAGG	ACACATTGGGGGTAGGAACA	183	60
<i>GATm</i>	GACCTGGTCTTGTGCTCTCC	GGGATGACTGGTGTGGAGG	103	60
<i>gDNA</i>	CGTGATCGTAGCGTCTGGTT	GGTCGCGGTGTGGGCATTTG	108	62
<i>Hsl</i>	GCTGGAGGAGTGTTTTTTTGC	AGTTGAACCAAGCAGGTCACA	63	60
<i>mGPDH</i>	TGCCCCGATACTACAGCCCT	GCGTATCAGTGGTGCCAGCA	117	63
<i>mtDNA</i>	ACATGCAAACCTCCATAGACCGG	TCCTGCTGAGTCCCGTGGG	131	63
<i>NRF-1</i>	ACAGATAGTCCTGTCTGGGGAAA	TGGTACATGCTCACAGGGATCT	99	61
<i>Octn2</i>	AAGACCTGCAGGAAGCTGAA	TCCTTGTTTTTTCGTGGGTGT	127	57
<i>PGC-1α</i>	ACTATGAATCAAGCCACTACAGAC	TTCATCCCTCTTGAGCCTTTCG	148	61
<i>Pm20d1</i>	GGCTTCATCTATGGCCGGGG	ACACCTCCTCATCATGGCCCA	139	63
<i>PRDM16</i>	CTACAGGGTAGAAAAGCGGAAGG	TGGTTTCTATGGCTGACATCTGG	110	63
<i>Tbp</i>	CCCACAACCTCTCCATTCTCAAAC	ACAGCCAAGATTCACGGTAGATAC	174	65
<i>Tbx1</i>	GGCAGGCAGACGAATGTTC	TTGTCATCTACGGGCACAAAG	101	60
<i>Tfam</i>	AAGACCTCGTTCAGCATATAACATT	TTTTCCAAGCCTCATTTACAAGC	104	60
<i>TFIIb</i>	TGGAGATTTGTCCACCATGA	GAATTGCCAAACTCATCAAACT	66	56
<i>Tmem26</i>	ACCCTGTCATCCCACAGA	TGTTTGGTGGAGTCCTAA	119	55
<i>UCP1</i>	ACAGTACCCAAGCGTACCAAGC	TGTTCAAAGCACACAAACATGATG	135	63

T_a, temperature of annealing; *Acadl*, acyl-coenzyme A dehydrogenase, long-chain; *Atgl*, adipose triglyceride lipase; *ATP2A1*, ATPase, Ca⁺⁺ transporting, cardiac muscle, fast twitch 1; *Cact*, mitochondrial carnitine/acylcarnitine translocase; *CD137*, tumor necrosis factor receptor superfamily, member 9; *CKMT2*, creatine kinase, mitochondrial 2; *COX IV*, cytochrome c oxidase subunit IV; *CPT1a*, carnitine palmitoyltransferase 1a; *CPT1b*, carnitine palmitoyltransferase 1b; *CrAT*, carnitine acetyltransferase; *Cyt c*, cytochrome c; *DIO2*, type 2 iodothyronine deiodinase; *eNOS*, endothelial nitric oxide synthase; *FGF21*, fibroblast growth factor 21; *GAMT*, guanidinoacetate methyltransferase; *GAPDH*, glyceraldehyde 3-phosphate dehydrogenase; *GATm*, glycine amidinotransferase; *gDNA*, genomic DNA (chromosome 5 *Actb*); *mGPDH*, glycerol phosphate dehydrogenase 2; *mtDNA*, mitochondrial DNA; *NRF1*, nuclear respiratory factor 1; *Octn2*, organic cation transporter 2; *PGC-1α*, peroxisome proliferator-activated receptor γ coactivator 1α; *Pm20d1*, peptidase M20 domain containing 1; *PRDM16*, PR domain containing 16; *Tbp*, TATA box binding protein; *Tbx1*, T-box 1; *Tfam*, transcription factor A; *TFIIb*, general transcription factor IIB; *Tmem26*, transmembrane protein 26; *UCP1*, uncoupling protein 1.

Supplementary Table 6 — Plasma analysis in mice fed with SFA-CAA and SFA-EAA diet for 6 weeks at room temperature

	U	SFA-CAA		SFA-EAA		<i>P</i>
		mean	SEM	mean	SEM	
Alanine amino transferase (ALT)	IU/L	46.5	6.0	27.0	6.5	0.16
Aspartate amino transferase (AST)	IU/L	104.0	25.0	98.0	26.0	0.88
γ-Glutamyl transferase (GGT)	IU/L	3.0	0.4	3.2	0.3	0.62
Alkaline phosphatase (ALK)	IU/L	5.0	0.1	5.1	0.5	0.23
Creatin kinase (CK)	IU/L	1713.5	716.5	644.5	112.5	0.28
Glucose	mg/dL	151.5	9.5	159.0	36.0	0.86
P	mg/dL	6.7	0.4	4.6	0.3	0.06
Ca	mg/dL	4.0	0.2	4.3	0.1	0.08
Total bilirubin	mg/dL	0.3	0.1	0.2	0.1	0.49
Total protein	g/dL	5.0	0.5	5.2	0.2	0.33
Albumin	g/dL	3.1	0.1	3.2	0.1	0.70
Urea nitrogen (BUN)	mg/dL	18.5	1.5	15.5	0.5	0.20
Creatinine	mg/dL	0.2	0.1	0.2	0.1	0.10
Na	mEq/L	211.0	1.0	218.0	2.0	0.50
K	mEq/L	6.4	0.6	5.6	0.4	0.38
Na/K ratio	-	33.3	3.0	39.1	2.8	0.29
Cl	mEq/L	79.0	1.0	81.0	1.0	0.29
Amylase	IU/L	333.5	3.5	327.5	16.5	0.76
Total bile acids	μmol/L	8.2	2.4	12.0	1.1	0.29
Insulin	ng/mL	0.5	0.01	0.48	0.06	0.10
TSH	pg/mL	352.5	50.3	376.2	48.6	0.12
Thyroxine (T4)	ng/mL	57.2	1.8	47.8	0.8	0.06

Blood samples were collected by submandibular venipuncture, during the same time interval after a fasting period of 5 h. Clinical chemistry parameters and hormone levels in mice fed with SFA-CAA or SFA-EAA for 6 weeks at room temperature. Values are expressed as the mean ± SEM. *P* values are shown as differences between dietary regimens (*n* = 4 mice per group).

Supplementary Table 7 — Spearman's correlation analysis

	BW		Adiposity		GTT		Liver weight		Liver OCR	
	Spearman	<i>P</i>	Spearman	<i>P</i>	Spearman	<i>P</i>	Spearman	<i>P</i>	Spearman	<i>P</i>
Alanine	-0.11	0.70	-0.16	0.56	0.07	0.81	-0.32	0.25	0.35	0.20
Arginine	0.47	0.08	0.57	0.03	-0.26	0.35	0.45	0.089	-0.51	0.054
Aspartate	0.03	0.92	-0.05	0.86	-0.13	0.64	-0.20	0.48	0.24	0.39
Asparagine	0.08	0.79	0.20	0.47	0.08	0.77	-0.16	0.58	0.24	0.38
Glutamate	-0.70	0.004	-0.54	0.04	-0.02	0.95	-0.74	0.002	0.58	0.02
Glutamine	-0.02	0.940	0.13	0.64	0.19	0.50	-0.14	0.62	0.30	0.27
Glycine	0.22	0.44	0.27	0.33	0.06	0.84	-0.18	0.53	0.06	0.82
Histidine	-0.77	0.001	-0.64	0.01	0.08	0.79	-0.85	0.0001	0.61	0.02
Isoleucine	-0.55	0.03	-0.44	0.101	0.34	0.22	-0.19	0.51	0.24	0.40
Leucine	-0.47	0.08	-0.52	0.05	0.12	0.67	-0.17	0.55	0.19	0.49
Lysine	-0.29	0.30	-0.06	0.83	0.05	0.86	-0.47	0.08	0.41	0.13
Methionine	0.18	0.52	0.32	0.24	-0.39	0.15	0.13	0.65	-0.26	0.35
Phenylalanine	-0.07	0.80	0.04	0.88	-0.52	0.16	-0.08	0.77	-0.11	0.70
Proline	0.17	0.54	-0.01	0.96	-0.33	0.24	0.05	0.86	-0.10	0.71
Serine	0.14	0.63	0.13	0.64	0.13	0.65	-0.27	0.33	0.08	0.77
Threonine	-0.67	0.006	-0.69	0.0048	0.07	0.81	-0.40	0.14	0.45	0.09
Tyrosine	0.49	0.06	0.68	0.005	-0.22	0.43	0.19	0.50	-0.45	0.10
Valine	-0.61	0.016	-0.63	0.0125	-0.04	0.88	-0.30	0.28	0.18	0.53

Amino acid plasma levels were correlated with body weight (BW), adiposity [*i.e.*, epididymal white adipose tissue (eWAT) weight], glucose homeostasis [*i.e.*, glucose tolerance test (GTT)], and liver parameters [liver weight and mitochondrial oxygen consumption rates (OCR)] measured after consumption of diets for six weeks. Positively and negatively Spearman correlation coefficient (*r*) with *P* values are shown.

Supplementary Table 8 — Influence of diets on energy metabolism

	2 weeks					6 weeks				
	SFA-CAA		SFA-EAA		<i>P</i>	SFA-CAA		SFA-EAA		<i>P</i>
	<i>n</i> = 7		<i>n</i> = 7			<i>n</i> = 6		<i>n</i> = 6		
VO ₂ (mL/h)	99.54	2.75	94.59	1.38	0.13	75.83	1.55	87.75	1.77	0.001
VO ₂ _{BW} (mL/h/kg)	3691.33	128.13	3714.70	40.13	0.87	3341.69	36.69	3677.50	49.52	0.001
VO ₂ _{LM} (mL/h/kg)	4946.10	125.13	4697.21	77.21	0.12	4307.04	56.77	4652.97	81.41	0.009
EE (kcal/h)	0.44	0.03	0.45	0.01	0.51	0.38	0.01	0.44	0.01	0.001
EE _{BW} (kcal/h/kg)	18.21	0.64	18.18	0.20	0.97	17.20	0.29	18.08	0.22	0.042
EE _{LM} (kcal/h/kg)	24.40	0.62	22.99	0.40	0.08	22.87	0.40	25.45	0.59	0.007
Movement (counts/h)	14368.0	1131.9	15243.8	1549.1	0.65	21291.4	1369.9	19331.0	1148.7	0.334
	HFD-CAA		HFD-EAA		<i>P</i>	HFD-CAA		HFD-EAA		<i>P</i>
	<i>n</i> = 5		<i>n</i> = 6			<i>n</i> = 5		<i>n</i> = 6		
VO ₂ (mL/h)	115.10	5.92	108.07	3.56	0.32	104.41	3.29	117.80	4.10	0.04
VO ₂ _{BW} (mL/h/kg)	2463.27	76.77	2548.40	125.01	0.58	2281.02	60.83	2569.29	95.39	0.04
VO ₂ _{LM} (mL/h/kg)	4836.70	149.38	4655.35	111.35	0.36	4401.70	87.59	5060.49	201.82	0.02
EE (kcal/h)	0.57	0.03	0.51	0.02	0.18	0.50	0.02	0.58	0.02	0.02
EE _{BW} (kcal/h/kg)	11.89	0.37	12.16	0.60	0.71	11.76	0.42	13.56	0.53	0.03
EE _{LM} (kcal/h/kg)	23.34	0.74	22.21	0.53	0.25	20.92	0.48	23.02	0.52	0.02
Movement (counts/h)	12648.6	1766.2	14106.7	1362.8	0.55	16573.9	3628.7	15348.0	3088.3	0.80

Average 24 h oxygen consumption (VO₂) and energy expenditure (EE) of mice fed for 2 or 6 weeks with SFA-CAA, SFA-EAA, HFD-CAA, or HFD-EAA. VO₂ is expressed as mL/h/mouse, while EE is expressed as EE kcal/mouse/h. VO₂_{BW}, VO₂_{LM}, EE_{BW}, and EE_{LM} are normalized by body weight (BW) and lean mass (LM), respectively. Average 24 h total movement is reported as activity number of x and y-axis infrared photobeam breaks (counts) per h. Values are reported as mean ± SEM. A separate experiment of indirect calorimetry was performed for each diet type (SFA or HFD) and time interval (2 or 6 weeks); thus *P* values are shown as differences between the single diet at the same time interval.

Supplementary Table 9 — Relative abundance of microbial taxa at the genus level in gut microbiota of mice fed with SFA, SFA-CAA, and SFA-EAA diets for 6 weeks

Taxa (Genus)	SFA		SFA-CAA		SFA-EAA		SFA vs SFA-CAA		SFA vs SFA-EAA		SFA-EAA vs SFA-CAA	
	mean	SD	mean	SD	mean	SD	<i>P</i>	FDR <i>P</i>	<i>P</i>	FDR <i>P</i>	<i>P</i>	FDR <i>P</i>
Acinetobacter	0.00160	0.00237	0.00605	0.00712	0.00122	0.00176	0.1545	0.6018	0.8398	1.0000	0.2257	0.4657
Actinomyces	0.00071	0.00189	0.00022	0.00065	0.00000	0.00000	0.8072	0.9213	0.2636	0.5979	0.3173	0.4657
Adlercreutzia	0.17391	0.12530	0.17204	0.07809	0.07314	0.04231	0.7898	0.9213	0.3284	0.6408	0.0963	0.3627
AF12	0.00200	0.00530	0.00000	0.00000	0.00000	0.00000	0.2636	0.6018	0.2636	0.5979	0.9422	1.0000
Akkermansia	14.8044	12.7593	22.3752	12.8806	29.2526	14.5437	0.2481	0.6018	0.0129	0.2420	0.0413	0.2913
Allobaculum	3.37911	4.59730	2.75129	3.48290	13.48335	5.82501	0.9292	0.9969	0.0005	0.0299	0.0003	0.0322
Anaerobacillus	0.00302	0.00660	0.00021	0.00063	0.00040	0.00120	0.3384	0.6129	0.4119	0.6808	0.9422	1.0000
Anaerofustis	0.00391	0.00544	0.00101	0.00162	0.00000	0.00000	0.4273	0.6615	0.0402	0.3788	0.0682	0.3279
Anaeroplasma	0.00000	0.00000	0.00000	0.00000	0.38383	1.15148	0.9352	1.0000	0.3711	0.6408	0.3173	0.4657
Anaerotruncus	0.03695	0.02349	0.06746	0.04771	0.02135	0.00710	0.1974	0.6018	0.8590	1.0000	0.0821	0.3436
Bacillus	0.00098	0.00170	0.00461	0.00975	0.00168	0.00504	0.5740	0.7814	0.4941	0.7567	0.3043	0.4657
Bacteroides	22.5826	9.78316	15.1754	12.7729	9.58613	7.19427	0.1309	0.6018	0.1551	0.5979	0.4057	0.5659
Bifidobacterium	0.00048	0.00128	0.00065	0.00141	3.55909	5.40134	0.6816	0.8710	0.0033	0.0919	0.0021	0.0705
Bilophila	0.03123	0.02346	0.04572	0.03626	0.02270	0.02689	0.3743	0.6129	0.3711	1.0000	0.2899	0.4657
Blautia	0.00026	0.00069	0.00045	0.00135	0.00037	0.00112	0.9352	0.9969	0.9352	1.0000	0.9422	1.0000
Brachybacillus	0.00084	0.00222	0.00000	0.00000	0.00000	0.00000	0.2636	0.6018	0.2636	0.5979	0.9422	1.0000
Brevundimonas	0.00227	0.00410	0.00000	0.00000	0.00033	0.00099	0.1037	0.6018	0.3384	0.6408	0.3173	0.4657
Candidatus arthromitus	0.08854	0.23426	0.00000	0.00000	0.00261	0.00782	0.2636	0.6018	0.8072	0.9915	0.3173	0.4657
Chryseobacterium	0.00212	0.00231	0.00191	0.00451	0.00195	0.00226	0.3408	0.6129	0.7758	0.9915	0.2143	0.4657
Citrobacter	0.00000	0.00000	0.00133	0.00205	0.00000	0.00000	0.1008	0.6018	0.3711	1.0000	0.0682	0.3279
Coprobacillus	0.00026	0.00069	0.00986	0.01145	0.01212	0.02616	0.0262	0.4632	0.1772	0.5979	0.4673	0.6362
Coprococcus	0.99149	0.48498	0.89902	0.51838	1.39069	1.62534	0.3743	0.6129	0.2863	0.6105	0.0821	0.3436
Corynebacterium	0.00084	0.00222	0.00000	0.00000	0.00000	0.00000	0.2636	0.6018	0.2636	0.5979	0.9422	1.0000
Curvibacter	0.00000	0.00000	0.00000	0.00000	0.00037	0.00112	0.9352	1.0000	0.3711	0.6408	0.3173	0.4657
Dehalobacterium	0.74957	0.39910	0.93851	0.51006	0.36417	0.26029	0.3284	0.6129	0.1551	0.5979	0.0696	0.3279
Delftia	0.00198	0.00212	0.00510	0.00374	0.00171	0.00157	0.0237	0.4632	0.2646	0.5979	0.1455	0.3797
Desulfovermiculus	0.00073	0.00193	0.00021	0.00063	0.00000	0.00000	0.8072	0.9213	0.2636	0.5979	0.3173	0.4657
Desulfovibrio	0.06065	0.16047	0.06638	0.13276	0.00000	0.00000	0.7844	0.9213	0.2636	0.5979	0.1468	0.3797
Dorea	0.11502	0.04637	0.22963	0.29937	0.07852	0.10686	0.2863	0.6105	0.1097	0.5392	0.0588	0.3279

Enterobacter	0.00088	0.00233	0.00083	0.00166	0.00000	0.00000	0.7844	0.9213	0.2636	0.5979	0.1468	0.3797
Enterococcus	0.02707	0.02873	0.07036	0.07258	0.01009	0.00541	0.0263	0.4632	0.7223	0.9490	0.0025	0.0705
Exiguobacterium	0.00000	0.00000	0.00135	0.00405	0.00000	0.00000	0.3711	0.6129	0.3711	1.0000	0.3173	0.4657
Facklamia	0.00000	0.00000	0.00086	0.00173	0.00000	0.00000	0.1931	0.6018	0.3711	1.0000	0.1468	0.3797
Flexispira	0.00052	0.00138	0.00000	0.00000	0.00000	0.00000	0.2636	0.6018	0.2636	0.5979	0.9422	1.0000
Helicobacter	2.03057	3.76863	0.05608	0.16305	0.64017	1.55791	0.4915	0.7006	0.7328	0.9518	0.2494	0.4657
Kocuria	0.00212	0.00240	0.00445	0.00515	0.00134	0.00236	0.3140	0.6129	0.6860	0.9252	0.1826	0.4128
Lactobacillus	0.72554	1.34432	0.69220	0.53928	4.34119	5.90851	0.0410	0.4632	0.0045	0.1009	0.0082	0.1316
Lactococcus	4.00347	6.68594	0.18356	0.10794	0.17767	0.37243	0.0004	0.0429	0.0019	0.0705	0.0963	0.3627
Leuconostoc	0.00044	0.00116	0.00175	0.00216	0.00069	0.00119	0.1772	0.6018	0.4278	0.6808	0.5946	0.7576
Microbacterium	0.00000	0.00000	0.00000	0.00000	0.00060	0.00121	0.9352	1.0000	0.1931	0.5979	0.1468	0.3797
Mitsuokella	0.00084	0.00222	0.00000	0.00000	0.00000	0.00000	0.2636	0.6018	0.2636	0.5979	0.9422	1.0000
Mucispirillum	0.16651	0.28652	1.17904	1.33989	0.83264	1.28583	0.1662	0.6018	0.0907	0.4917	0.9397	1.0000
Ochrobactrum	0.00210	0.00555	0.00043	0.00086	0.00037	0.00112	0.7844	0.9213	0.8072	0.9915	0.6267	0.7782
Odoribacter	1.77718	2.90161	6.42061	5.90189	1.58773	1.40149	0.0209	0.4632	0.2481	0.5979	0.0963	0.3627
Oscillospira	10.68389	5.36761	9.71479	4.11254	4.71619	3.33414	0.4772	0.6913	0.1826	0.5979	0.0821	0.3436
Parabacteroides	22.55795	16.67867	17.79957	9.94875	12.68725	6.17695	0.7898	0.9213	0.8590	1.0000	0.6501	0.7986
Paracoccus	0.00000	0.00000	0.00041	0.00124	0.00026	0.00079	0.3711	0.6129	0.3711	0.6408	0.9422	1.0000
Paraprevotella	0.00000	0.00000	0.07330	0.15711	0.00000	0.00000	0.1931	0.6018	0.0907	1.0000	0.1468	0.3797
Prevotella	0.00000	0.00000	0.00000	0.00000	0.00470	0.00986	0.9352	1.0000	0.1931	0.5979	0.1468	0.3797
Propionibacterium	0.00000	0.00000	0.00112	0.00270	0.00000	0.00000	0.1931	0.6018	0.0907	1.0000	0.1468	0.3797
Pseudomonas	0.00084	0.00222	0.00157	0.00340	0.00205	0.00231	0.6816	0.8710	0.1249	0.5880	0.1558	0.3828
Pseudoxanthomonas	0.00000	0.00000	0.00071	0.00153	0.00000	0.00000	0.1931	0.6018	0.0907	1.0000	0.1468	0.3797
Rhodococcus	0.00000	0.00000	0.00078	0.00175	0.00034	0.00101	0.1931	0.6018	0.3711	0.6408	0.6267	0.7782
Roseburia	0.00000	0.00000	0.07609	0.18854	0.00019	0.00056	0.1931	0.6018	0.3711	0.6408	0.4656	0.6362
Rothia	0.00084	0.00222	0.00000	0.00000	0.00000	0.00000	0.2636	0.6018	0.2636	0.5979	0.9422	1.0000
Ruminococcus	3.52180	2.12668	4.36248	2.04570	2.51037	2.05136	0.4772	0.6913	0.7898	0.9915	0.5453	0.7166
Salana	0.00071	0.00189	0.00000	0.00000	0.00026	0.00079	0.2636	0.6018	0.9352	1.0000	0.3173	0.4657
Serratia	0.00674	0.00730	0.01162	0.00746	0.00393	0.00279	0.1301	0.6018	0.5901	0.8286	0.1735	0.4000
SMB53	0.00942	0.01527	0.00168	0.00361	0.00267	0.00414	0.0689	0.6018	0.5690	0.8139	0.2257	0.4657
Sphingobacterium	0.00000	0.00000	0.00100	0.00299	0.00000	0.00000	0.3711	0.6129	0.0907	1.0000	0.3173	0.4657
Staphylococcus	0.01643	0.01300	0.02407	0.01899	0.00347	0.00406	0.4499	0.6870	0.0725	0.4494	0.0182	0.1884
Streptococcus	0.02200	0.03673	0.01224	0.00764	0.00430	0.00477	0.3733	0.6129	0.5264	0.7736	0.1478	0.3797
Sutterella	0.81837	1.05663	3.53592	7.35599	6.87912	6.33044	0.2481	0.6018	0.0005	0.0299	0.0041	0.0920
Turicibacter	1.69882	3.94318	0.29104	0.58247	1.44283	2.06308	0.0854	0.6018	0.6877	0.9252	0.0692	0.3279
Other	4.50579	3.64642	5.96632	3.69171	3.50317	4.03575	0.9352	0.9969	0.1059	0.9915	0.9422	0.1884

[Prevotella]	0.51313	1.16283	0.89861	0.25728	0.68826	0.98051	0.0179	0.4632	0.2431	0.5979	0.1730	0.4000
[Ruminococcus]	2.46031	0.66873	2.98674	3.69159	1.07346	0.92375	0.9292	0.9969	0.0330	0.3387	0.0233	0.1884
Unassigned	0.12355	0.31389	2.87607	2.19429	0.20738	0.25575	0.1309	0.6018	0.1059	0.9915	0.1124	0.3797

Data are presented as percentage (mean $\pm$ SD). The non-parametric Kruskal Wallis test was used for the comparison of relative abundances of microbial taxa between groups, and the resulting *P* values were corrected (Bonferroni correction) for multiple testing controlling the false discovery rate (FDR *P* value).

Supplementary Table 10 — Amino acid amounts in SFA-EAA diet compared to the amino acid requirements of mice

SFA-EAA (RD number: D14032501)				
Ingredients	g/kg diet	Amino acids	Grams in formula ^a	NRC Guidelines (g/kg diet) ^b
Protein		Leucine	59.77	7.0
Casein	200	Valine	29.91	5.0
		Lysine	31.32	4.0
Carbohydrates		Isoleucine	29.78	4.0
Corn Starch	452	Threonine	17.01	4.0
Maltodextrin	75	Histidine	7.46	2.0
Sucrose	173	Cysteine/cystine	7.21	
Cellulose	50	Phenylalanine	5.3	7.6
		Methionine	2.82	5.0
Fat		Tyrosine	1.99	
Soybean oil	25	Tryptophan	1.11	1.0
Lard	20	Alanine	0.4	
Mineral Mix S10026	10	Arginine	0.44	
CaHPO ₄	13	Aspartic acid	0.9	
CaCO ₃	5.5	Glutamic acid	2.82	
Potassium Citrate	16.5	Glycine	0.22	
Vitamin Mix	10	Proline	1.31	
Choline Bitartrate	2	Serine	0.74	

^aTotal amino acid amount derived from casein (13 g) and EAAM; ^bessential amino acid amounts for mice as recommended by the U.S. National Research Council (NRC) (Nutrient Requirements of Laboratory Animals. Fourth Revised Edition, 1995).