

Supplementary files

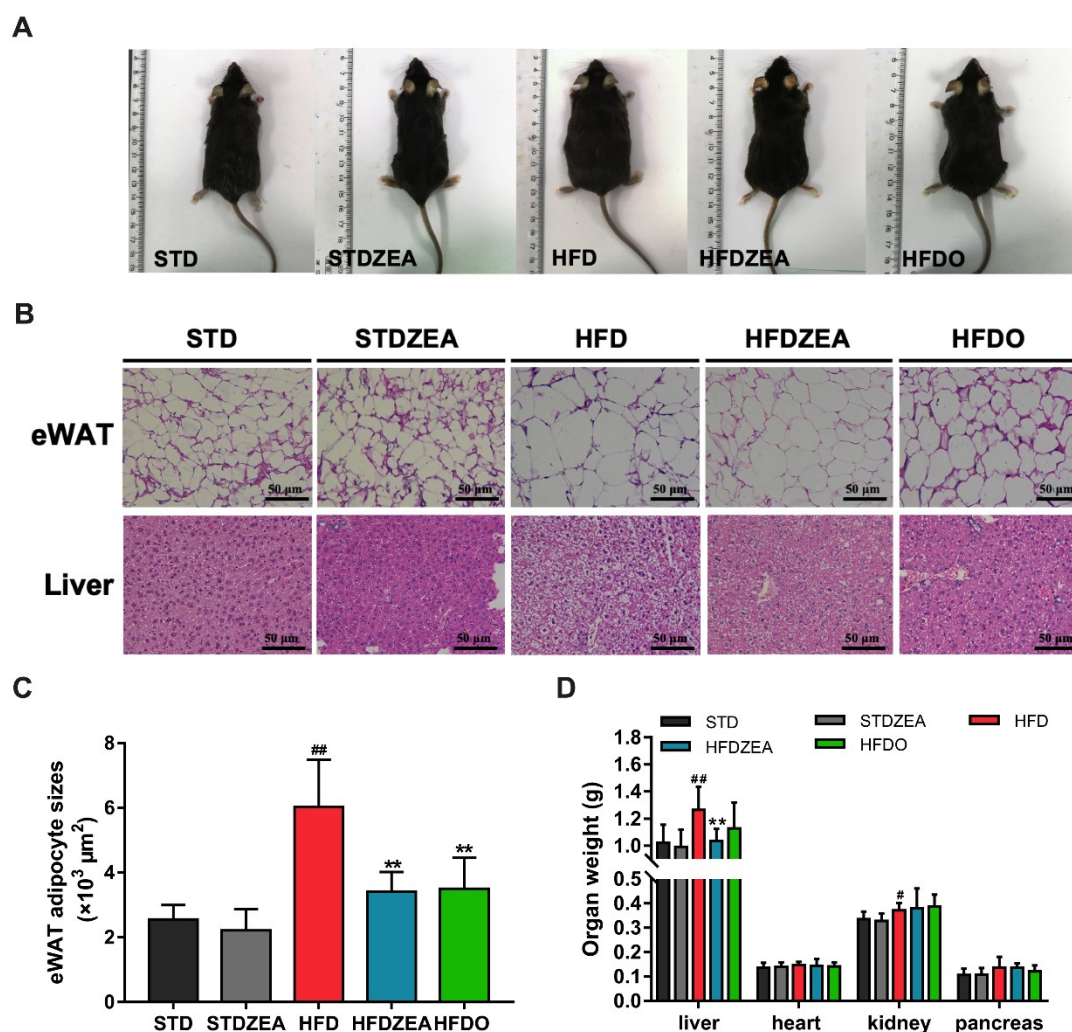


Fig. S1 ZEA improves eWAT adipocyte hypertrophy, reduces liver weight and lipid deposition in HFD-fed mice. (A) Whole-body image of mice in different groups. (B) H&E staining of eWAT and liver (scale bar, 50 μm). (C) EWAT adipocyte sizes. (D) Organ weight of liver, heart, kidney, and pancreas ($n = 12$). [#] $P < 0.05$, ^{##} $P < 0.01$ STDZEA, HFD vs. STD; ^{**} $P < 0.01$ HFDZEA, HFDO vs. HFD.

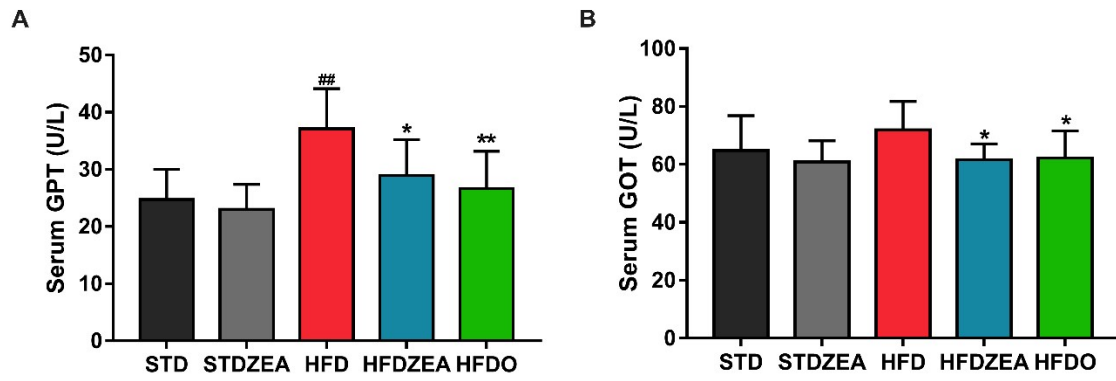


Fig. S2 ZEA improves serum GPT and GOT in HFD-fed mice. (A) Serum GPT. (B) Serum GOT. (n = 8). ^{##}*P* < 0.01 STDZEA, HFD vs. STD; ^{*}*P* < 0.05, ^{**}*P* < 0.01 HFDZEA, HFDO vs. HFD.

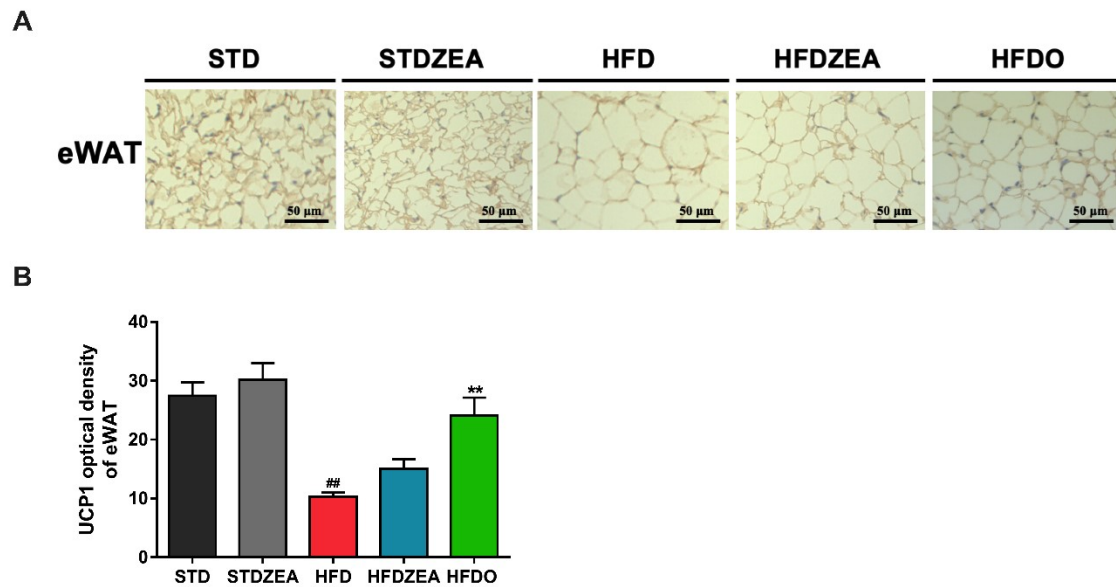


Fig. S3 ZEA can not induce UCP1 expression in eWAT. (A) Immunohistochemical staining with UCP1 antibody performed on eWAT (scale bar = 50 μm). (B) UCP1 optical density of eWAT. ^{##}*P* < 0.01 STDZEA, HFD vs. STD; ^{**}*P* < 0.01 HFDZEA, HFDO vs. HFD.

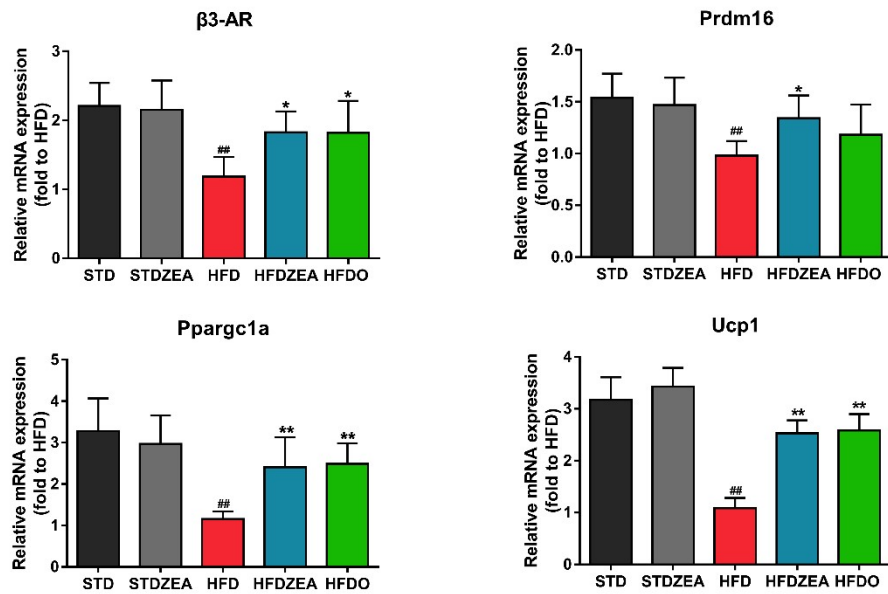
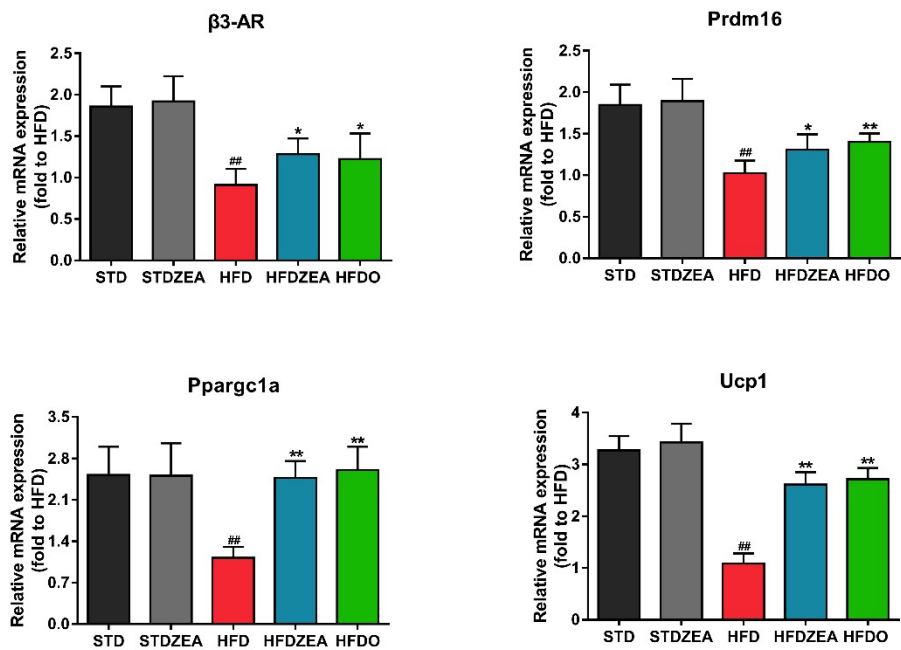
A**iWAT****B****BAT**

Fig. S4 ZEA induces the expression of $\beta 3$ -ar and key thermogenic genes in iWAT and BAT.

The mRNA expression of $\beta 3$ -ar, Prdm16, Pgc-1a, Ucp1 in iWAT (A) and BAT (B). (n = 6). ##P <

0.01 STDZEA, HFD vs. STD; *P < 0.05, **P < 0.01 HFDZEA, HFDO vs. HFD.

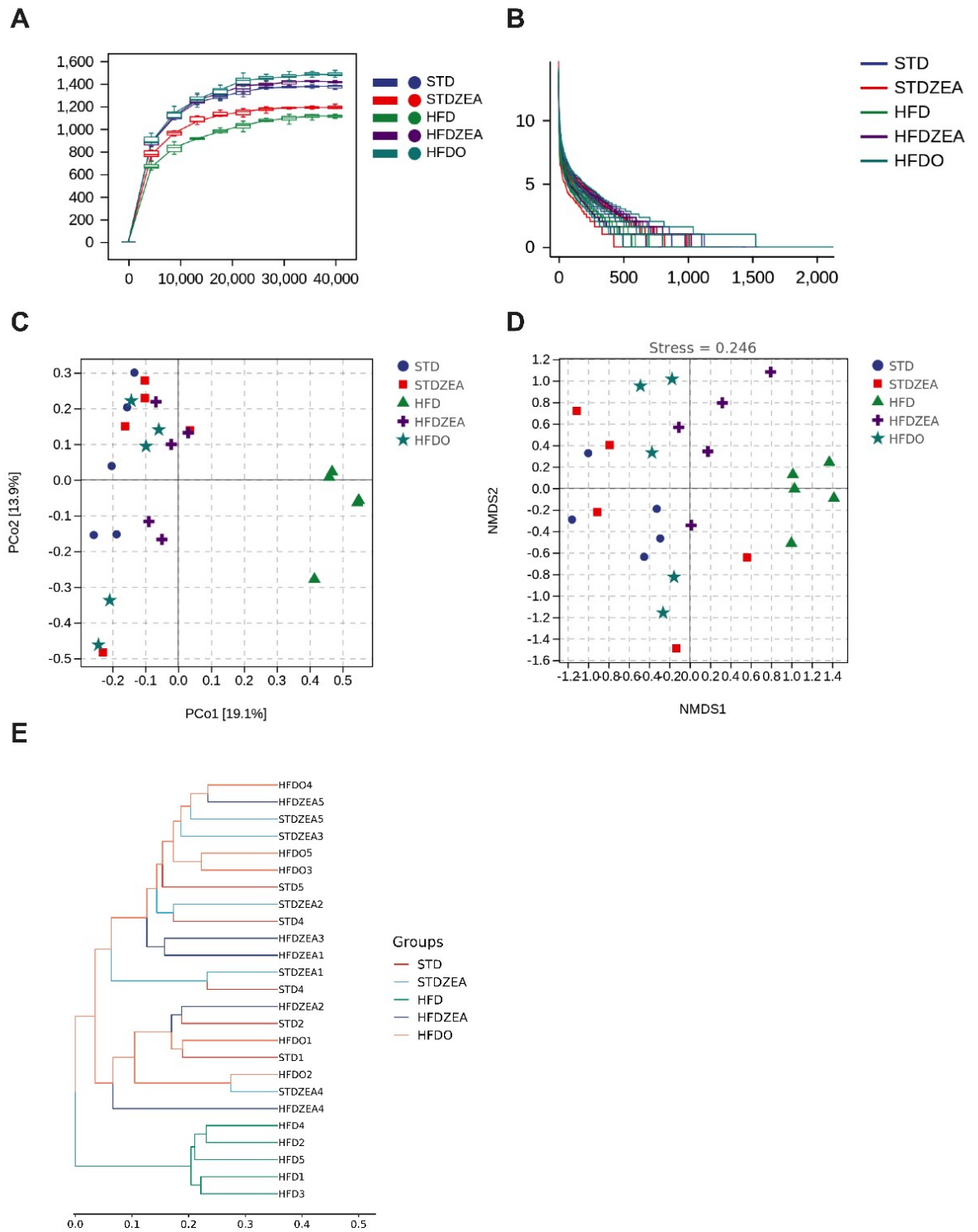


Fig. S5 ZEA reverses the microbial structure destroyed by HFD. (A) Rarefaction curves. (B)

Rank abundance curves. (C) PCoA plot. (D) NMDS plot. (E) UPGMA analysis. (n = 5).

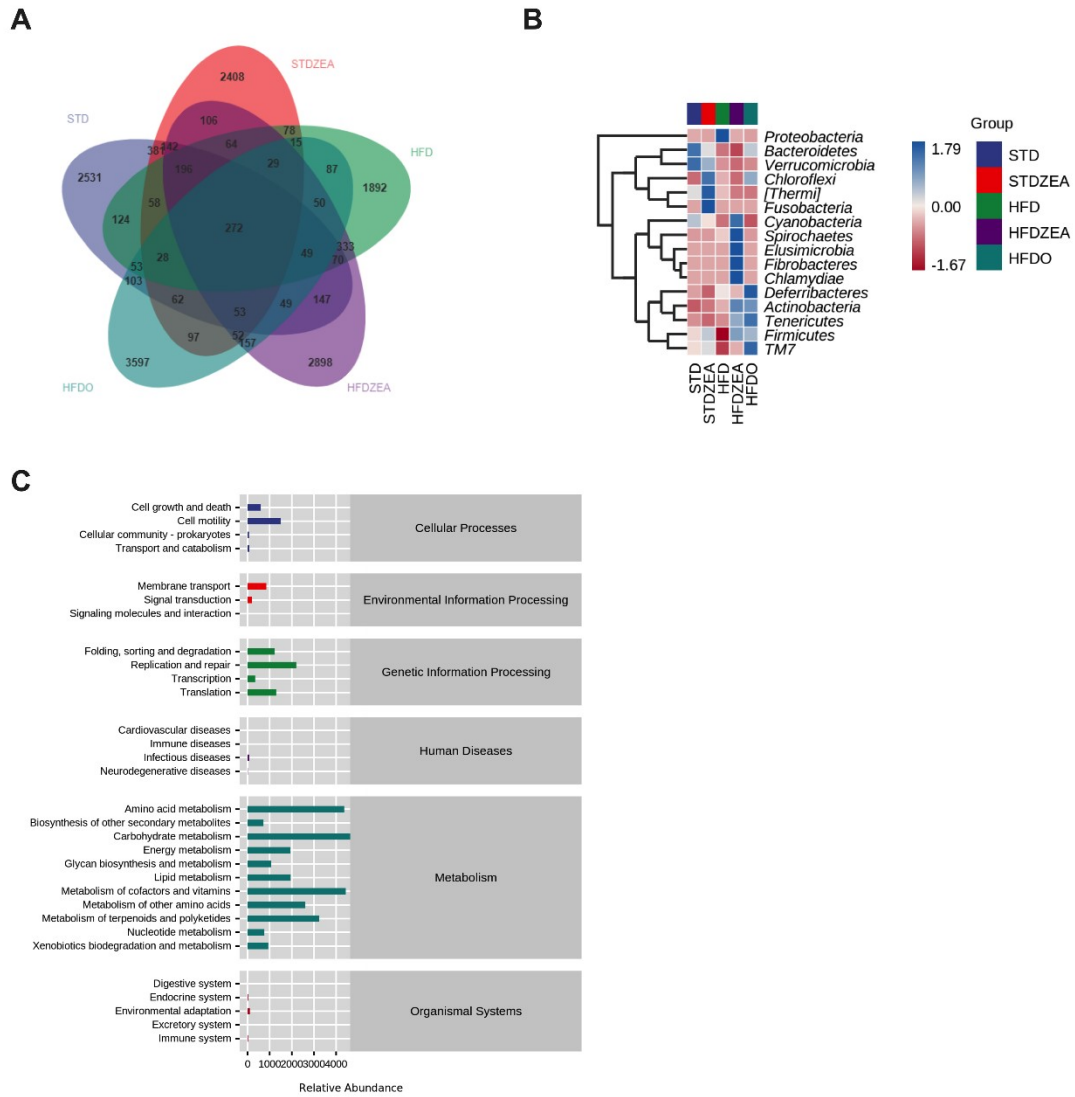


Fig. S6 ZEA improves gut microbiota dysbiosis and enriches lipid metabolism-related KEGG pathways. (A) OTU-Venn analysis. (B) The heat map clustering analysis at the level of bacterial phylum level. (C) Analysis of KEGG enrichment pathways between HFDZEA and HFD groups at level 2. (n = 5).

Table S1 Composition and ingredients of different diets

STD (D12450B)	HFD (D12492)
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Composition	gm%	kcal%	gm%	kcal%
Protein	19	20	26	20
Carbohydrate	67	70	26	20
Fat	4	10	35	60
Total	-	100	-	100
kcal/gm		3.8		5.2
Ingredient	gm	kcal	gm	kcal
Casein, 80 Mesh	200	800	200	800
L-Cystine	3	12	3	12
Corn Starch	315	1260	-	-
Maltodextrin 10	35	140	125	500
Sucrose	350	1400	68.8	272
Cellulose, BW200	50	-	50	-
Soybean Oil	25	225	25	225
Lard	20	180	245	2205
Mineral Mix S10026	10	-	10	-
DiCalcium Phosphate	13	-	13	-
Calcium Carbonate	5.5	-	5.5	-
Potassium Citrate, 1 H ₂ O	16.5	-	16.5	-
Vitamin Mix V10001	10	40	10	40
Choline Bitartrate	2	-	2	-
FD&C Yellow Dye #5	0.05	-	-	-

FD&C Blue Dye #1	-	-	0.05	-
Total	1055.05	4057	773.85	4057

Table S2 Primer sequences used for the Real-time qPCR

Primer name	Forward (5'-3')	Reverse (5'-3')
β 3-AR	TGATGGCTATGAAGGTGCG	AAAATCCCCAGAAGTCCTGC
Prdm16	CAGTGGGGAGAGAGGACAGA	ACGGATGTACTIONTGGAGCCAGC
Pgc-1 α	CACCAAACCCACAGAAAACAG	GGGTCAGAGGAAGAGATAAAGTTG
Ucp1	GCATTCAGAGGCAAATCAGC	GCCACACCTCCAGTCATTAAG
Nrf1	GCTAATGGCCTGGTCCAGAT	CTGCGCTGTCCGATATCCTG
Tfam	CACCCAGATGCAAACTTTCAG	CTGCTCTTTATACTTGCTCACAG
Nampt	GAATGTCTCCTTCGGTTCTGG	TCAGCAACTGGGTCCTTAAAC
Cyt-C	AAGGGAGGCAAGCATAAGAC	ATTCTCCAAATACTCCATCAGGG
Sirt1	CTCTGAAAGTGAGACCAGTAGC	TGTAGATGAGGCAAAGGTTCC
Pex3	AGTTTGCAGTGAGACACCTAG	AGTCCGCTGTAGTTTTCTGG
Pex16	CCGTTCTATGACCGCTTCTC	GGAGGGCAAGTAGTCCATGA
Pex19	AGCATCATGCAGAACCTCCT	TGCTGCTGCTGGTACTTCTC
Pmp 70	TCTGCCTACTCCATAAGCGG	CACCACAGCTCGCTCTTTCT
Cpt1	ACCACTGGCCGCATGT	CTCCATGGCGTAGTAGTTGCT
Cpt2	CAGCACAGCATCGTACCCA	TCCCAATGCCGTTCTCAAAT
Acadm	AGGGTTTAGTTTTGAGTTGACG	CCCCGCTTTTGTTCATATTCCG

Acox1	CAAGACCCAAGAGTTCATT	TTCAGGTAGCCATTATCCA
β -actin	CATCCGTAAAGACCTCTATGCCAAC	ATGGAGCCACCGATCCACA

Table S3 List of Antibodies

Antibodies	Source	Catalogue #
β 3-AR	Abcam	ab94506
PRDM16	Abclonal	A18633
PGC-1 α	Proteintech Group Inc	#20658
UCP1	Abcam	ab10983
ATGL	Cell Signaling Technology	#2439
CGI-58	Santa Cruz Biotechnology	sc100468
phospho (Ser563)-HSL	Cell Signaling Technology	#4139
HSL	Cell Signaling Technology	#4107
SIRT1	Cell Signaling Technology	#2496
PMP70	Abcam	ab3421
