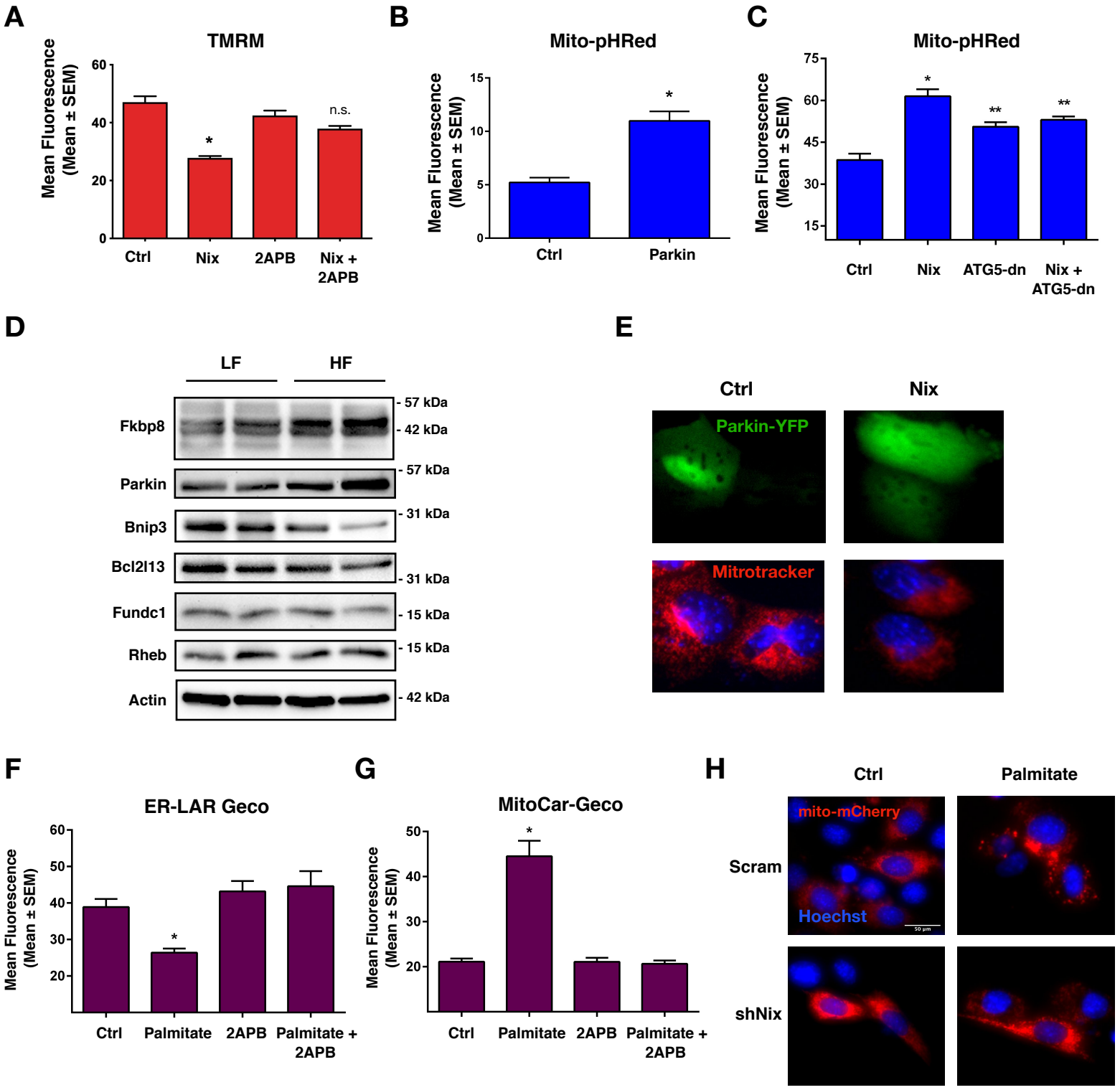
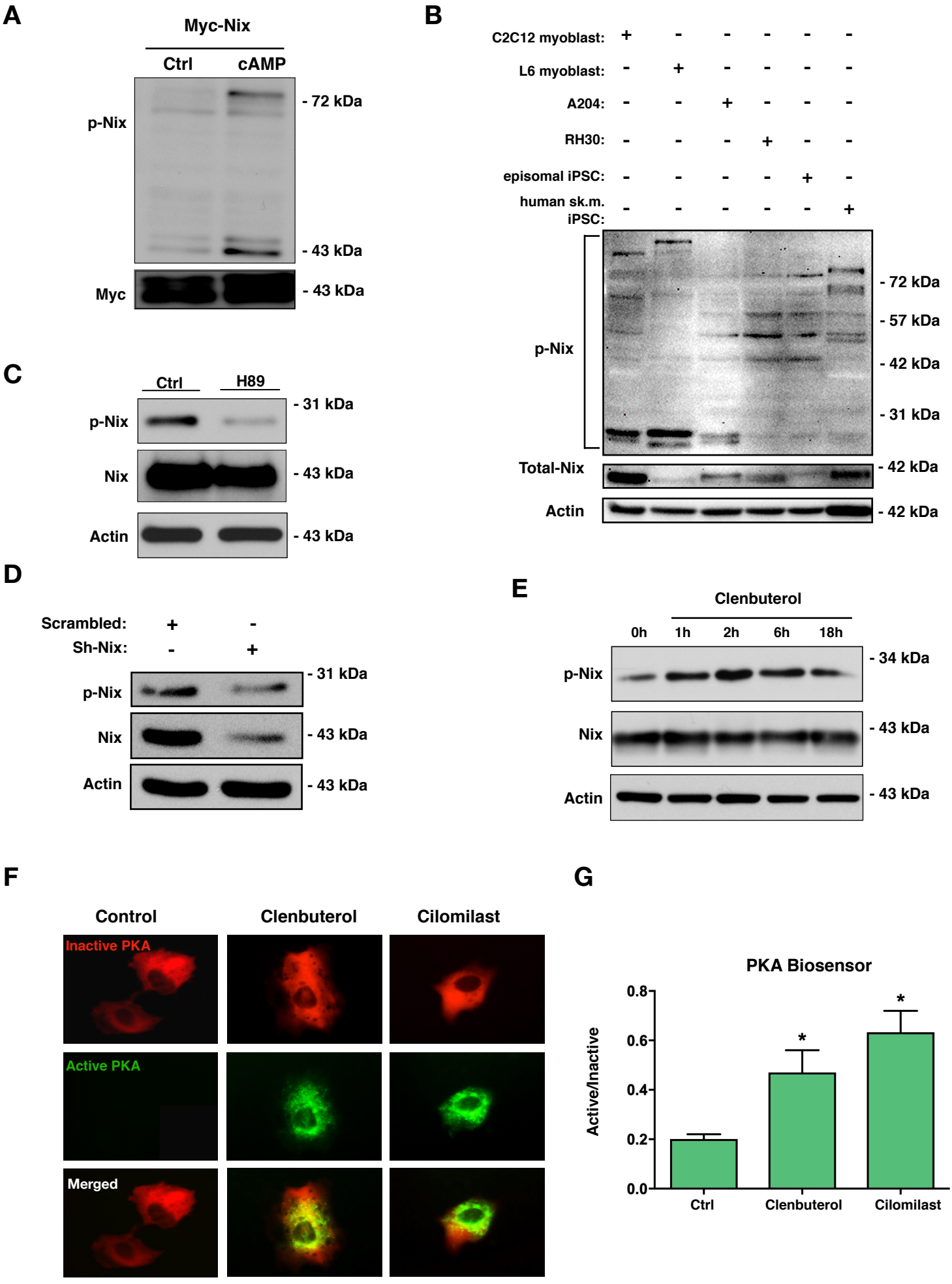
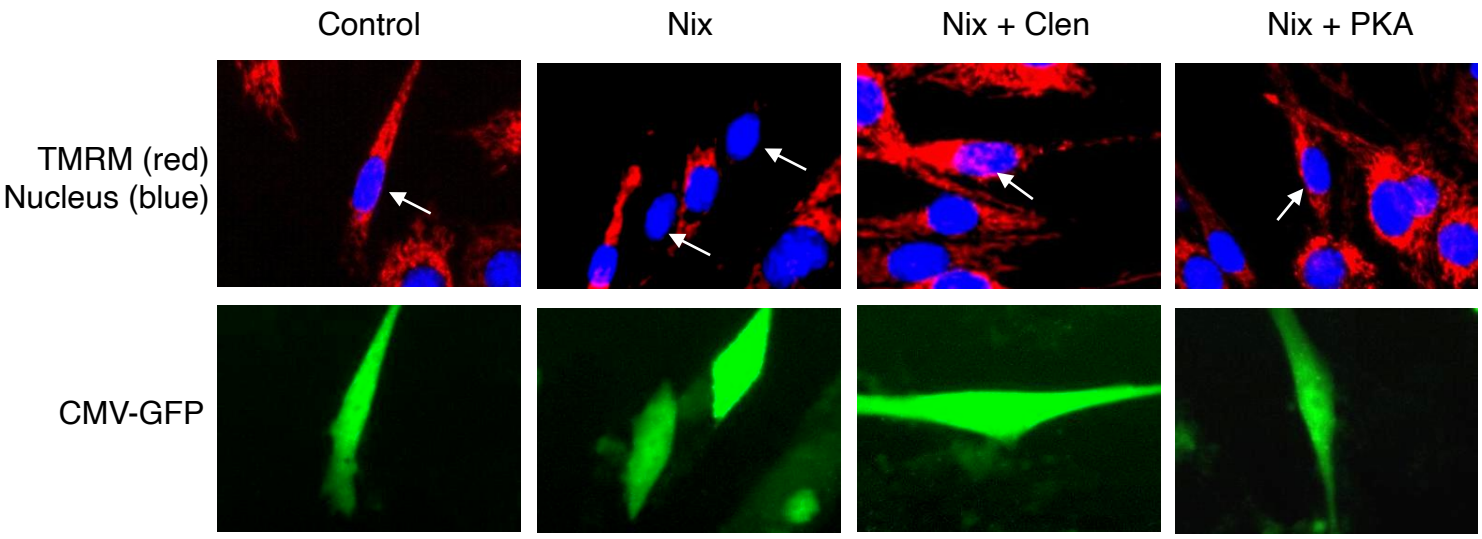
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3.



Supplemental Figure 1. ***Palmitate-induced mitochondrial dysfunction.***
A) C2C12 myoblast cells were transfected with Myc-Nix or an empty vector. Cells were treated with 2-aminoethoxydiphenyl borate (2APB 10μM, 1h), or DMSO as a vehicle control. Cells were stained for TMRM and quantified. B) C2C12 myoblasts cells were transfected with HA-Parkin or an empty vector, along with the mitophagy biosensor mito-pHRed. C) C2C12 myoblast cells were transfected Myc-Nix, a dominant-negative ATG5, or an empty vector, along with mito-pHRed. D) Western blot analysis of rat soleus muscle (n=2) exposed to high fat (HF) or low fat (LF) diet for 12-weeks. Protein extracts were analyzed as indicated. E) C2C12 myoblasts cells were transfected with Myc-Nix or an empty vector, along with fluorescent protein YFP-Parkin. Cells were stained with Mitotracker and Hoechst and imaged by standard fluorescence microscopy. F-G) C2C12 myoblasts cells were transfected with ER-Lar-Geco (f) or MitoCar-Geco (g) and an empty vector, followed by overnight treatment with palmitate conjugated to 2% albumin in low glucose media. Control cells were treated with 2% albumin alone. Following palmitate treatment, cells were treated with 2APB, as described in (a). H) C2C12 myoblasts were transfected with shNix, or a scrambled control shRNA, along with mito-mCherry. Cells were treated overnight with palmitate conjugated to 2% albumin in low glucose media. Control cells were treated with 2% albumin alone. Cells were stained with Hoechst and imaged by standard fluorescence microscopy. Data are represented as mean ± S.E.M. *$P < 0.05$ compared with control, while **$P < 0.05$ compared with treatment, determined by 1-way ANOVA.

Supplemental Figure 2. ***PKA Phosphorylation of Nix.***
A) C2C12 myoblast cells were transfected with Myc-Nix or an empty vector and treated with c-AMP analogue (1mM, 1h) or vehicle control. Protein extracts were immunoblotted, as indicated. B) Protein extract of multiple cell lines (as indicated) were immunoblotted for endogenous phospho-Nix. C) C2C12 myoblast cells were treated with PKA inhibitor, H89 (10 μM, 1h) or vehicle control. Protein extracts were immunoblotted, as indicated. D) C2C12 myoblasts were transfected with shNix, or a scrambled control shRNA. Protein extracts were immunoblotted, as indicated. E) C2C12 myoblast cells were treated with 500 nM clenbuterol or vehicle for multiple time points. Protein extracts were immunoblotted, as indicated. F) C2C12 myoblast cells were transfected with PKA biosensor (pPHT-PKA), and treated with clenbuterol (500nM), cilomilast (10μM) or vehicle for 2 h. Cells were imaged by standard fluorescence microscopy. G) Quantification of fluorescent images in (f) by measuring the ratio of green (active) to red (inactive) fluorescent signal. Data are represented as mean ± S.E.M. *$P < 0.05$ compared with control, determined by 1-way ANOVA.

Supplemental Figure 3. ***Nix-induced mitochondrial depolarization.***
A) C2C12 myoblast cells were transfected with Nix, PKA or CMV-GFP as a control. Cells were treated with clenbuterol (500nM, 2h) or a vehicle control. Cells were stained for TMRM and Hoechst and imaged by standard fluorescence. Red active or inactive mitochondria are indicated by arrows.

Table 1: Metabolomics Table for Selected Muscle Triglycerides

TG	HF diet (Fold of Control)
TG(22:3/22:6/22:6)	474.16094524
TG(21:0/22:0/22:3)	225.30109372
TG(20:0/22:0/22:6)	252.24362556
TG(13:0/18:3/22:2)	0.73820095
TG(13:0/16:0/18:3)	0.00002156
TG(13:0/15:1/17:2)	12.42594972
TG(13:0/14:0/18:2)	15.80467647
TG(13:0/14:0/18:2)	0.03724308
TG(12:0/19:1/19:1)	0.00010212
TG(12:0/17:0/17:0)	0.00000017
TG(12:0/16:0/20:1)	0.00454675
TG(12:0/15:1/16:0)	0.09477939
TG(12:0/15:1/16:0)	226.25782085
TG(12:0/14:1/18:4)	451.47187440
TG(12:0/14:1/18:0)	0.03885087
TG(12:0/14:1/15:1)	303.10081212
TG(12:0/14:1/15:1)	5701844.90556225
TG(12:0/13:0/22:5)	27.28222775
TG(12:0/12:0/20:0)	215.98445754
TG(12:0/12:0/19:1)	14.43848324
TG(12:0/12:0/18:0)	16.99116745
TG(12:0/12:0/14:1)	378.78275633

Table 2: Metabolomics Table for Selected Muscle Diacylglycerides

DG	HF diet (Fold of Control)
DG(O-16:0/18:1)	302.8633348
DG(22:3/22:6)	315.3130896
DG(22:3/22:2)	0.765006279
DG(22:2/24:1)	685.4931195
DG(22:2/24:0)	393.766622
DG(21:0/22:6)	271.0025641
DG(20:5/22:4)	380753.4087
DG(20:4/22:6)	6176.598903
DG(20:4/20:4)	16.2638058
DG(20:2/24:0)	0.021997727
DG(20:2/22:0)	0.643402435
DG(20:2/14:1)	244.0439805
DG(20:2/20:2)	16.8409959
DG(20:1/18:0)	388.6799158
DG(20:0/22:1)	208.6105653
DG(20:0/20:0)	0.3638602
DG(19:0/19:0)	12.66929387
DG(18:4/24:1)	255.600736
DG(18:4/22:6)	499.6432217
DG(18:2/18:1)	19.57482869
DG(18:1/24:1)	0.040373546
DG(17:0/17:0)	0.00292789
DG(16:1/16:1)	347.202625
DG(16:0/20:0)	306.2108676
DG(16:0/18:0)	8408.447173
DG(16:0/16:0)	346.1952462
DG(15:0/16:0)	0.046122772
DG(12:0/20:0)	9857.486705

Table 3: Metabolomics Table for Selected Muscle Cerimides

Cer	HF diet (Fold of Control)
α-Galactosyl Ceramide	0.002657066
Lactosylceramide (d18:1/22:0)	16.50345981
GlcCer(d18:2/23:0)	7.268878339
GlcCer(d18:2/23:0)	3059839.875
GlcCer(d18:2/23:0)	2728569.373
GlcCer(d18:2/21:0)	557.2568299
GlcCer(d18:2/21:0)	12.48381689
GlcCer(d18:1/26:0)	4.72854E-07
GlcCer(d18:0/24:0)	3.28482E-06
GlcCer(d16:2/20:0)	19.35893404
GlcCer(d15:2/22:0)	0.409136853
GlcCer(d15:2/22:0)	328.6126366
GlcCer(d15:2/22:0)	34.65256394
GlcCer(d15:2/20:0)	8118.391671
GlcCer(d15:2/18:0)	293.9558449
GlcCer(d15:1/22:0)	15.98534449
GlcCer(d15:1/20:0)	371.4533676
GlcCer(d15:1/20:0)	324.6849811
GlcCer(d14:1/18:1)	348.5725632
Dihydroceramide C2	556.0446801
Ceramide (d18:1/26:0)	205.005966
Cer(t18:0/16:0)	5829.65025
Cer(t18:0/16:0)	14.57712641
Cer(t18:0/16:0)	216.174369
Cer(d18:2/23:0)	0.030951015
Cer(d18:2/20:1)	270.0500114
Cer(d18:2/20:1)	297.7411079
Cer(d18:2/20:1)	11.30894339
Cer(d18:2/18:1)	0.944696921
Cer(d18:2/14:0)	16.71667118
Cer(d18:2/14:0)	282.8826293
Cer(d18:1/22:1)	0.020334332
Cer(d18:1/20:0)	2.15693E-06
Cer(d18:1/16:0)	367.3849827
Cer(d18:1/16:0)	190.163429
Cer(d18:0/17:0)	225.1782813
Cer(d18:0/15:0)	828.6573179
Cer(d18:0/13:0)	6573251.966
Cer(d18:0/12:0)	117007904.1
Cer(d16:2/24:0)	213.6848341
Cer(d16:2/20:1)	122962.2496
Cer(d16:1/23:0)	271.2296103

Cer(d15:2/22:0)	0.803124732
Cer(d14:2/20:0)	423.0099666
Cer(d14:2/18:1)	0.028376394
Cer(d14:2/18:0)	10.79612912
Cer(d14:2/18:0)	1.51852E+11
Cer(d14:1/26:0)	450314.2601
Cer(d14:1/24:0)	15.3688333
Cer(d14:1/22:1)	298.2445388
Cer(d14:1/22:0)	509.2305146
C-6 NBD Ceramide	450.8602007
AV-Ceramide	283.9401451
PE-Cer(d16:1/23:0)	13621.34993
PE-Cer(d16:1/18:0)	14.52539596
PE-Cer(d14:1/25:0)	344.1520018
PE-Cer(d14:1/23:0)	10237.21749
CerP(d18:1/24:1)	140907.4644
CerP(d18:1/18:0)	273.3677637

Table 4: Gene expression Array Table for Selected Muscle Cardiolipins

CL	HF diet (Fold of Control)
CL(18:2/18:2/18:2/18:2)	0.002684369
CL(22:6/20:3/18:2/18:2)	ND in HF condition
CL(18:2/18:2/18:2/18:3)	ND in HF condition
CL(22:1/22:1/22:1/14:1)	ND in HF condition
CL(14:1/14:1/14:1/15:1)	ND in HF condition

Table 5: Gene expression Array Table for Selected Muscle Phosphatidic Acids

PA	HF diet (Fold of Control)
PA(20:0/22:6)	19842.77007
PA(20:0/18:2)	509.6983175
PA(22:6/14:1)	0.05364954
PA(20:5/22:6)	0.000120273
PA(20:5/18:3)	245.1649605
PA(20:5/18:3)	13.27455068
PA(20:5/18:3)	0.526316154
PA(20:5/18:3)	300.8537298
PA(20:3/21:0)	518.1851107
PA(20:2/21:0)	296.0700657
PA(20:1/22:0)	243.7446545
PA(19:0/20:0)	12388.91034
PA(18:4/20:5)	12.8543701
PA(18:4/18:4)	314.6167207
PA(18:4/18:3)	0.072812107
PA(16:0/21:0)	366.4071164
PA(15:1/22:4)	335.3509184
PA(15:0/20:3)	206.8535602
PA(14:1/17:2)	0.708617217
PA(14:0/15:0)	4534.707717
PA(14:0/12:0)	257.1628321

Table 6: Gene expression Array Table for Selected Muscle Genes

Gene	HF diet (Fold of Control)
Atg12	0.82
Atg16l1	0.97
Atg3	0.86
Atg5	0.73
Atg7	1.13
Atp6v1g2	0.39
Bax	1.05
Bcl2	1.08
Bcl2a1	0.7
Bcl2l1	0.7
Bcl2l11	0.91
Becn1	0.84
Igf1	1.13
Igf1r	1.25
Tnf	0.69
Tnfrsf11b	0.69
Tnfrsf1a	0.94
Tp53	1.32
Ulk1	1.03
Actb	1.08
Ldha	1.03
Cs	0.8
Foxo3	0.87
Hdac5	0.91
Mb	0.48
Mef2c	0.91
Mstn	1.51
Myf5	1.37
Myod1	1.25
Myog	0.89
Myh1	0.38
Myh2	0.04
Nfkb1	1.04
Pdk4	0.51
Pparg	0.85
Ppargc1a	0.65
Ppargc1b	0.98
Rhoa	0.97
Slc2a4	0.95
Tgfb1	1.19
Tnnc1	0.02
Tnni2	1.02

Tnnt1	0.02
Tnnt3	1.06
Fasn	0.18
Gys1	0.95
Hk2	0.8
Il6	0.58
Insr	0.89
Irs1	1.2
Irs2	0.96
Mtor	0.97
Slc27a1	0.66
Srebf1	1.14
Srebf2	1.17
Bnip3	0.84
Cox18	0.91
Cpt1b	0.82
Cpt2	0.78
Dnm1l	0.73
Fis1	0.82
Gpx1	0.51
Mfn1	0.74
Mfn2	0.86
Opa1	0.92
Taz	0.91
Timm44	0.96
Tomm40	1.02
Tomm22	0.94
Ucp2	1.11
Ucp3	0.8
Acly	0.48
Aldoa	1.01
Idh2	0.74
Mdh1	0.63
Mdh2	0.91