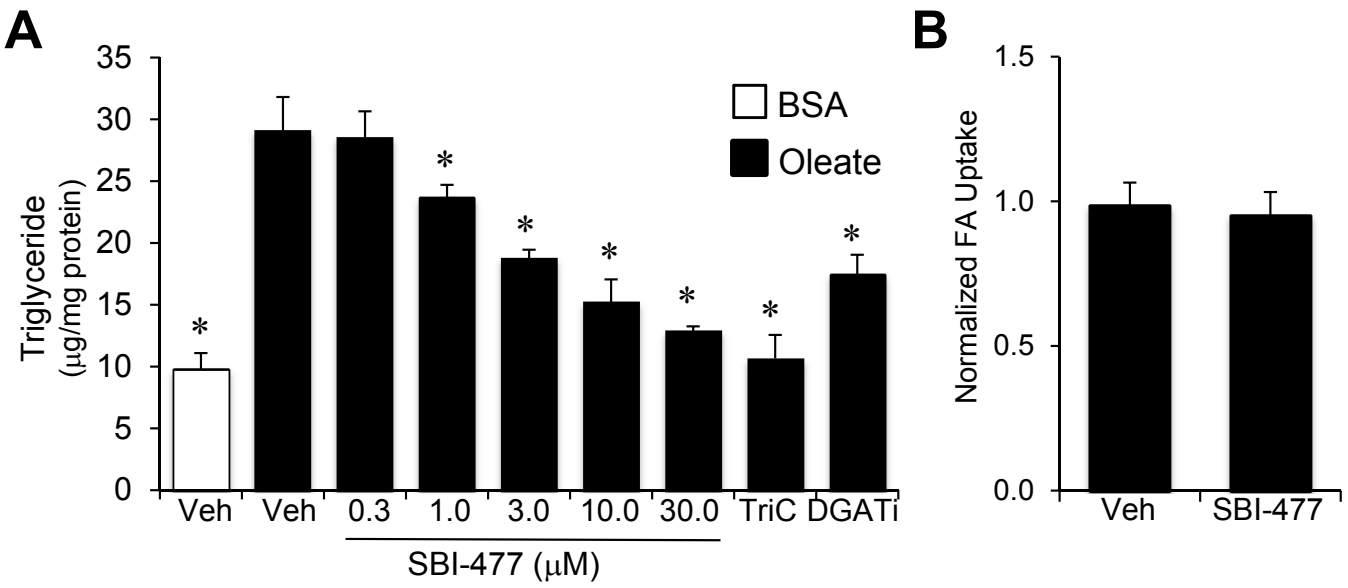
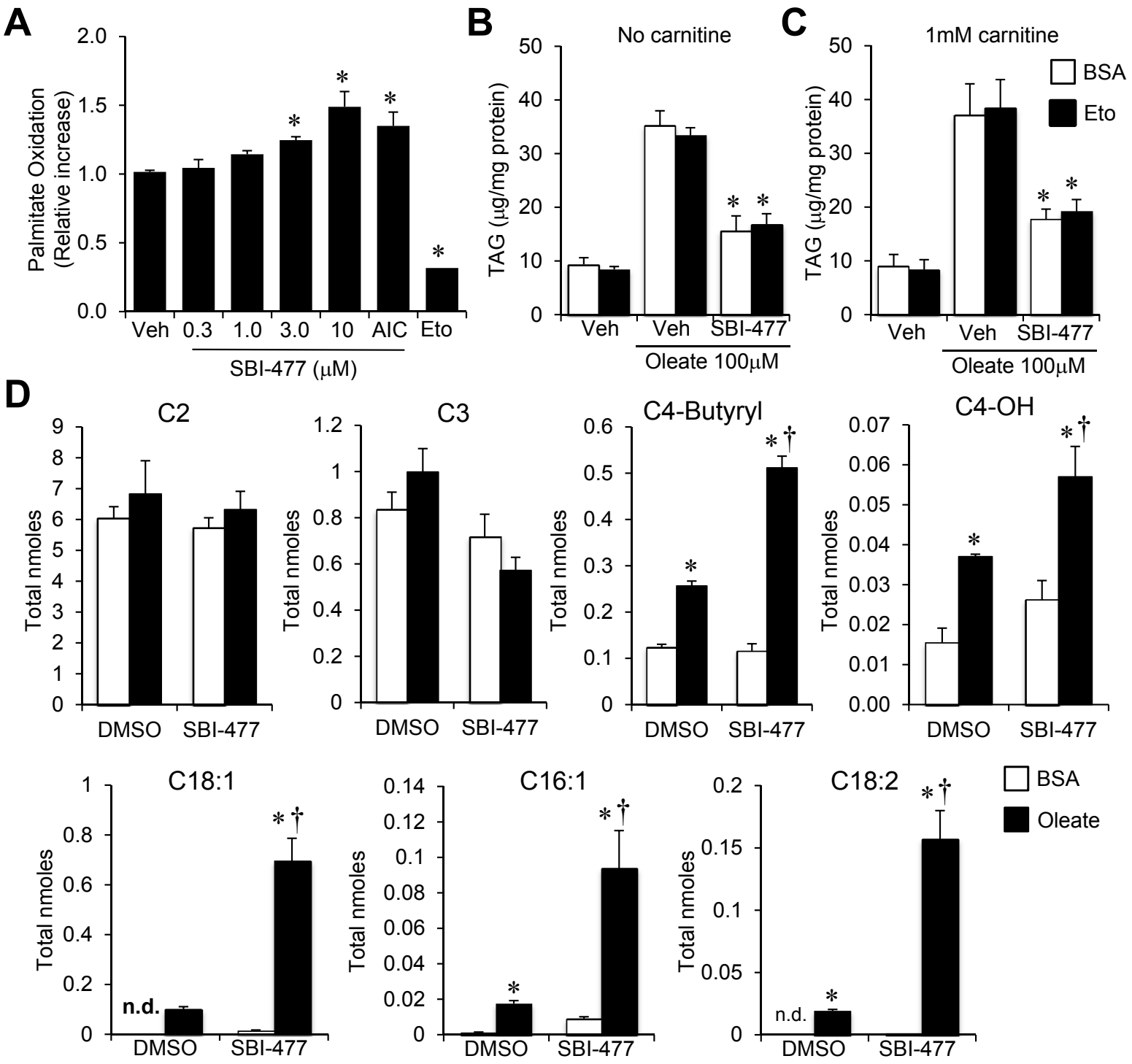
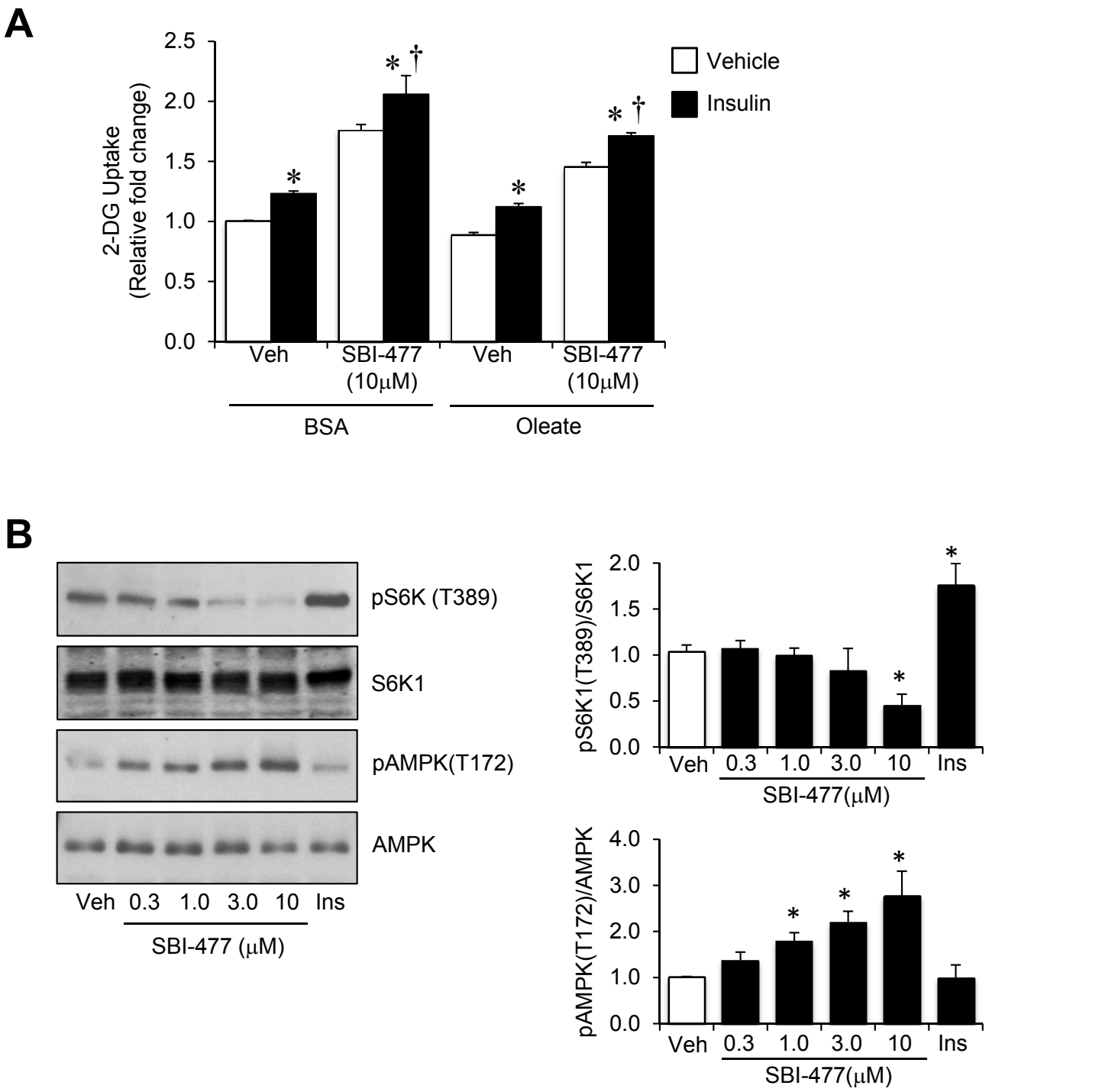
SUPPLEMENTAL DATA



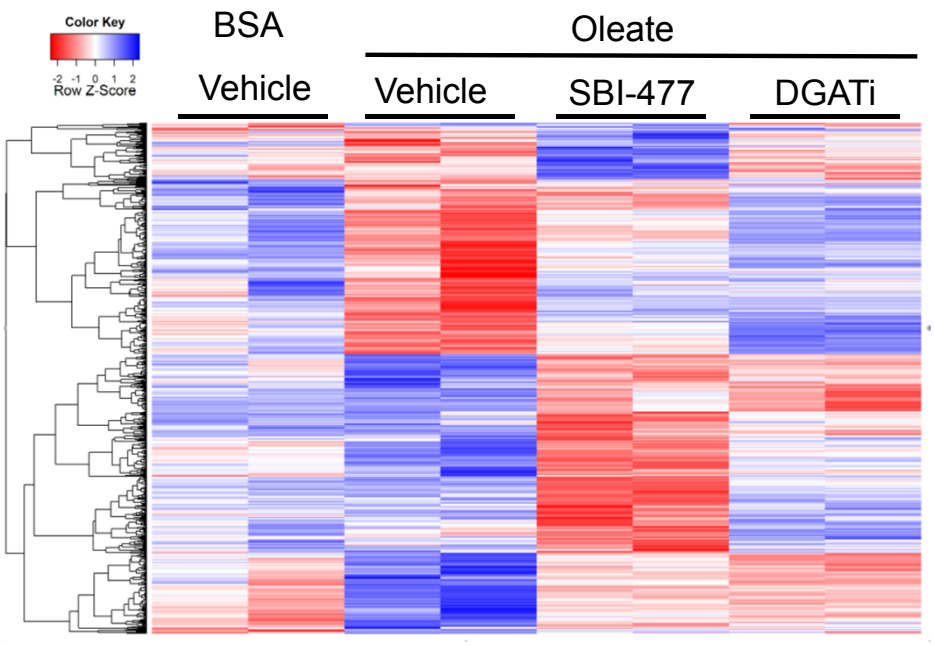
Supplemental Figure 1. Dose-dependent inhibition of myocyte TAG accumulation by SBI-477. (A) Primary human myotubes were incubated with the indicated concentrations of SBI-477, 1μM triascin C, 1μM A922500 (DGAT inhibitor) or vehicle control with 100μM oleate for 24 hours. Fatty acid free bovine serum albumin (BSA) was included as non-lipid loading control. Bars represent mean triglyceride level normalized to total protein ± SD. *p<0.001 vs. oleate/vehicle control by one-way ANOVA with Bonferroni post hoc test. (B) Primary human myotubes were incubated with 10μM SBI-477 or vehicle control for 24 hours. Fatty acid (FA) uptake was measured as cellular uptake of ^{3}H-oleate. Bars represent fatty acid uptake normalized to the vehicle control.



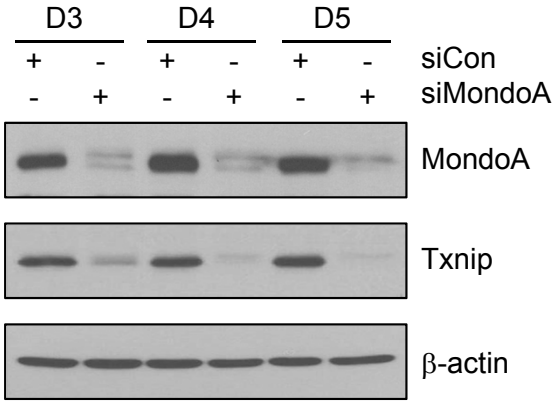
Supplemental Figure 2. SBI-477 increases FAO rates in human skeletal myotubes. (A) Human skeletal myotubes were incubated with the indicated concentration of SBI-477, AICAR (1mM) or etomoxir (50μM) for 24 hours. FAO rates were determined by ^{3}H-palmitate oxidation (n=5). AICAR and etomoxir were included as controls for activation and inhibition of FAO, respectively. *p<0.01 vs. vehicle. Total cellular triglyceride levels were determined following incubation with SBI-477 (10μM) for 24 hours with etomoxir (50μM) in the absence (B) or presence of 1mM carnitine (C) (n=4). *p<0.05 vs. vehicle control. (D) Quantitative metabolite profiling was performed on human skeletal myotubes following incubation with 10μM SBI-477 in the presence of 100μM oleate (black bars) or BSA non-lipid loading control (white bars). Levels of carnitine ester intermediates of mitochondrial FAO (C4-butyryl, C4-OH, C18:1, C16:1 and C18:2) are shown. *p<0.05 vs. DMSO/BSA, †p<0.05 vs. DMSO/Oleate. *p<0.05 vs. oleate-loaded vehicle. The data represent mean ± SD. All statistical significance determined by one-way ANOVA with Bonferroni post hoc test.



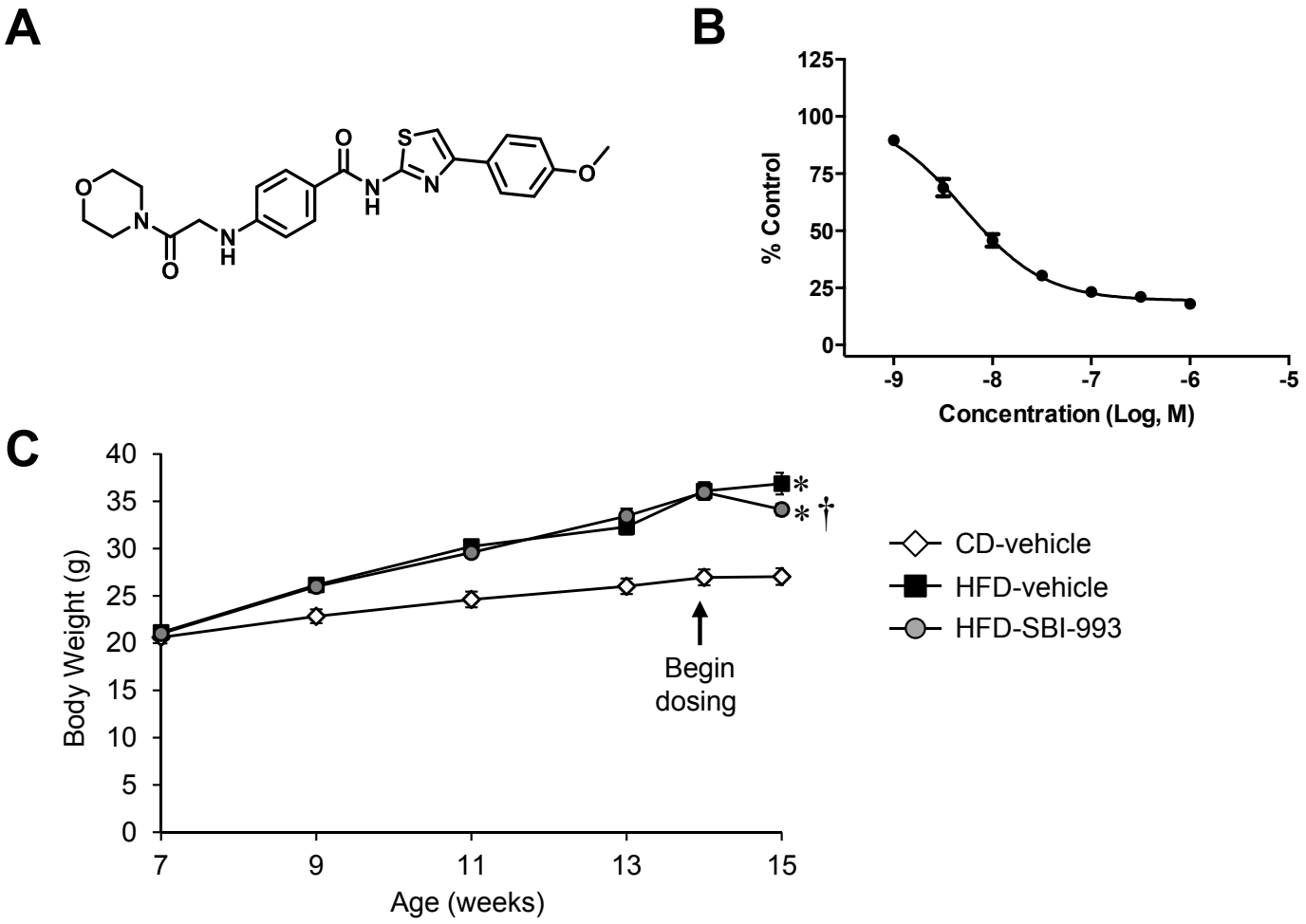
Supplemental Figure 3. SBI-477 increases glucose uptake in oleate-loaded skeletal myotubes (A) SBI-477 increases glucose uptake in oleate-loaded human skeletal myotubes. Basal and insulin-stimulated [^{3}H]2-DG uptake was measured in human skeletal myotubes following treatment with SBI-477 for 24 hours in the presence of 100μM oleate or BSA as a control. *p<0.05 vs. vehicle, †p<0.05 vs. vehicle/insulin by one-way ANOVA with Bonferroni post hoc test. (B) Western blot analysis (left) of human myotubes treated with SBI-477 for 24 hours was performed to determine activation of S6K and AMPK using the indicated phospho-specific antibodies. Insulin treatment (100nM) for 30 minutes was used as positive control. Quantitation (right) of the Western blot data is shown (n=4-5). *p<0.05 vs. Vehicle by one-way ANOVA with Bonferroni post hoc test. The data represent mean ± SD.



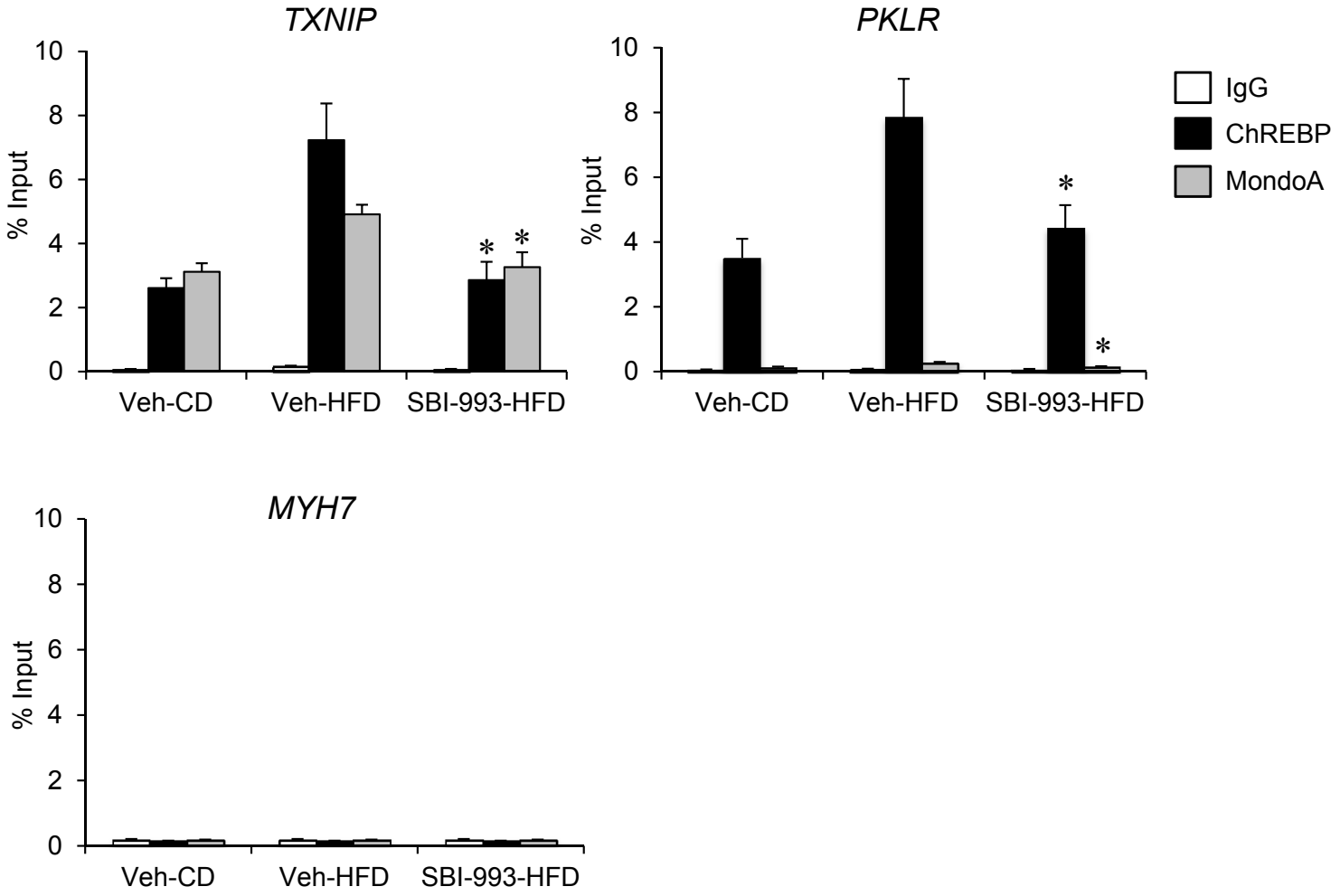
Supplemental Figure 4. Heat map representation of transcriptomic data in skeletal myotubes treated with SBI-477. SBI-477 elicits a distinct transcriptomic signature in oleate-loaded human skeletal myotubes. A heat map was generated using all significantly differentially expressed transcripts from microarray analysis performed on human myotubes treated with 100μM oleate and 10μM SBI-477, 1μM A922500 (DGAT inhibitor) or vehicle control as indicated at the top. Relative expression across all samples for each transcript is denoted by blue (increased) and red (decreased) colors as indicated in the color key. A cluster dendogram is shown on the left according to expression pattern across the treatment groups.



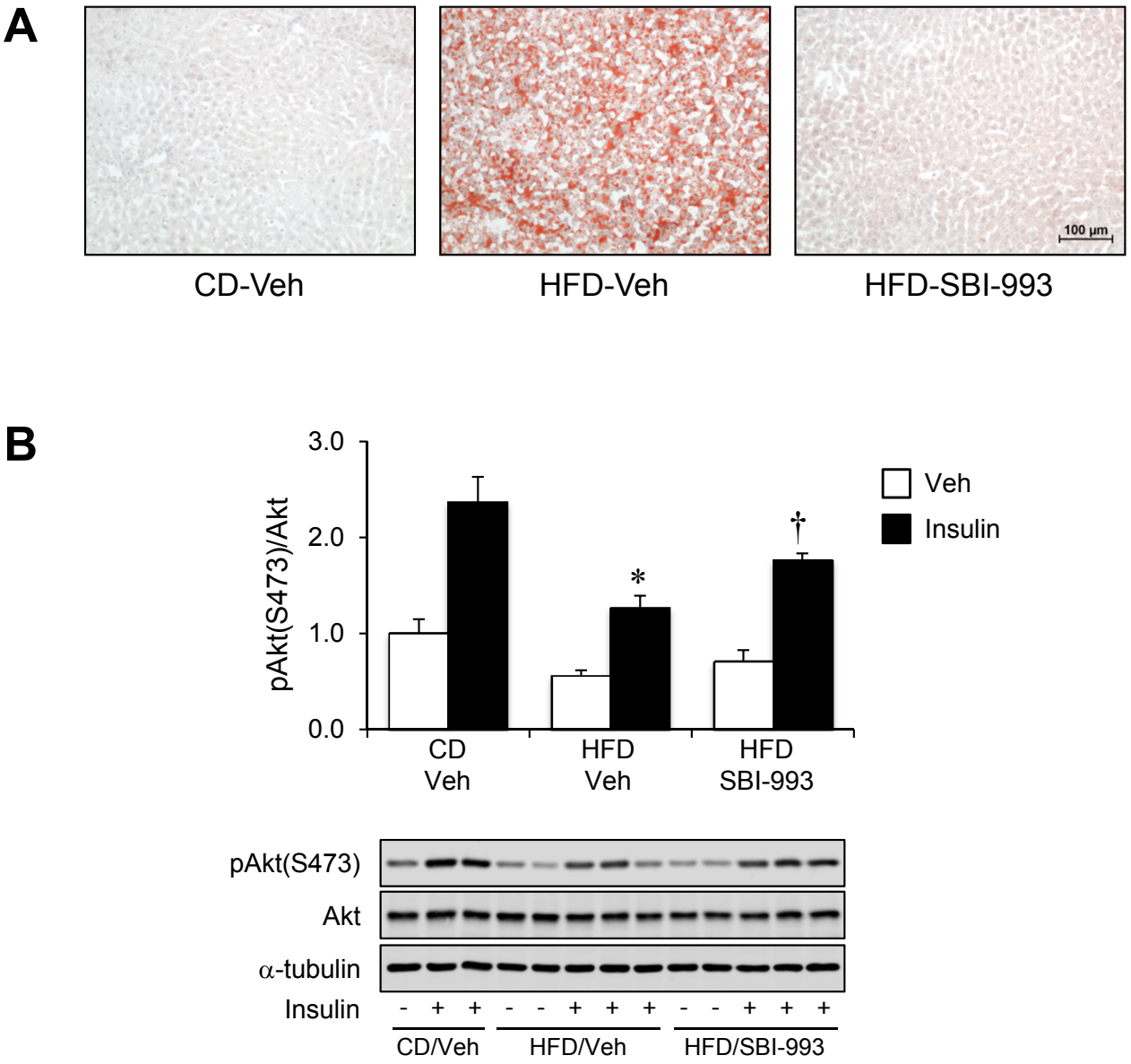
Supplemental Figure 5. Effective MondoA protein knockdown in human skeletal myotubes. MondoA and TXNIP protein levels as determined by immunoblotting on day 3, 4, and 5 after siMondoA or non-targeting control siRNA (siCon) knockdown in human myotubes is shown.



Supplemental Figure 6. SBI-993 inhibits triglyceride accumulation in vitro and in vivo. (A) The chemical structure of SBI-993 is shown. (B) A dose-response curve for the inhibition of triglyceride accumulation for SBI-993 in human skeletal myotubes is shown. (C) Body weight of mice over the study beginning at 7 weeks of age and start of high-fat diet (HFD) (n=6-10). Data represents mean ± SEM. *p<0.05 vs. CD-vehicle; †p<0.05 vs. HFD-vehicle by one-way ANOVA with Bonferroni post hoc test.



Supplemental Figure 7. SBI-993 reduces ChREBP and MondoA occupation on target gene promoter elements in liver. ChIP-qPCR analysis was performed with nuclear extracts from liver tissue of mice following 1 week administration of SBI-993 or a vehicle control. Antibodies directed against ChREBP (black bars), MondoA (gray bars) or an IgG (open bars) control were used. Occupation of carbohydrate response elements from the *Txnip* and pyruvate kinase (*Pklr*) gene promoters is shown as % input. Occupation of an unrelated region within intron 26 of the *Myh7* gene was used a negative control. **p*<0.05 vs. Veh-HFD for indicated antibody by one-way ANOVA followed by Bonferroni post hoc test. CD, control diet; HFD, high fat diet.



Supplemental Figure 8. SBI-993 inhibits hepatic steatosis. (A)Representative micrograph images of Oil Red O stained liver sections are shown for control diet-vehicle, high-fat diet-vehicle, and high-fat diet-SBI-993 treated groups show reduced hepatic steatosis following SBI-993 treatment. The images are representative of staining from 6 individual mice. (B) Western blot analysis of liver whole cell lysates from mice receiving an acute insulin challenge (1.5U/kg for 10 minutes) to examine insulin signaling using phosphorylated Akt (S473). Top panel is quantification of western blot analysis (n=4-6/condition). Representative western blots are shown in the bottom panel. *p<0.05 vs. CD/vehicle and † p<0.05 vs. HFD/vehicle by one-way ANOVA with Bonferroni post hoc test. CD, control diet, HFD, high-fat diet.

Supplemental Table 1. Lipidomics results from oleate-loaded human myotubes

Lipid (nmol/mg protein)	MASS	DMSO (N=3)	SBI-477 (N=3)
Phosphatidylethanolamine (PE)			
P16:0-18:2	698.512	0.02±0.02	0.07±0.03
P18:1-16:0/P16:0-18:1	700.528	0.47±0.11	0.58±0.17
D16:0-18:2/D16:1-18:1	714.507	0.14±0.03	0.19±0.08
D16:0-18:1	716.523	0.5±0.02	0.44±0.14
P16:1-20:4/ P14:0-22:5	720.497	0.31±0.06	0.33±0.05
P16:0-20:4	722.512	3.38±0.29	3.52±0.11
P16:0-20:3/P18:1-18:2	724.528	0.26±0.05	0.22±0.11
P18:1-18:1/P18:0-18:2/P16:0-20:2	726.544	0.98±0.05	0.9±0.08
P18:0-18:1/P16:0-20:1	728.559	0.4±0.11	0.37±0.09
A18:0-18:1	730.575	0.13±0.05	0.09±0.02
D16:1-20:4	736.492	0.49±0.08	0.44±0.1
D16:0-20:4/D18:2-18:2	738.507	0.17±0.06	0.23±0.04
D18:1-18:2/D16:0-20:3	740.523	0.21±0.06	0.13±0.04
D18:0-18:2/D18:1-18:1/D16:0-20:2	742.539	9.17±1.19	9.75±1.12
D18:0-18:1/D16:0-20:1	744.554	1.35±0.14	1.25±0.05
P16:0-22:6/D18:0-18:0/P18:2-20:4	746.512	1.14±0.15	1.13±0.14
P18:1-20:4/P16:0-22:5	748.528	3.97±0.48	3.98±0.14
P18:0-20:4/P16:0-22:4/P18:1-20:3	750.544	3.97±0.56	3.93±0.2
P18:0-20:3	752.559	0.36±0.03	0.27±0.09
P20:1-18:1/P18:1-20:1	754.575	0.35±0.04	0.31±0.05
D16:0-22:6	762.507	0.21±0.03	0.12±0.06
D18:1-20:4D/16:0-22:5	764.523	1.63±0.15	1.64±0.1
D18:0-20:4/D16:0-22:4	766.539	4.61±0.35	4.73±0.43
D18:0-20:3/D18:1-20:2/D16:0-22:3	768.554	0.52±0.1	0.52±0.03
P18:2-22:6/D18:1-20:1	770.570	0.51±0.09	0.29±0.06*
P18:1-22:6/D18:0-20:1	772.528	1.04±0.03	1.05±0.15
P18:0-22:6/P18:1-22:5/D18:0-20:0	774.544	1.17±0.23	1.31±0.03
P18:0-22:5/P18:1-22:4	776.559	1.04±0.18	1.09±0.1
P18:0-22:4/P20:0-20:4/P18:1-22:3	778.575	0.67±0.11	0.61±0.1
A20:0-20:4/P18:0-22:3	780.591	0.33±0.05	0.25±0.05
P18:0-22:2	782.606	0.27±0.01	0.2±0.05
D18:1-22:6	788.523	0.92±0.11	0.88±0.11
D18:0-22:6/D18:1-22:5	790.539	0.68±0.04	0.81±0.06*
D18:0-22:5/D18:1-22:4	792.554	0.77±0.13	0.94±0.09
D20:0-20:4/D18:0-22:4	794.570	0.54±0.07	0.46±0.07
	Sum	42.66±3.32	43.03±1.79
Ceramide (CER)			
N16:0	536.504	0.19±0.02	0.25±0.03
N18:1	562.520	0.02±0.01	0.03±0.01
N18:0	564.536	0.01±0	0.03±0*
N22:0	620.598	0.03±0.01	0.04±0.01
N24:2	644.598	0.01±0	0.01±0
N24:1	646.614	0.16±0.02	0.16±0.03
N24:0	648.629	0.11±0.01	0.15±0.02
	Sum	0.53±0.04	0.66±0.09
Phosphatidylinositol (PI)			
16:0-18:2	833.518	0.1±0.01	0.16±0.03
16:0-18:1	835.534	0.05±0.01	0.07±0.01
16:0-20:4	857.518	0.04±0.01	0.05±0.01
18:1-18:2	859.534	0.04±0.02	0.05±0.01
18:0-18:2	861.549	0.93±0.19	1.12±0.14

18:0-18:1	863.565	0.28±0.05	0.26±0.07
18:0-18:0	865.581	0.01±0.01	0.01±0
18:2-20:4/16:0-22:6	881.518	0.01±0	0.01±0
18:1-20:4	883.534	0.54±0.14	0.71±0.07
18:0-20:4	885.549	1.12±0.19	1.22±0.18
18:0-20:3	887.565	0.18±0.06	0.13±0.05
18:0-20:2	889.581	0.05±0.02	0.03±0.01
18:1-22:6	907.534	0.02±0.01	0.02±0.01
18:0-22:6	909.549	0.05±0.01	0.06±0.01
18:0-22:5	911.565	0.07±0.01	0.09±0.01
18:0-22:4	913.581	0.03±0.01	0.04±0.02
18:0-22:3	915.596	0.01±0	0±0
	Sum	3.53±0.67	4.03±0.6
Cardiolipin (CL)			
18:2-18:1-16:1-16:1	698.973	0.03±0.02	0.08±0.03
18:1-18:1-16:1-16:1	699.981	0.05±0.02	0.09±0.03
18:2-18:2-18:2-16:1	710.973	0.01±0	0.01±0.01
18:2-18:2-18:2-16:0	711.981	0.13±0.05	0.17±0.03
18:2-18:1-18:1-16:1 18:2-18:2-18:1-16:0	712.988	0.32±0.07	0.39±0.05
16:1-18:1-18:1-18:1	713.996	0.2±0.06	0.27±0.03
18:2-18:2-18:2-18:2	723.981	0.01±0.01	0.02±0.01
18:2-18:2-18:2-18:1	724.988	0.09±0.05	0.14±0.03
18:2-18:2-18:1-18:1	725.996	0.34±0.05	0.39±0.04
18:2-18:2-18:1-18:0 18:2-18:1-18:1-18:1	727.004	0.34±0.07	0.37±0.03
18:1-18:1-18:1-18:1	728.012	0.45±0.07	0.49±0.03
18:2-18:2-18:2-20:3	736.988	0±0	0.01±0.01
18:2-18:2-18:2-20:2	737.996	0.03±0.02	0.05±0.02
18:1-18:2-18:2-20:2	739.004	0.03±0.02	0.06±0.02
	Sum	2.03±0.51	2.53±0.27
Phosphatidylserine (PS)			
16:0-16:1	732.482	0.03±0.01	0.04±0.01
16:0-16:0	734.498	0.01±0	0.01±0.01
P16:0-18:0	746.534	0.02±0	0.02±0.01
16:0-18:2	758.498	0.11±0.01	0.12±0.02
16:0-18:1	760.513	0.74±0.11	0.77±0.03
16:0-18:0	762.529	0.05±0.01	0.04±0.01
P18:0-18:1	772.550	0.03±0	0.04±0.01
P18:0-18:0	774.565	0.19±0.03	0.2±0.02
18:2-18:2	782.498	0.01±0.01	0.03±0.01*
18:1-18:2	784.513	0.09±0.02	0.1±0.02
18:0-18:2	786.529	1.57±0.2	1.65±0.11
18:0-18:1	788.545	3.68±0.39	3.45±0.14
18:0-18:0	790.560	0.08±0.03	0.06±0.05
P18:0-20:4	794.534	0.01±0	0.01±0
P18:0-20:3	796.550	0.02±0.01	0.02±0
P18:0-20:2	798.565	0.01±0	0.01±0
P18:0-20:1	800.581	0.04±0.01	0.04±0.01
P18:0-20:0	802.597	0.04±0.01	0.04±0.01
18:1-20:4	808.513	0.1±0.01	0.11±0.01
18:0-20:4	810.529	0.8±0.08	0.76±0.05
18:0-20:3	812.545	0.56±0.07	0.49±0.05
18:0-20:2	814.560	0.6±0.05	0.43±0.01*
18:0-20:1	816.576	0.13±0.01	0.11±0.01
P20:0-20:3/P18:0-22:3	824.581	0.01±0	0.02±0.01
P20:0-20:2/P18:0-22:2	826.597	0.02±0.01	0.02±0
P20:0-20:1/P18:0-22:1	828.612	0.02±0.01	0.02±0.01
18:1-22:6	832.513	0.03±0.01	0.05±0.01

18:0-22:6	834.529	0.25±0.02	0.29±0.04
18:0-22:5	836.545	0.25±0.03	0.3±0.03
20:0-20:4/18:0-22:4	838.560	0.39±0.03	0.41±0.01
20:0-20:3/18:0-22:3	840.576	0.09±0.01	0.08±0.01
20:0-20:2/18:0-22:2	842.592	0.35±0.04	0.18±0.03*
20:0-20:1/18:0-22:1	844.607	0.09±0.02	0.07±0.01
20:1-22:6	860.545	0.01±0	0.01±0
20:0-22:6	862.560	0.02±0.01	0.01±0
20:0-22:5	864.576	0.03±0.01	0.03±0
20:0-22:4	866.592	0.02±0.01	0.01±0
20:0-22:3	868.607	0.01±0	0±0.01
20:0-22:2	870.623	0.03±0.01	0.01±0
20:0-22:1	872.639	0.03±0.01	0.03±0
	Sum	10.55±1.07	10.08±0.48
Phosphatidylglycerol (PG)			
16:0-18:2	745.502	0.05±0.03	0.08±0
16:0-18:1	747.518	0.06±0.02	0.09±0.01
18:1-18:2	771.518	0.09±0.01	0.13±0*
18:1-18:1	773.533	1.53±0.1	1.45±0.05
18:0-18:1	775.549	0.1±0.02	0.11±0.01
18:1-20:4	795.518	0.16±0.01	0.29±0.01*
18:1-22:6	819.518	0.51±0.04	0.56±0.03
18:0-22:6	821.533	0.2±0.03	0.25±0
	Sum	2.7±0.19	2.95±0.1
Phosphatidic acid (PA)			
16:0-16:1PA	645.450	2.35±1.03	4±1.47
16:0-16:0PA	647.466	0.15±0.05	0.28±0.16
16:0-18:2PA	671.466	34.09±1.39	40.43±3.45*
16:0-18:1PA	673.481	24.2±5.6	30.91±2.76
18:0-18:2/18:1-18:1PA	699.497	100.35±15.44	113.6±17.14
18:0-18:1PA	701.513	8.44±2.18	8.78±0.9
18:0-20:4PA	723.497	8.63±3.69	6.73±2.74
18:0-20:2PA	727.528	8.5±2.95	7.53±5.34
	Sum	186.7±27.04	212.26±27.76
Sphingomyelin (SM)			
N14:1	679.537	0.02±0.01	0.02±0
N14:0	681.552	0.57±0.03	0.44±0.04
N15:0	695.568	0.26±0.04	0.25±0.03
N16:1	707.568	1.02±0.08	0.85±0.06
N16:0	709.584	6.22±0.51	4.64±0.33*
N17:1	721.584	0.06±0.01	0.06±0.01
N17:0	723.599	0.28±0.04	0.2±0.01
N18:2	733.584	0.04±0.01	0.06±0.02
N18:1	735.599	0.53±0.07	0.49±0.04
N18:0	737.615	0.31±0.01	0.22±0.01
N20:1	763.631	0.03±0	0.03±0.01
N20:0	765.646	0.06±0.01	0.05±0
N21:0	779.662	0.01±0	0.01±0
N22:2	789.646	0.03±0	0.03±0.01
N22:1	791.662	0.32±0.03	0.25±0.04
N22:0	793.677	0.42±0.01	0.34±0.03*
N23:2	803.662	0.02±0	0.02±0
N23:1	805.677	0.15±0.01	0.12±0.02
N23:0	807.693	0.11±0.01	0.07±0.01*
N24:3	815.662	0.07±0.01	0.06±0.01
N24:2	817.677	0.73±0.06	0.44±0.02*
N24:1	819.693	2.3±0.12	1.17±0.13*

N24:0	821.709	0.62±0.09	0.44±0.06*
N25:1	833.709	0.07±0	0.03±0
N25:0	835.724	0.03±0	0.02±0.01
N26:2	845.709	0.05±0.01	0.02±0
N26:1	847.724	0.03±0.01	0.02±0.01
	Sum	14.36±1.08	10.34±0.83
Phosphatidylcholine (PC)			
D14:0-16:1	710.531	0.07±0	0.06±0.01
D14:0-16:0	712.547	0.37±0.05	0.3±0.02
A16:0-16:0	726.599	0.08±0.04	0.1±0.01
D16:1-16:1/D14:1-18:1	736.547	0.21±0.02	0.22±0.03
D16:1-16:0/D14:1-18:0	738.563	1.93±0.24	1.6±0.17
D16:0-16:0	740.578	0.99±0.14	0.85±0.09
P16:0-18:1/P18:1-16:0	750.599	0.51±0.04	0.46±0.02
P16:0-18:0/P18:0-16:0/A18:1-16:0/A16:0-18:1	752.615	0.66±0.09	0.62±0.07
A16:0-18:0	754.630	0.42±0.05	0.32±0.03*
D16:1-18:2	762.563	0.15±0.01	0.16±0.01
D16:0-18:2	764.578	5.23±0.58	4.92±0.38
D16:0-18:1	766.594	8.31±1.46	6.64±1.68
D16:0-18:0	768.609	0.31±0.02	0.28±0.03
P16:0-20:4/P20:4-16:0/P18:2-18:2	772.583	0.8±0.12	0.62±0.08
P18:0-18:1/P18:1-18:0	778.630	0.76±0.09	0.64±0.02
A18:0-18:1/P18:0-18:0	780.646	0.42±0.05	0.33±0.03
D18:2-18:3/D16:1-20:4	786.563	0.13±0.01	0.1±0.01*
D18:2-18:2/D16:0-20:4	788.578	0.73±0.06	0.64±0.04
D18:1-18:2/D16:0-20:3	790.594	1.4±0.15	1.07±0.09*
D18:0-18:2/D18:1-18:1	792.609	32.56±1.78	22.96±3.53*
D18:0-18:1	794.625	3.3±0.38	2.38±0.19
P18:1-20:4	798.599	0.52±0.08	0.39±0.06
P18:0-20:4	800.615	0.69±0.09	0.59±0.07
P18:0-20:1/P16:0-22:1	806.661	0.36±0.04	0.26±0.01*
D16:0-22:6/D18:2-20:4	812.578	0.25±0.02	0.2±0.01*
D18:1-20:4/D16:0-22:5	814.594	3.29±0.34	2.32±0.19*
D18:2-20:2/D18:0-20:4	816.609	1.52±0.17	1.13±0.13*
D18:0-20:3	818.625	0.52±0.08	0.35±0.03*
D18:0-20:2/P18:2-22:6	820.641	1.53±0.18	0.72±0.05*
D18:0-20:1/P18:1-22:6	822.656	0.1±0.01	0.05±0.01*
D18:1-22:6/D18:2-22:5	838.594	0.3±0.04	0.23±0.02*
D18:0-22:6	840.609	0.38±0.04	0.34±0.05
D18:0-22:5	842.625	0.52±0.06	0.39±0.03*
D18:0-22:4/D20:0-20:4/D20:2-20:2	844.641	0.13±0.02	0.08±0.01*
	Sum	69.46±3.71	52.35±6.99*
Lyso Phosphatidylcholine (LPC)			
P16:0	502.327	0.02±0	0.02±0
A16:0	504.343	0.07±0.01	0.08±0.05
16:1	516.307	0.09±0.02	0.12±0.03
16:0	518.322	0.16±0.01	0.19±0.03
P18:1	528.343	0.02±0.01	0.02±0
P18:0	530.359	0.03±0	0.04±0.01
18:2	542.322	0.04±0.01	0.03±0.01
18:1	544.338	0.81±0.1	0.84±0.17
18:0	546.354	0.08±0.01	0.08±0.01
P20:4	550.327	0.01±0.01	0.01±0
20:4	566.322	0.31±0.04	0.29±0.07
20:3	568.338	0.03±0.01	0.03±0.01
20:2	570.354	0.02±0.01	0.02±0.01

20:1	572.369	0.05±0.01	0.05±0.01
22:6	590.322	0.03±0.01	0.03±0.01
22:5	592.338	0.03±0.01	0.04±0.01
22:4	594.354	0.12±0.02	0.15±0.01
22:3	596.369	0.02±0	0.02±0.01
22:1	600.401	0.01±0	0.01±0
	Sum	1.96±0.23	2.05±0.29
Acyl Carnitine (CAR)			
16:1	398.327	1.08±0.19	1.24±0.41
16:0	400.343	0.72±0.35	0.93±0.31
18:1	426.358	11.28±3.54	15.82±1.1
18:0	428.374	1.97±0.46	2.5±0.45
	Sum	15.05±4.22	20.49±2.25
Diacylglycerol (DAG)			
D12:0-16:0/D14:0	598.504	0.02±0	0.02±0.01
D16:0-16:2	650.535	0.03±0	0.02±0*
D16:0-16:1	652.551	0.12±0.01	0.07±0*
D16:0/D14:0-18:0	654.567	0.08±0.01	0.07±0.02
D16:0-17:1	666.567	0.04±0	0.03±0*
D16:0-17:0	668.582	0.01±0	0.01±0
D16:1-18:2	676.551	0.03±0	0.02±0*
D16:0-18:2/D16:1-18:1/D17:1	678.567	0.67±0.03	0.39±0.01*
D16:0-18:1/D16:1-18:0	680.582	0.69±0.06	0.43±0.01*
D16:0-18:0	682.598	0.23±0.05	0.21±0.07
D16:0-19:2/D17:0-18:2	692.582	0.08±0.01	0.05±0*
D16:0-19:1/D17:0-18:1	694.598	0.03±0	0.02±0
D16:0-20:4/D16:1-20:3/D18:2	702.567	0.03±0	0.02±0
D18:1-18:2/D16:0-20:3	704.582	0.19±0.01	0.12±0*
D18:1/D18:0-18:2/D16:0-20:2	706.598	4.46±0.26	2.32±0.0*9
D18:1-18:0/D16:0-20:1	708.614	0.43±0.06	0.31±0.01*
D18:0	710.629	0.33±0.07	0.3±0.1
D18:0-19:1/D17:0-20:1	722.629	0.06±0.02	0.09±0.01
D18:1-20:4/D16:0-22:5	728.582	0.11±0.01	0.08±0*
D18:0-20:4/D18:1-20:3/D16:0-22:4	730.598	0.23±0.03	0.16±0*
D18:0-20:3/D18:1-20:2	732.614	0.17±0.02	0.1±0.01*
D18:0-20:2/D18:1-20:1/D18:2-20:0	734.629	0.23±0.02	0.09±0*
D18:0-20:1	736.645	0.02±0	0.01±0
D18:1-22:6	752.582	0.06±0	0.03±0*
D18:0-22:6/D18:1-22:5	754.598	0.08±0	0.05±0.01*
D18:0-22:5/D18:1-22:4/D20:1-20:4	756.614	0.08±0.01	0.06±0*
D18:0-22:4/D18:1-22:3/D20:0-20:4	758.629	0.05±0.01	0.02±0*
D18:0-22:3	760.645	0.02±0.01	0.01±0
	Sum	8.57±0.66	5.11±0.31*
Triacylglycerol (TAG)			
C46:2	781.690	0.12±0.02	0.15±0.02
C46:1/C47:8	783.705	0.13±0.03	0.15±0.02
C46:0/C47:7	785.721	0.11±0.03	0.16±0.01
C47:0/C48:7	799.737	0.08±0.02	0.1±0.01
C48:6	801.658	0.04±0.01	0.05±0.01
C48:5	803.674	0.07±0	0.1±0.01
C48:4	805.690	0.07±0.02	0.08±0.01
C48:3	807.705	0.08±0.01	0.08±0.01
C48:2/C49:9	809.721	0.18±0.01	0.11±0.01
C48:1/C49:8	811.737	0.14±0.01	0.1±0.01
C48:0/C49:7	813.752	0.1±0.01	0.12±0.02
C49:2/C50:9	823.737	0.07±0.01	0.06±0.01
C49:1/C50:8	825.752	0.08±0.01	0.08±0

C49:0/C50:7	827.674	0.04±0.01	0.04±0.01
C50:6	829.690	0.07±0.02	0.07±0.01
C50:5	831.705	0.08±0.01	0.08±0
C50:4	833.721	0.1±0.01	0.08±0.01
C50:3/C51:10	835.737	0.86±0.08	0.48±0.02*
C50:2/C51:9	837.752	1.66±0.2	0.68±0.02*
C50:1/C51:8	839.768	0.29±0.01	0.2±0.01*
C50:0/C51:7	841.784	0.09±0.02	0.11±0.02
C51:2/C52:9	851.768	0.36±0.05	0.19±0.01*
C51:1/C52:8	853.690	0.24±0	0.13±0*
C51:0/C52:7	855.705	0.09±0.02	0.07±0
C52:6	857.721	0.08±0.03	0.06±0.01
C52:5	859.737	0.21±0.01	0.18±0.01
C52:4/C53:11	861.752	0.85±0.11	0.49±0.02*
C52:3/C53:10	863.768	10.29±1.1	4.76±0.08*
C52:2/C53:9	865.784	6.26±0.43	2.91±0.05*
C52:1/C53:8	867.799	0.55±0.04	0.25±0.02*
C53:3/C54:10	877.784	0.62±0.08	0.33±0.02*
C53:2/C54:9	879.705	1.56±0.04	0.78±0.01*
C53:1/C54:8	881.721	0.96±0.05	0.49±0.03*
C53:0/C54:7	883.737	0.26±0.03	0.16±0.01*
C54:6	885.752	0.45±0.05	0.34±0.02*
C54:5/C55:12	887.768	1.06±0.08	0.71±0.02*
C54:4/C55:11	889.784	3.33±0.17	1.77±0.03*
C54:3/C55:10	891.799	52.82±3.29	21.73±0.18*
C54:2/C55:9	893.815	6.2±0.63	2.76±0.06*
C54:1/C55:8	895.831	0.24±0.05	0.16±0.03*
C55:2/C56:9	907.737	7.3±0.1	3.25±0.11*
C55:1/C56:8	909.752	1.36±0.07	0.67±0.02*
C55:0/C56:7	911.768	1.13±0.12	0.68±0.04*
C56:6	913.784	2.97±0.22	1.99±0.06*
C56:5/C57:12	915.799	3.39±0.48	1.84±0.01*
C56:4/C57:11	917.815	2.34±0.25	0.99±0.01*
C56:3/C57:10	919.831	3.54±0.29	1.13±0.1*
C56:2/C57:9	921.846	0.65±0.07	0.28±0.01*
C56:1/C57:8	923.862	0.08±0.02	0.08±0.02
C57:6/C58:13	927.799	0.16±0.02	0.13±0.01
C57:5/C58:12	929.815	0.45±0.03	0.37±0.01
C57:4/C58:11	931.737	0.43±0.01	0.23±0.01*
C57:3/C58:10	933.752	0.39±0.03	0.19±0*
C57:2/C58:9	935.768	0.6±0.03	0.26±0.01*
C57:1/C58:8	937.784	1.1±0.04	0.59±0.03*
C57:0/C58:7/C59:14	939.799	1.4±0.08	0.85±0.05*
C58:6/C59:13	941.815	1.47±0.08	0.92±0.03*
C58:5/C59:12	943.831	0.86±0.09	0.44±0.01*
C58:4/C59:11	945.846	0.3±0.04	0.15±0.01*
C58:3/C59:10	947.862	0.51±0.02	0.23±0.02*
	Sum	121.31±8.09	56.62±0.45*
TAG Fatty Acyl Chain content (FA)			
226(14:1)	226.193	1.09±0.07	1.03±0.04
228(14:0)	228.209	2.16±0.12	1.39±0.12*
252(16:2)	252.209	1.91±0.19	1.44±0.11*
254(16:1)	254.225	16.91±1.81	8.24±0.13*
256(16:0)	256.240	9.04±0.49	5.25±0.05*
278(18:3)	278.225	1.87±0.05	1.71±0.1*
280(18:2)	280.240	5.81±0.51	4±0.2*
282(18:1)	282.256	252.07±16.57	109.71±0.81*

284(18:0)	284.272	22.01±1.71	10.76±0.25*
302(20:5)	302.225	4.08±0.41	2.39±0.15*
304(20:4)	304.240	14.6±0.67	7.93±0.28*
306(20:3)	306.256	6.61±0.29	3.77±0.06*
308(20:2)	308.272	7.13±1.13	5.2±0.29*
310(20:1)	310.287	10.3±0.93	5.26±0.19*
312(20:0)	312.303	5.51±0.94	2.91±0.14*
328(22:6)	328.240	3.47±0.39	2.42±0.08*
330(22:5)	330.256	3.28±0.16	2.51±0.04*
332(22:4)	332.272	3.48±0.42	2.76±0.15*
334(22:3)	334.287	2.87±0.16	1.92±0.25*
336(22:2)	336.303	2.29±0.19	1.64±0.09*
338(22:1)	338.318	2.84±0.35	1.94±0.05*
340(22:0)	340.334	2.41±0.54	1.99±0.05*
	Sum	381.74±24.83	186.17±1.81*

*$p<0.05$ vs. DMSO control

Supplemental Table 2. Lipidomic results from non-oleate loaded human myotubes.

Lipid (nmol/mg protein)	MASS	DMSO (N=3)	SBI-477 (N=3)
Phosphatidylethanolamine (PE)			
P16:0-18:2	698.512	0.22±0.05	0.19±0.03
P18:1-16:0/P16:0-18:1	700.528	1.21±0.6	1.12±0.39
D16:1-18:2	712.492	0.12±0.06	0.11±0.04
D16:0-18:2/D16:1-18:1	714.507	0.64±0.31	0.73±0.26
D16:0-18:1	716.523	1.43±0.33	1.27±0.14
P16:1-20:4/ P14:0-22:5	720.497	1.29±0.49	0.87±0.24
P16:0-20:4	722.512	5.85±1.01	6.16±0.79
P16:0-20:3/P18:1-18:2	724.528	0.72±0.12	0.61±0.12
P18:1-18:1/P18:0-18:2/P16:0-20:2	726.544	0.95±0.18	0.77±0.08
P18:0-18:1/P16:0-20:1	728.559	0.82±0.56	0.58±0.03
A18:0-18:1	730.575	0.22±0.21	0.16±0.05
D14:1-22:6	732.460	0.12±0.07	0.03±0.02
D16:2-20:4/D14:1-22:5	734.476	0.26±0.12	0.18±0.07
D16:1-20:4	736.492	1.52±0.7	1.24±0.46
D16:0-20:4/D18:2-18:2	738.507	0.82±0.23	0.67±0.17
D18:1-18:2/D16:0-20:3	740.523	0.48±0.1	0.48±0.07
D18:0-18:2/D18:1-18:1/D16:0-20:2	742.539	2.79±1.05	2.96±0.82
D18:0-18:1/D16:0-20:1	744.554	1.99±0.74	1.84±0.38
P16:0-22:6/D18:0-18:0/P18:2-20:4	746.512	1.56±0.54	1.55±0.31
P18:1-20:4/P16:0-22:5	748.528	4.84±0.88	5.15±0.69
P18:0-20:4/P16:0-22:4/P18:1-20:3	750.544	4.75±0.77	5.17±0.76
P18:0-20:3	752.559	0.97±0.33	0.77±0.15
P20:1-18:1/P18:1-20:1	754.575	0.29±0.09	0.22±0.04
P18:1-20:0	756.591	0.18±0.11	0.08±0.04
D16:1-22:6	760.492	0.26±0.12	0.22±0.06
D16:0-22:6	762.507	1±0.46	0.65±0.2
D18:1-20:4D/16:0-22:5	764.523	2.42±0.51	2.47±0.25
D18:0-20:4/D16:0-22:4	766.539	5.61±0.43	6±0.73
D18:0-20:3/D18:1-20:2/D16:0-22:3	768.554	1.21±0.08	0.87±0.1*
P18:2-22:6/D18:1-20:1	770.570	0.24±0.11	0.23±0.08
P18:1-22:6/D18:0-20:1	772.528	0.84±0.21	0.81±0.18
P18:0-22:6/P18:1-22:5/D18:0-20:0	774.544	0.99±0.34	1.06±0.2
P18:0-22:5/P18:1-22:4	776.559	0.84±0.34	0.98±0.28
P18:0-22:4/P20:0-20:4/P18:1-22:3	778.575	0.58±0.21	0.64±0.17
A20:0-20:4/P18:0-22:3	780.591	0.3±0.14	0.21±0.09
P18:0-22:2	782.606	0.17±0.1	0.09±0.05
D18:1-22:6	788.523	2.01±0.39	2.21±0.79
D18:0-22:6/D18:1-22:5	790.539	0.63±0.12	0.78±0.15
D18:0-22:5/D18:1-22:4	792.554	1.16±0.47	1.16±0.11
D20:0-20:4/D18:0-22:4	794.570	1.83±0.86	1.41±0.27
D20:0-20:3/D18:0-22:3	796.586	0.34±0.09	0.2±0.02
D20:0-20:2/D18:0-22:2	798.601	0.08±0.02	0.08±0.04
	Sum	54.54±6.29	52.96±3.91
Ceramide			
N16:0	536.504	0.39±0	0.45±0.04
N18:0	564.536	0.04±0	0.05±0.01
N22:0	620.598	0.04±0.02	0.06±0.02
N23:0	634.614	0.01±0	0.02±0.01
N24:2	644.598	0.02±0	0.02±0.01
N24:1	646.614	0.18±0.03	0.22±0.02
N24:0	648.629	0.18±0.04	0.21±0.05
	Sum	0.86±0.07	1.02±0.15
Phosphatidylinositol (PI)			
16:1-16:1	805.487	0±0	0.01±0.01

16:0-16:1	807.502	0.05±0	0.05±0.01
16:0-16:0	809.518	0.01±0.01	0±0.01
16:1-18:2/16:0-18:3	831.502	0.03±0	0.06±0.01*
16:0-18:2	833.518	0.25±0.02	0.39±0.04*
16:0-18:1	835.534	0.4±0.04	0.55±0.02*
16:0-18:0	837.549	0.02±0.01	0.04±0.01
16:1-20:4	855.502	0.02±0.01	0.01±0.01
16:0-20:4	857.518	0.28±0.08	0.32±0.05
18:1-18:2	859.534	0.15±0.04	0.2±0.03
18:0-18:2	861.549	0.43±0.08	0.64±0.08*
18:0-18:1	863.565	0.26±0.06	0.38±0.03*
18:0-18:0	865.581	0.01±0	0.02±0
18:2-20:4/16:0-22:6	881.518	0.04±0.01	0.05±0.01
18:1-20:4	883.534	0.91±0.15	1.34±0.07*
18:0-20:4	885.549	4.59±1.09	5.56±1.57
18:0-20:3	887.565	0.76±0.31	0.73±0.14
18:0-20:2	889.581	0.07±0.05	0.04±0.04
18:1-22:6	907.534	0.03±0.01	0.07±0.02*
18:0-22:6	909.549	0.14±0.03	0.23±0.02*
18:0-22:5	911.565	0.19±0.02	0.35±0.01*
18:0-22:4	913.581	0.14±0.02	0.23±0.02*
18:0-22:3	915.596	0.03±0.03	0.02±0.01
	Sum	8.81±0.85	11.29±1.46
Cardiolipin			
16:1-16:1-16:1-18:1	685.965	0.09±0.07	0.11±0.08
18:2-18:2-16:1-16:1	697.965	0.03±0.02	0.05±0.03
18:1-18:2-16:1-16:1	698.973	0.2±0.12	0.25±0.12
18:1-18:1-16:1-16:1	699.981	0.25±0.2	0.32±0.22
18:2-18:2-18:2-16:0	711.981	0.19±0.02	0.24±0.01*
18:2-18:1-18:1-16:1 18:2-18:2-18:1-16:0	712.988	0.35±0.14	0.45±0.13
16:1-18:1-18:1-18:1	713.996	0.15±0.08	0.19±0.07
18:2-18:2-18:2-18:1	724.988	0.12±0.01	0.14±0.04
18:2-18:2-18:1-18:1	725.996	0.21±0.02	0.25±0.05
18:2-18:2-18:1-18:0 18:2-18:1-18:1-18:1	727.004	0.11±0.03	0.14±0.02
18:1-18:1-18:1-18:1	728.012	0.02±0	0.02±0.02
18:1-18:2-18:2-20:2	739.004	0.03±0	0.04±0.01*
	Sum	1.74±0.66	2.2±0.51
Phosphatidylserine (PS)			
16:0-16:1	732.482	0.05±0.02	0.08±0.01*
P16:0-18:0	746.534	0.04±0.02	0.07±0.02
16:0-18:2	758.497	0.23±0.08	0.31±0.02
16:0-18:1	760.513	1.91±0.75	2.18±0.48
16:0-18:0	762.529	0.06±0.03	0.07±0.01
P18:0-18:1	772.549	0.07±0.02	0.1±0.02
P18:0-18:0	774.565	0.44±0.11	0.61±0.18
18:2-18:2	782.497	0.05±0.04	0.07±0.01
18:1-18:2	784.513	0.22±0.12	0.32±0.04
18:0-18:2	786.529	2.44±1.34	2.51±0.33
18:0-18:1	788.544	8.48±3.39	9.7±2.8
18:0-18:0	790.560	0.18±0.06	0.21±0.05
P18:0-20:1	800.581	0.08±0.06	0.09±0.02
P18:0-20:0	802.596	0.1±0.03	0.11±0.04
18:2-20:4	806.497	0.05±0.03	0.05±0.01
18:1-20:4	808.513	0.27±0.09	0.26±0.02
18:0-20:4	810.529	2.17±0.58	1.99±0.56
18:0-20:3	812.544	1.57±0.89	1.51±0.22
18:0-20:2	814.560	0.51±0.46	0.52±0.13
18:0-20:1	816.575	0.35±0.16	0.35±0.09
P20:0-20:3/P18:0-22:3	824.581	0.03±0.04	0.06±0.01

P20:0-20:2/P18:0-22:2	826.596	0.1±0.06	0.1±0.02
18:1-22:6	832.513	0.07±0.07	0.12±0.06
18:0-22:6	834.529	0.79±0.54	0.96±0.05
18:0-22:5	836.544	0.73±0.45	1.03±0.11
20:0-20:4/18:0-22:4	838.560	1.21±0.54	1.76±0.4
20:0-20:3/18:0-22:3	840.575	0.29±0.24	0.27±0.04
20:0-20:2/18:0-22:2	842.591	0.33±0.24	0.33±0.05
20:0-20:1/18:0-22:1	844.607	0.33±0.13	0.32±0.15
20:0-22:6	862.560	0.02±0.01	0.04±0.01
20:0-22:5	864.575	0.04±0.02	0.07±0.02
20:0-22:4	866.591	0.04±0.01	0.05±0.02
20:0-22:3	868.607	0.05±0.05	0.05±0.02
20:0-22:2	870.622	0.12±0.08	0.09±0.02
20:0-22:1	872.638	0.14±0.05	0.14±0.05
	Sum	23.58±10.69	26.51±5.29
Phosphatidylglycerol (PG)			
16:1-18:2	743.486	0.01±0.02	0.01±0.01
16:0-18:2	745.502	0.15±0.14	0.16±0.12
16:0-18:1	747.518	0.35±0.21	0.32±0.1
18:2-18:2	769.502	0.04±0.05	0.04±0.04
18:1-18:2	771.518	0.21±0.18	0.19±0.12
18:1-18:1	773.533	0.66±0.58	0.51±0.29
18:0-18:1	775.549	0.25±0.15	0.21±0.04
18:2-20:4/16:0-22:6	793.502	0.05±0.01	0.12±0.02*
18:1-20:4	795.518	0.14±0.07	0.31±0.12*
18:1-22:6	819.518	0.43±0.17	0.44±0.12
18:0-22:6	821.533	0.19±0.08	0.21±0.07
	Sum	2.49±1.65	2.51±1.04
Phosphatidic acid (PA)			
16:1-16:1PA	643.434	0.02±0.01	0.02±0
16:0-16:1PA	645.450	0.06±0.02	0.05±0
16:0-16:0PA	647.466	0.02±0.01	0.01±0
16:0-18:2PA	671.466	0.09±0.04	0.09±0.01
16:0-18:1PA	673.481	0.13±0.06	0.13±0.01
18:0-18:2/18:1-18:1PA	699.497	0.08±0.05	0.09±0.03
18:0-18:1PA	701.513	0.05±0.02	0.07±0.02
18:0-20:4PA	723.497	0.06±0.02	0.07±0.01
	Sum	0.5±0.22	0.53±0.06
Sphingomyelin (SM)			
N14:1	679.537	0.07±0.05	0.05±0.02
N14:0	681.552	0.51±0.2	0.57±0.08
N15:0	695.568	0.19±0.02	0.23±0.02*
N16:1	707.568	1.43±0.67	1.4±0.34
N16:0	709.584	4.62±2.17	4.39±0.76
N17:1	721.584	0.04±0.01	0.05±0.01
N17:0	723.599	0.15±0.02	0.15±0.04
N18:2	733.584	0.02±0.03	0.01±0
N18:1	735.599	0.3±0.15	0.28±0.07
N18:0	737.615	0.22±0.07	0.25±0.03
N22:2	789.646	0.02±0.02	0.03±0.01
N22:1	791.662	0.18±0.06	0.25±0.03
N22:0	793.677	0.21±0.08	0.29±0.05
N23:1	805.677	0.07±0.01	0.13±0.02*
N23:0	807.693	0.05±0.02	0.08±0.01
N24:3	815.662	0.06±0.02	0.1±0.04
N24:2	817.677	0.57±0.35	0.68±0.22
N24:1	819.693	1±0.58	1.23±0.35
N24:0	821.709	0.34±0.14	0.41±0.06

N25:1	833.709	0.03±0.01	0.04±0.01
N25:0	835.724	0.02±0.01	0.03±0
N26:2	845.709	0.02±0.02	0.03±0.02
N26:1	847.724	0.02±0.02	0.03±0.01
N26:0	849.740	0.01±0.01	0.01±0
	Sum	10.13±4.76	10.68±1.98
Phosphatidylcholine (PC)			
D14:0-16:1	710.531	0.59±0.35	0.54±0.1
D14:0-16:0	712.547	1.28±0.83	0.83±0.04
P16:0-16:1/P16:1-16:0/P18:1-14:0	722.568	0.11±0.03	0.14±0.01
P16:0-16:0/A16:0-16:1	724.583	0.42±0.25	0.38±0.06
A16:0-16:0	726.599	0.35±0.22	0.29±0.03
D16:1-16:2/D14:1-18:2	734.531	0.06±0	0.09±0.03
D16:1-16:1/D14:1-18:1	736.547	1.08±0.41	1.18±0.22
D16:1-16:0/D14:1-18:0	738.563	3.86±1.66	3.55±0.28
D16:0-16:0	740.578	2.04±1.09	1.38±0.09
P16:0-18:1/P18:1-16:0	750.599	0.66±0.34	0.65±0.08
P16:0-18:0/P18:0-16:0/A18:1-16:0/A16:0-18:1	752.615	1.3±0.76	1.3±0.09
A16:0-18:0	754.630	0.13±0.05	0.08±0.01
D14:1-20:3/D16:2-18:2/D14:0-20:4	760.547	0.17±0.03	0.3±0.02*
D16:1-18:2	762.563	0.62±0.33	0.63±0.11
D16:0-18:2/D16:1-18:1	764.578	5.72±2.94	6.12±1.72
D16:0-18:1/D16:1-18:0	766.594	11.73±5.61	11.27±1.86
D16:0-18:0	768.609	0.63±0.33	0.49±0.04
P16:0-20:4/P20:4-16:0/P18:2-18:2	772.583	0.64±0.2	0.63±0.09
A16:0-20:4	774.599	0.48±0.24	0.51±0.18
P18:1-18:1	776.615	0.27±0.2	0.27±0.05
P18:0-18:1/P18:1-18:0	778.630	0.79±0.56	0.64±0.08
A18:0-18:1/P18:0-18:0	780.646	0.64±0.4	0.46±0.04
D18:2-18:3/D16:1-20:4	786.563	0.43±0.07	0.42±0.03
D18:2-18:2/D16:0-20:4	788.578	1.72±0.69	1.68±0.32
D18:1-18:2/D16:0-20:3	790.594	2.21±0.99	2.16±0.45
D18:0-18:2/D18:1-18:1	792.609	6.53±3.38	6.26±1.72
D18:0-18:1	794.625	4.37±3.42	2.74±0.21
P18:1-20:4	798.599	0.42±0.22	0.49±0.15
P18:0-20:4	800.615	0.52±0.08	0.69±0.11
P18:2-20:1/P20:1-18:2/A18:0-20:4	802.630	0.35±0.19	0.33±0.06
P18:1-20:1/P16:1-22:1	804.646	0.09±0.08	0.05±0.03
P18:0-20:1/P16:0-22:1	806.661	0.15±0.12	0.1±0.07
D16:0-22:6/D18:2-20:4	812.578	0.43±0.11	0.4±0.03
D18:1-20:4/D16:0-22:5	814.594	1.89±0.63	1.84±0.12
D18:2-20:2/D18:0-20:4/D18:1-20:3	816.609	1.77±0.48	1.67±0.17
D18:0-20:3	818.625	0.66±0.57	0.46±0.19
D18:0-20:2/P18:2-22:6	820.641	0.19±0.18	0.1±0.03
D18:0-20:1/P18:1-22:6	822.656	0.01±0.02	0.01±0
D18:1-22:6/D18:2-22:5	838.594	0.16±0.06	0.18±0.03
D18:0-22:6	840.609	0.32±0.14	0.34±0.02
D18:0-22:5	842.625	0.27±0.09	0.36±0.03
D18:0-22:4/D20:0-20:4/D20:2-20:2	844.641	0.1±0.04	0.15±0.02
	Sum	56.18±28.37	52.19±8.33
Lyso Phosphatidylcholine (LPC)			
P16:0	502.327	0.06±0.01	0.1±0.03
A16:0	504.343	0.12±0.09	0.17±0.08
16:1	516.307	0.08±0.01	0.14±0.02*
16:0	518.322	0.2±0.01	0.3±0.03*
P18:2	526.327	0±0	0±0
P18:1	528.343	0.03±0.01	0.06±0.03
P18:0	530.359	0.06±0	0.1±0.03*
18:2	542.322	0.01±0	0.03±0*

18:1	544.338	0.2±0.01	0.37±0.03*
18:0	546.354	0.13±0.03	0.26±0.09
20:4	566.322	0.03±0	0.06±0.02*
20:3	568.338	0.01±0	0.01±0
20:2	570.354	0.01±0.01	0.03±0.01
20:1	572.369	0.04±0	0.07±0.02
22:6	590.322	0±0	0.01±0.01
22:5	592.338	0.01±0	0.02±0*
22:4	594.354	0.08±0.02	0.16±0.05
22:3	596.369	0.01±0	0.02±0.01
22:2	598.385	0±0.01	0±0
22:1	600.401	0.01±0.01	0.01±0.01
	Sum	1.1±0.05	1.92±0.2
Diacylglycerol (DAG)			
D12:0-16:0/D14:0	598.504	0.03±0.01	0.03±0.02
D16:0-18:2	650.535	0.04±0.01	0.04±0
D16:0-18:1	652.551	0.18±0.04	0.14±0.02
D16:0/D14:0-18:0	654.567	0.26±0.01	0.19±0.04
D16:1-17:1	664.551	0.01±0	0.01±0
D16:0-17:1	666.567	0.05±0.01	0.04±0.01
D16:0-17:0	668.582	0.03±0	0.03±0.01
D16:1-18:2	676.551	0.02±0.01	0.03±0
D16:0-18:2/D16:1-18:1/D17:1	678.567	0.24±0.07	0.24±0.02
D16:0-18:1/D16:1-18:0	680.582	0.53±0.15	0.42±0.04
D16:0-18:0	682.598	0.45±0.07	0.43±0.11
D16:0-19:2/D17:0-18:2	692.582	0.02±0	0.03±0
D16:0-19:1/D17:0-18:1	694.598	0.03±0	0.03±0
D16:0-19:0/D17:0-18:0	696.614	0.03±0.01	0.03±0.01
D16:0-20:4/D16:1-20:3/D18:2	702.567	0.05±0.01	0.04±0
D18:1-18:2/D16:0-20:3	704.582	0.07±0.02	0.08±0
D18:1/D18:0-18:2/D16:0-20:2	706.598	0.28±0.11	0.27±0.04
D18:1-18:0/D16:0-20:1	708.614	0.19±0.04	0.17±0.01
D18:0	710.629	0.49±0.07	0.51±0.11
D18:0-19:1/D17:0-20:1	722.629	0.05±0	0.05±0.02
D16:0-22:6/D18:2-20:4/D18:3-20:3	726.567	0.05±0.01	0.05±0.02
D18:1-20:4/D16:0-22:5	728.582	0.08±0.01	0.09±0.01
D18:0-20:4/D18:1-20:3/D16:0-22:4	730.598	0.32±0.09	0.28±0.11
D18:0-20:3/D18:1-20:2	732.614	0.09±0.02	0.05±0*
D18:0-20:2/D18:1-20:1/D18:2-20:0	734.629	0.03±0.02	0.02±0
D18:0-20:1	736.645	0.01±0	0.01±0*
D18:0-20:0	738.661	0.01±0	0.01±0
D18:1-22:6	752.582	0.04±0.01	0.03±0.01
D18:0-22:6/D18:1-22:5	754.598	0.03±0	0.03±0
D18:0-22:5/D18:1-22:4/D20:1-20:4	756.614	0.03±0	0.03±0.01
D18:0-22:4/D18:1-22:3/D20:0-20:4	758.629	0.02±0.01	0.02±0
D18:0-22:3	760.645	0±0	0±0
	Sum	3.8±0.28	3.44±0.43
Triacylglycerol (TAG)			
C46:2	781.690	0.28±0.08	0.26±0.06
C46:1/C47:8	783.705	0.26±0.1	0.22±0.06
C46:0/C47:7	785.721	0.26±0.08	0.22±0.04
C47:0/C48:7	799.737	0.13±0.07	0.12±0.04
C48:6	801.658	0.07±0.02	0.06±0.01
C48:5	803.674	0.11±0.05	0.1±0.03
C48:4	805.690	0.12±0.04	0.11±0.03
C48:3	807.705	0.09±0.05	0.11±0.04

C48:2/C49:9	809.721	0.15±0.07	0.14±0.07
C48:1/C49:8	811.737	0.2±0.1	0.17±0.07
C48:0/C49:7	813.752	0.18±0.06	0.13±0.03
C49:2/C50:9	823.737	0.06±0.02	0.06±0.04
C49:1/C50:8	825.752	0.08±0.03	0.07±0.01
C49:0/C50:7	827.674	0.05±0.02	0.05±0.02
C50:6	829.690	0.05±0.03	0.04±0.02
C50:5	831.705	0.07±0.03	0.06±0.03
C50:4	833.721	0.07±0.02	0.09±0.05
C50:3/C51:10	835.737	0.13±0.11	0.17±0.1
C50:2/C51:9	837.752	0.26±0.15	0.31±0.14
C50:1/C51:8	839.768	0.31±0.15	0.22±0.08
C50:0/C51:7	841.784	0.12±0.03	0.1±0.02
C51:2/C52:9	851.768	0.07±0.03	0.08±0.02
C51:1/C52:8	853.690	0.08±0.03	0.08±0.02
C51:0/C52:7	855.705	0.07±0.02	0.07±0.02
C52:6	857.721	0.08±0.03	0.08±0.02
C52:5	859.737	0.1±0.03	0.15±0.04
C52:4/C53:11	861.752	0.1±0.06	0.19±0.08
C52:3/C53:10	863.768	0.21±0.14	0.3±0.18
C52:2/C53:9	865.784	0.39±0.27	0.4±0.2
C52:1/C53:8	867.799	0.2±0.1	0.14±0.06
C53:3/C54:10	877.784	0.07±0.02	0.09±0.03
C53:2/C54:9	879.705	0.05±0.02	0.06±0.02
C53:1/C54:8	881.721	0.07±0.04	0.08±0.02
C53:0/C54:7	883.737	0.07±0.01	0.09±0*
C54:6	885.752	0.07±0.02	0.16±0.0*2
C54:5/C55:12	887.768	0.14±0.02	0.23±0.04*
C54:4/C55:11	889.784	0.13±0.09	0.22±0.11
C54:3/C55:10	891.799	0.22±0.18	0.27±0.16
C54:2/C55:9	893.815	0.17±0.08	0.15±0.06
C54:1/C55:8	895.831	0.08±0.06	0.07±0.04
C55:2/C56:9	907.737	0.05±0.03	0.06±0.01
C55:1/C56:8	909.752	0.05±0.02	0.08±0.01
C55:0/C56:7	911.768	0.09±0.02	0.14±0.01*
C56:6	913.784	0.12±0.05	0.22±0.04
C56:5/C57:12	915.799	0.16±0.07	0.2±0.04
C56:4/C57:11	917.815	0.09±0.06	0.11±0.07
C56:3/C57:10	919.831	0.08±0.07	0.07±0.05
	Sum	6.09±2.84	6.62±2.23
TAG Fatty Acyl Chain content (FA)			
228(14:0)	228.209	0.85±0.29	0.85±0.14
254(16:1)	254.225	1.04±0.65	1.56±0.7
256(16:0)	256.240	2.39±0.89	2.35±0.56
278(18:3)	278.225	0.47±0.12	0.53±0.09
280(18:2)	280.240	0.71±0.24	0.94±0.34
282(18:1)	282.256	3±1.84	3.92±1.84
284(18:0)	284.272	1.35±0.53	1.35±0.46
304(20:4)	304.240	0.33±0.14	0.49±0.3
306(20:3)	306.256	0.41±0.22	0.57±0.06
308(20:2)	308.272	0.95±1.14	0.85±0.76
310(20:1)	310.287	1.07±0.05	0.86±0.41
312(20:0)	312.303	0.53±0.11	0.72±0.1*
326(21:0)	326.318	0.44±0.11	0.43±0.02
328(22:6)	328.240	0.45±0.15	0.43±0.08
330(22:5)	330.256	0.74±0.18	0.65±0.06
332(22:4)	332.272	0.7±0.24	0.64±0.1
334(22:3)	334.287	0.76±0.19	0.68±0.19

336(22:2)	336.303	0.43±0.12	0.44±0.07
338(22:1)	338.318	0.51±0.22	0.5±0.15
340(22:0)	340.334	0.57±0.18	0.56±0.19
	Sum	17.71±7.56	19.33±5.81

*p<0.05 vs. DMSO control

Supplemental Table 3. Triglyceride remodeling induced by SBI-477.

Pathway	BSA/Vehicle	BSA/SBI-477	Oleate/Vehicle	Oleate/SBI-477
K1	0.96±0.03	0.50±0.08*	0.25±0.02	0.36±0.03*
K2	0.04±0.03	0.44±0.10*	0.75±0.02	0.63±0.03*
K3	0.00	0.05±0.03	0.00	0.01±0.02

*$p<0.05$ vs. Vehicle

Supplemental Table 4. Primer sequences for qRT-PCR.

Human Gene	**Forward (5' to 3')**	**Reverse (5' to 3')**
ACACA (ACC1)	GTCAATCTTGAGGGCTAGGTCT	CTGGTTCAGCTCCAGAGGTT
ACACB (ACC2)	CAGGTGGGCCTATGAGATGT	GGACGTAATGATCCGCCATCTT
ARRDC4	TCCCACCTGTTACTCCATCC	CACTAATGGCAGTTCGAGCA
DGAT1	GTGGCTTCAGCAACTACCGT	CAGGAACAGAGAAACCACCTG
DGAT2	GCTCTACTTCACTTGGCTGGT	CAGCAGGTTGTGTGTCTTCAC
ELOVL6	GCAGTCAGTTTGTGACCAGG	GATCAGCTTCTGCTTCCTCAGA
FASN	GATGCCTCCTTCTTCGGAGT	CCTCGGAGTGAATCTGGGTT
GPAM (GPAT1)	TCAAGAGCGAGATGTGCATAAG	CCATCAGGGTTTAATTCAGCAG
MLXIP (MondoA)	GCTCACCAAGCTCTTCGAGT	GCCGGATCTTGTCTCTCCAC
MLXIPL (ChREBP)	GTGTCTCCCAAGTGGAAGAATTT	GCTCTTCCTCCGCTTCACAT
SCD	CTTCTCTCACGTGGGTTGGC	CATCAGCAAGCCAGGTTTGTAG
TXNIP	AGTTTCCTGCATGTTCATTCCT	CCACAATTCGGGAACATGTATT
RPLP0 (36B4)	TCTACAACCCTGAAGTGCTTGAT	GATAGAATGGGGTACTGATGCAA
Mouse Gene	**Forward (5' to 3')**	**Reverse (5' to 3')**
Acaca (ACC1)	GGCCAGTGCTATGCTGAGAT	ATCACACAGCCAGGGTCAAG
Acacb (ACC2)	CGCTCACCAACAGTAAGGTGG	GCTTGGCAGGGAGTTCCTC
Arrdc4	CAGCCTCCTCAGAAGTGGAAT	TCAGACGGAAGCTGAAAGCG
Dgat1	GTGCACAAGTGGTGCATCAG	CAGTGGGATCTGAGCCATCA
Dgat2	GCATTTGACTGGAACACGCC	CTGGTGGTCAGCAGGTTGTG
Elovl6	CGTAGCGACTCCGAAGATCA	AGCGTACAGCGCAGAAAACA
Fasn	CCAAGCAGGCACACACAATG	GTTCGTTCCTCGGAGTGAGG
Gpam (GPAT1)	ACAGTTGGCACAATAGACGTTT	CCTTCCATTTCAGTGTTGCAGA
G6pc	GCTGGAGTCTTGTCAGGCAT	GCCGCTCACACCATCTCTTA
Pklr (LPK)	GCTAGGAGCACCAGCATCAT	TGGGAGAAGTTGAGTCGTGC
Mlx	CATGGACTCCCTCTTCCAGTC	TGATGAAGGACACCGATCACA
Mlxip (MondoA)	TGCTACCTGCCACAGGAGTC	GACTCAAACAGTGGCTTGATGA
Mlxipl (ChREBP)	CAGCATCGATCCGACACTCA	CGGATCTTGTCCCGGCATAG
Pepck	AGTTCGTGGAAGGCAAT	GTGAGAGCCAGCCAACA
Scd1	CCAAGCTGGAGTACGTCTGG	CAGAGCGCTGGTCATGTAGT
Txnip	GTCTCAGCAGTGCAAACAGACTT	GCTCGAAGCCGAACTTGTACTC
Rplp0 (36B4)	TGGAAGTCCAACTACTTCCTCAA	ATCTGCTGCATCTGCTTGGAG

Supplemental Table 5. Primer sequences for ChIP-qRT-PCR.

Human promoter	Forward (5' to 3')	Reverse (5' to 3')
TXNIP ChoRE	CCGGGCAGCCAATGGGAG	GCAGGAGGCGGAAACGTCTC
ARRDC4 ChoRE	CGGAGATAACCCTGTTCCGC	CAGGCCGTTTACTGGCTGA
Impa2 MEF2	CTATCGGATGGTCAGCTTCAA	GCACTGGCTTCTCATGTTTATC

Mouse promoter	Forward (5' to 3')	Reverse (5' to 3')
Txnip ChoRE	GCCTGGTAAACAAGGGCCAA	GCTGCCGGAAACGGCTTATA
Pklr ChoRE	GATCCAGGCTCTGCAGACAG	CAGCTAGCATCTCTCTTGCCA
Myh7 Intron 26	TGCATACAGACTTGGTGAATAG	GAACAGTGTGAAGACTCCTATG

SUPPLEMENTAL METHODS

Fatty acid oxidation (FAO) assay

Primary human skeletal myotubes were grown and differentiated in 24-well plates. Cells were treated with the indicated concentration of SBI-477 for 24 hours. Following compound treatment, cells were rinsed three times with PBS and then incubated in 125μM [^{3}H]-palmitic acid (60 Ci/mmol) bound to fatty acid free albumin containing 1mM carnitine for 2 hours at 37°C. The cell medium was transferred to a tube containing cold 10% trichloroacetic acid (TCA). The tubes were centrifuged at 8,500 x g for 10 minutes at 4°C. The supernatant was immediately removed, mixed with 6N NaOH, and applied to ion-exchange resin (DOWEX 1; Sigma-Aldrich). The eluate was collected, measured by liquid scintillation analyzer (PerkinElmer) and normalized to total protein amount. The amount of cell protein was measured by Micro BCA protein assay kit (Thermo Scientific).

Lipidomics

Primary human myotubes were treated with BSA or 100μM oleate with 10μM SBI-477 or a vehicle control for 24 hours. The cells were added to 300μL PBS in an Eppendorf tube and homogenized for 1 minute using a disposable soft tissue homogenizer. A 25μL aliquot was used to determine the protein content (BCA protein assay kit, Thermo Scientific, Rockford, IL). The remaining homogenate was accurately transferred into a disposable glass culture test tube, and a mixture of lipid internal standards was added prior to lipid extraction for quantification of all reported lipid species. Lipid extraction was performed by using a modified Bligh and Dyer procedure as described previously (Wang and Han, 2014). Each lipid extract was resuspended into a volume of 500μL of chloroform/methanol (1:1, v/v) per mg of protein and flushed with

nitrogen, capped, and stored at −20 °C for lipid analysis. For ESI direct infusion analysis, lipid extract was further diluted to a final concentration of ~500 fmol/μL, and the mass spectrometric analysis was performed on a QqQ mass spectrometer (Thermo TSQ VANTAGE, San Jose, CA) equipped with an automated nanospray device (TriVersa NanoMate, Advion Bioscience Ltd., Ithaca, NY).

Glycogen synthesis assay

Differentiated primary human skeletal myotubes were treated with SBI-477 as described in the glucose uptake assay. Following incubation with SBI-477, cells were serum starved in α-MEM for 1 hour. The medium was replaced with α-MEM supplemented with [14C]-D-glucose (1 μCi/mL) for three hours and then treated in the absence or presence of insulin (100nM) for 30minutes. After incubation, the cells were washed three times with ice-cold PBS and then lysed in 30% potassium hydroxide (KOH) containing 6mg/mL glycogen. For glycogen precipitation, the samples were added to ice-cold 100% ethanol for 18 hours at 4°C. The samples were centrifuged at 8,500 x g for 10 minutes at 4°C and the supernatant was discarded. After one wash with 75% ethanol, the glycogen precipitate was dissolved in distilled water. [14C]-D-glucose incorporated to glycogen was counted by liquid scintillation analyzer (PerkinElmer) and normalized to total protein.

SBI-477 and SBI-993 Synthesis

Synthesis route for SBI-477

A round bottom flask was charged with ethyl 4-hydroxy-3-methoxybenzoate (4.95 g, 1.0 eq) and acetonitrile (100 mL). To this solution was then sequentially added NEt_3 (5.27 mL, 1.5 eq) and

chloromethyl methylether (2.01 mL, 1.05 eq); the resulting solution was stirred at 50 °C. Additional Et_3N and MOMCl were added as necessary to drive the reaction to completion. Upon completion, the solution was cooled to room temperature and partially concentrated. The solution was then diluted with EtOAc and sequentially washed with aqueous ammonium chloride, water, and brine. The organic portion was dried over sodium sulfate and concentrated in vacuo to give ethyl 3-methoxy-4-(methoxymethoxy)benzoate (6.06 g) as a pale yellow oil which was used without further purification. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.57 (dd, J = 8.5, 2.0 Hz, 1H), 7.51 (d, J = 2.0 Hz, 1H), 7.09 (d, J = 8.4 Hz, 1H), 5.21 (s, 2H), 4.29 (q, J = 7.2 Hz, 2H), 3.86 (s, 3H), 3.43 (s, 3H), 1.31 (t, J = 7.2 Hz, 3H).

A round bottom flask was charged with ethyl 3-methoxy-4-(methoxymethoxy)benzoate (6.06 g, 1.0 eq), THF (30 mL) and water (15 mL). To this solution was then added LiOH (2.4 g, 4.0 eq) and the resulting mixture was warmed to 65 °C. Upon completion of the reaction (by LCMS), the solution was cooled to room temperature, treated with a 10% citric acid solution and thrice extracted with EtOAc. The combined organic portions were washed with brine and dried over sodium sulfate. Concentration *in vacuo* gave 3-methoxy-4-(methoxymethoxy)benzoic acid (3.99 g, 75% yield) as a white solid which was pure by NMR. mp = 158 – 160 °C. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.76 (dd, J = 8.5, 1.9 Hz, 1H), 7.66 (d, J = 2.0 Hz, 1H), 7.23 (d, J = 8.4 Hz, 1H), 5.34 (s, 2H), 3.98 (s, 3H), 3.55 (s, 3H).

A vial was charged with 4-(4-methoxyphenyl)thiazol-2-amine (412 mg, 1.0 eq), triethylamine (558 μL, 2.0 eq), and acetonitrile (~0.4 M). To this solution was then added 3-methoxy-4-(methoxymethoxy)benzoic acid (509 mg, 1.2 eq). Finally, HATU (912 mg, 1.0 eq) was added and the vial was warmed to 75 °C. Stirring at 75 °C was continued until full consumption of the carboxylic acid by LCMS. The solution was then cooled to room

temperature, diluted with EtOAc, and then sequentially washed with water and brine. Concentration in vacuo gave an oil that was used without further purification. This oil was directly dissolved in THF (16 mL) and treated with 1N HCl (4 mL). The solution was then warmed to 65 °C and stirred until the acetal deprotection was complete by LCMS (45 min). Upon completion, the solution was cooled to room temperature, diluted with EtOAc and sequentially washed with saturated $NaHCO_3$, water, and brine. After drying over sodium sulfate, the material was concentrated and purified on silica gel (10-50% hex/EtOAc gradient) to give 4-hydroxy-3-methoxy-N-(4-(4-methoxyphenyl)thiazol-2-yl)benzamide as a white solid (441 mg, 62% yield). ^{1}H NMR (500 MHz, Chloroform-d) δ 7.76 (d, J = 8.8 Hz, 2H), 7.57 (d, J = 2.0 Hz, 1H), 7.45 (dd, J = 8.3, 2.1 Hz, 1H), 7.04 (s, 1H), 7.01 (d, J = 8.3 Hz, 1H), 6.95 (d, J = 8.8 Hz, 2H), 3.99 (s, 3H), 3.85 (s, 3H).

A vial was charged with 4-hydroxy-3-methoxy-N-(4-(4-methoxyphenyl)thiazol-2-yl)benzamide (441 mg, 1.0 eq) and DMF (0.1 M). Potassium carbonate (343 mg, 2.0 eq) was added, followed by 2-chloro-1-morpholinoethan-1-one (163 μL, 1.0 eq). This solution was then warmed to 85 °C in a microwave reactor for 4 hours. The solution was partially concentrated and then partitioned between EtOAc and water. The aqueous layer was further extracted with EtOAc and the combined organic layers were dried over sodium sulfate. Purification on silica gel (10-100% hex/EtOAc gradient) gave 3-methoxy-N-(4-(4-methoxyphenyl)thiazol-2-yl)-4-(2-morpholino-2-oxoethoxy)benzamide (398 mg, 66%) as a white solid. ^{1}H NMR (500 MHz, methanol-d_4) δ 7.87 (d, *J* = 8.7 Hz, 2H), 7.69 (s, 1H), 7.69 – 7.62 (m, 1H), 7.26 (s, 1H), 7.08 (d, *J* = 8.4 Hz, 1H), 6.96 (d, *J* = 8.8 Hz, 2H), 4.94 (s, 2H), 3.97 (s, 3H), 3.83 (s, 3H), 3.69 (m, 4H), 3.61 (m, 4H). ^{13}C NMR (125 MHz, methanol-d_4) δ 166.10, 164.42, 159.76, 158.80, 151.04,

150.15, 149.73, 127.58, 127.38, 126.00, 120.52, 114.28, 112.98, 111.43, 106.61, 68.53, 67.02, 66.95, 56.21, 55.53, 46.18, 42.80.

Synthesis route for SBI-993

The commercially available 4-((2-ethoxy-2-oxoethyl)amino)benzoic acid (1.21 g, 1.0 eq) was dissolved in acetonitrile (0.3 M) and treated with triethylamine (3.0 mL, 4.0 eq) and a 4-(4-methoxyphenyl)thiazol-2-amine hydrobromide (1.56 g, 1.0 eq). To this mixture was then added HATU (3.09 g, 1.5 eq). The resulting solution was warmed to 75 °C and stirred until the reaction was complete (~ 48 h). At this time the solution was cooled to room temperature and diluted with EtOAc. The solution was then sequentially washed with water (2x) and brine, dried over sodium sulfate, and then concentrated in vacuo. Purification on silica gel (hex/EtOAc gradient 10%-100%) gave an impure mixture which could be dissolved in DMF and precipitated by the addition of water. This solid was dissolved in THF (12 mL) and water (4 mL) and treated with LiOH (308 mg, 4.0 eq). Saponification was completed within one hour of stirring at room temperature. The solution was then diluted with EtOAc and washed with a 10% citric acid solution followed by brine. Drying on sodium sulfate and concentration gave a colored solid which was then washed with a dichloromethane and methanol solution to give the intermediate carboxylic acid as an off-white solid (571 mg). This carboxylic acid (570 mg, 1.0 eq) was dissolved in DMF (0.25 M). Triethylamine (415 µL, 2.0 eq) and morpholine (518 µL, 4.0 eq) were then added. Lastly, HATU (680 mg, 1.2 eq) was added and the solution was stirred at room temperature for 2 h. Water was then slowly added to the reaction which then formed a precipitate. This precipitate was collected via filtration and washed with a mixture of THF and methanol to cleanly give N-(4-(4-methoxyphenyl)thiazol-2-yl)-4-((2-morpholino-2-

oxoethyl)amino)benzamide (422 mg) as a white solid. 1H NMR (500 MHz, DMSO-d_6) δ 12.22 (s, 1H), 7.95 (d, J = 8.7 Hz, 2H), 7.88 (d, J = 8.8 Hz, 2H), 7.44 (s, 1H), 7.01 (d, J = 8.8 Hz, 2H), 6.76 (d, J = 8.8 Hz, 2H), 6.48 (t, J = 5.2 Hz, 1H), 4.05 (d, J = 5.1 Hz, 2H), 3.80 (s, 3H), 3.61 (m, 4H), 3.56 – 3.47 (m, 4H).

SUPPLEMENTAL REFERENCES

Wang, M., and Han, X. Multidimensional mass spectrometry-based shotgun lipidomics. *Methods Mol Biol*. 2014;1198, 203-220.