SUPPLEMENTARY DATA

Supplementary Table 1. Used primer sequences

Gene		Sequences (5'→3')
β-actin	F	GAC GTT GAC ATC CGT AAA G
	R	CAG TAA CAG TCC GCC T
GAPDH	F	CAA GGT CAT CCA TGA CAA CTT TG
	R	GGC CAT CCA CAG TCT TCT GG
SPTLC1	F	AGT GGT GGG AGA GTC CCT TT
	R	CAG TGA CCA CAA CCC TGA TG
SPTLC2	F	GGA TAC ATC GGA GGC AAG AA
	R	ACC TGG TGT TCT CAG CCA AC
SPHK1	F	TGT GAA CCA CTA TGC TGG GTA
	R	CAG CCC AGA AGC AGT GTG
SPHK2	F	AGA CGG GCT GCT TTA CGA G
	R	CCT GCT CAA ACC CGC CAT
aP2	F	GAA CCT GGA AGC TTG TCT TCG
	R	ACC AGC TTG TCA CCA TCT CG
C/EBPα	F	GAA CAG CAA CGA GTA CCG GGT A
	R	GCC ATG GCC TTG ACC AAG GAG
PPARγ	F	GAG TGT GAC GAC AAG ATT TG
	R	GGT GGG CCA GAA TGG CAT CT
PEPCK	F	CCC CTT GTC TAT GAA GCC CTC A
	R	GCC CTT GTG TTC TGC AG
SREBP-1c	F	CGG AAG CTG TCG GGG TAG
	R	GGC CAG AGA AGC AGA AGA GA
GLUT4	F	AGA GTC TAA AGC GCC T
	R	CCG AGA CCA ACG TGA A
FAS	F	CAG ATG ATG ACA GGA GAT GGA A
	R	CAC TCA CAC CCA CCC AGA
DGAT1	F	GTG CCA TCG TCT GCA AGA TTC
	R	GCA TCA CCA CAC ACC AAT TCA G
DGAT2	F	CTG TCA CCT GGC TCA ACA GA
	R	TAT CAG CCA GCA GTC TGT GC
HMG CoR	F	GGG AAC TAT TGC ACC G
	R	GTA GCC GCC TAT GCT C
LpL	F	TCT GTA CGG CAC AGT GG
	R	CCT CTC GAT GAC GAA GC
CPT1α	F	CCA TCC TGT CCT GAC AAG GTT TAG
	R	CCT CAC TTC TGT TAC AGC TAG CAC
CPT1β	F	GGT CCC ATA AGA AAC AAG ACC TCC
	R	CAG AAA GTA CCT CAG CCA GGA AAG
ACOX1	F	ACG CCA CTT CCT TGC TCT TC
	R	AGA TTG GTA GAA ATT GCT GCA AA
LCAD	F	TTT CCT CGG AGC ATG ACA TTT T
	R	GCC AGC TTT TTC CCA GAC CT
MCAD	F	AAC ACT TAC TAT GCC TCG ATT GCA
	R	CCA TAG CCT CCG AAA ATC TGA A
S1PR1	F	ATG GTG TCC ACT AGC ATC CC
	R	CGA TGT TCA ACT TGC CTG TGT AG
S1PR2	F	ACA GCA AGT TCC ACT CAG CAA
	R	CTG CAC GGG AGT TAA GGA CAG
S1PR3	F	ACT CTC CGG GAA CAT TAC GAT
	R	CCA AGA CGA TGA AGC TAC AGG
CD36	F	ATT GGT CAA GCC AGC T
	R	TGT AGG CTC ATC CAC TAC
ACC	F	CGC CAA CAA TGG TAT TGC AGC
	R	TCG GAT TGC ACG TTC ATT TCG

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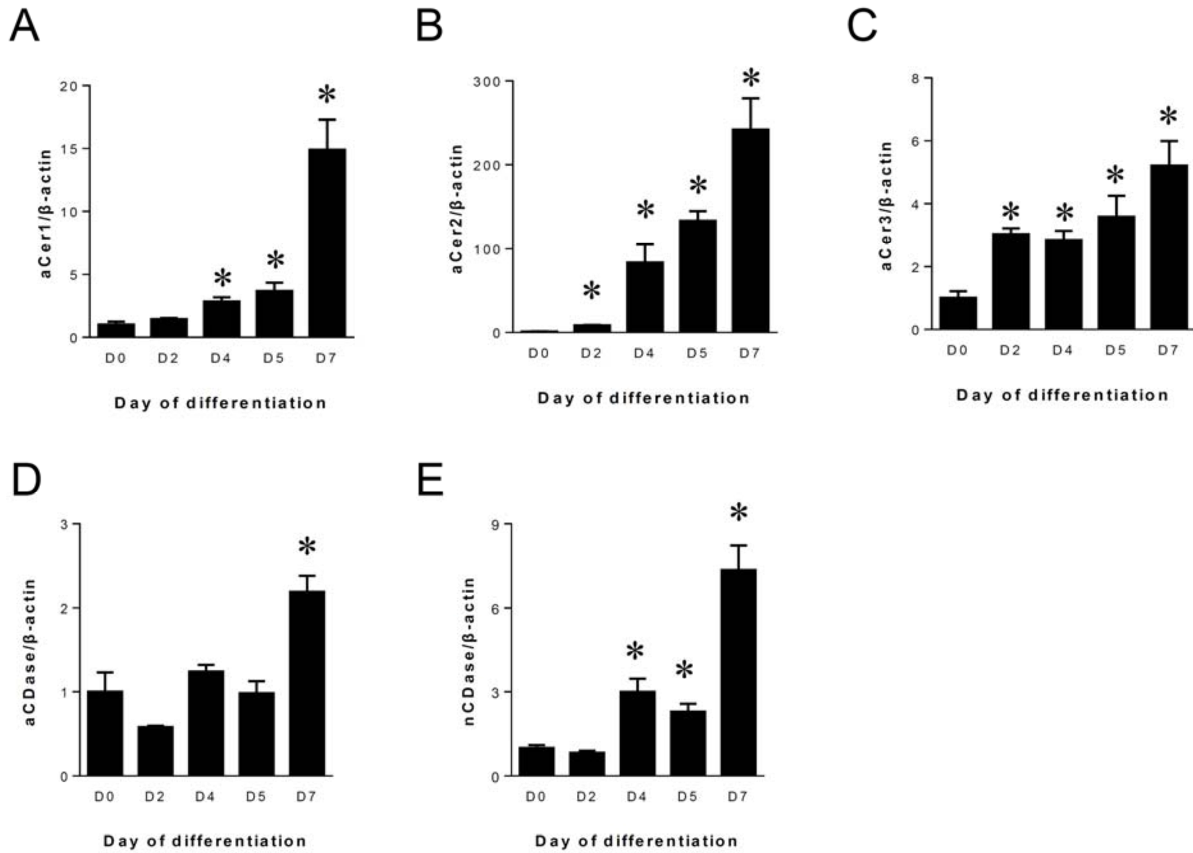
SUPPLEMENTARY DATA

Supplementary Figure S1. Expression of alkaline ceramidase (aCer) 1/2/3 (A-C), acid ceramidase (aCDase) (D) and neutral ceramidase (nCDase) (E). 3T3-L1 cells were differentiated for 7 days and harvested at the indicated days (D0, D2, D4, D5, and D7). qRT-PCR was performed to measure the mRNA expression. Data are presented as the mean ± SEM, *$p < 0.05$ vs. D0 of 3T3-L1 control, n=3.



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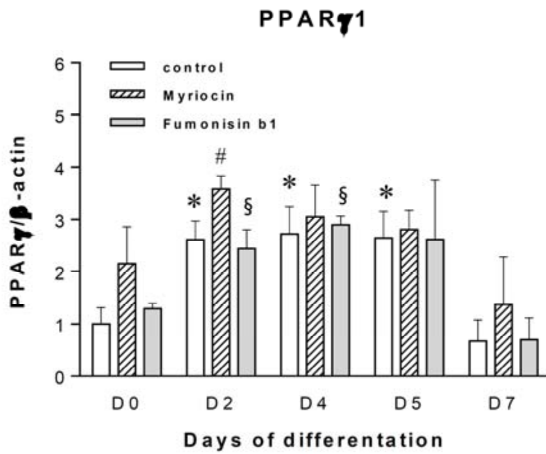
SUPPLEMENTARY DATA

Supplementary Figure S2. Pharmacological inhibition of SPT by myriocin and fumonisin B1 did not alter adipogenesis in 3T3-L1 cells. 3T3-L1 preadipocytes were treated with 10 µM myriocin or 10 µM Fumonisin B1 and differentiated as described in *Materials and Methods*. Expression of PPARγ1 (A), aP2 (B), and C/EBPα (C) were measured by qRT-PCR. Data are presented as the mean ± SEM, *p < 0.05 vs. D0 of 3T3-L1 control, #p < 0.05 vs. D0 of myriocin-treated cells, §p < 0.05 vs. D0 of fumonisin B1-treat cells. n=3.

A



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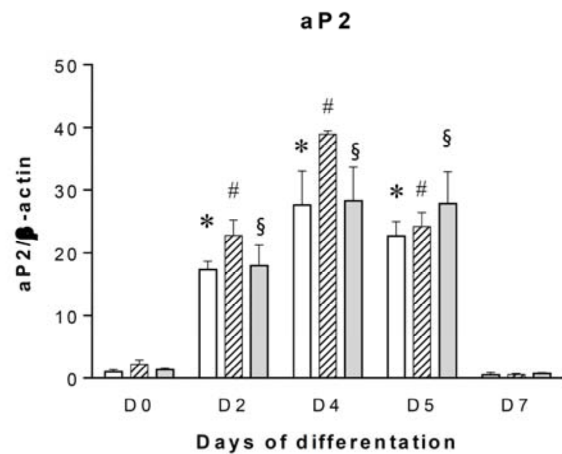

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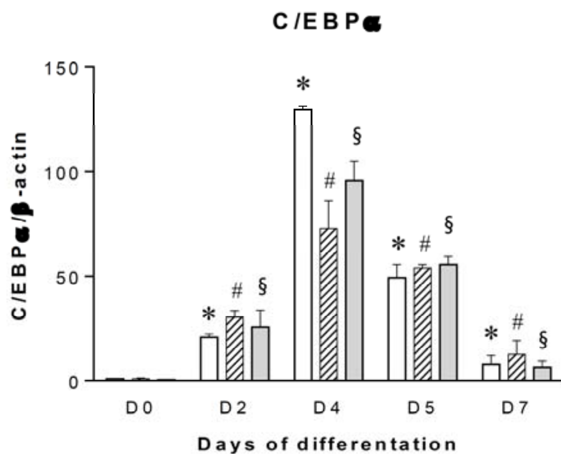

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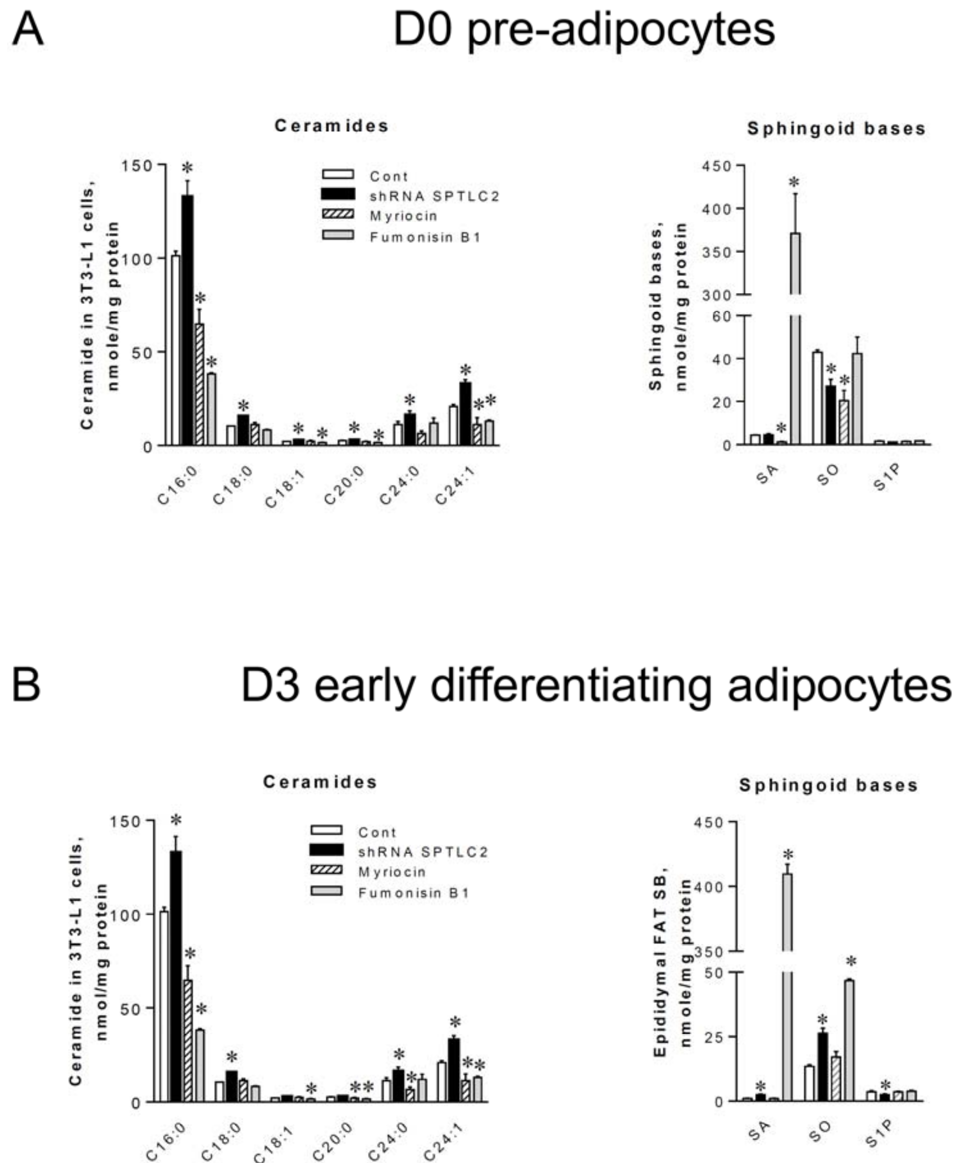
SUPPLEMENTARY DATA

Supplementary Figure S3. Sphingolipid profile in 3T3-L1. 3T3-L1 cells treated with 10 µM myriocin or fumonisin and SPTLC shRNA cells were differentiated with insulin cocktail as described in RESEARCH DESIGN AND METHODS. Myriocin and fumonisin B1 were treated one day before initiation of differentiation and treatment was continued until day 7 (D7). Cells were harvested and ceramide and sphingoid bases were were analyzed by LC/MS/MS at day 0 (D0) and day 3 (D3) after addition of insulin cocktail. Data are presented as the mean ± SEM. *p < 0.05 vs. the control 3T3-L1 cells (Cont). n=3..



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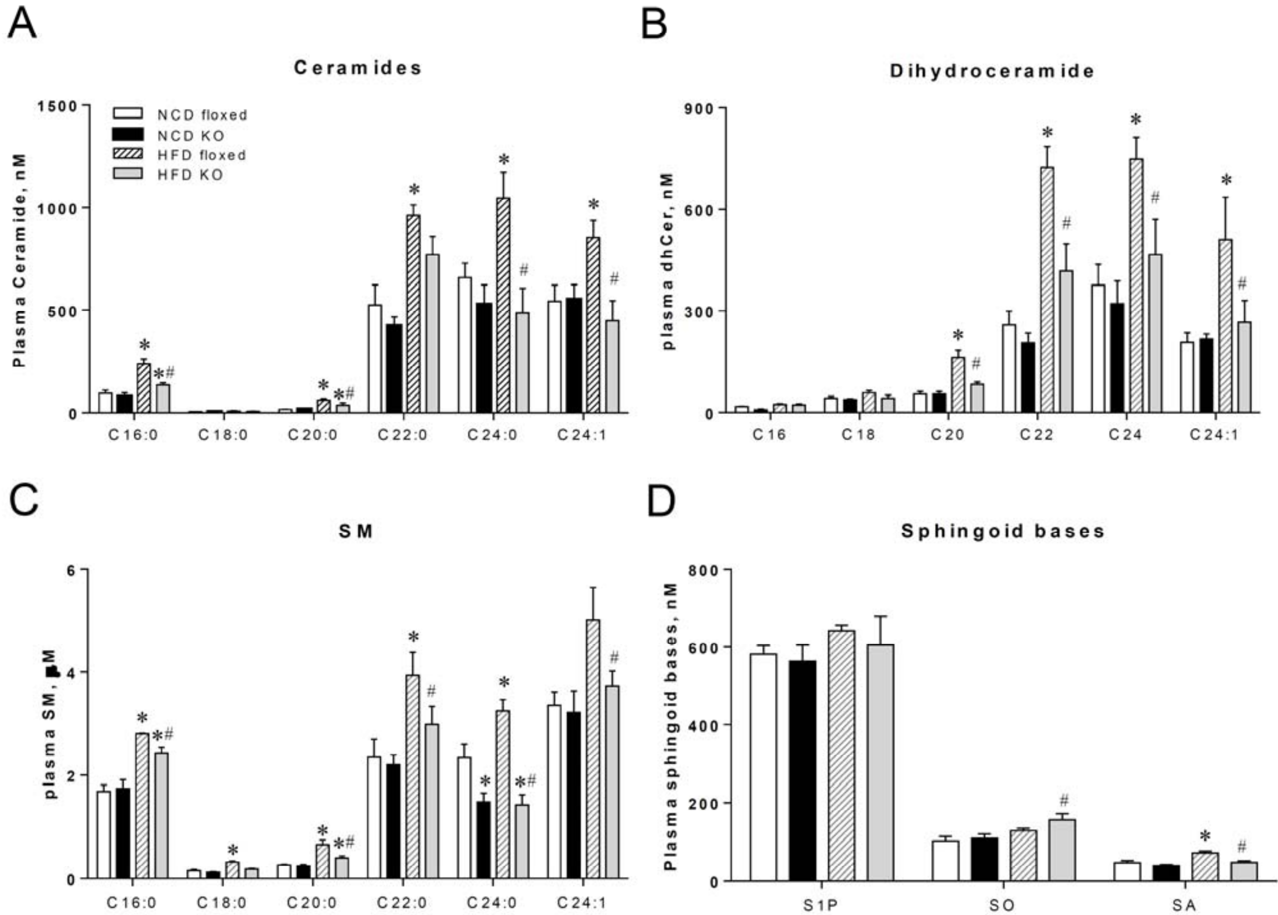
SUPPLEMENTARY DATA

Supplementary Figure S4. Plasma sphingolipid profile. Mice were fed a normal chow diet (NCD) or a high-fat diet (HFD, 45 kcal % fat) for 8 weeks and plasma samples were collected. Ceramide (A), dihydroceramide (B), sphingomyelin (C), sphingoid bases (D) were analyzed by LC/MS/MS. Data are presented as the mean ± SEM. *$p < 0.05$ vs. floxed mice fed a NCD, #$p < 0.05$ vs. floxed mice fed a HFD, n=5-6.



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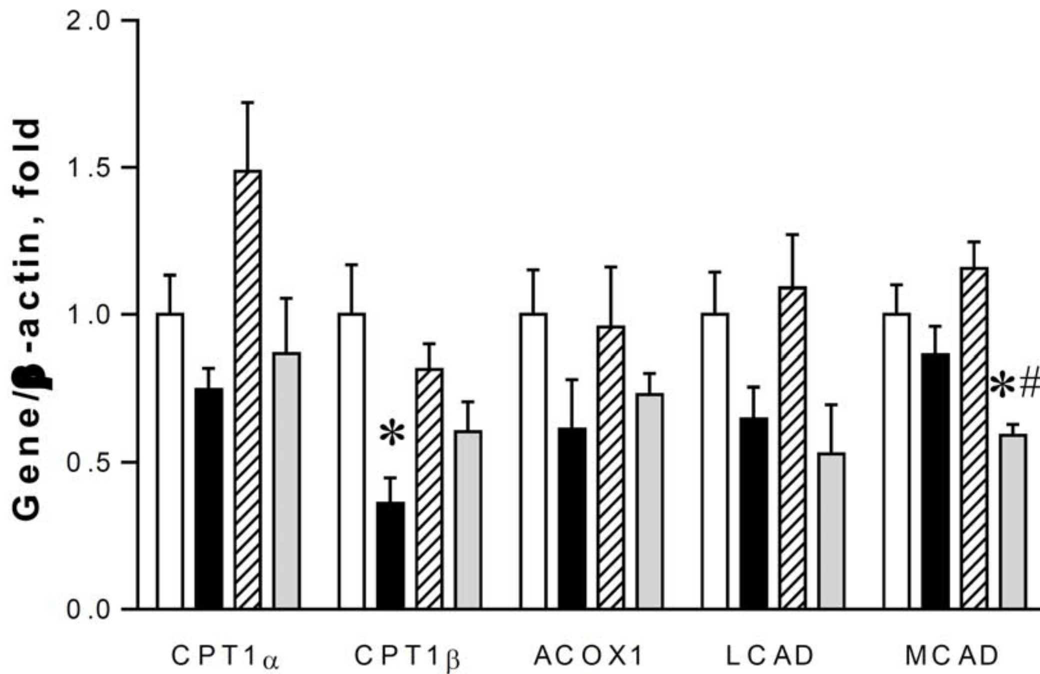
SUPPLEMENTARY DATA

Supplementary Figure S5. Quantitative RT-PCR analyses of TG biosynthetic genes. Mice were fed a normal chow diet (NCD) or a high-fat diet (HFD, 45 kcal % fat) for 8 weeks and livers were isolated from floxed and aSPTLC2 mice. mRNA were isolated and expression was measured by real-time PCR. Data are presented as the mean ± SEM. *p < 0.05 vs. floxed mice fed a NCD, #p < 0.05 vs. floxed mice fed a HFD, n=5-6.



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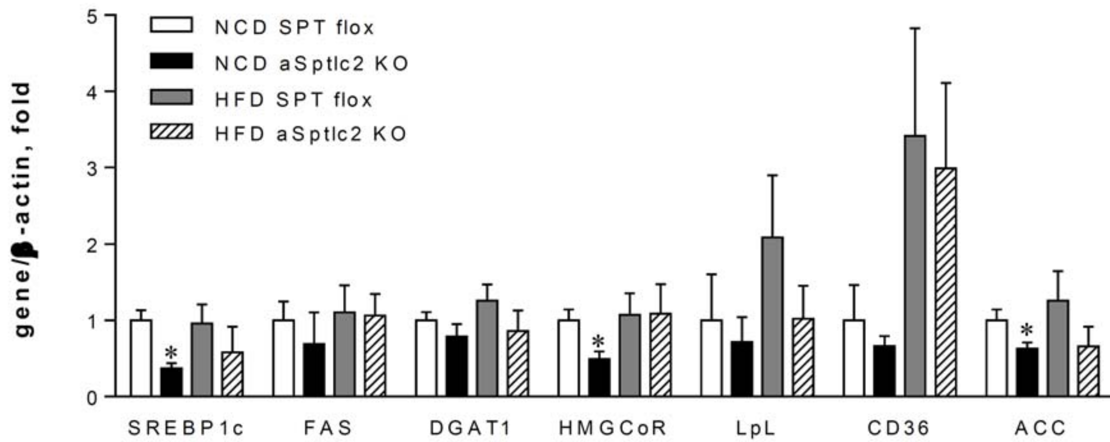
SUPPLEMENTARY DATA

Supplementary Figure S6. Expression of lipid synthesizing genes in mouse skeletal muscles after normal chow diet (NCD) or high-fat diet (HFD) feeding. The floxed and aSPTLC2 KO mice were fed a HFD (45 kcal % fat) for 8 weeks and their muscle tissues isolated. Quantitative RT-PCR analysis was performed to examine the expression of lipid biosynthetic genes. Data are presented as the mean ± SEM, *$p < 0.05$ vs. floxed mice fed a NCD, n=5-6.



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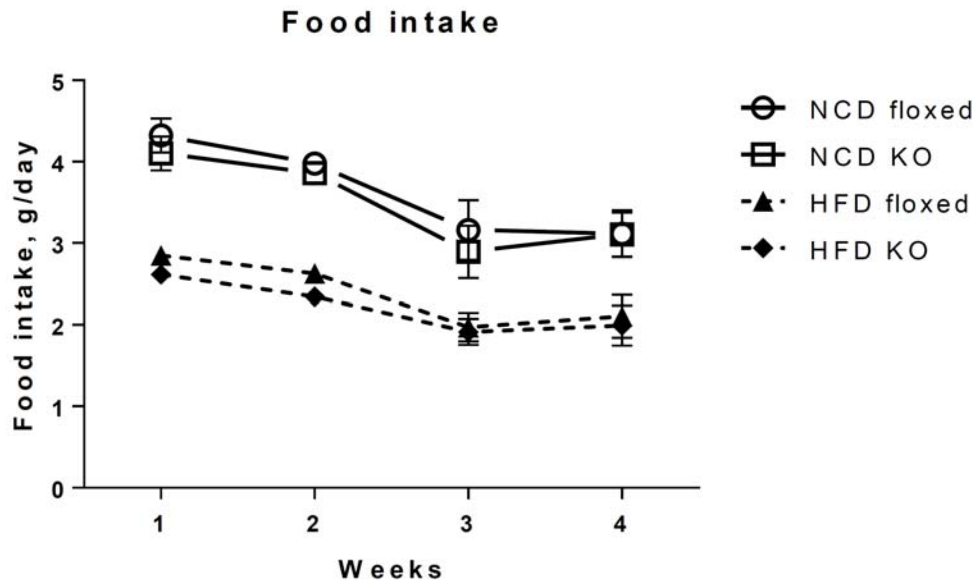
SUPPLEMENTARY DATA

Supplementary Figure S7. Food intake of the floxed and aSPTLC2 KO mice. During feeding with a normal chow diet (NCD) or a high-fat diet (HFD, 60 kcal % fat), food intake was measured every week for 4 weeks. Data are presented as the mean ± SEM, n=10-14.



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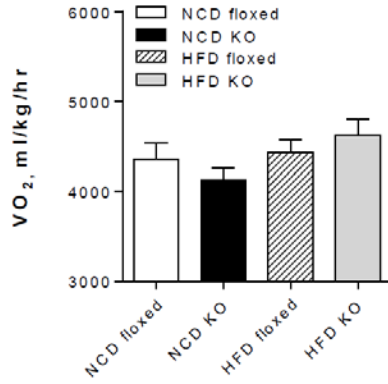
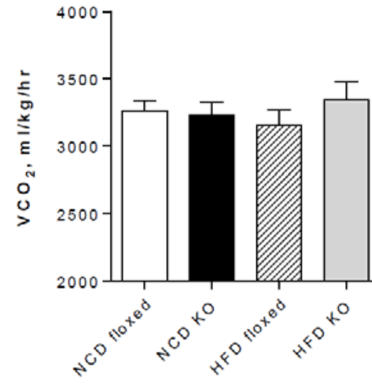
SUPPLEMENTARY DATA

Supplementary Figure S8. Metabolic changes in the floxed and aSPTLC2 KO mice. Basal oxygen consumption (VO_2) (A), carbon dioxide production rate (VCO_2) were recorded after 4 weeks of normal chow diet (NCD) and high-fat diet (HFD, 60 % kcal) using a Comprehensive Laboratory Animal Monitoring System. Data are presented as the mean ± SEM, *p < 0.05 vs. floxed mice fed a NCD, n=10.





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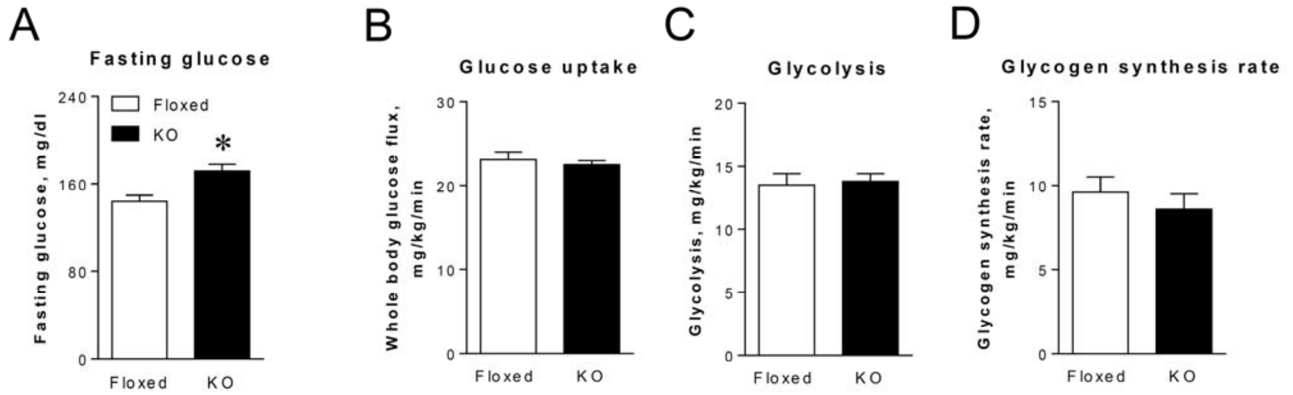
SUPPLEMENTARY DATA

Supplementary Figure S9. Additional hyperinsulinemic euglycemic clamp and glucose flux results. After 6 weeks of HFD and overnight fasting, the fasting glucose was measured (A) and the whole body glucose uptake (B), glycolysis (C), and glycogen synthesis rates (D) were measured, as described in the Supplementary Materials and Methods. Independently data are presented as the mean ± SEM, *p < 0.05 vs. floxed mice fed a HFD, n=10-14.



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