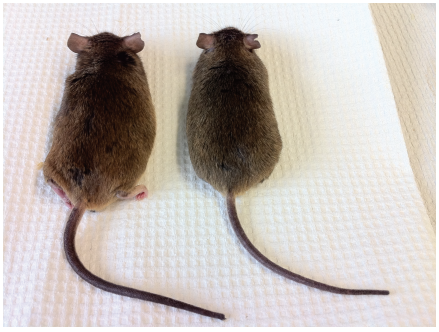
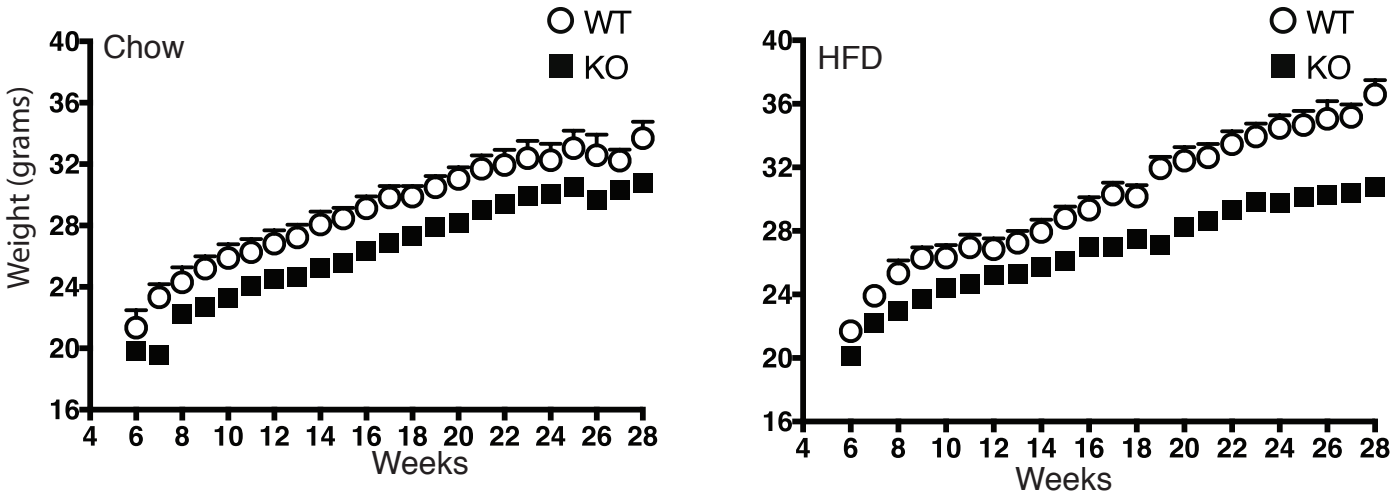
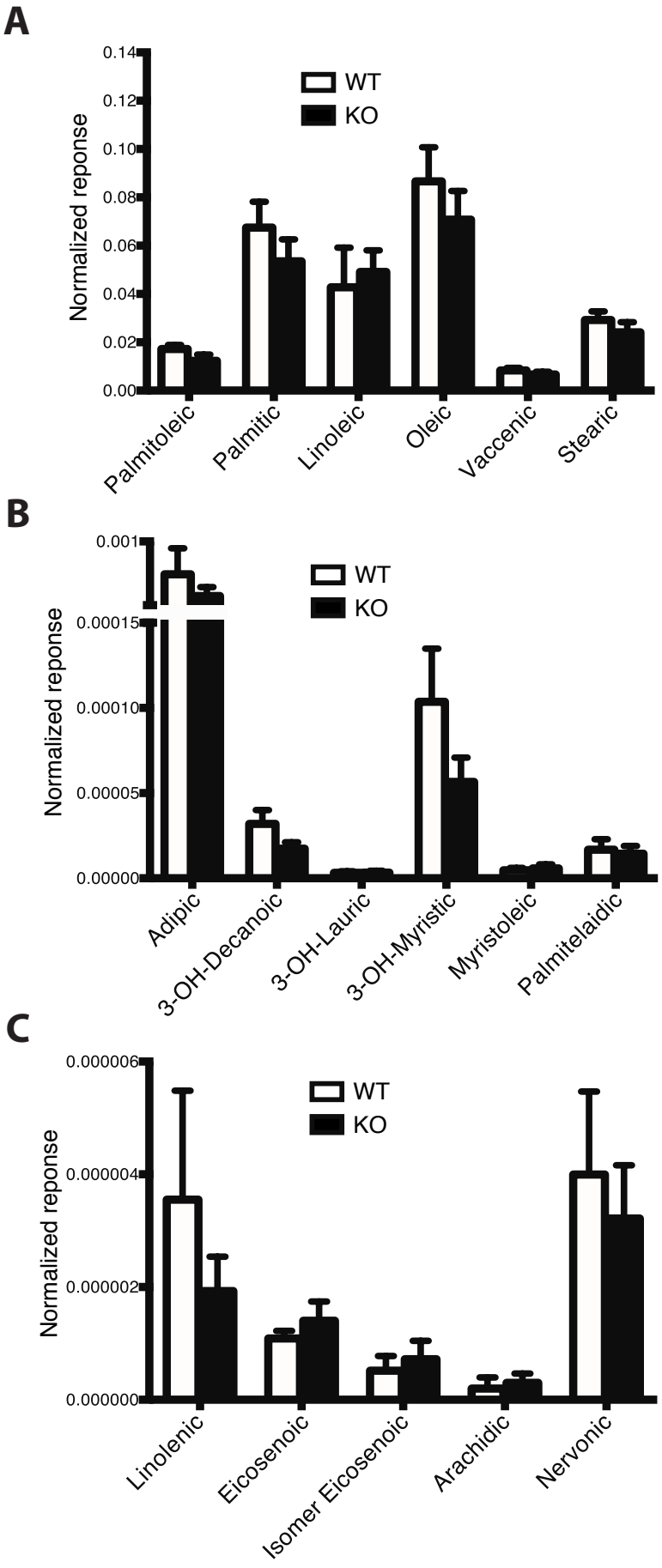
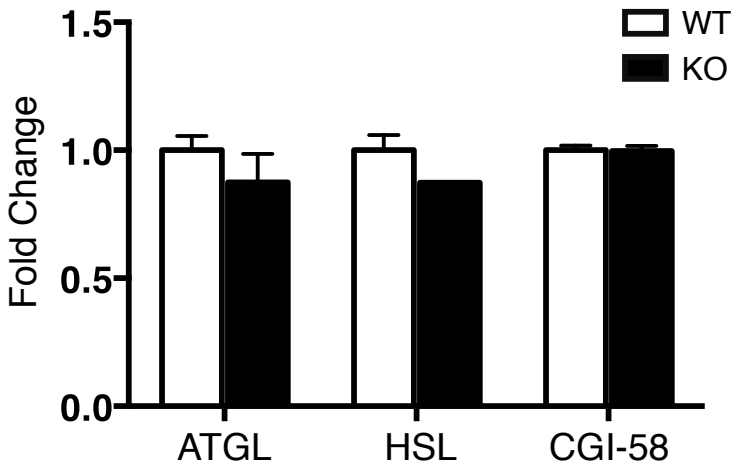
A

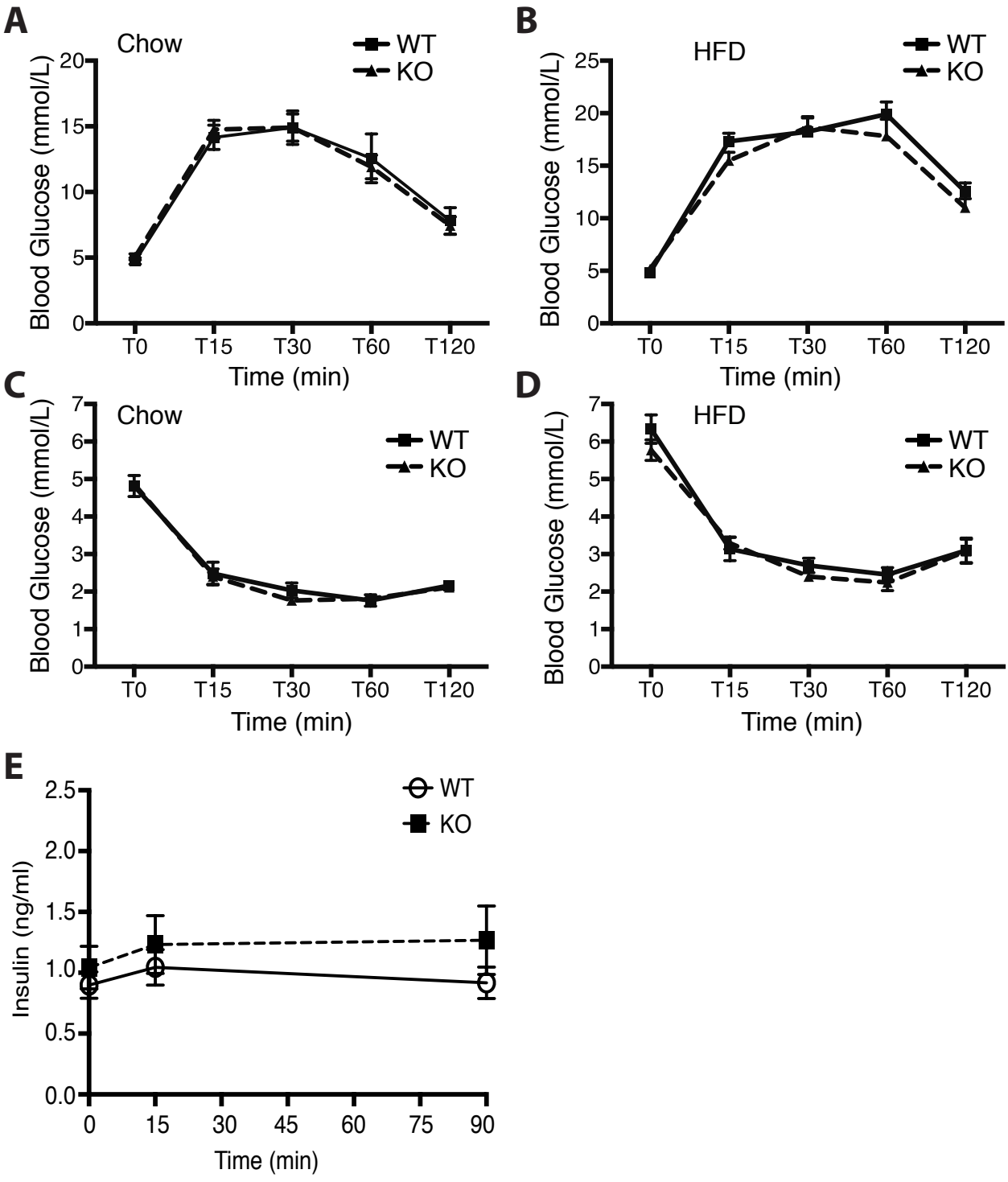


B


Chow
WT
KO
Weight (grams)
40
36
32
28
24
20
16
4 6 8 10 12 14 16 18 20 22 24 26 28
Weeks
HFD
WT
KO
40
36
32
28
24
20
16
4 6 8 10 12 14 16 18 20 22 24 26 28
Weeks


A
Normalized reponse
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
WT
KO
Palmitoleic
Palmitic
Linoleic
Oleic
Vaccenic
Stearic
B
Normalized reponse
0.001
0.00015
0.00010
0.00005
0.00000
WT
KO
Adipic
3-OH-Decanoic
3-OH-Lauric
3-OH-Myristic
Myristoleic
Palmitelaidic
C
Normalized reponse
0.000006
0.000004
0.000002
0.000000
WT
KO
Linolenic
Eicosenoic
Isomer Eicosenoic
Arachidic
Nervonic


1.5
1.0
0.5
0.0
Fold Change
WT
KO
ATGL
HSL
CGI-58


A
Chow
WT
KO
Blood Glucose (mmol/L)
0
5
10
15
20
T0
T15
T30
T60
T120
Time (min)
B
HFD
WT
KO
Blood Glucose (mmol/L)
0
5
10
15
20
25
T0
T15
T30
T60
T120
Time (min)
C
Chow
WT
KO
Blood Glucose (mmol/L)
0
1
2
3
4
5
6
7
T0
T15
T30
T60
T120
Time (min)
D
HFD
WT
KO
Blood Glucose (mmol/L)
0
1
2
3
4
5
6
7
T0
T15
T30
T60
T120
Time (min)
E
WT
KO
Insulin (ng/ml)
0.0
0.5
1.0
1.5
2.0
2.5
0
15
30
45
60
75
90
Time (min)

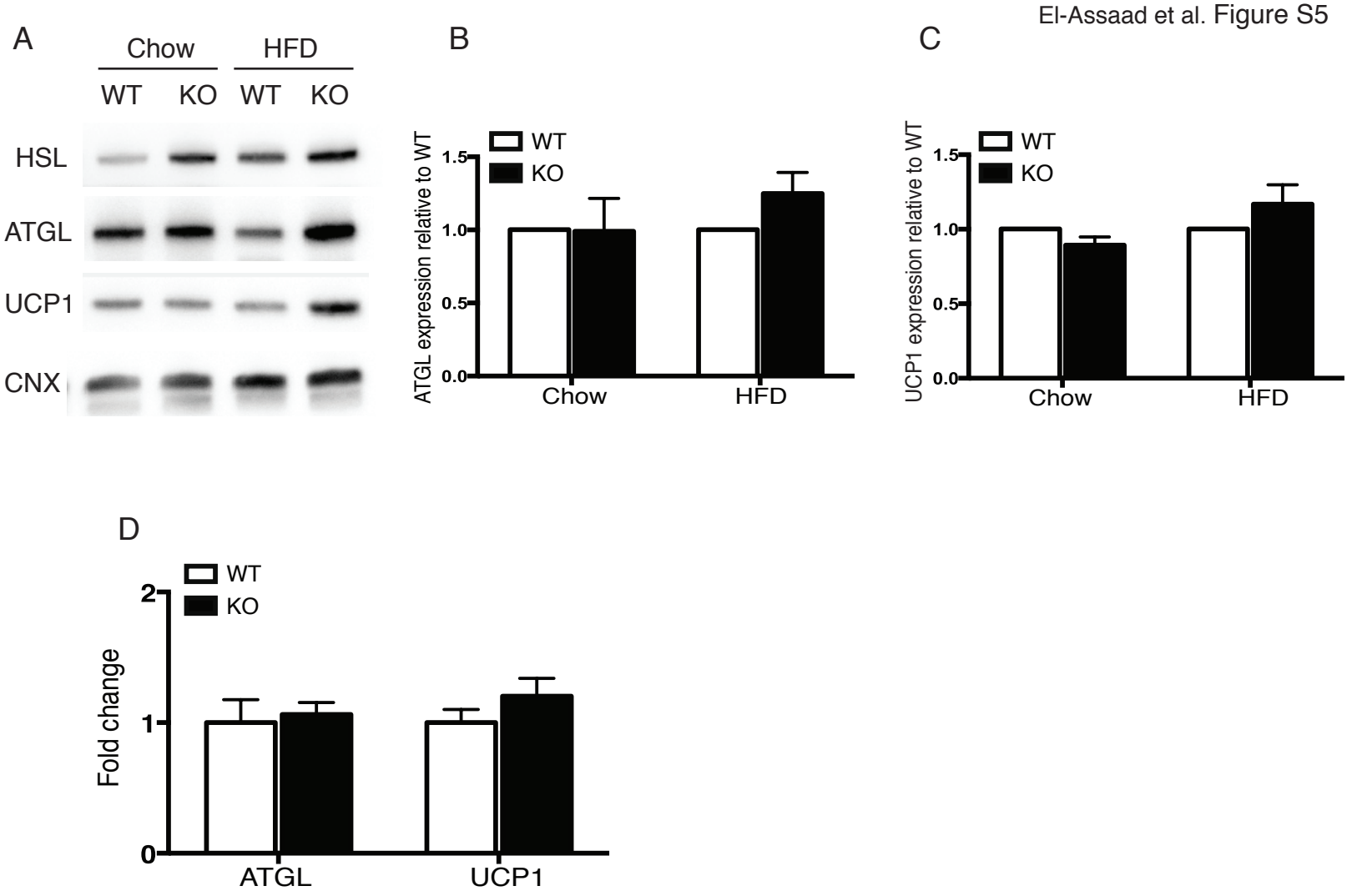

A
Chow
HFD
WT
KO
WT
KO
HSL
ATGL
UCP1
CNX
B
WT
KO
ATGL expression relative to WT
1.5
1.0
0.5
0.0
Chow
HFD
C
WT
KO
UCP1 expression relative to WT
1.5
1.0
0.5
0.0
Chow
HFD
D
WT
KO
Fold change
2
1
0
ATGL
UCP1

Table S1 quantitative RT-PCR primers

Gene	Primer (Forward)	Primer (Reverse)
G0S2	GCTCAGATGGAAAGTGTGCAG	TGGCGGCTGTGAAAGGGCTGC
PPARγ	CCAGAGCATGGTGCCTTCGCT	CAGCAACCATTGGGTCAGCTC
Fatp1	CGCTTTCTGCGTATCGTCTGCAAG	AAGATGCACGGGATCGTGTCT
L-Fabp4	GGGAAGAAAATCAAACTCACCATC	AGTTGTCACCATTTTATTGTCACC
SCD1	AGCTGGTGATGTTCCAGAGG	TGAGCACCAGAGTGTATCGC
Acadm	ACTTCGTGGCTTCGTCTAGA	GAGCAGGTTTCAAGATCGCA
Acadl	TTTCCGGGAGAGTGTAAGGA	ACTTCTCCAGCTTTCTCCCA
SREBP1c	ACGGAGCCATGGATTGCACATTTG	AGGCTGTAGGATGGTGAGTGG
Cidea	TGCTCTTCTGTATCGCCCAGT	GCCGTGTTAAGGAATCTGCTG
Elovl3	TCCGCGTTCTCATGTAGGTCT	GGACCTGATGCAACCCTATGA
PGC-1α	CCCTGCCATTGTTAAGACC	TGCTGCTGTTCCTGTTTTC
UCP1	ACTGCCACACCTCCAGTCATT	CTTTGCCTCACTCAGGATTGG
ECH1	GGTCAGCCGCGTGTTCCAAGATA	GTAGTCAAGGCTCTCGTCCACAG
Cox8b	CATAGTCGTTGGCTTCATGGTTC	GGCAGATGGACATGTTTAATGAA
Tfam	GCTGCCAGGCTTTCCTAGATGAA	TTCTATGCAAGAAAAACCTATGC
DiO2	GCAGTTAGGACTGAAGTGTTAGT	TACATGCCTCAATGCTGATGCTG
phyH	CGAGTACAGCCTGAGCTACACAG	TGATAAAAAGAGAACATCATCTC
HSL	CAGCGGTAGAGAATAGCATCATC	CTCTGCTCTCGTCATGTCACCTG
CGI-58	TGTGCAGGACTCTTACTTGGCAGT	GTTTCTTTGGGCAGACCGGTTTCT
C/EBPβ	GCAAGAGCCGCGACAAG	GGCTCGGGCAGCTGCTT
C/EBPα	GAACAGCAACGAGTACCGGGTA	GCCATGGCCTTGACCAAGGAG
Perilipin A	GGCCTGGACGACAAAACC	CAGGATGGGCTCCATGAC
ATGL	GAGACCAAGTGGAACATC	GTAGATGTGAGTGGCGTT

Supplementary methods

Generation of G0S2-knockout mice To generate G0S2-deficient mice, we screened a mouse 129S6/SvEv BAC library (RPCI-22, Taconic). G0S2-positive clones were mapped using restriction enzyme digestion and sequencing. Approximately 9kb of mouse genomic DNA was inserted into pBS and a HindIII/Xho1 fragment containing the coding exon (exon 2 encodes the entire open reading frame) of *G0S2* was excised and replaced by a Neo cassette. The TK negative selection marker was then added and the 9kb construct linearized by Asp718 digestion followed by electroporation of AB2.2 embryonic stem (ES) cells. A schematic of the targeting strategy is shown in figure 1A. Following selection, ES clones were screened by Southern blotting with a mouse *G0S2*-specific probe and targeted ES clones injected into C57BL/6 blastocysts. Germline transmission of the targeted allele was confirmed by Southern blotting. Primers used for genotyping are AAGAACGCCAAAGCCAGTC and CTTGATTGCTCGCACAGCCT for wildtype gene and CTGCTAAAGCGCATGCTC and CATCAGGCTATCTTCTTGGC for knockout gene.

Real Time Quantitative PCR analysis Total RNAs were extracted either from adipocytes using the RNeasy lipid tissue mini kit or from liver tissues using the Allprep DNA/RNA/Protein kit (Qiagen). 1ug RNA was reverse-transcribed to cDNA (QuantiTect Reverse Transcription Kit, Qiagen). Primers used are listed in Supplemental table 1. QRTPCR analysis was performed on the Eppendorf realplex using the QuantiTect SYBR Green (Qiagen). Gene expression analysis was determined using the delta CT method and normalized to cyclophilin.

Tolerance tests IPGTT and IPITT were performed on conscious mice after overnight fast. A 20% glucose solution (2g of glucose/kg of body weight) or insulin (0.75 mU/g of body weight) was administered intraperitoneally. Blood samples (~ 50-60 ul) were collected into tubes containing 10mM EDTA for

measurements of glycemia and insulin. Insulin levels were measured using an ELISA Kit (Millipore).

Triglyceride content Triglyceride content in mice sera and liver extracts were performed using a triglyceride kit (BioVision) according to the manufacturer's instructions.

Triglyceride lipase activity Triglyceride lipase activity assay was performed as described previously in [20] on visceral fat extracts of 22 weeks HFD fed-mice.

GCMS Free fatty acids extracted from approximately 20 mg visceral fat tissue from *wt* and *G0S2*$^{-/-}$ mice on chow diet were measured using gas chromatography coupled to mass spectrometry. Folch extraction [20] was performed followed by a series of acid-base extractions. The final extraction was evaporated to dryness under nitrogen gas. The dried samples were then dissolved in anhydrous pyridine and transferred to autoinjector vials where 70 ul of MTBSTFA (N-Methyl-N-tert-butyldimethylsilyltrifluoroacetamide, Sigma) were added. Samples were heated at 70^{o}C for 1 hour and were then loaded into the GC-MS machine for fatty acid analysis. Responses were normalized to the internal standard D27-myristic acid and were expressed per mg tissue.

Supplementary figure Legends

Fig. S1 Characterization of G02$^{-/-}$ mice. (A) A picture of wt (left) and G0S2 knockout mice (Right) on 22 weeks HFD. (B) raw data of mice body weight both on chow and 22-week HFD.

Fig. S2 FFA profile in *wt* and *G0S2*$^{-/-}$ visceral fat. Lipid extracts were subjected to gas chromatography coupled to mass spectrometry analysis. Data are expressed as means ± SEM. n = 6 per genotype.

Fig. S3 Expression levels of key lipolytic genes. mRNA levels of ATGL, HSL and CGI-58 in visceral fat of 22-week HFD-fed mice measured by quantitative real time-PCR and were normalized to cyclophilin.

Fig. S4 Effects of *G0S2* deletion on glucose homeostasis. Blood glucose levels during IPGTT in fasted *wt* and *knockout* female mice on (A) chow diet and (B) HFD. Blood glucose levels during IPITT in fasted *wt* and *knockout* female mice on (C) chow diet and (D) HFD. (E) Insulin levels measured during IPGTT on male mice. Values represent means ± SEM. n= 6 for *wt* and 9 for *knockout* mice on chow diet; n= 6 for *wt* and 10 for *knockout* mice on HFD.

Fig. S5 Expression levels of lipases and UCP1 in BAT from mice fed either chow or 22-week HFD (A) a representative western blot of HSL, ATGL and UCP1 done on BAT extracts from chow and HFD mice. (B, C) Quantitation of protein levels of ATGL and UCP1 normalized to calnexin. (D) mRNA levels of ATGL and UCP1 in BAT of mice on HFD. n=4 per genotype for western blot on each diet condition; and n=5 per genotype for quantitative RT-PCR.