**Supplementary Figure 1.**

*Central leptin signaling induces STAT3 activation and Socs3 expression in WAT.***, B**. MBH leptin increased STAT3 Y705 phosphorylation **(A)** and total STAT3 **(B)** (Figure still needs star) and STAT3 phosphorylation levels in WAT. **C.** *Socs3* mRNA expression in WAT was increased 3 fold by MBH leptin. **D.** *IL6* mRNA expression in WAT. *P<0.05.

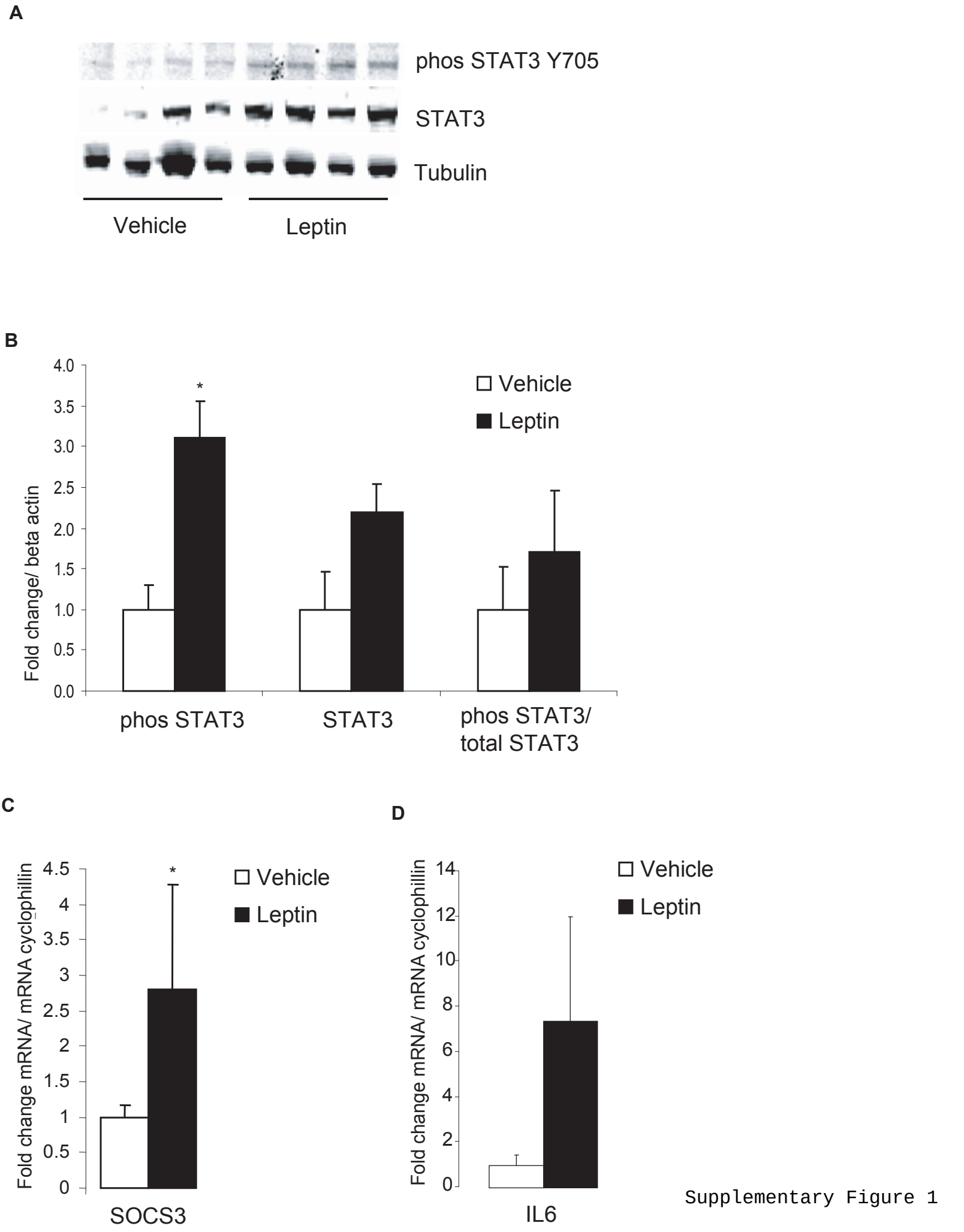
**Supplementary Figure 2.**

*Suppression of WAT lipogenesis by MBH leptin depends on the suppression of EC tone.* **A.** Experimental protocol: rats underwent pancreatic basal-insulin clamps during a 6 hrs infusion of MBH leptin. At the beginning of the study a single IP injection of the CB1 agonist Win 55,212-2 was administered at a dose of 3mg/kg. **B.** Lipogenic enzyme expression was analyzed by Western blot and quantified **(C)**. MBH leptin was unable to suppress Fas, Acc and Atpcl as well as Hsl activation in WAT when the suppression of WAT EC tone was prevented by systemic CB1 activation.

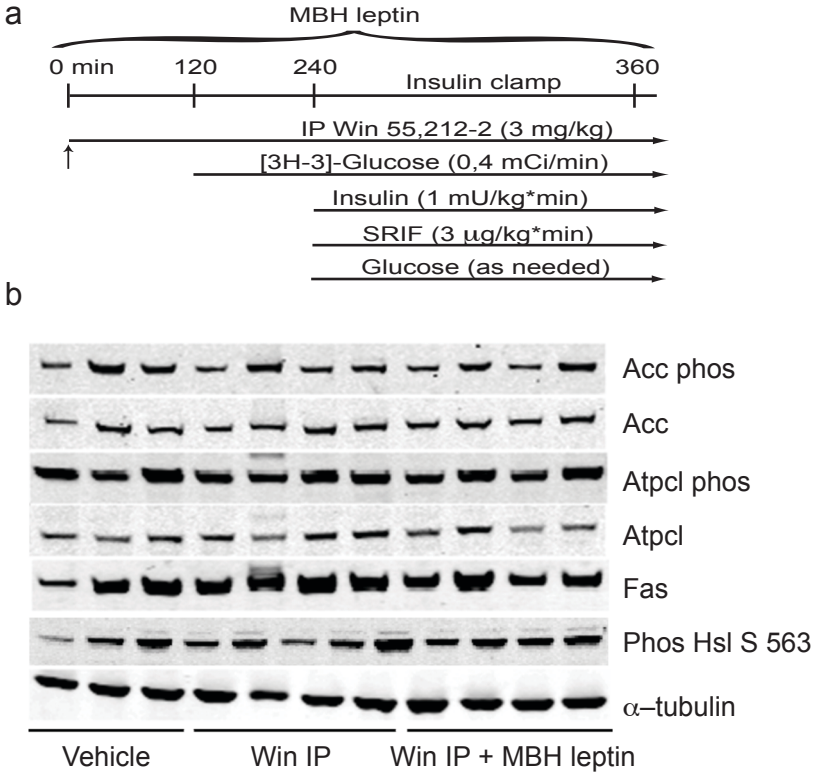
**Supplementary Figure 3.** *Surgical denervation of WAT*. SD rats underwent surgical denervation of the right testis fat (TF) depot and were sacrificed one week later. **A.** Weight in grams of the innervated vs the denervated TF depot and **B.** of the subcutaneous inguinal (IF) fat depot (innervation intact). **C.** Norepinephrine levels were markedly suppressed in the denervated TF depot indicating the success of the surgical

sympathectomy. **D.** AEA and 2AG are not suppressed by MBH leptin after surgical denervation of WAT. *P<0.05. ***P* < 0.01.

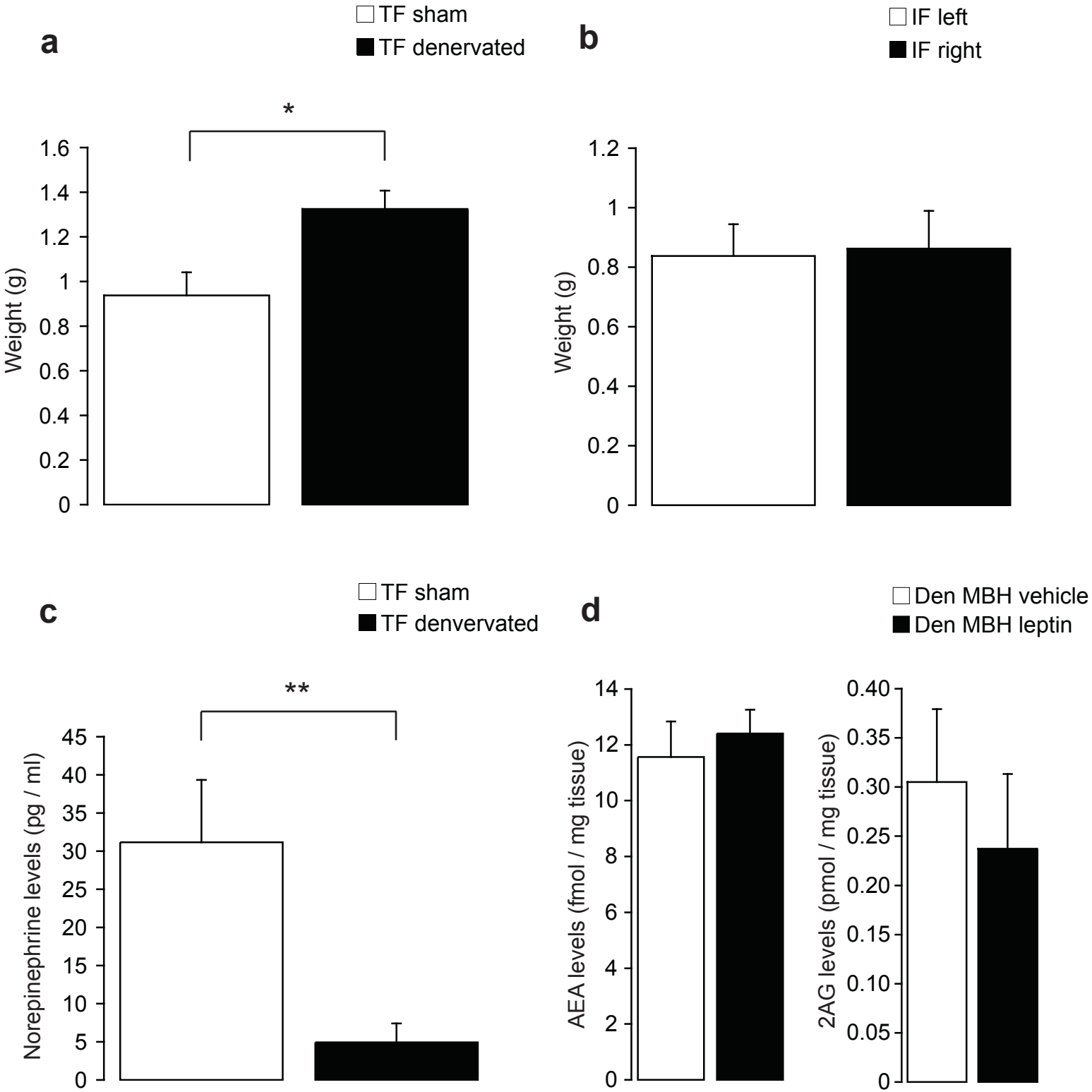
**Supplementary Figure 4.** *Control of WAT lipogenesis by central leptin is lost after pharnacological sympathectomy.* SD rats underwent pharmacological sympathectomy by local injection of 6-OHDA into the epididymal neurovascular strand. One week later vehicle or leptin was infused into the MBH of SD rats for 3 hrs at which time the animals were sacrificed. **A.** Pharmacological sympathectomy abolished the suppression of WAT lipogenesis by MBH leptin. **B.** The suppression of anadamide by MBH leptin was lost after pharmacological sympathectomy.

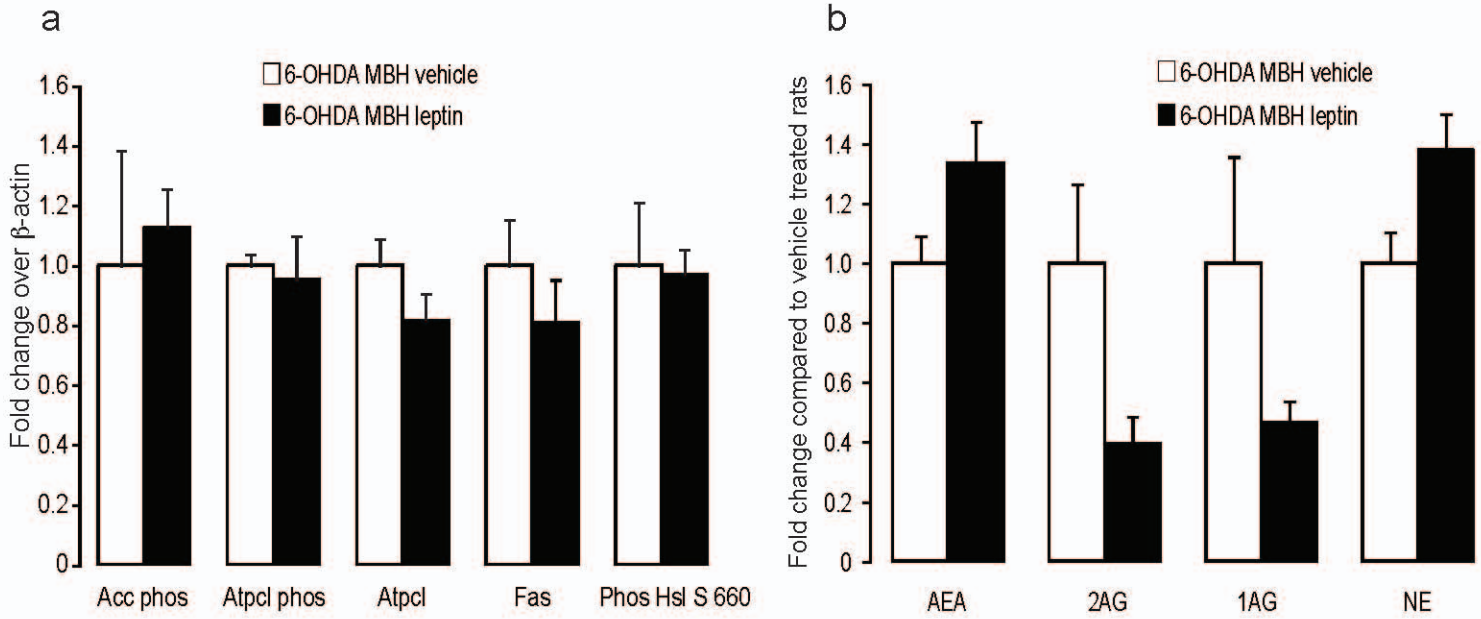



Supplementary Figure 1




Supplementary Figure 2


a
TF sham
TF denervated
*
Weight (g)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
b
IF left
IF right
Weight (g)
1.2
1
0.8
0.6
0.4
0.2
0
c
TF sham
TF denervated
**
Norepinephrine levels (pg / ml)
45
40
35
30
25
20
15
10
5
0
d
Den MBH vehicle
Den MBH leptin
AEA levels (fmol / mg tissue)
14
12
10
8
6
4
2
0
2AG levels (pmol / mg tissue)
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0




Supplementary Figure 4

***Supplementary Table 1***. *Sequences of QPCR Primers*

| | |
|---|---|
| ACCα | 5'- TGA GGA GGA CCG CAT TTA TC-3' |
| | 5'- GCA TGG AAT GGC AGT AAG GT-3' |
| ATGL | 5'- TGG GTG ACC ATC TAC CTT CC-3' |
| | 5'- CCC AGT GAG AGG TTG TTT CG-3' |
| CypA | 5'- TAT CTG CAC TGC CAA GACTGAGTG-3' |
| | 5'- CTT CTT GCT GGT CTT GCC ATT CC-3' |
| FAS | 5'- ATG GGA AGG TGT CTG TGC ACA T-3' |
| | 5'- TGT GGA TGA TGT TGA TGA TA-3' |
| G6Pase | 5'- TCC TGG GAC AGA CAC ACA AG-3' |
| | 5'- CTT TCC TCA AAC AGC CCT GT-3' |
| HSL | 5'- CTC ATG GAT CCT CTT CTA CCA CTG A-3' |
| | 5'- GCC TTC TGG TCT GAG TCA AAA TG-3' |
| PCK1 | 5'- CTT CTC TGC CAA GGT CAT CC-3' |
| | 5'- GAG CCA GCC AAC AGT TGT CA-3' |
| PPARγ | 5'- TTC GCC AAG GTG CTC CAG AA-3' |
| | 5'- AGA GGGTGA AGG CTC ATA T-3' |
| SCD1 | 5'- CTA CAA GCC TGG CCT CCT GC-3' |
| | 5'- GGA CCC CAG GGA AAC CAG GA-3' |
| SREBP-1c | 5'- AGC TCA CGG TAC CAG CAA T-3' |
| | 5'- GTA GGA AGA CCC TCC TCA TA-3' |
| 18S | 5'- AGG GTT CGA TTC CGG AGA GG-3' |
| | 5'- CAA CTT TAA TAT ACG CTA TTG G-3' |
| FAAH | 5'- TGCTTGGGAGACCTGATCTTA-3' |
| | 5'- CAGTTTCCACAGCTTTTCAGC-3' |
| SOC3 | 5'- CCT CCA GCA TCT TTG TCG GAA GAC-3' |
| | 5'- TAC TGG TCC AGG AAC TCC CGA ATG-3' |
| PGC1-a | 5'- TATGAGAAGCGGGAGTCTGAA-3' |
| | 5'- GCGTTGTGTCAGGTCTGATTT-3' |

## Methods

**Animals.** We housed 10-week old male Sprague Dawley rats (Charles River Breeding Laboratories, Wilmington, MA, USA) in individual cages in a temperature and light (12 hrs light/dark cycle) controlled facility. Animals were stereotaxically fit with indwelling intrahypothalamic cannulae 2 weeks prior to euglycemic clamp study, followed one week later by intra-carotid arterial and intra-jugular venous catheterization for infusion and blood sampling respectively. Animals were allowed to recover for 5 days after last surgery and needed to have reached pre-surgical body weights prior to studies.
Mice with the *Lepr*$^{s1138}$ mutation on C57BL/6 background have been described(1) and were provided by Martin Myers, University of Michigan, Ann Arbor. C57BL/6 *db/db* animals were obtained from the Jackson Laboratory (Bar Harbor, Maine) or were generously provided by Dr Ruth Harris.

**Pair feeding studies.** Up to 4 weeks of age the body weight of s/s and db/db mice does not differ from that of wt mice after which both mutant strains become progressively hyperphagic and obese. To assess the consequences of lifelong deficiency of all LRb signaling and the isolated obliteration of the STAT3 pathway of LRb signaling *db/db* and *s/s* mice were pair-fed to the daily food intake of wild type mice starting at 4 weeks of age (average of 3.8gm/day, testdiet #5001). At 11 weeks of age *s/s* and *db/db* mice were studied by MRI followed by clamp studies (see also Figure 4 a).

**Implantation of chronic cannulae and intrahypothalamic infusions**

Stereotaxic coordinates were selected according to the Rat Brain Atlas of Paxinos & Watson and experimentally modified in order to fit the anatomy of Sprague-Dawley rats. Briefly, animals were placed in a stereotaxic frame (Harvard/American Scientific Institution, Holliston, MA) under ketamine/xylazine anesthesia. The MBH was targeted bilaterally using a dual-guide, 26-g cannula system (Plastics One, Roanoke, VA, Cat. #: G235G-0.8/9.6) directed to stereotaxic coordinates 3.3 mm posterior to bregma and 9.6 mm below the surface of the skull. The center-center distance between each guide of the cannula was 0.8mm. Leptin and vehicle (artificial CSF) The anatomical placement of the bilateral cannulae was initially validated by histochemistry and the sampling of specific

nuclei after infusion of tracer (H3 glucose). Routinely, we infused 1 ul of food dye after the sacrifice of the animal immediately before the brain was removed, followed by anatomical sampling of either the whole hypothalamus or a wedge resection of the MBH.

**Denervation procedures**

For surgical denervation the vascular strand innervating the epigonadal fat pad was identified after laparotomie. The fascia containing the nerve bundel was separated from the vessels and dissected, followed by local application of phenol. Pharmacological sympathectomy was performed by injection of 6-OHDA into the nerve bundel innervating the epigonadal fat pad.

**Euglycemic/Hyperinsulinemic Clamp Studies**

Pancreatic-basal insulin clamp studies in rats were performed as described(2), experimental protocol see (**Fig. 2a** and **Supplementary Fig. 2a** online).

**Analytical procedures.** Plasma glucose was measured by the glucose oxidase method (Glucose Analyzer II; Beckman Instruments, Inc., Fullerton, CA, USA). Endocannabinoids were extracted from 100 mg of tissue and measured by HPLC-MSD as described(3) in with the following modification. After the final evaporation step 40-90 µl of clear oil remained which was extracted with 60 µl of ice cold methanol. After methanol evaporation, ECs were determined by HPLC-MSD. Catecholamines were determined as described(4).

**RNA Extraction and Quantitative Real-Time RT-PCR**.

Total RNA was obtained from frozen tissue (~85-100 mg) using Trizol (Invitrogen, Carlsbad, CA, USA) reagent (**Fig. 2b and c**) or using RNeasy Lipid Tissue Mini kit (Valencia, CA, USA; **Fig. 4** and **Supplementary Fig. 1** online) according to the manufacturer's instructions. Following treatment with DNase I (Invitrogen, Carlsbad, CA, USA), purified RNA was used as template for first-strand cDNA synthesis using Superscript III (Invitrogen, Carlsbad, CA, USA). Quantitative real-time RT-PCR was run either using LC-Fast Start DNA SYBR Green I chemistry (Roche Diagnostics,

Indianapolis, IN, USA) on a LightCycler 2.0 platform (Roche Diagnostics, Indianapolis, IN, USA) (**Fig. 2b and c**) or using SYBR GreenER qPCR SuperMix (QIAGEN, Valencia, CA 91355, USA) on a 7900HT sequence detection system (Applied Biosystems, Foster City, CA 94404, USA) (**Fig. 4** and **Supplementary Fig. 1** online).

Forward and reverse primer pairs were as listed (**Supplementary Table 1** online).

**Western Blot Analyses**. The indicated tissues were homogenized in 20 mM MOPS, 2 mM EGTA, 5 mM EDTA, 30 mM sodium fluoride, 40 mM beta-glycerophosphate, 10 mM sodium pyrophosphate, 2 mM orthovanadate, 0.5 % NP-40 and complete protease inhibitor cocktail (Roche, Indianapolis, IN, USA) and centrifuged at 12,000 g for 15 min and the supernatant was harvested while carefully avoiding the lipid layer on top. Protein concentration was measured with a BCA protein quantification kit (Pierce, Rockford, IL, USA). Protein extracts were separated on 4-12% NuPAGE (Invitrogen, Carlsbad, CA, USA) gels and were blotted onto Immobilon FL PVDF (Millipore, Billerica, MA, USA). Membranes were blocked at room temperature for 1 hr in Odyssey LI-COR Blocking Buffer (LI-COR, Lincoln, NE, USA) and incubated in primary antibodies against Acc, phosphor- STAT3 Y705, phospho-Akt S473, Akt, phospho-Jnk1/2, (Cell Signaling Technology, Inc., Beverly, MA, USA), STAT3, (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and Gapdh (Research Diagnostics, Concord, MA, USA) in 1:1 Blocking Buffer/TBS-T overnight at 4˚C. Following four consecutive 5 min washes in TBS-tween 20 (0.1%), blots were incubated with Alexa Fluor 680 donkey anti-goat IgG, Alexa Fluor 680 anti-mouse IgG (Molecular Probes, Eugene, OR, USA) or IR Dye 800 conjugated goat anti-rabbit IgG (Rockland, Gilbertsville, PA, USA) for 1 hr at room temperature in blocking buffer containing 0.1% TBS-T and 0.1% SDS. After three washes in TBS-T and a final wash in TBS the blots were scanned using the LI-COR Odyssey (LI-COR, Lincoln, NE, USA) and quantified using Odyssey 2.0 software based on direct fluorescence measurement (Figures 3, 5,6, Supplementary Figures 1 and 2) or developed by using HRP coupled secondary antibodies (Figures 1 and 2).

**[14C]-palmitate storage in WAT TGs.** Infusates were prepared daily in quantities sufficient to perform experiments in two rats. 150 ul of ethanol containing ~8 x $10^7$ dpm $^{14}C$ - palmitate was added drop-wise to 0.6 ml of continuously stirred 4% (w:v) essentially FFA-free BSA (Sigma, St. Louis, MO) in normal saline. The infusate was made up to a final volume of ~2 ml by addition of normal saline. At t = 0 min, an MBH infusion of leptin or aCSF was initiated. A primed-continuous infusion of [14C]-palmitate (GE Healthcare Bio-Sciences Corp, Piscataway, NJ, USA; 20 µCi bolus; 0.4 µCi/min) was initiated at t =180 min and was maintained until animals were sacrificed at t=240 min. In some initial studies we obtained sample for the [14C]-palmitate specific activity every 5 min for the last 60 min of the study. Steady state was reached after ~10 min of [14C]-palmitate infusion. In the subsequent studies, the serum specific activity of [14C]-palmitate was determined at the end of the study after Folch extraction and divided by the FFA concentration in the same sample. Incorporated [14C]-palmitate was determined after Folch extraction of adipose tissue and was expressed as dpm/gm of tissue x SA.

**Statistics.** All values are presented as the mean+/-SE. Comparisons among groups were made using analysis of variance followed by unpaired, nonparametric Student's t test. Differences were considered statistically significant at $p < 0.05$. The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Albert Einstein College of Medicine and Mount Sinai School of Medicine.

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