Supplemental Experimental Procedures

Fatty Acids Analysis

Tissue was homogenized in 6 mL methanol/Dichloromethane (1:1) solution. 0.5 mL of the the solution was mixed with 20 μL surrogate internal standard mix, 0.5 mL phosphate buffer solution (PBS) and 50 μL 45% KOH. The solution was heated to 60° C for 90 min, in order to release acyl chains from the esterified FA by hydrolysis. After cooling at room temperature, 1 mL isooctane was added for liquid-liquid extraction (repeated three times). Isooctane extracts were dried at room temperature with a gentle N2 stream. 50 μL of pentafluorobenzoyl bromide (PFBBr) solution (1% acetone) and 50 μL triethylamine solution (1% acetone) were added for derivatization of the carboxylic moiety of the FA. After 25 minutes at room temperature, 1 mL of isooctane was added and transferred into a 1.6 mL glass vial for further injection into a 7590A GC/5795C MSD Agilent instrument (Agilent Inc, Palo Alto, USA), equipped with negative chemical ionization. Quantification was carried out by internal standard calibration, using d_3-12:0 (lauric acid), d_3-18:0 (stearic acid) and d_4-24:0 (lignoceric acid) carboxylic acids, added as a surrogate mix of 500 ng mL^{-1} of each in isopropyl alcohol.

Estradiol quantitiation

Estradiol was isolated from plasma using solid-phase extraction and analyzed by HPLC-MS using electrospray ionization in the negative mode.

Sphingolipid quantitation

Sphingolipids levels were quantitated using LC/MS/MS methodology. Briefly, in an Eppendrof polypropylene microcentrifuge tube, flash frozen hypothalamic tissue samples were homogenized in 500 μM of cold PBS using a sonic tissue disruptor fitted with a microprobe. 50 μL of homogenate were aliquoted and reserved for protein content determination. The remaining homogenate was added to 2 mL of organic extraction solvent (isopropanol:ethyl acetetate, 15:65;

v:v). The microcentrifuge tube was rinsed with 2x500 µL of cold PBS. Immediately afterwards, 20 µL of internal standard solution was added (Avanti Polar Lipids, AL Ceramide/Sphingoid Internal Standard Mixture II diluted 1:4 in ethanol). The mixture was vortexed and sonicated in an ultrasonic bath for 40 minutes at 40 °C. Then the samples were allowed to cool to room temperature and two-phase liquid extraction was performed. The supernatant was transferred to a new tube and the pellet was re-extracted. Supernatants were combined and evaporated under nitrogen. The dried residue was reconstituted in 200 µL of HPLC solvent B (methanol/ formic acid 99:1; vol:vol containing 5 mM ammonium formate) for LC-MS/MS analysis. Lipid separation was achieved on a 2.1 (i.d.) x 150 mm Kinetex C8, 2.6 micron core-shell particle column (Phenomenex, Torrance, CA).

Sphingolipids quantitation from cell culture

N43 cells were cultured in 6 mm plates (500,000 cells/plate) and grown for 24 hours before treatment with medium containing 2% charcoal:dextran stripped fetal bovine serum. Cells were pretreated overnight with E2 (10^{-8}M) followed by PA (100 µM) for 8 hours. Following the treatment supernatant was collected and cells were washed with ice cold PBS and scraped in 3 mL of 80% methanol. Supernatant and cells were rapidly frozen in liquid nitrogen and stored at -80 °C. Sphinglolipid measurements was performed as previously indicated.

Plasma Collection

Blood was collected from anesthetized mice through eye bleeding and centrifuged twice at 8,000 rpm at 4 °C to collect plasma. Plasma aliquots were stores at -80 °C.

ELISA

Mouse IL1β (Thermo Scientific) and TNFα (R&D Systems, Minneapolis, MN) ELISA Kits were performed following the kit instructions. Protein lysates were prepared as previously described.

β-Hydroxybutyrate (Calbiochem) measurement was performed on plasma samples according to manufacturer's instruction.

Genotyping for XY chromosome

DNA extraction and genotyping were done using the REDExtract-N-AMP Tissue PCR Kit (Sigma). Briefly, between 24-48 hours after birth, pups were numbered with a permanent marker to provide identification. Following a snip of the tail, the clippings were digested in 31.25 µL tissue preparation and extraction solution for 10 minutes at room temperature and then heated to 95 ºC for 5 minutes. After cooling to 4º C, 25 µL of neutralization solution was added and the samples vortexed. PCR was then carried out using a Thermocycler (Applied Biosystems) and the PCR products separated by gel electrophoresis and visualized.

For genotyping the following primers (Sigma) were used:

MYOF: 5' TTACGTCCATCGTGGACAGCAT 3'

MYOR: 5' TGGGCTGGGTGTTAGTCTTAT 3'

YMPT FP1: 5' CTGGAGCTCTACAGTGATGA 3'

YMTRP1: 5' CAGTTACCAATCAACACACATCAC 3'

Cell Culture

Unless otherwise specified, culture media and supplements were purchased from Gibco-Invitrogen (Carlsbad, USA) and plasticware from Corning (Corning, USA).

Primary hypothalamic cells (astrocytes and neurons) were prepared from 1-3 day old C57BL/6 male and female pups. Pups were divided by sex based on genotyping for XY chromosome as outlined above.

Primary hypothalamic neurons were prepared using the papain dissociation system (Worthington

Biochemical Corporation, Lakewood, NJ, USA) according to the manufacturer's instructions. Primary neurons were cultured in phenol-free Neurobasal® medium containing B-27® serum-free supplement, 5% horse serum, 100 mg/L sodium pyruvate, 100 units/mL penicillin G sodium and 100 µg/mL streptomycin sulfate (37 °C, 5% CO_2). Cells were seeded in 24-well plates previously coated with Ploy-L-lysine (Sigma) and experiments were performed 3-5 days after preparation of primary cells. Cells were grown for 24 hours before treatments with medium containing 2% charcoal:dextran stripped fetal bovine serum and treated with the same medium plus 10^{-8} M E2 (Sigma), 100 µM PA-BSA, a combination of E2 and PA-BSA (with 1 hour pre-treatment of E2 before the addition of PA-BSA) or vehicle. Cells were collected following 8 hours of treatments.

Primary hypothalamic astrocytic cultures were prepared under sterile conditions and triturated in DMEM/F-12 containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. The suspension was centrifuged and the pellet resuspended in this medium. Cells were grown in 75 cm^3 culture flasks at 37°C and 5% CO_2. On the ninth day, the flasks were placed in a 37°C shaking incubator (SI-300, Jeoi Tech; Medline Scientific, Oxford, United Kingdom) at 280 rpm for 16 hours. The cells were then harvested (0.05% trypsin/EDTA solution; Biochrom AG, Berlin, Deutchland), resuspended in DMEM/F-12 plus 10% FBS and 1% antibiotics/antimycotics, and centrifuged for 5 minutes at 1000 rpm. For protein extraction and gene expression assays, cells were seeded at a concentration of 3×10^5 cells/ml in 6-well culture plates previously treated with poly-L-lysine hydrobromide (10 µg/ml; Sigma) and grown for 24 hours. The medium was changed to DMEM F-12 plus 1% antibiotics/antimycotics (without FBS) for 24 hours before treatments. Primary astrocytes were treated with the same medium plus 10^{-8} M E2 (Sigma), 50 µM PA-BSA, a combination of E2 and PA-BSA (with 1 hour pre-treatment of E2 before the addition of PA-BSA) or vehicle. These concentrations of E2 and PA-BSA were chosen after performing a dose-response curve. Cells were collected following 8 hours of treatments. In each experiment, treatments were done in duplicate or triplicate; each experiment was repeated 3-4 times (n= 3 to 4).

For stearic acid and linoleic acid treatment, N43 hypothalamic neuronal cells were grown for 24 hours before treatments with medium containing 2% charcoal:dextran stripped fetal bovine serum. Cells were treated for 8 hours with 50 μM stearic acid (18:0) or linoleic acid (18:2) (Matreya, Pleasant Gap, PA, USA) conjugated with BSA. Doses were chosen based on the lowest possible concentrations and relative to their abundance in the HFD.

Western blot

For protein extraction mouse hypothalamic tissue was homogenized in a 20 mM Tris buffer (pH 7.4) containing 150 mM NaCl, 1% Triton X-100, 10 mM EDTA, Complete® protease inhibitor cocktail and PhosSTOP® phosphatase inhibitor cocktail (Roche Applied Science). Tissue extracts were then centrifuged at 12,000 G at 4 ºC and supernatants were collected.

Primary astrocyte cultures were processed with a lysis buffer containing 25 mM HEPES, 150 mM KCl, 2mM of EDTA, 0.1% Igepal, 1 mM PMSF, 10 μM benzamidine and leupeptin and 0.5 mM DTT. The samples were frozen and stored at -80ºC for 24 hours. They were then centrifuged for 20 minutes at 12,000 G at 4ºC. The supernatant was removed, frozen, lyophilized, and then resuspended in 40 μl sterile water and stored at -80ºC.

The primary antibodies used were ERα (MC-20, Santa Cruz, Dallas, TX, USA) and PGC-1α (4C1.3, Calbiochem, Darmstadt, Germany). β-actin (Santa Cruz), glyceraldehyde 3-phosphate dehydrogenase (GAPDH; Anaspec, Fremont, CA,USA) or α-tubulin antibodies (Sigma) were used as loading controls.

Bands were quantified using a LiCor Odyssey scanner. Boxes were manually placed around each band of interest, which returned near-infrared fluorescent values of raw intensity with intra-lane background subtracted using Odyssey 3.0 analytical software (LiCor). Data were normalized to control values.

Adenoiral constructs and infections

Adenoviruses expressing FLAG-ERα (AdERα) and GFP were constructed as follows: N-terminal FLAG-tagged hERα fragments were inserted into the pADTrack-CMV shuttle vector. Empty shuttle vector was used for control virus (AdGFP). Cloned shuttle vectors were digested with *PmeI* and then electroporation was performed for homologous recombination between shuttles and AdEasy-1 viral vector in AdEasier electrocompetent cells (Agilent Technologies, Santa Clara, CA, USA). Viral constructs were linearized with *PacI* and amplified using AD 293 cells (Agilent Technologies). Viruses were purified by CsCl density gradient ultracentrifugation and dialyzed with 10% glycerol/1X PBS solution.

Fluorescence microscopy on cells

For fluorescence microscopy determinations on cells, cells cultured on coverslips were fixed in paraformaldehyde (4% w:v) for 15 min at room temperature. Cells were permeabilized in 0.01% Triton and blocked in 3% BSA-PBS (Fischer Scientific) for 1 hour. Cells were incubated with the primary antibodies overnight (ERα, Santa Cruz and GFAP, Abcam) followed by the respective secondary antibodies (Alexa Fluor®, Life Technologies) for 1 hour. Confocal fluorescence images were captured using a Leica TCS SP2 confocal fluorescence microscope.

Co-localization analysis

ERα and PGC-1α co-localization analysis was performed using IMARIS (Bitplane). The co-localization channel was generated using IMARIS Coloc.

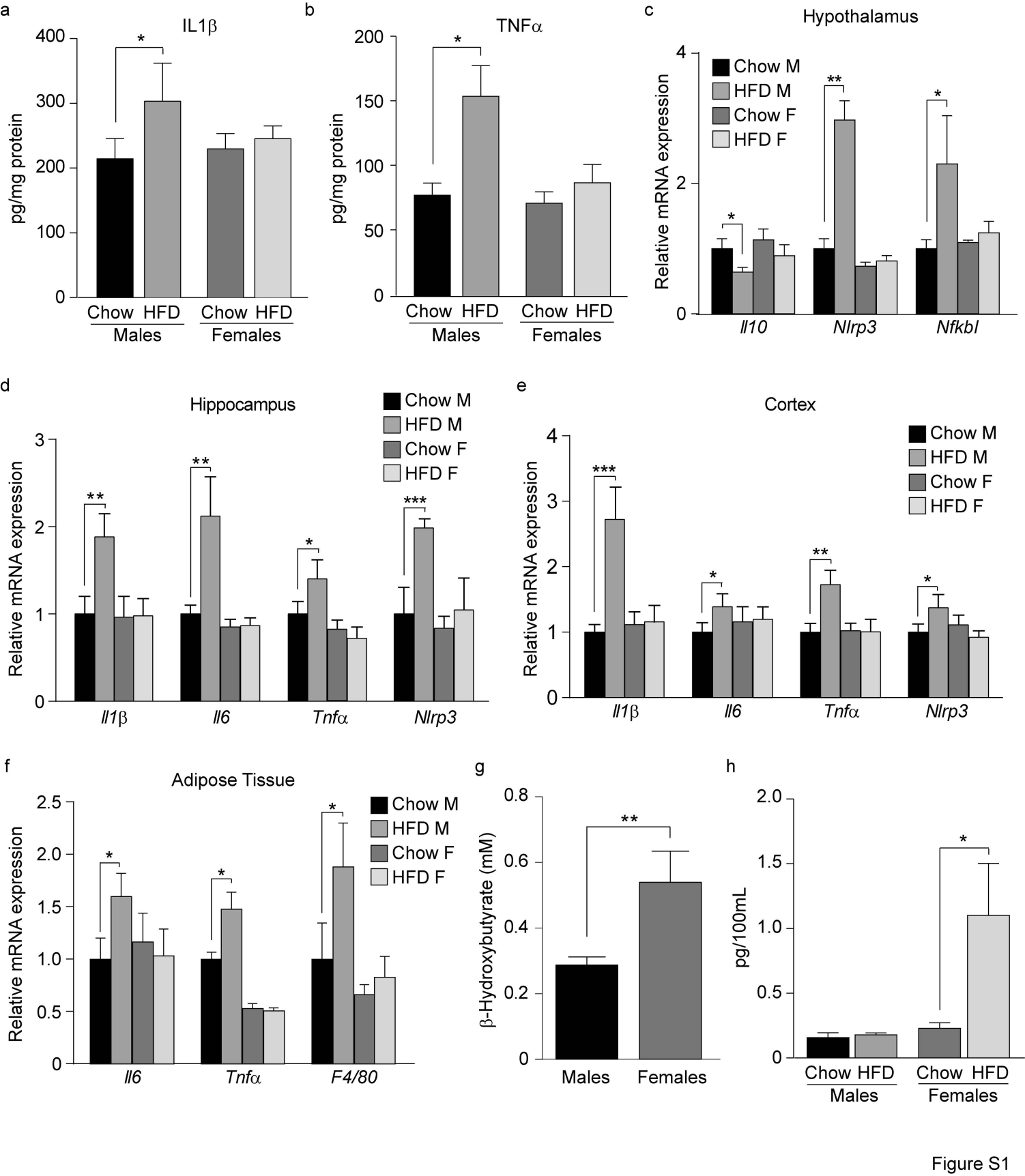
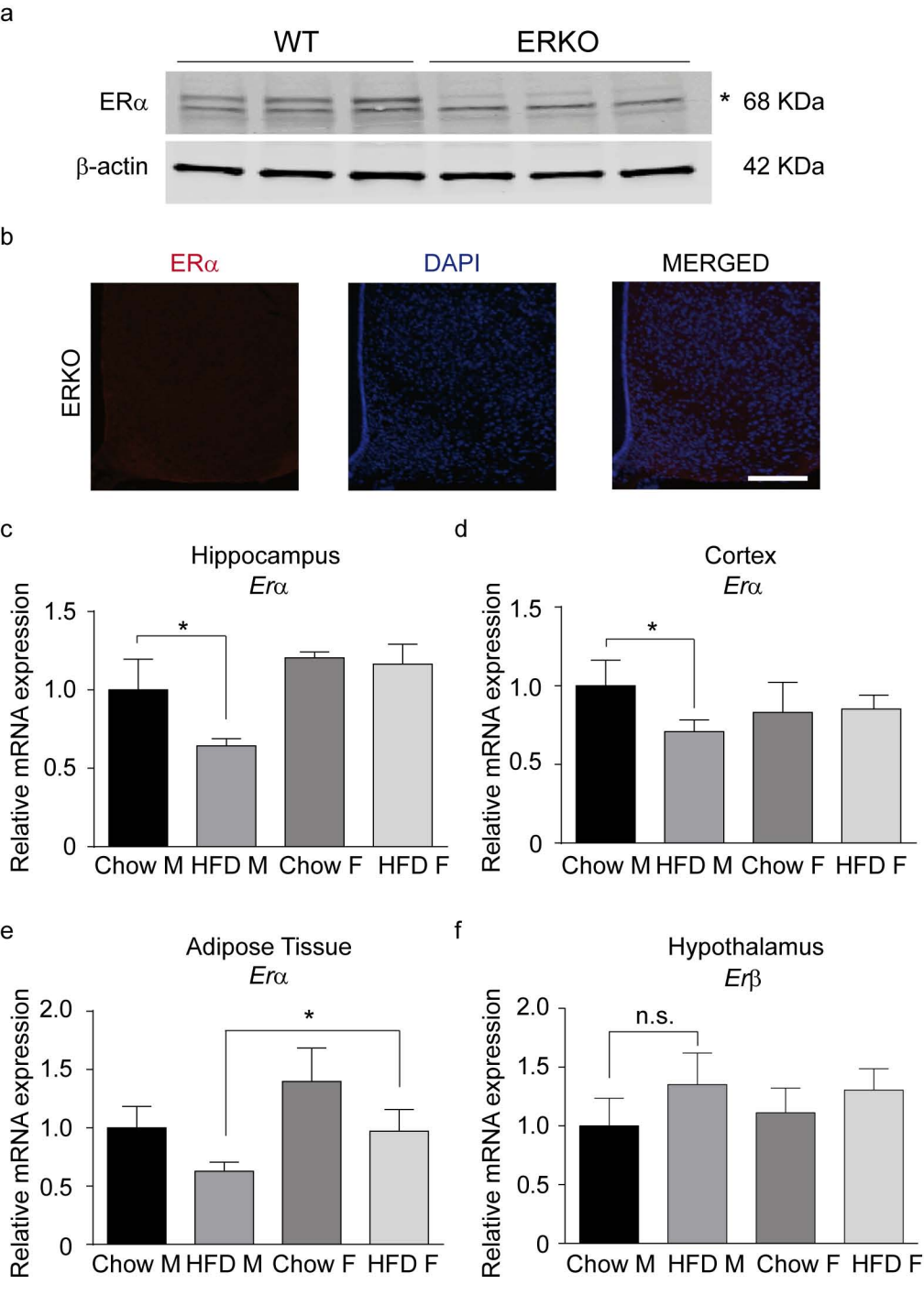

a
IL1β
pg/mg protein
400
300
200
100
0
*
Chow HFD
Males
Chow HFD
Females
b
TNFα
pg/mg protein
200
150
100
50
0
*
Chow HFD
Males
Chow HFD
Females
c
Hypothalamus
Relative mRNA expression
4
2
0
Chow M
HFD M
Chow F
HFD F
*
**
*
Il10
Nlrp3
Nfkbl
d
Hippocampus
Relative mRNA expression
3
2
1
0
Chow M
HFD M
Chow F
HFD F
**
**
*
***
Il1β
Il6
Tnfα
Nlrp3
e
Cortex
Relative mRNA expression
4
3
2
1
0
Chow M
HFD M
Chow F
HFD F
***
*
**
*
Il1β
Il6
Tnfα
Nlrp3
f
Adipose Tissue
Relative mRNA expression
2.5
2.0
1.5
1.0
0.5
0
Chow M
HFD M
Chow F
HFD F
*
*
*
Il6
Tnfα
F4/80
g
β-Hydroxybutyrate (mM)
0.8
0.6
0.4
0.2
0
**
Males
Females
h
pg/100mL
2.0
1.5
1.0
0.5
0
*
Chow HFD
Males
Chow HFD
Females

Figure S1


a
WT
ERKO
ERα
* 68 KDa
β-actin
42 KDa
b
ERα
DAPI
MERGED
ERKO
c
Hippocampus
Erα
Relative mRNA expression
1.5
1.0
0.5
0
*
Chow M
HFD M
Chow F
HFD F
d
Cortex
Erα
Relative mRNA expression
1.5
1.0
0.5
0
*
Chow M
HFD M
Chow F
HFD F
e
Adipose Tissue
Erα
Relative mRNA expression
2.0
1.5
1.0
0.5
0
*
Chow M
HFD M
Chow F
HFD F
f
Hypothalamus
Erβ
Relative mRNA expression
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1.5
1.0
0.5
0
n.s.
Chow M
HFD M
Chow F
HFD F

Figure S2

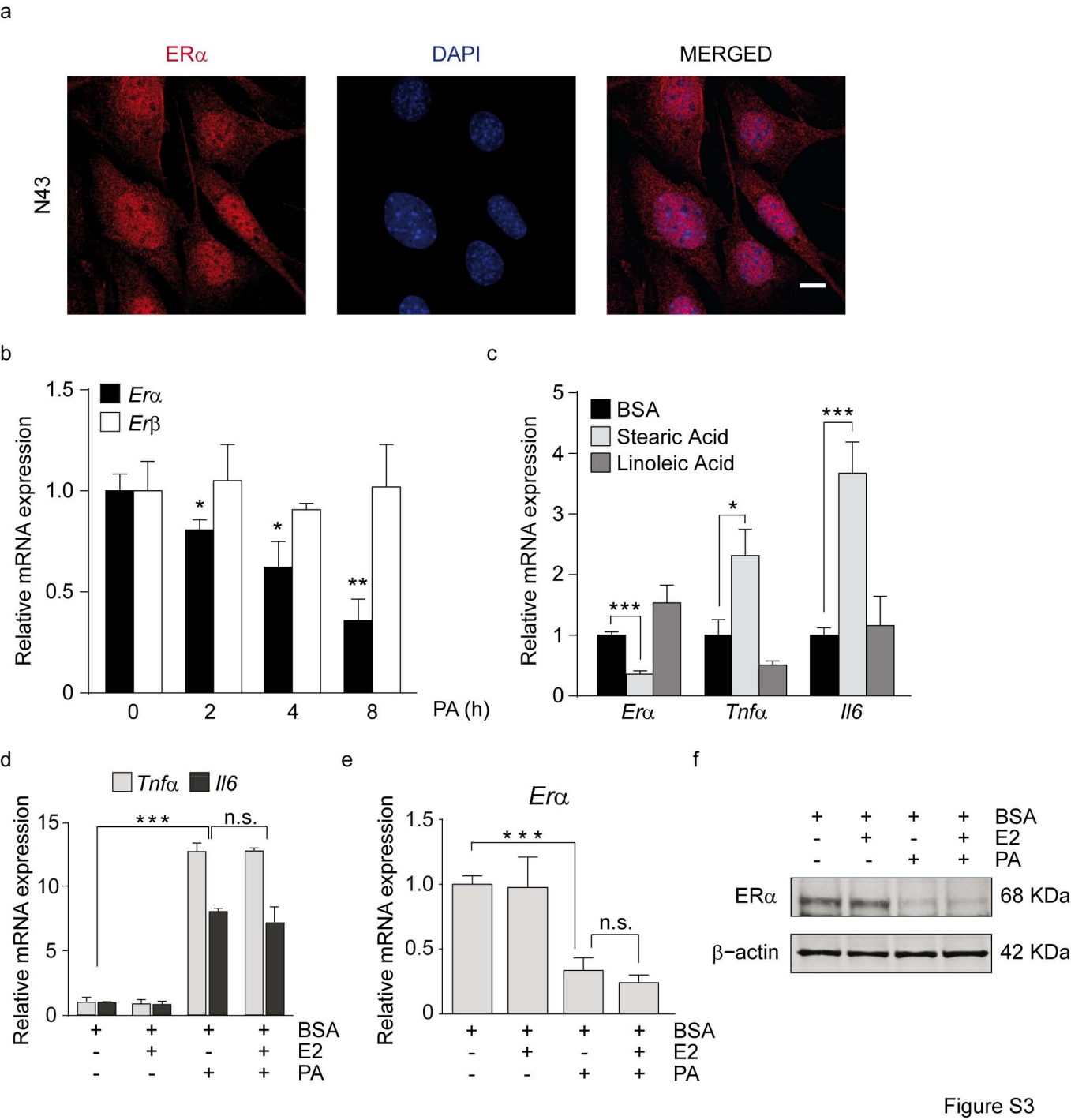
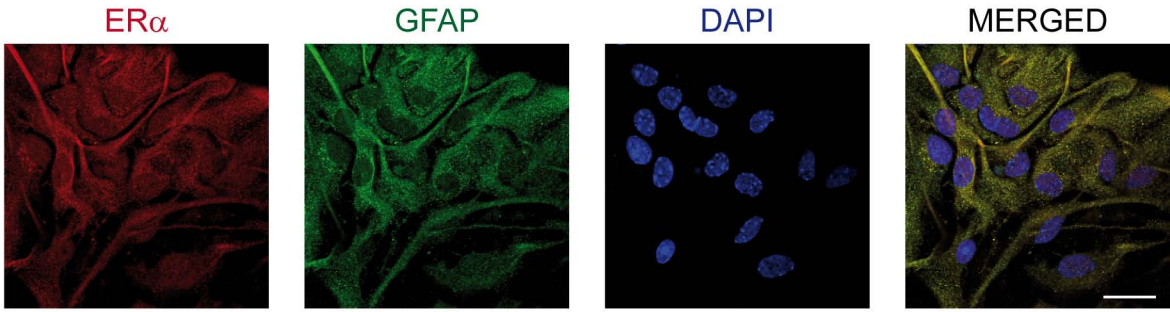

a
ERα
DAPI
MERGED
N43
b
Erα
Erβ
Relative mRNA expression
PA (h)
c
BSA
Stearic Acid
Linoleic Acid
Relative mRNA expression
Erα
Tnfα
Il6
d
Tnfα
Il6
Relative mRNA expression
n.s.
BSA
E2
PA
e
Erα
Relative mRNA expression
n.s.
f
ERα
β−actin
68 KDa
42 KDa

Figure S3

a


ERα
GFAP
DAPI
MERGED

b

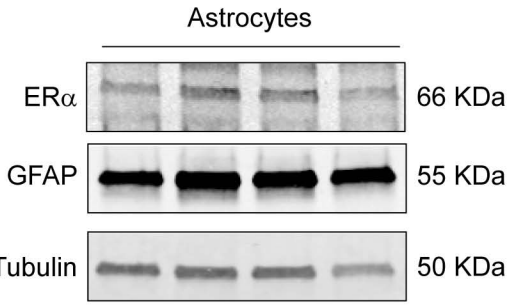

Astrocytes
ERα
66 KDa
GFAP
55 KDa
β-Tubulin
50 KDa

Figure S4

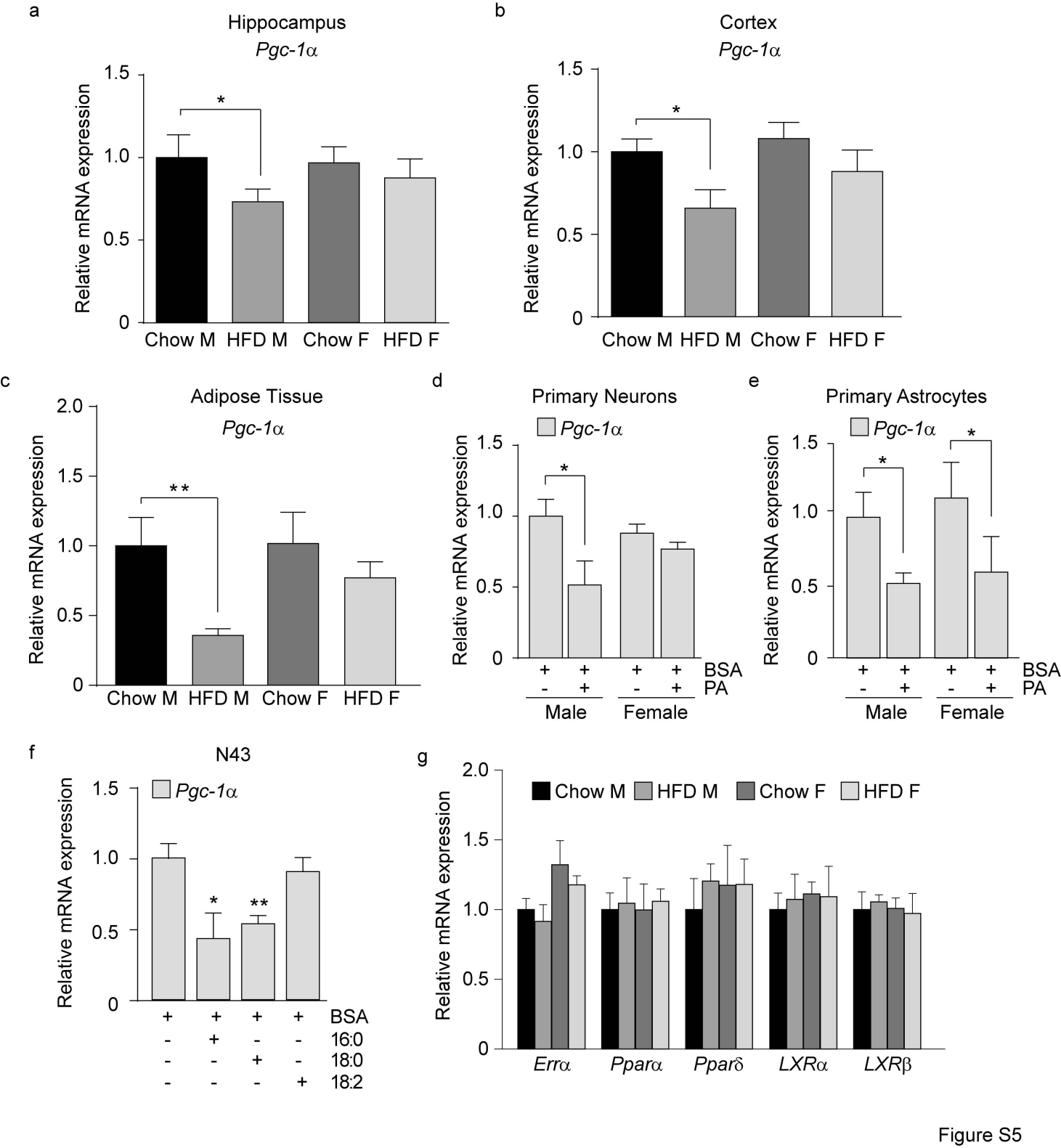

a
Hippocampus
Pgc-1α
Relative mRNA expression
1.5
1.0
0.5
0
*
Chow M
HFD M
Chow F
HFD F
b
Cortex
Pgc-1α
Relative mRNA expression
1.5
1.0
0.5
0
*
Chow M
HFD M
Chow F
HFD F
c
Adipose Tissue
Pgc-1α
Relative mRNA expression
2.0
1.5
1.0
0.5
0
**
Chow M
HFD M
Chow F
HFD F
d
Primary Neurons
Pgc-1α
Relative mRNA expression
1.5
1.0
0.5
0
*
+ + + + BSA
- + - + PA
Male
Female
e
Primary Astrocytes
Pgc-1α
Relative mRNA expression
1.5
1.0
0.5
0
*
*
+ + + + BSA
- + - + PA
Male
Female
f
N43
Pgc-1α
Relative mRNA expression
1.5
1.0
0.5
0
*
**
+ + + + BSA
- + - - 16:0
- - + - 18:0
- - - + 18:2
g
Chow M
HFD M
Chow F
HFD F
Relative mRNA expression
2.0
1.5
1.0
0.5
0
Errα
Pparα
Pparδ
LXRα
LXRβ

Figure S5

Supplemental Figure Legends

Figure S1, Related to Figure 1: Chronic HFD exposure increases inflammation in male mice (a-b) Determination of tissue concentration of IL1β (a) and TNFα (b) by ELISA in the hypothalamus of WT male and female mice fed chow or HFD for 16 weeks. n=8/group. (c) mRNA levels of *Il10, Nlrp3 and Nfkb* in hypothalamic tissue. Chow M and F, n=9; HFD M, n=10; HFD F, n=12. (d-f) mRNA levels of the indicated genes in hippocampal (d) cortical (e) and visceral adipose tissue (f). n=4/group. (g) Determination of plasma concentration of β-hydroxybutyrate in males and females. n=8/group. (h) plasma concentration of 17-β estradiol. Chow M and F, n=6; HFD M, n=7; HFD F, n=5. All data are presented as mean ±SEM, $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$.

Figure S2, Related to Figure 2:Chronic HFD exposure decreases ERα in the CNS of male mice. (a)Validation of MC-20 ERα antibody by western blot. Representative immunoblot of ERα protein in the hypothalamus of WT and ERKO male mice. β-actin was used as a loading control. n=3/group. *= non-specific band. (b) Validation of MC-20 ERα antibody by immunofluorescence. Representative confocal image showing non-specific binding for ERα immunoreactivity in the ARC of the hypothalamus in ERKO mice. (c-d) mRNA levels of ERα in hippocampal (c), cortical (d) and visceral adipose tissues (e). n=4/group. (f) mRNA levels of *Erβ* in hypothalamic tissue. Chow M and F, n=9; HFD M, n=10; HFD F, n=12. All data are presented as mean ±SEM, and $^{*}p < 0.05$.

Figure S3, Related to Figure 3: ERα is decreased in N43 cells following PA treatment. (a) ERα expression in N43 cells. N43 cells were stained with the validated ERα antibody to confirm ERα protein expression in the N43 hypothalamic cell line. Nuclei were counterstained with

DAPI. Scale bar = 10 μm. (b) mRNA expression for *Erα* and *Erβ* in N43 cells following PA treatment (100 μM) at the indicated time points. n=3. (c) mRNA levels in N43 cells for the indicated genes following stearic and linoleic acid treatment for 8 h (50 μM). n=3. (d-f) N43 cells were treated for 8 hours with vehicle (BSA), E2-BSA and PA-BSA alone or in combination. (d) mRNA levels of inflammatory markers in N43 cells. (e) mRNA for *Erα* and (f) protein levels of ERα in N43 cells following the aforementioned treatments. n=3. All data are presented as mean±SEM. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$.

Figure S4, Related to Figure 5: ERα expression in primary astrocytes. (a) Primary male astrocytes were stained with the previously validated ERα antibody to confirm the protein expression in these cells. Scale bar=10 μm. (b) Representative immunoblot of GFAP and ERα protein in primary astrocytes. α-tubulin was used as a loading control. n=4.

Figure S5, Related to figure 7: PA treatment decreases PGC-1α expression. (a-c) mRNA levels of *Pgc-1α* in hippocampal (a), cortex (b) and visceral adipose tissues (c) from male and female mice fed chow or HFD. n=4/group. (d-e) mRNA levels of *Pgc-1α* in male and female primary hypothalamic neurons (d), and primary male and female astrocytes (e). Cells were treated either with BSA or with PA. RNA was extracted 8 hours later to measure *Pgc-1α* expression by qPCR. Primary neurons, n=8; primary astrocytes; n = 3-4/sex. (f) mRNA levels of *Pgc-1α* in N43 cells following 8 h of the indicated treatments. n=3. 16:0 = Palmitic Acid, 18:0 Stearic Acid, 18:2 = Linoleic Acid. (g) mRNA levels of the indicated nuclear receptors in hypothalamic tissue. Chow M and F, n=9; HFD M, n=10; HFD F, n=12. All data are presented as mean ±SEM, and $^{*}p < 0.05$ and $^{**}p < 0.01$.

Primers used for qPCR.

Assay	TaqMan or ID Forward (5' – 3')	Reverse (5' – 3')
B2M	Mm00437762_m1	
Erα	Mm00433148_m1	
Erβ	Mm00599821_m1	
Gfap	Mm01253033_m1	
IL6	Mm00446190_m1	
IL1β	Mm00434228_m1	
Mrpl32	Mm00777741_sH	
Nlrp3	Mm00840904_m1	
Nfkb	Mm00476361_m1	
Pgc-1α	Mm01208835_m1	
Tnfα	Mm00443260_m1	
36B4	cacctggtctaggacccgagaag	ggtgcctctggaagattttcg
Errα	ctcagctctctacccaaacgc	ccgcttggtgatctcacactc
F4/80	ctttggctatgggcttccagtc	gcaaggaggacagagtttatcgtg
Hprt	aagcctaagatgagcgcaag	ttactaggcagatggccaca
Il10	gctggacaacatactgctaacc	atttccgataaggcttggcaa
Lxrα	tctggagacgtcacggaggta	cccggttgtaactgaagtcctt
Lxrβ	ctcccacccacgcttacac	gccctaacctctctccactca
Pparα	acaaggcctcagggtacca	gccgaaagaagcccttacag
Pparδ	acgcacccttttgtcatcca	ttccacaccaggcccttct

Abbreviations: ARC, arcuate; CNS, central nervous system; E2, 17β-estradiol; BSA, bovine serum albumin; ER, estrogen receptor; ERKO, ERα total body knockout mice; FBS, fetal bovine serum; FS, fractional shortening; GADPH, glyceraldehyde 3-phosphate dehydrogenase; GFAP, glial fibrillar acidic protein; GFP, green fluorescent protein; HFD, high fat diet; IL, interleukin; KO, knock-out; LVEDd, Left Ventricular End-Diastolic Diameter; LVEDs, Left Ventricular End-Systolic Diameter; OGTT, oral glucose tolerance tests; PA, palmitic acid; PBS, phosphate-buffered saline; PFBBr, pentafluorobenzoyl bromide; PGC-1α, peroxisome proliferator-activated receptor gamma, coactivator 1 alpha; RT, real time; siRNA, small interfering RNA; TNFα, tumor necrosis factor α; UNR, unrelated; WT, wild-type.