**SUPPLEMENTARY INFORMATION**

**Material and methods**

***Neuro2A cells.***

Neuro2a (N2A) cells, derived from mouse neurons, were cultivated in DMEM/HamF12 (1:1), supplemented with 10% (v/v) fetal bovine serum (FBS) and 100 U/mL streptomycin/penicillin. Cells were cultivated at 37 °C in a humidified atmosphere containing 5% CO2. N2a were subjected to neurites differentiation before treatments by incubation with media containing 2% FBS for 24 h. Cells were treated with 50 ng/mL of IL6 for 60 min. For protein extraction, cells were resuspended in lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100) containing protease and phosphatase inhibitor cocktails (Roche). Lysates were incubated on ice for 15 min and centrifuged at 12.000×g for 10 min at 4 °C. Supernatants were collected and stored for immunoblotting analysis.

***Animals***

C57BL6/J, IL6 knockout (IL6KO) mice, and α2A/α2C-adrenoceptor knockout (α2ACKO) male mice were used. C57BL6/J was obtained from the University of Campinas Central Breeding Center. IL6-KO and α2ACKO were from the University of São Paulo. Male wild-type (WT) and congenic α2A/α2C ARKO 1 mice with C57Bl6/J genetic backgrounds were from the University of São Paulo.

Mice were kept in individual cages with controlled temperature (22-24 oC) and light and dark cycles (12h). They had *ad libitum* access to standard rodent chow and water. All experimental protocols were subjected to the University of Campinas Animal Ethics Use Committee (# 2849-1). The number of animals used in each experiment was described in the legend of figures.

***Physical exercise protocol***

Four animals per turn were acclimated to the water for three consecutive days, ten minutes a day, in plastic containers with 40cm length, 30 cm wide, and 45 cm depth. The water temperature was maintained at approximately 33°C during the entire protocol. Afterward, part of the acclimated animals was subjected to a single bout of exercise. The swimming protocol was composed of four sessions of 30 minutes with five minutes intervals in between, for a total of two hours, as previously described 2.

***Serum IL6 determination***

IL6 serum levels were determined by using an ELISA kit (Pierce Endogen, Rockford, IL), following the manufacture's recommendations.

***Cannula implantation***

Animals were anesthetized with Ketamine (100mg) and Diazepam (0.07mg) (0.2mL/100g body weight). The procedure started when corneal and paw pain reflexes were abolished. Animals were positioned at the stereotaxic, and a 1 cm intraparietal incision was performed after the cranial trichotomy and antisepsis. Afterward, the periosteum was divulsed, and with the exposed skullcap, the bregma was visualized for the stereotaxic coordinates. Cannula implantation was aimed at the third ventricle of mice weighing 25 to 30g (coordinates: AP(antero-posterior): -1.8mm, L(lateral): 0.0mm, V(vertical): -5.0mm) according to the stereotaxic atlas.

***Intracerebroventricular microinjections***

*IL6 recombinant*- Icv IL6 recombinant microinjection (200ng) from Calbiochem (San Diego, CA, USA) was performed between 5:00 and 6:00 pm.

*IL-6 neutralizing antibody*- Animals were randomly selected for icv microinjection containing saline or rabbit antiserum against IL6 (IL6 AB). Anti-IL6 AB (100ng) from Santa Cruz Biotechnology was performed 30 minutes before and immediately after the exercise protocol.

*PD98059-* Mice were randomly selected for icv microinjection containing saline or PD98059, a selective, cell-permeable inhibitor of the MEK. PD98059 (60µM) was performed 30 minutes before the icv IL6 recombinant microinjection.

***VMH IL6R lentivirus transfection***

Five different shRNA–based lentiviral clones (The RNAi Consortium – TRC; titer 106) targeting Interleukin 6 receptor (IL6R) TRCN 0000057 (LV1), TRCN 0000059 (LV2), TRCN 0000089 (LV3), TRCN 0000093 (LV4), TRCN 0000094 (LV5) or scramble (SHC 016 V, pLKO.1-puro non-mammalian shRNA control) (SCR) from Sigma-Aldrich (St Louis, MO, USA) were tested for IL6R knockdown in preliminary experiments, as previously described3. Mice were submitted to stereotaxic surgery (Ultra Precise–model 963, Kopf). Lentiviral shRNA particle (LV5) was administered bilaterally (1 μL/min) into the ventromedial hypothalamus (VMH) following the coordinates: AP (antero-posterior): - 1.7mm, L(lateral): -0.5mm, DV (dorsoventral) -5.5mm. The injection of Evan blue and dissection of the region of interest provided the anatomical control of stereotaxic procedure.

***Reagents and antibodies***

Reagents for SDS-PAGE and immunoblotting were from Bio-Rad Laboratories (Hercules, CA). Tris, aprotinin, ATP, dithiothreitol, phenylmethylsulfonyl fluoride, Triton X-100, Tween 20, glycerol, and BSA (fraction V) were from Sigma Chemical Co. (St. Louis, MO). Nitrocellulose paper (BA85, 0.2 mm) was from Schleicher & Schuell (Keene, NH). Ketamine hydrochloride was from Cristália (Itapira SP, Brazil). The chemiluminescent kit was from Thermo Scientific (Rockford, IL, EUA). Antibodies anti-IL6 (M-19) and anti-α-tubulin (B-7) were from Santa Cruz Biotechnology and anti-pERK 1/2 (Thr202/Tyr204) and anti-pAMPK (Thr 172) were from Cell Signaling Biotechnology, the antibody anti- pACC (Ser79/Ser212) and α-tubulin were from Millipore and Sigma respectively. Secondary antibodies were from Thermo Scientific. Recombinant IL-6 was from Calbiochem (San Diego, CA, USA). PD 98059 was from LC Laboratory (Woburn, MA). Routine reagents were purchased from Sigma Chemical Co. (St. Louis, MO), unless otherwise specified. The doses administrated in each experimental group are given below.

***Immunoblotting***

After the respective treatments, animals were anesthetized. Samples from the hypothalamus and soleus muscle were obtained, minced coarsely, and homogenized immediately in solubilization buffer containing (mM) 100 Tris (pH 7.6), 1% Triton X-100, 10 Na3VO4, 100 NaF, 10 Na4P2O7, 4 EDTA, 150 NaCl, 0.1 mg aprotinin, and 35 mg phenylmethylsulfonyl fluoride per milliliter, using a polytron PTA 20S generator (model PT 10/35; Brinkmann Instruments, Westbury, NY) operated at 44 maximum speed for 30 seconds and clarified by centrifugation. All the samples from all groups were subjected to SDS-PAGE and blotted onto a nitrocellulose membrane. The membranes were incubated for 12 hours at 4°C with primary antibody after blockade with 5% non-fat milk in TBST (10 mmol/L Tris; 150 mmol/L NaCl; 0.02% Tween 20) for 90 minutes at room temperature. After the secondary antibody incubation, the signal was detected for 60 minutes in a 3% non-fat milk TBST solution and treatment with two mL of SuperSignal® West Pico Chemiluminescent Substrate and exposition to photosensitive RX film from Kodak or visualized using G:Box from Syngene. Band intensities were quantified by optical densitometry by using UN-SCAN-IT gel 7.1 (Silk Scientific, Inc.) The Ponceau staining from Sigma-Aldrich (Saint Louis, MO, USA) was also utilized to monitor the loading control for each sample.

***mRNA isolation and real-time PCR***

Total RNA was extracted using Trizol reagent (Life Technologies), according to the manufacturer's recommendations. Total RNA was rendered genomic DNA free by digestion with Rnase-free Dnase (RQ1, Promega, Madison, WI, USA). Real-time PCR and mRNA isolation were performed using a commercial kit: GAPD (4352338E) for mouse, taqman gene expression assays for mouse interleukin-6 (IL6) (Mn00446190\_m1) and using the following primers.

*Cpt1β*: 5’TCGAATCAAGAATGGCATCCT, 5’GAGATGTCCACCTTGCAGTAGTTG.

*Parα*:5’TTAGAGGAGAGCCAAGTTGAAGTTC, 5’GCAGGCCACAGAGCGCTAA.

***Oxygen consumption determination***

O2 consumption was measured using an indirect open circuit calorimeter (CLAMS - Oxymax Deluxe System; Columbus Instruments, Columbus, OH, USA). Mice were adapted 1 day before the experiment. Measurements were performed for 3 hours after icv IL6 microinjection.

***Palmitate oxidation in soleus muscle***

To evaluate the palmitate oxidation in the skeletal muscle of mice, 0.2 μCi/ml [1-14C] palmitate was used. The soleus muscle samples were transferred to a flask containing filter paper saturated with 0.3 mL of phenylethylamine. The flasks were gently shaken for 1 hour at 37°C, and the center wells were carefully removed and transferred to specific vials for liquid scintillation determination.

***Mass spectrometry analysis for fatty acid determination***

For fatty acid profile determination, 150 μL of tissue homogenates were taken in a screw cap glass tube containing 25 μg internal standard (tridecanoic acid - C13:0). After that, 1 mL of 0.5 M NaOH-methanol was added, and the sample was boiled at 100 °C for 15 min. After cooling the samples, 2 mL of BF3-methanol was added, and the sample was boiled at 100°C for 20 s. The sample was again cooled to room temperature, and 1 mL of isooctane was added. The tubes were shaken, and 5 mL of saturated NaCl solution was added. After phase separation, the supernatant was collected and evaporated with nitrogen gas to concentrate the fatty acid methyl esters (FAME). FAME was resuspended in 50 μL hexane for chromatographic analysis4. Chromatographic analyses were performed using a gas chromatograph-mass spectrometer (model GCMS-QP2010 Ultra; Shimadzu). A fused silica capillary column Stabilwax (length, 30 m; internal diameter, 0.25 mm; thickness, 0.25 μm; Restek, USA) was used to inject 1 μL of the sample at 250 ºC. High-grade pure helium (He) was used as the carrier gas with a constant flow rate of 1.3 mL/min with a split injection of 2:1. The oven temperature was programmed from 80 to 175 °C at a rate of 5 °C/min, followed by another gradient of 3 °C/min to 230 °C, which was maintained for 10 min. Mass conditions were as follows: ionization voltage, 70 eV; ion source temperature, 200 ºC; full scan mode in the 35–500 mass range with 0.2 s/scan velocity.

***Immunofluorescence staining***

*Ad-libitum* fed animals were perfused with 4% paraformaldehyde transcardially. The brains were immersed in 10% sucrose (w/v) and, after 24h, transferred to 30% sucrose (w/v). After that, embedded in OCT compound (Sakura Finetek, Torrance, CA) and cut into 20 µm coronal sections using a cryostat. The sections were incubated in blocking solution in TPBS (0.2% of Triton X-100) and 5% BSA for 2h at 21°C and then incubated overnight at 4°C in mouse anti-NeuN (Millipore, MAB377, 1:300), anti-IL-6Rα (Santa Cruz Biotechnology, sc 660, 1:200), anti-phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP® Rabbit mAb #4370 (Cell Signaling) and anti-SF-1 antibody (A-1): (Santa Cruz Biotechnology, sc-393592, 1:200). After washing in PBS, sections were placed in secondary donkey anti-mouse FITC (Santa Cruz Biotechnology, sc 2010, 1:500) and goat anti-rabbit Alexa546 (Thermo Fischer, CA, USA, 1:500) for 2h and mounted with Vectashield Mounting Medium with DAPI (#H-1200 Vector Laborataries, Inc., Burlingame, CA, USA). The images were acquired by a confocal laser microscope (LSM 780, Zeiss, Jena, Germany). Digital/electronic zoom (eZoom) of highlighted areas was performed when necessary.

***Arc and VMH cell counting***

To determine the ERK1/2 phosphorylation in VMH of mice transfected with IL6R shRNA (LV5) or Scramble, the sections from the immunofluorescence assay were used. The images were obtained, isolating only the Arc or VMH in the images. Five images per group were counted blindly by two independent researchers, using the Image J software 1.48v. The average of each image was used to obtain the group mean.

***Hypothalamic VMH dissection*.**

VMH was quickly dissected in a stainless-steel matrix with razor blades from *ad-libitum* fed animals, as described previously5, and frozen in liquid nitrogen for further protein analysis.

***Denervation experiment***

For the denervation experiments, mice were anesthetized with Ketamine (100mg) and Diazepam (0.07mg) (0.2mL/100g body weight). The procedure started when corneal and paw pain reflexes were abolished. The denervation and the cannulae implantation surgeries were performed in the same procedure. The left hindlimb muscles were carefully denervated by excising approximately 4 mm of the sciatic nerve. The animals were used in the experiment 48 h later, and the contralateral paw was used as control.

***BXD hypothalamus microarray processing***

Correlation analyses were performed using hypothalamic mRNAs of BXD inbred family (Hypothalamus Affy MoGene 1.0 ST (Nov10) and EPFL/LISP BXD CD Muscle Affy Mouse Gene 1.0 ST (Dec11) RMA) 6, excepted for figure S2B. These data sets are accessible on Genetwork (<http://www.genenetwork.org>) 7. The Pearson's and Spearman's correlation graphs were built using PrismGraph, and the heat map graph was obtained using the Gene-E software.

For figure 2B, we downloaded the log2-transformed, RMA-normalized 8, rescaling BXD hypothalamus microarray intensity data from the 89 BXD mouse hypothalami from Andreux 6 from the NCBI Gene Expression Omnibus (GEO accession number GSE36674). A complete overview of the data and its preprocessing pipeline can be found at [GeneNetwork.org](http://gn1.genenetwork.org/webqtl/main.py?FormID=sharinginfo&GN_AccessionId=317) 7 (GN Accession ID GN317). These 89 samples come from 50 different murine BXD strains, whereby tissues from two mice of the same strain and sex were pooled. We regressed these preprocessed gene expressions to select outcome firing-rate-related genes on the preprocessed gene expressions of *Il6* through ordinary least squares regression. The *p*-values associated with the effect of preprocessed *Il6* expression were corrected with the Benjamini-Hochberg false discovery rate procedure and reported as *q*-values.

***GTEx hypothalamus normalized gene expression processing***

GTEXv5 human brain hypothalamus Refseq (Sep 15) RPKM Log2 and GTEXv5 human muscle-skeletal Refseq (Sep 15)9 were used, except for figure 2SC. These data sets are accessible on Genetwork (http://www.genenetwork.org). The Pearson's and Spearman's correlation graphs were built using PrismGraph, and the heat map graph was obtained using the Gene-E software. For figure S2C, we accessed the normalized, log-transformed (with offset 1) RNA-Seq gene read counts in the hypothalami of 170 deceased human subjects from the Genotype-Tissue Expression (GTEx) project 9 version 8 through accession number dbGaP phs000424.v8.p2 (see <https://www.gtexportal.org/home/documentationPage> for a complete overview of the data and its processing pipeline). We regressed these preprocessed gene expressions in GTEx hypothalamus for the human homologs of the firing-rate-related genes on the *IL6* preprocessed gene expressions through ordinary least squares regression. The p-values associated with *IL6* expression were corrected with the Benjamini-Hochberg false discovery rate procedure and reported as *q*-values.

All individual values used in bioinformatics analysis are described in supplemental tables.

***Statistical analysis***

The results were expressed as mean ± SEM. Immunoblot results were presented as direct comparisons between the groups. Data were analyzed by Student's t test or one-way ANOVA, as appropriate, with *post hoc* Bonferroni or Tukey tests for multiple unpair-wise comparisons of the means. The level of significance adopted was *p*<0.05. STATISTICA 6.0 software was used for the analysis. Pearson and Spearman's correlations were used for the bioinformatics analysis, and the r and *p* values are described in the figures or figure legends. The number of animals/samples used in each experiment is described in the figure legends.

***Additional references***

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**Supplementary figures legends**

**Figure S1. Fractions of fatty acid in skeletal muscle and co-localization of IL6R in neurons of hypothalamic nuclei.** (A) Determination of fatty acid fractions using mass spectrometry analysis in the gastrocnemius muscle 3 h after recombinant IL6 icv microinjection. Unpaired *t*-test was. (\*, *p*<0.05, *vs* saline). Immunostaining was performed to evaluate the co-localization of IL6 receptor (red) and NeuN (green) in (B) arcuate nucleus and (C) paraventricular nucleus. White arrows indicate the co-localization. Scale bars 50 μm (n=5). Digital/eletronic zoom (eZoom) was performed in the right panels (scale bars 50 μm). (D) co-localization of IL6 receptor (red) and SF-1 (green) in VMH. Red arrows indicate IL6R and yellow arrows indicate SF-1 staining. Scale bars 50 μm (n=5). Digital/eletronic zoom (eZoom) was performed in the right panel (scale bars 50 μm).

**Figure S2. Association between IL6/ERK signaling and firing-rate related genes.** (A) Immunostaining was performed to evaluate the co-localization of IL6 receptor (red) and pERK1/2 (green) in VMH of mice. White arrows indicate the co-localization. Scale bars 50 μm (n=5). Digital/eletronic zoom (eZoom) was performed in the right panel (scale bars 50 μm). **(B, C)** *Il6* gene expression correlates positively with a set of firing-rate-related genes in the hypothalamus, both in a dataset of 50 BXD mouse strains (89 samples) and in the Genotype-Tissue Expression (GTEx) dataset of 170 deceased humans. The dark green lines show the best fit for linear relationships between the log2-transformed normalized expressions for each outcome gene and *Il6* (panel B) and between the GTEx normalized log-transformed gene expressions for each outcome gene and *IL6* (panel C). The corresponding 95% confidence bands are shown in light green. r² denotes the coefficient of determination, the proportion of the variation in the preprocessed outcome gene expressions that can be explained by the preprocessed gene expression of *Il6* (panel B) or *IL6* (panel C). *q* denotes the Benjamini-Hochberg false discovery rate-adjusted *p*-values. Males are shown in blue, females in pink.

**Figure S3. Evaluation of -adrenergic receptors in BXD in mice.** (A) Distribution of hypothalamic *Il6* gene expression in BXD cohort (34 strains). (B) Families containing the lowest (blue) and highest (red) in terms of hypothalamic *Il6* gene expression were studied. *t*-test was performed, \*\**p*<0.01 *vs* Low group). (C) Heatmap graph using hypothalamic and muscle transcripts from Low and High groups.

**Figure S4. Evaluation of hypothalamus and skeletal muscle in response to acute exercise.** (A) Experimental design. ERK1/2 phosphorylation in; (B) whole hypothalamus lysate, immediately after acute exercise (n=4). (C) Phospho- ACCSer79/Ser212 and phospho- AMPKThr172 in the soleus muscle 3 hours after the exercise protocol (n=4). *t*-test was performed, \*\**p*<0.01 *vs* Rest WT group and \*\*\*\**p*<0.0001 *vs* Rest WT group). (D) Experimental design involving wild-type and IL6-null mice (IL6KO). (E) Hypothalamic ERK1/2 phosphorylation immediately after acute exercise (n=4) (J) ACCSer79/Ser212 and AMPKThr172 phosphorylation in soleus muscle 3 h after the exercise protocol (n=4). *t*-test was performed, ns, non-significative).

**Figure S5. Effect of anti-IL6 antibody microinjection on fatty acid oxidation in muscle in exercised mice.** (A) Experimental design. Microinjections of anti-IL6 antibody (100ng), were performed 30 minutes and immediately after the exercise protocol. The soleus muscle was removed 3 hours later. (B) Palmitic acid oxidation (n=6-8, \*\*\*, *p*<0.001 *vs* saline and ###, *p*<0.001 *vs* exercise+saline). A one-way analysis of variance was performed.

**Figure S6. Effects of IL6R lentiviral transfections.**

1. Five IL6R lentiviral sequences were injected into the third ventricle of mice. The hypothalamic samples were removed seven days after the lentivirus transfections, and the Western blot was performed using an anti-IL6R antibody (n=3). (B) Daily food intake evaluation after bilateral microinjection of Scramble or IL6R lentivirus (TRCN94) in VMH (n=3). (C) Determination of fatty acid fractions using mass spectrometry analysis in the gastrocnemius muscle 3 h after the exercise session (n=4-6). One-way analysis of variance was performed, \*, *p*<0.05 *vs* scramble and \*\*, *p*<0.01 *vs* scramble and ns, non-significative. (D) Illustration of the neuromuscular circuitry involving IL6/ERK axis in the ventromedial nucleus of the hypothalamus and alpha-adrenergic receptors and AMPK/ACC signaling, driving fatty acid oxidation in the skeletal muscle.