SUPPLEMENTAL FIGURES

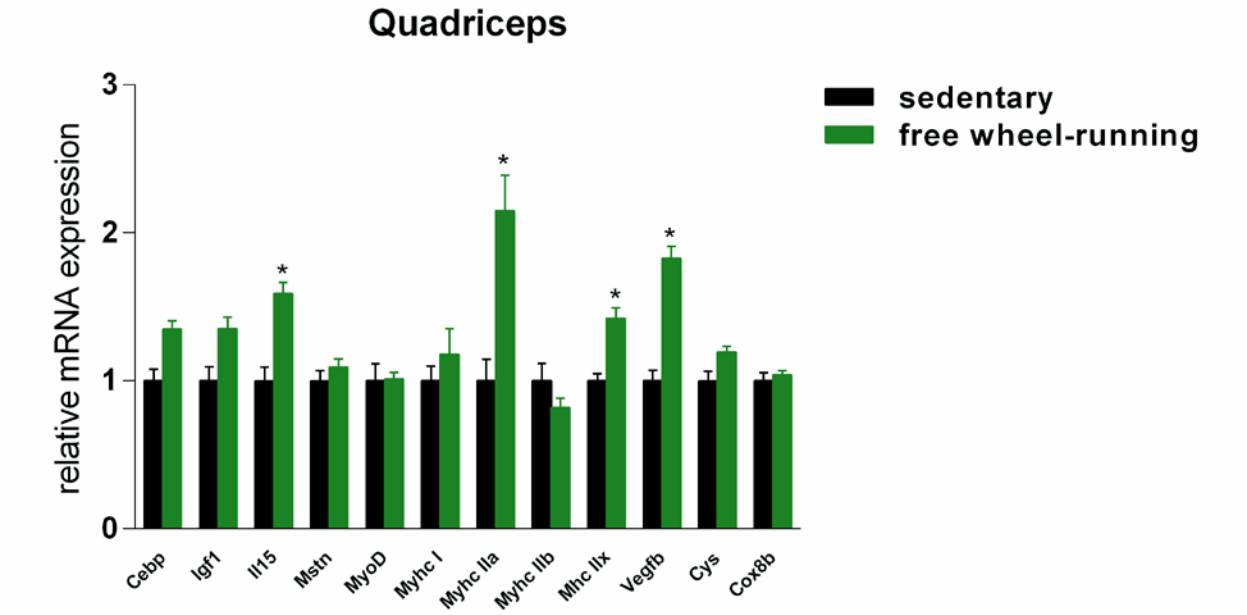

Figure S1: Endurance exercise induces hippocampal *Fndc5* gene expression (related to Figure 1).
Male six week old C57/Bl6 wild type mice were individually housed in cages with access to a running-wheel (free wheel-running) or without (sedentary). Mice were exercised for 30 days and sacrificed approximately 10 h after their last bout of exercise. The quadriceps muscle (quadriceps) was harvested. The brain was retrieved and the hippocampus was dissected out. mRNA was prepared and gene expression was assessed by qPCR. Data are shown as mRNA levels relative to Rsp18 expression, expressed as mean ± SEM.

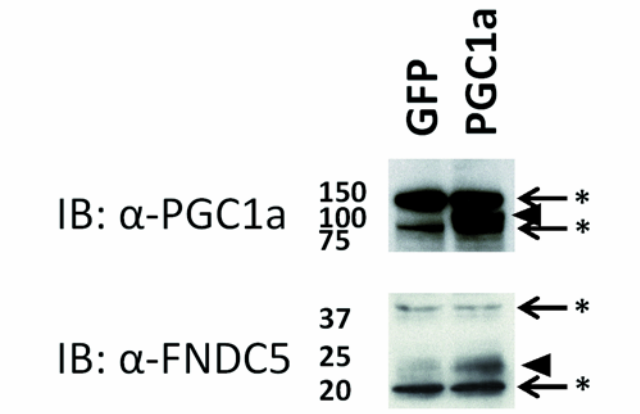

Figure S2: Neuronal FNDC5 protein levels are regulated by a PGC-1α (related to Figure 3).
Primary cortical neurons at DIV 6 were transduced with either PGC-1α or GFP adenovirus. Forty-eight hours later, whole cell lysates were harvested and analyzed by immunoblotting. * = unspecific band. Intensity of unspecific bands and Ponceau staining were used to assess equal loading.

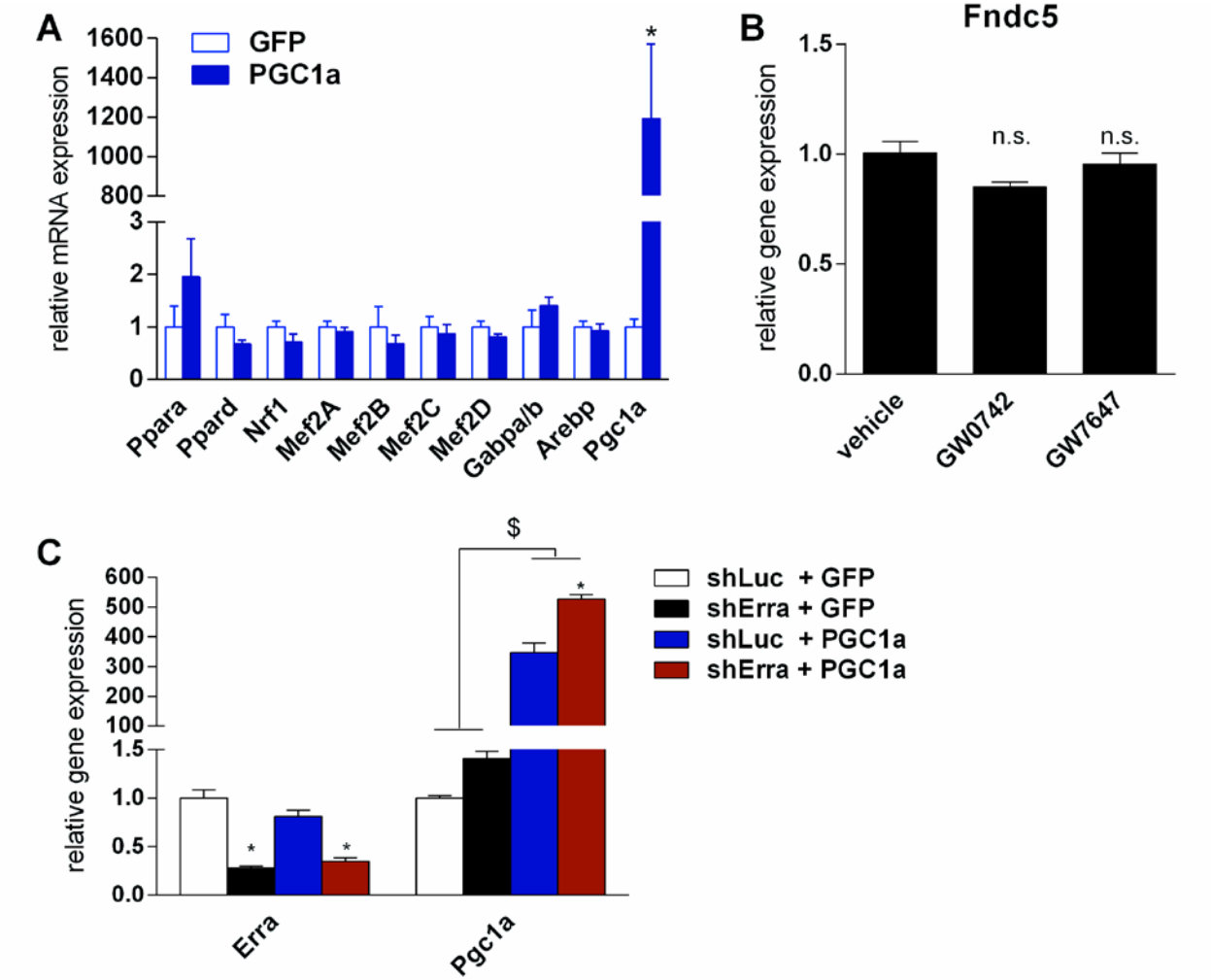

Figure S3: ERRα is a key interacting transcription factor with PGC-1α for regulating *Fndc5* gene expression in neurons (related to Figure 4).

(A) Primary cortical neurons at DIV 7 were transduced with either PGC-1α or GFP adenovirus. Forty-eight hours later mRNA was prepared and gene expression was assessed by qPCR. Data are shown as mRNA levels relative to Rsp18 expression, expressed as mean ± SEM. *P < 0.05 compared to corresponding GFP expressing control group.

(B) Primary cortical neurons at DIV 7 were treated with either GW7647 (1 μM), a potent and highly selective PPARα agonist, GW0742 (1 μM), a potent and highly selective PPARδ or vehicle for overnight. mRNA was prepared and gene expression was assessed by qPCR. Data are shown as mRNA levels relative to Rsp18 expression, expressed as mean ± SEM. *P < 0.05 compared to vehicle only group.

(C) Primary cortical neurons at DIV 4 were transduced with lentivirus carrying shRNA hairpins against either Erra or luciferase (Luc) as control. Three days later were cells were transduced with either PGC-1α or GFP adenovirus. Forty-eight hours later mRNA was prepared and gene expression was assessed by qPCR. Data are shown as mRNA levels relative to Rsp18 expression, expressed as mean ± SEM. *P < 0.05 compared to corresponding shLuc expressing control group. $P < 0.05 compared to corresponding GFP expressing control group.

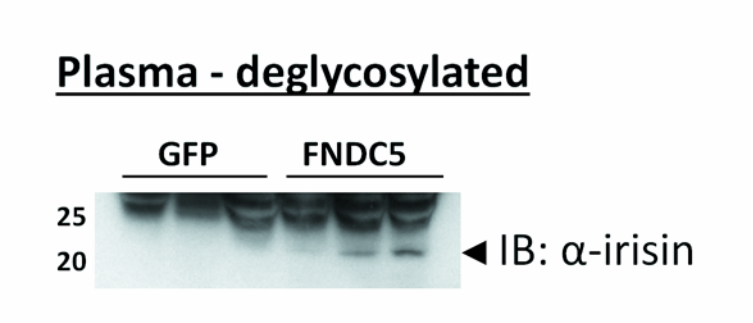

Figure S4: Peripheral delivery of FNDC5 by adenoviral vectors increases Bdnf expression in the hippocampus (related to Figure 6).
Five week old male wild-type BALB/c mice were injected with GFP- or FNDC5-expressing adenoviral particles intravenously. Animals were sacrificed seven days later. Plasma samples were collected, depleted from albumin/IgG, deglycosylated, and subjected to WB analysis.

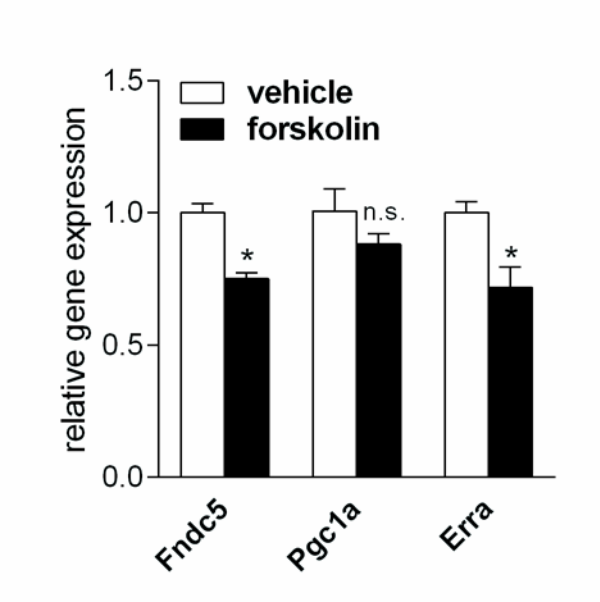

Figure S5: PGC-1α/FNDC5/BDNF pathway in primary hippocampal neurons (related to Figure 7).
Primary hippocampal neurons at DIV 7 were treated with either forskolin (10 µM), a stimulator intracellular cAMP levels, or vehicle for overnight. mRNA was prepared and gene expression was assessed by qPCR. Data are shown as mRNA levels relative to Rsp18 expression, expressed as mean ± SEM. *P < 0.05 compared to vehicle only group.

SUPPLEMENTAL TABLE 1 (related to Experimental procedures).

Primer	Sequence
mRps18 QS	CAT GCA GAA CCC ACG ACA GTA
mRps18 AS	CCT CAC GCA GCT TGT TGT CTA
mFndc5QS	atgaaggagatggggaggaa
mFndc5QA	gcggcagaagagagctataaca
mPGC-1aQS	TGATGTGAATGACTTGGATACAGACA
mPGC-1aQA	GCTCATTGTTGTACTGGTTGGATATG
mErra QS	CACTACGGTGTGGCATCCTG
mErra AS	ACAGCTGTACTCGATGCTCC
mErrb QS	AACCGAATGTCGTCCGAAGAC
mErrb AS	GTGGCTGAGGGCATCAATG
mErrg QS	ATGGATTCGGTAGAACTTTGCC
mErrg AS	CTTCTTCGTAGTGCAGGGAAAA
mBdnf QS	TGGCCCTGCGGAGGCTAAGT
mBdnf AS	AGGGTGCTTCCGAGCCTTCCT
mIgf1 QS	TGGATGCTCTTCAGTTCGTG
mIgf1 AS	GTCTTGGGCATGTCAGTGTG
mNpas4QS	CTGCATCTACACTCGCAAGG
mNpas4QA	GCCACAATGTCTTCAAGCTCT
mc-FosQS	ATGGGCTCTCCTGTCAACACAC
mc-FosQA	ATGGCTGTCACCGTGGGGATAAAG
mArcQS	TACCGTTAGCCCCTATGCCATC
mArcQA	TGATATTGCTGAGCCTCAACTG
mZif268QS	TATGAGCACCTGACCACAGAGTCC
mZif268QA	CGAGTCGTTTGGCTGGGATAAC

QS qPCR-sense
QA qPCR-antisense

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Reagents

Recombinant human BDNF was purchased from PeproTech. Recombinant human GDNF and CNTF and forskolin were obtained from Sigma. Recombinant mouse IGF-1 was obtained from R&D Systems. Recombinant mouse NGF and K252a were obtained from EMD Millipore. Nifedipine, XCT 790, DY131, GW7647, and GW0742 were purchased from Tocris. Recombinant irisin (human, rat, mouse, canine) was obtained from Phoenix Pharmaceuticals (Burlingame, CA).

Primers used for qPCR

All primers used are listed with their sequences in Supplemental Table 1.

Animal studies

All animal experiments were performed according to procedures approved by the IACUC of Dana-Farber Cancer Institute and the BIDMC. Generation and characterization of the Pgc1a total body KO (*Pgc1a*$^{-/-}$) mice have been described previously (Lin et al., 2004). Mice were kept under 14-hour light/10-hour dark cycles at constant temperature (22°C) with free access to food and water. Mice were fed either a standard diet (Rodent Diet 8664, Harlan Teklad). For free wheel running exercise, six week old male wild type C56/Bl6 mice (Jackson Laboratory) were housed individually with stainless steel running wheels. Sedentary controls were housed without wheels. Mice were exercised for 30 days and sacrificed approximately 10 h after their last bout of exercise and the indicated tissues were harvested. For the tissue panel 13 weeks old male C57/Bl6 mice were used. For the developmental time-course pups were sacrificed at the indicated time-points and brains were harvested for total RNA. For RNA expression studies, animals were sacrificed and tissues harvested and stored at –80°C until analysis.

Cell culture

Primary cortical and hippocampal neurons were isolated as described in great detail previously (Bartlett and Banker, 1984). Briefly, cortices and hippocampi were dissected from E16-E18 embryos, dissociated with trypsin (Sigma) and DNAse (Roche) and plated on poly-L-lysine-coated (Sigma) plates. Dissociated neurons were cultured in Neurobasal Media supplemented with B27, GlutaMAX™ (Life Technologies), and Penicillin-Streptomycin (Cellgro).

RNA preparation and expression analysis

Cells or tissues were lysed and homogenized in TRIzol (Invitrogen). Total RNA was subsequently isolated using the RNeasy Mini or Micro Kit (Qiagen). First-strand cDNA was generated using the High Capacity cDNA Reverse Transcription Kit (Life Technologies), and qPCR was performed using SYBR Green Master Mix in a 7900HT Real-Time PCR system (Applied Biosystems). mRNA quantities were normalized to Rsp18 after determination by the comparative Ct method (Schmittgen and Livak, 2008).

Protein Extraction and Western Blot Analysis

Cell lysates were prepared with RIPA buffer supplemented with complete protease inhibitor cocktails. For generation of conditioned media cells were washed three times with PBS and plain neurobasal with glutamine and antibiotics but without B27 supplement was added. Cells and media were collected the next morning. The conditioned media was spun twice at low speed and then concentrated in spin-filter columns with a molecular weight cut-off of 3KDa (Millipore). Deglycosylation was performed using Protein Deglycosylation Mix (New England Biolabs). Blood was collected in lithium heparinized tubes (BD Biosciences) and plasma was separated by centrifugation. Albumin and IgG was removed using the ProteoExtract-kit (Millipore). Then the samples were concentrated using Ultra-2 Centrifugal Filter (Millipore) and deglycosylated with PNGase F (New England Biolabs).For western blot analyses, 80-100 µg protein was subjected to SDS-PAGE under reducing conditions, transferred, and blotted with anti-PGC-1α mouse (4C1.3) antibody (Calbiochem / EMD Millipore,) and anti-FNDC5 (Irisin) rabbit polyclonal antibody (Adipogen). Equal loading was assessed by Ponceau staining (Sigma-Aldrich).

Forced expression and knockdown of target genes

Generation and delivery of the PGC-1α, GFP, and FNDC5 adenovirus has been detailed describes before (Bostrom et al., 2012; Lustig et al., 2011). Primary cortical neurons were transduced at the indicated time-points and were harvested 48 hrs later for RNA isolation. For knockdown studies, primary cortical neurons were transduced with viral supernatants from HEK293T cells transfected with pLKO.1 vector (TRC) containing the specified shRNAs at the indicated time-points. Cells were harvested four days later for total RNA. To produce lentiviral supernatants HEK293T cells, cultured in DMEM with 10% FBS, were transfected using Lipofectamine2000™ (Life Technologies) with the specified shRNA plasmid and the packing plasmid psPAX2 and pMD2.G in a 2:1:1 ratio. After an overnight incubation, media was exchanged to neurobasal media supplemented as described above and supernatants were harvested 24 hrs later.

Cell viability assay

Cell viability of cultured neurons was assessed using CellTiter-Glo® Luminescent Cell Viability Assay (Promega, Madison, WI) according to the manufacturer's instructions. Luminescence of cell lysates was measured using the FLUOstar Omega plate reader (BMG LABTECH, Offenburg, Germany).

Analysis of the murine *Fndc5* promoter for Erra transcription factor binding sites

The genomic sequence of the murine *Fndc5* gene and 6kb of its upstream promoter was retrieved from the USCS Genome browser (www.genome.ucsc.edu; assembly mm9). This genomic sequence was searched for the canonical Erra transcription factor binding motif: TGACCTT. This motif had been identified and established in previous studies (Charest-Marcotte et al., 2010; Mootha et al., 2004; Wang et al., 2012).

Peripheral delivery of FNDC5 by adenoviral vectors.

High titer GFP- or FNDC5-expressing adenoviral particles were obtained by ViraQuest Inc. (North Liberty, IA). Five week old male wild-type BALB/c mice were injected with GFP- or FNDC5-expressing adenoviral particles (10^{11}/animal) intravenously. Animals were sacrificed seven days later and the indicated tissues were harvested for gene expression analyses using qPCR.