Supplementary Material

Extended Materials and Methods

Tissue Culture

293T HEK (ATCC) and mHypoE 42 (Cellutions Biosystems) cell lines were cultured with DMEM supplemented with 1% Pen/Strep, 10% Fetal Bovine Serum (all from Life Technologies), and maintained at 37ºC in humidified incubator with 5% CO₂. Adult primary neuronal hypothalamic cells were isolated as previously described (Brewer and Torricelli, 2007). Briefly, 4 hypothalami, per 6-well plate, from adult mice were punched out under sterile conditions, and neurons isolated using Papain Dissociation System (Worthington), following the manufacturer’s protocol. Primary neurons were cultured on Poly-D-Lysine coated plates with Neurobasal medium, supplemented with B27, 2mM GlutaMAX, 1% Pen/Strep (all from Life Technologies) for a period of 2-3 weeks, with no more than 50% of the medium replaced twice a week.

Preparation and validation of CRE-inducible AP-1 factors and galanin shRNA containing LV

FLAG-tagged AP-1 factors were amplified by PCR from mouse cDNA with HiFi Hotstart Readymix (KAPA) using the following primer sets:

ΔFosB FLAG (F):
5’-
CTCGGATCCATGGACTACAAGGACGACGATGACAAGGGGTGGTTCTGGTTTTCAAGCTTTTC
CCGGAGAC-3’

ΔFosB FLAG (R):
5’-AGTGGATCCTTACTCGGCCAGCGGGCCTGG-3’

Δ2ΔFosB FLAG (F):
5’-
CTCGGATCCATGGACTACAAGGACGACGATGACAAGGGGTGGTTCTGGTTCTGGCCAGTCCAGGG
GGCAGCCA-3’

Δ2ΔFosB FLAG (R):
5’-AGTGGATCCTTACTCGGCCAGCGGGCCTGG-3’
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DNJunD FLAG (F):
5' -
CTCGGATCCATGGACTACAAGGACGACGATGACAAGGGTGGTTCTGGTCAAAGCCAGCTG
GGTGCGGCC - 3'

DNJunD FLAG (R):
5' -AGTGGATCTCCTCAGTACGCCGGACCTGGTG - 3'

FosB FLAG (F):
5' -
CTCGGATCCATGGACTACAAGGACGACGATGACAAG
GGTGGTTCTGGTTTTCAAGCTTTTC
CCGGAGAC - 3'

FosB FLAG (R):
5' -AGTGGATCTCCTCAGAGCAAGAAGGGAGGG - 3'

FLAG inserts were ligated into subcloning PCR2.1TOPO vector (Life Technologies), excised with BamHI restriction enzyme (NEB), purified and then inserted with T4 ligase to BamHI cut-open pTomo vector (Addgene, #26291). Insert orientation was verified by sequencing.

To test the efficiency and CRE-specificity of the cloned constructs, they were transfected into 293T HEK cells, in the presence or absence of CAG-CRE. Western blots show that all four vectors exhibit detectable expression of FLAG and of their respective proteins, which are “silent” in the absence of CRE (Fig. S1B), confirming their CRE-inducibility. Moreover, CRE led, as expected, to a reduction in the RFP signal intensity with an unchanged GFP signal after transduction of AP1 LVs into either 293T HEK cells (Fig. S1C) or primary neurons isolated from the hypothalami of AgRP-CRE or POMC-CRE mice (Fig. S1D). qPCR analysis further confirmed the expression of specific AP1 factors in LV infected primary hypothalamic neurons. Having demonstrated the CRE-specific inducibility of our vectors, we then optimized the stereotaxic injection protocols and validated the specific anatomical region of the brain where our AP1 antagonists are expressed. For this purpose, the neuron-specific CRE mice were crossed with the reporter R26R-Brainbow2.1 mice, to allow individual neurons to be distinguished by fluorescent labels. These mice were then stereotaxically VHT-injected with CRE-inducible AP1 LVs and subjected to histological examination. Supporting previous findings, red fluorescence showed AgRP and POMC neurons being present mostly in the ARC region. Bilateral lentiviral vector injection, seen as yellow label
with a proportion of green, was shown to be delivered in the vicinity of neuronal cells of interest (Fig. S1G). Examination of several sections anterior and posterior to the injection site showed no fluorescence, confirming that the viral particles do not spread to large and undefined areas and remain restricted to the injection site. To show neuron-specific expression of our constructs we performed immunostaining of brain sections in mice injected with FLAG-tagged ΔFosB LV. FLAG was colocalized with AgRP or POMC in AgRP-CRE or POMC-CRE mice, respectively, and we did not observe FLAG staining in non-targeted neuronal cells (we find no green signal in the absence of red), demonstrating, as expected, neuron-specific ΔFosB protein expression (Fig. S2A). Furthermore, ΔFosB signal was colocalized with AgRP or POMC in primary hypothalamic neurons isolated from AgRP-CRE or POMC-CRE mice, respectively (Fig. S2B). Finally, to exclude a potential promoter “escape” in vivo, we injected AP-1 LV to the VHT of C57BL (lacking CRE) mice and found no effect on energy expenditure, glucose metabolism, or bone density (Fig. S3). Taken together these data supported the ability of our tools to selectively target individual populations of VHT neurons, allowing their further evaluation in the AgRP- and POMC-CRE mice models.

Empty pTomo and ΔFosB FLAG pTomo were subsequently used to generate constructs carrying scrambled or galanin shRNA. First, scrambled shRNA and 3 galanin shRNA oligos were ligated to pSilencer3.1-H1 neo vector (Life Technologies). The following oligo sets were used:

Scrambled shRNA:
5'-
GATCCCCCTAAGGTTAAGTCGCCCTCTTTCAAGAGAGAGGGCGACTTAACCTTAGGTTTTTTGG AAA-3'
3'-
GGCGGATTCACAATTCAGCGGAGAAGTTCTCTCTCCGCTGAATTGGAATCCAAAAACCTTT TCGA-5'

Galanin shRNA1:
5'-
GATCCCCAGATCATTTAGCGACAAGTTCAAGAGACTTGTCGCTAAATGATCTGTTTTTTGGAA A-3'
3'-
GGCGTCTAGTAAATCGCTGTTCAAGTTCTCTCTCAACAGCGATTTACTAGACAAAAAACCTTTTC GA-5'
Galanin shRNA2:
5’-
GATCCGGAGACCAGGAAGTGTTGATTCTCAAGAGAACTCAACACTTCTGGTCTCTCTTTTTTG
AAA-3’
3’-
GCCTCTGGTCCTCTCAACAACTAAAGTTCTTATGTTGAAAGGACCAGAGAAAAAACCTTTTC
GA-5’

Galanin shRNA3:
5’-
GATCCGCAACATTGTCCGCACTATATTCAAGAGATATAGTGCGGACAATGTTGCTTTTTTG
AAA-3’
3’-
GCGTTGTAACAGGCGTGATATAAGTTCTCTATATCACGCCTGTTACAACGAAAAAACCTTTTC
GA-5’

H1 promoter and shRNA sequence were excised with EcoRI restriction enzyme and subcloned into EcoRI linearized Empty pTomo or ΔFosB FLAG pTomo vectors. Sequenced and validated constructs were packaged into third generation LV system (Addgene) using 293T HEK cells, as previously described (Tiscornia et al., 2006). Viral titer was assessed and particle aliquots were stored at -80°C for further use.

**Western blot evaluation of CRE-inducible AP-1 factors expression**

293T HEK cells were grown to 50% confluence and transfected with AP-1 FLAG tagged pTomo constructs in the presence of CAG-CRE (Addgene) or empty vector control. 48 hours post-transfection, cells were lysed using SBN buffer: 1 mM EDTA, 50 mM Tris-HCl, 150 mM NaCl, 10% Glycerol, 1% NP40, pH 7.5 containing protease inhibitor cocktail (Sigma-Aldrich). Following 15 minutes incubation on ice, cell lysates were collected, centrifuged at 12,000 g for 15 min and supernatants were stored at -20°C. Total protein quantification was performed using BCA protein assay kit (Thermo Scientific). Equal amounts of protein (50 μg) were heated in 1X loading buffer containing 1% DTT at 100°C for 5 min, subjected to 10-15% SDS-PAGE, and transferred to nitrocellulose membrane using semi-dry TransBlot Turbo (BioRad). The following primary antibodies were used in 1:1000 dilution in 5% bovine serum albumin in PBS blocking solution: mouse anti-FLAG (Sigma, #F7425), rabbit anti-deltaFosB (Cell Signaling, #9890), rabbit anti-FosB (Cell Signaling, #2263), rabbit anti-JunD (AbCam, #ab28837)
Respective secondary anti-mouse HRP (Cell Signaling, #7056) and anti-rabbit HRP antibodies (Cell Signaling, #7074) were used at 1:10,000 in 5% dry milk in PBS. Signal was developed with Western Lightning Plus ECL (PerkinElmer).

Immunohistochemical analysis of ΔFosB expression in primary neurons and brain sections
Primary hypothalamic neurons were isolated using Papain Dissociation System (Worthington) and maintained in poly-D-lysine coated plates with Neurobasal medium (Life Technologies), supplemented with Glutamax and B27 (Life Technologies). For IHC, cells were washed, fixed in 4% paraformaldehyde, and incubated with rabbit anti-ΔFosB (Cell Signaling, #9890) and goat anti-AgRP (AbCam, #ab32882) or goat anti-POMC (AbCam, #ab32893), using secondary anti-rabbit Alexa Fluor 488 (ThermoFisher, #A11034), and anti-goat Alexa Fluor 405 antibodies (AbCam, #ab175664). CRE-inducible ΔFosB LV were injected in VHT of AgRP-CRE or POMC-CRE mice and sacrificed 4 days post-operatively. Briefly, animals were subjected to cardiac perfusion with 4% PFA, brains removed, soaked first overnight at 4% PFA and then overnight at 30% sucrose, embedded in OCT compound (Tissue Tek) and sectioned on cryostat at -20C, at 20 μm. Frozen sections were fixed with 4% PBS and subjected to IHC staining with primary rabbit anti-FLAG (AbCam, #ab1162), mouse anti-AgRP (AbCam, #ab89114) or mouse anti-POMC (AbCam, #ab73092) and secondary anti-rabbit Alexa Fluor 488 (ThermoFisher, #A11034) and anti-mouse Alexa Fluor 568 (ThermoFisher, #A11031).

mRNA quantification with real-time PCR
Total RNA was extracted using TRI-reagent PureLink RNA Mini Kit (Life Technologies) and reverse transcribed using PrimeScript RT Master Mix (Clontech). cDNA was diluted 1:25 and used for real-time qPCR with FastStart Universal SYBR Green Master with Rox (Roche) and StepOnePlus Real-Time PCR System (Applied Biosystems). Gene expression was normalized to one of the house-keeping genes, HPRT, GAPDH, or 36B4, and the data were analyzed using comparative $2^{-\Delta\Delta CT}$ method.

FACS sorting of AgRP and POMC neurons
AgRP-CRE (or POMC-CRE) mice were crossed with reporter BrainbowRosa26-2.1 marking all AgRP (or POMC) neurons single, unique color (predominantly red). Recombined mice were then VHT injected with GFP-AAV or ΔFosB-AAV (green), primary hypothalamic neurons isolated and subjected to
BD FACS AriaII counting, analysis and sorting using PE-A filter for red fluorescence and FITC-A for green fluorescence. A population displaying both red and green constitutes AgRP (or POMC) neurons infected with AAV. 500 cells were collected and subjected to gene expression analysis by qPCR.

\( \Delta \text{FosB chromatin immunoprecipitation (ChIP) and galanin luciferase assays} \)

For \( \Delta \text{FosB ChIP}, \) mHypoE 42 cell line was transfected with \( \Delta \text{FosB FLAG pTomio} \) in combination with either CAG-CRE construct (Addgene) or empty-vector control \((n = 3)\). ChIP was performed using anti-FLAG antibodies (Sigma) and SimpleChIP Plus Enzymatic Chromatin IP kit (CellSignaling) according to manufacturer instructions. Specific primers were designed to detect short sequences from the evolutionary conserved regions (ECRs) positioned upstream of galanin transcription site (TSS), including promoter (1 kb upstream of TSS), ECR1 (2.5 kb upstream of TSS) and ECR2 (5.4 kb upstream of TSS):

- Galanin promoter (chr19:3414393-3414610): (F)CGCTGCTGCCGCTATTTATG, (R)CCTTGGGACTCGCAGGAG
- Galanin ECR1 (chr19:3416713-3416900): (F)ATGGACTCCTCAGCAATG, (R)TGGTGACCCATTTTGCTAGC
- Galanin ECR2 (chr19:3419379-3419587): (F)GTGACTCACCCAGCTCAGC, (R)TGGACAATGCTACGGGTCTG

For the galanin luciferase activation assay, two fragments of increasing length from TSS were subcloned into the luciferase dual reporter plasmid pEZX-PG04 (GeneCopoeia), both containing promoter region. Additionally, these constructs contained a CMV-driven alkaline phosphatase sequence (SEAP), used to assess transfection efficiency. The following regions were subcloned into Gal pEZX-PG04 constructs: Gal 1.0 kb (1008 kb upstream and 244 bp downstream of TSS) and Gal 2.5 kb (2500 kb upstream and 244 bp downstream of TSS). Gal-GTRE-WT-pEZX-PG04 construct was engineered by subcloning 3414264 to 3414526 (288 bp) galanin promoter region, containing the GTRE site (TGACGCGG) or mutant GTRE site (GGCAGCGG), using XhoI restriction digest. HEK-293 cells were cotransfected with Gal pEZX-PG04 constructs in combination with pcDNA vectors encoding the FosB isoforms (FosB and \( \Delta \text{FosB} \)) and JunD isoforms (JunD and DNJunD). The total amount of transfected DNA was maintained at 0.3μg and transfection efficiency was assessed by measuring SEAP luminescence. 36 hours following transfection, a dual luminescence assay for Gaussia luciferase and SEAP was performed according to the manufacturer’s instructions.
Central blockade of galanin receptors with M35

For the galanin receptor blocker, M35 (AnaSpec) study, in addition to ΔFosB or GFP AAV injection, C57BL mice underwent intrascapular implantation of osmotic pumps containing 10, 100, or 1000 μM of M35 (vehicle as control) connected by a catheter to a cannula (Brain Infusion Kit 2, Alzet). Cannulas were placed in the 3rd ventricle using the following stereotaxic coordinates: AP = 2.0, DV = -5.7, LAT = 0, infusing M35 for a period of 2 weeks, followed by metabolic and bone analysis. For the evaluation of injection site, animals were subjected to cardiac perfusion with 4% PFA, brains removed, soaked overnight in 30% sucrose and sectioned on cryostat at -20C. For histological evaluation of adipose tissue and bone, organs were harvested, fixed in 4% PFA, placed in paraffin blocks, sectioned, and subjected to standard H&E staining. Adipocyte area was assessed using Adiposoft plugin for ImageJ. For real time mRNA analysis, tissues were snap frozen in liquid nitrogen and stored at -80C until RNA extraction.

Glucose tolerance test and insulin tolerance test

Glucose tolerance test (GTT) was performed by administrating glucose (2.0 mg/g BW, Sigma) i.p. after a 16-hr fast. Blood glucose levels were monitored using glucose test strips and a glucometer (OneTouch ultra, LifeScan) at indicated times. Blood was also collected from tails using EDTA-treated microcapillaries and plasma insulin levels were measured using an EIA kit (ALPCO). For insulin tolerance test (ITT), mice were fasted for 4 hrs and injected insulin (1.0 mU/g BW, Lilly) intraperitoneally, and blood glucose levels were measured at indicated times. ITT data are presented as percentage of initial blood glucose concentration.

Fat pad H&E staining and adipocyte morphometry

Abdominal fat pads were removed, weighted and processed into formalin-fixed-paraffin-embedded blocks, cut into 4 μm sections, mounted on slides and stained using a standard H&E protocol. Sections were examined under light microscope (Nikon Eclipse E800 with FDX35 camera). ImageJ Adiposoft plug-in was used to measure adipocyte area. Plots under the images of H&E stained fat pads represent a distribution of all adipocytes and their respective areas (arbitrary units).

Insulin pancreatic β-cell immunohistochemistry
Pancreases were collected, fixed overnight in 4% PFA, embedded in paraffin and sectioned at 5 μm. Sections were immunostained using rabbit anti-insulin polyclonal antibody (Cell Signaling, #4590) and counterstained with hematoxylin. Quantitative histomorphometric analysis of islet area and number was performed using Image J software (National Institute of Health).

Data are available on request from the authors.
Supplementary Figures

**Figure S1. Generation of CRE-inducible AP-1 construct carrying LV. Related to Figure 1 and Figure 2.**

A. Experimental approach scheme, representing pTomo construct with CMV-promoter driven floxed RFP and stop codon, followed by FLAG tagged AP-1 factor (ΔFosB, Δ2ΔFosB, DNJunD, or FosB) and IRES GFP. These CRE-inducible constructs were packaged into LV and stereotaxically delivered to the...
VHT of neuronal promoter CRE mice, ensuring expression of AP-1 factor only in the neuron of interest (namely, AgRP, POMC, or SF-1). B. Western Blot 293T HEK cells transfected with constructs, in the absence or presence of CAG-CRE. C. Fluorescent imaging of constructs transfected into 293T HEK cells, in the absence or presence of CAG-CRE. D. Fluorescent imaging of primary hypothalamic neurons isolated from AgRP-CRE and POMC-CRE adult mice, and cultured for a week with Empty LV. Note the disappearance of RFP signal in selected AgRP and POMC neurons, as it is excised by CRE (pink arrows). E. Real-time PCR analysis of AP-1 factors mRNA expression following transduction of primary hypothalamic neuronal cultures isolated from AgRP-CRE or POMC-CRE mice with CRE-inducible AP-1 LV. Note that in the FosB group, both ΔFosB and FosB primers recognize FosB expression, since ΔFosB is a truncated version of FosB lacking 101 C-terminal amino acids (n=4, t-test *p<0.05). F. Stereotaxic injection coordinates. G. Neuronal promoter CRE mice crossed with reporter R26R-Brainbow2.1 and injected with Empty LV. Yellow blobs with specks of green indicate LV injection site, in the vicinity of bright red ARC or VMH neurons.
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A

\[ \Delta FosB \text{ LV in } \text{AgRP-CRE hypothalami IHC} \]

\begin{align*}
\text{anti-AgRP} & \quad \text{anti-FLAG} & \quad \text{merge} \\
\text{\textbf{40X}} & & & \\
\end{align*}

\[ \Delta FosB \text{ LV in POMC-CRE hypothalami IHC} \]

\begin{align*}
\text{anti-POMC} & \quad \text{anti-FLAG} & \quad \text{merge} \\
\text{\textbf{40X}} & & & \\
\end{align*}

B

primary neurons from AgRP-CRE IHC

\begin{align*}
\text{Bright field} & \quad \text{anti-AgRP (CFP)} & \quad \text{anti-\( \Delta \)FosB (GFP)} \\
\text{\textbf{40X}} & & & \\
\end{align*}

primary neurons from POMC-CRE IHC

\begin{align*}
\text{Bright field} & \quad \text{anti-POMC (CFP)} & \quad \text{anti-\( \Delta \)FosB (GFP)} \\
\text{\textbf{40X}} & & & \\
\end{align*}
Figure S2. Neuron specific expression of CRE-inducible AP-1 construct carrying LV. Related to Figure 1 and Figure 2. A. IHC staining of hypothalami of AgRP-CRE or POMC-CRE mice VHT injected with CRE-inducible FLAG tagged ΔFosB LV. White arrows point at neurons where AgRP (or POMC) is colocalized with ΔFosB, indicating specific neuronal expression. ΔFosB signal was not observed in other neuron types (no green without red) (n=4). Note that as expected as well, the neuromediators are localized in the cytosol when ΔFosB is expressed in the nucleus (see high magnification inserts). B. IHC staining of primary neurons isolated from AgRP-CRE or POMC-CRE mice injected with CRE-inducible FLAG tagged ΔFosB LV, showing specific ΔFosB expression in AgRP- or POMC-positive neurons, respectively. White arrows point at neurons where AgRP (or POMC) is colocalized with ΔFosB, indicating specific neuronal expression. Yellow arrows point at neurons negative for ΔFosB and neuropeptide of interest (n=4).
Figure S3. CRE-inducible ΔFosB LV has no effect on metabolism or bone in wild-type C57BL mice.

Related to Figure 1 and Figure 2. CRE-inducible AP-1 LV were validated with wild-type (non-CRE bearing) mice, by means of stereotaxic VHT delivery and assessment of energy metabolism and bone parameters 6 weeks post-operatively. A. Weight gain, feeding, locomotion, and calorimetric analysis of energy expenditure measured by CLAMS. B. Insulin tolerance test and glucose tolerance test. C. Bone parameters measured by micro-CT. Statistical analysis included ANOVA followed by Tukey-Kramer HSD test, p<0.05 comparing 2 groups (n=4 animals per group).
Figure S4. AP-1 blockade in AgRP, POMC, and SF1 neurons reduces β-cell islet size. Related to Figures 1, 2, and 7. Male 6-7 week old AgRP-CRE, POMC-CRE, or SF1-CRE mice were stereotaxically injected into VHT with CRE-inducible ΔFosB or FosB LV and pancreases assessed 8 weeks post-surgery (n=6-9 animals per group). The figure shows insulin immunohistochemistry, counterstained with
hematoxylin. The numbers indicate average area occupied by islets. Statistical analysis included ANOVA followed by Tukey-Kramer HSD test, p<0.05 comparing 3 groups.
Figure S5. Differential effects of AP1 expression in AgRP, POMC or SF1 neurons on bone dynamic parameters. Related to Figures 1, 2. Male 6-7 week old AgRP-CRE, POMC-CRE, or SF1-CRE mice were stereotaxically injected into VHT with CRE-inducible AP-1 (ΔFosB, Δ2ΔFosB, DNJunD, or FosB) LV, labeled with calcein and demeclocylin, and assessed 8 weeks post-surgery. The table shows tibiae histomorphometry. Statistical analysis included ANOVA followed by Tukey-Kramer HSD test, p<0.05 comparing 5 groups (n=3-9 animals per group).
Figure S6. AP-1 antagonism in AgRP neurons increases energy expenditure and elevates bone density. Related to Figure 1. Male 6-7 week old AgRP-CRE mice were stereotaxically injected into VHT with CRE-inducible AP-1 (ΔFosB, Δ2ΔFosB, DNJunD, or FosB) LV and metabolic and bone profiles were assessed 6 weeks post-surgically. A. Weight gain at 3 and 6 weeks. B. Calorimetric analysis of energy expenditure. C. Locomotion. D. Representative images of gross biopsy of abdominal fat pads. E. H&E stained sections of abdominal fat pads. Top left values and bottom left graphs correspond to adipocyte area mean and distribution. F. Bone parameters measured by micro-CT. Statistical analysis included ANOVA followed by Tukey-Kramer HSD test, p<0.05 comparing 5 groups (n=6-9 animals per group).
Figure S7. AP-1 antagonism in POMC neurons increases energy expenditure and elevates bone density. Related to Figure 2. Male 6-7 week old POMC-CRE mice were stereotaxically injected into VHT with CRE-inducible AP-1 (ΔFosB, Δ2ΔFosB, DNJunD, or FosB) LV and metabolic and bone profiles were assessed 6 weeks post-surgically. A. Weight gain at 3 and 6 weeks. B. Calorimetric analysis of energy expenditure. C. Locomotion. D. Representative images of gross biopsy of abdominal fat pads. E. H&E stained sections of abdominal fat pads. Top left values and bottom left graphs correspond to adipocyte area mean and distribution. F. Bone parameters measured by micro-CT. Statistical analysis included ANOVA followed by Tukey-Kramer HSD test, p<0.05 comparing 5 groups (n=6-9 animals per group).
Figure S8. AP-1 antagonism in AgRP and POMC neurons does not affect feeding. Related to Figures 1,2. Male 6-7 week old AgRP-CRE and POMC-CRE mice were stereotactically injected into VHT with CRE-inducible ΔFosB or Empty control LV. Feeding was monitored manually for a period of 6 weeks post-operatively, energy expenditure was assessed at 2 and 6 weeks post-operatively, normalized to lean body mass, as measured by DXA. A. Representative DXA images. B. Peripheral fat mass and lean mass. C. Body weight, feeding and calorimetric analysis of energy expenditure in AgRP-CRE mice. D. Body weight, feeding and calorimetric analysis of energy expenditure in POMC-CRE mice. Statistical analysis included student t-test, p<0.05 (n=4-5 animals per group).
Figure S9. Microarray heatmap of ENO2-\(\Delta\)FosB mice hypothalamic compared to control. Related to Figure 3. Heatmap displays only the genes regulated >2 fold and <0.35 fold (n=2)
Figure S10. FACS sorting of primary hypothalamic AgRP and POMC neurons. Related to Figure 3. AgRP-CRE (or POMC-CRE) mice were crossed with reporter BrainbowRosa26-2, marking all AgRP neurons single, unique color (predominantly red). Recombined mice were then injected with GFP-AAV or ΔFosB-AAV (green), primary hypothalamic neurons isolated and subjected to FACS. After eliminating cell debris (P1) and cells clusters (P2) by forward and side scatters fluorescence channels FITC-A (green) and PE-A (red) were applied and 4 distinct cells populations were sorted, as indicated above. Infected AgRP and POMC cells (P4) comprise about 0.6-0.7% of total population, respectively (n=4).
Figure S11. Galanin GTRE octamer (TGACGCGG) fails to activate luciferase transcription. Related to Figure 3. Gal promoter region sequence 3414552-34264 (288 bp) containing the GTRE octamer TGACGCGG (wild-type) or GGCAGCGG (mutant) was subcloned into luciferase pEZX-PG04 reporter vector. Gal-1kb-pEZX-PG04 (1008 kb upstream and 244 bp downstream of TSS) was used as positive control and empty pEZX-PG04 was used as negative control. Luciferase assay was performed using various combinations of AP-1 factors transfected into HEK293 cells with pEZX-PG04 constructs carrying Gal 1kb promoter or GTRE fragments.
Figure S12. Validation of CRE-inducible ΔFosB galanin shRNA LV in mHypoE42 cell line and ex vivo, upon injection into the VHT of neuronal promoter CRE mice. Related to Figure 5. A. Real time PCR analysis of galanin and its receptors mRNA levels in mHypoE42 cell line transfected with CRE-inducible AP-1 shRNA constructs, in the presence of CRE (n=3). Statistical analysis included t-test *p<0.05, **p<0.01. B. Real time PCR analysis of ΔFosB and galanin mRNA expression levels in the whole hypothalami of neuronal promoter CRE mice, VHT-injected with AP-1 shRNA LV (n=4). Statistical analysis included t-test *p<0.05 comparing GalshRNA group to respective scrambled control.
Figure S13. Silencing of galanin counteracts ΔFosB-enhanced glucose tolerance and insulin sensitivity in AgRP and POMC neurons. Related to Figure 5. CRE-inducible FLAG tagged ΔFosB pTomo or empty pTomo constructs were modified to incorporate H1 promoter and scrambled or galanin shRNA. These constructs were packaged into LV and stereotaxically delivered to VHT of neuronal promoter AgRP-CRE or POMC-CRE mice. 6 weeks post-injection glucose metabolism was assessed. A. GTT and ITT in AgRP mice. B. GTT and ITT in POMC mice; statistical analysis included ANOVA followed by Tukey-Kramer HSD test, comparing individual groups to Empty/sc control, *p<0.05 for ΔFosB group; &p<0.05 for Empty/GalshRNA group; #p<0.05 for ΔFosB/GalshRNA group (n=5-8).
Figure S14. Summary of metabolic and bone effects following AP1 alteration in VHT

AP1 transcriptional activity in VHT affects whole body metabolism and bone homeostasis. Targeted alteration of AP1 in AgRP and POMC neurons increases energy expenditure, glucose utilization, and bone density. These effects are dependent upon the local availability of the neurotransmitter galanin. The increase in bone mass is achieved via differential mechanisms: in AgRP neurons bone formation is increased and resorption is decreased, while in POMC neurons, both formation and resorption are increased. Although, AgRP and POMC neurons are commonly classified as orexigenic and anorexigenic, respectively, our data suggests that under certain conditions, such as altered AP1 transcription, both types of ARC neurons can function in accord to promote energy catabolism and bone anabolism. In contrast to ARC, targeted alteration of AP1 signaling in the VMH residing SF1 neurons, increases energy expenditure and glucose metabolism, but decreases bone mass, in a galanin-independent manner. These data suggest that energy and bone homeostasis are not inexorably linked, but are rather two mechanistically dissociated processes.