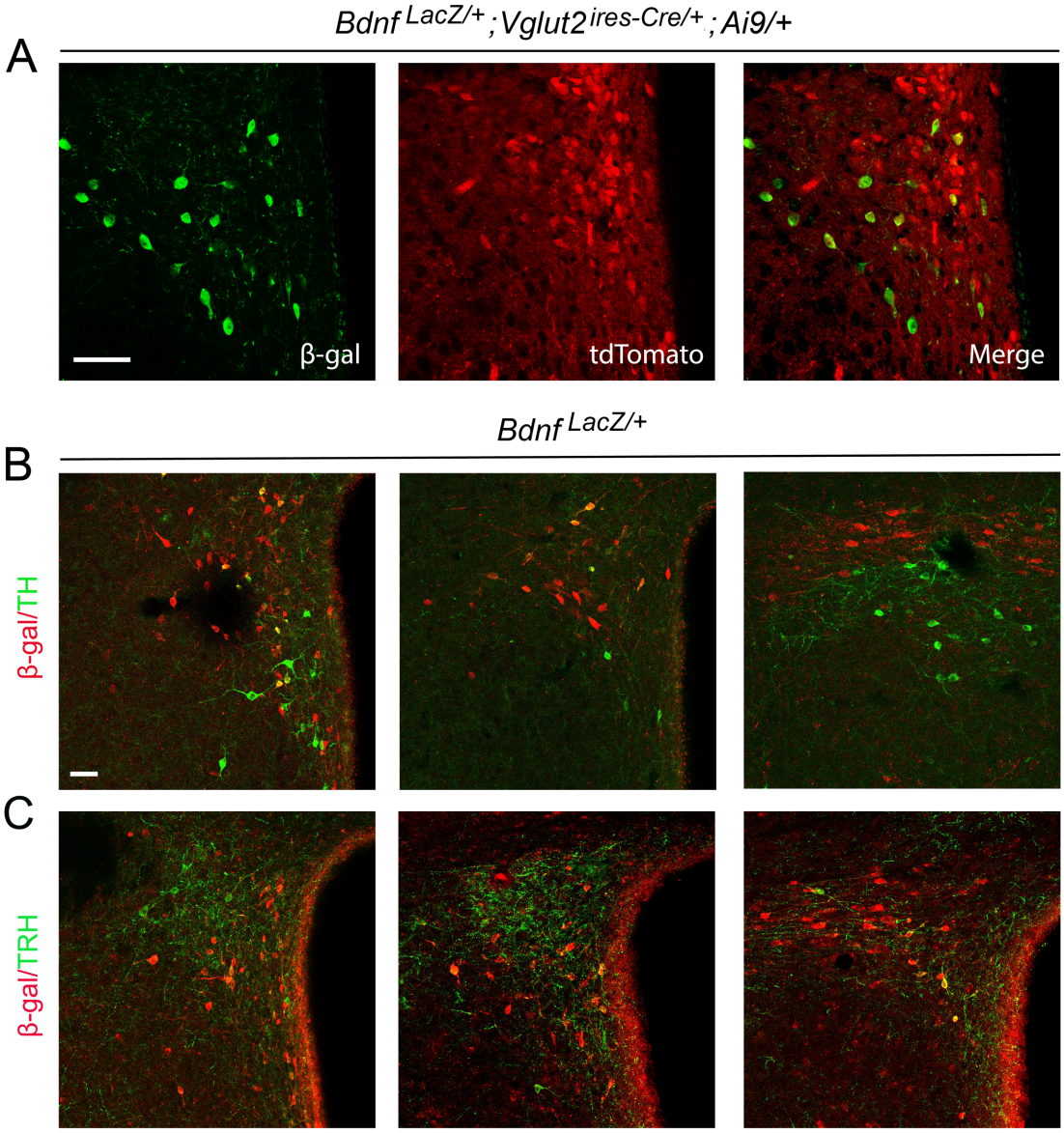


Figure S1, related to Figure 1. BDNF expression in the PVH

(A) Coexpression of BDNF with VGLUT2 in the PVH. Arrows denote representative neurons that express both BDNF and VGLUT2. The scale bar represents 50 μm.

(B) Coexpression of BDNF with tyrosine hydroxylase (TH) in the anterior PVH (left), medial PVH (middle), and posterior PVH (right). The scale bar represents 50 μm.

(C) Coexpression of BDNF with thyrotropin-releasing hormone (TRH) in the anterior PVH (left), medial PVH (middle), and posterior PVH (right). The scale bar represents 50 μm.

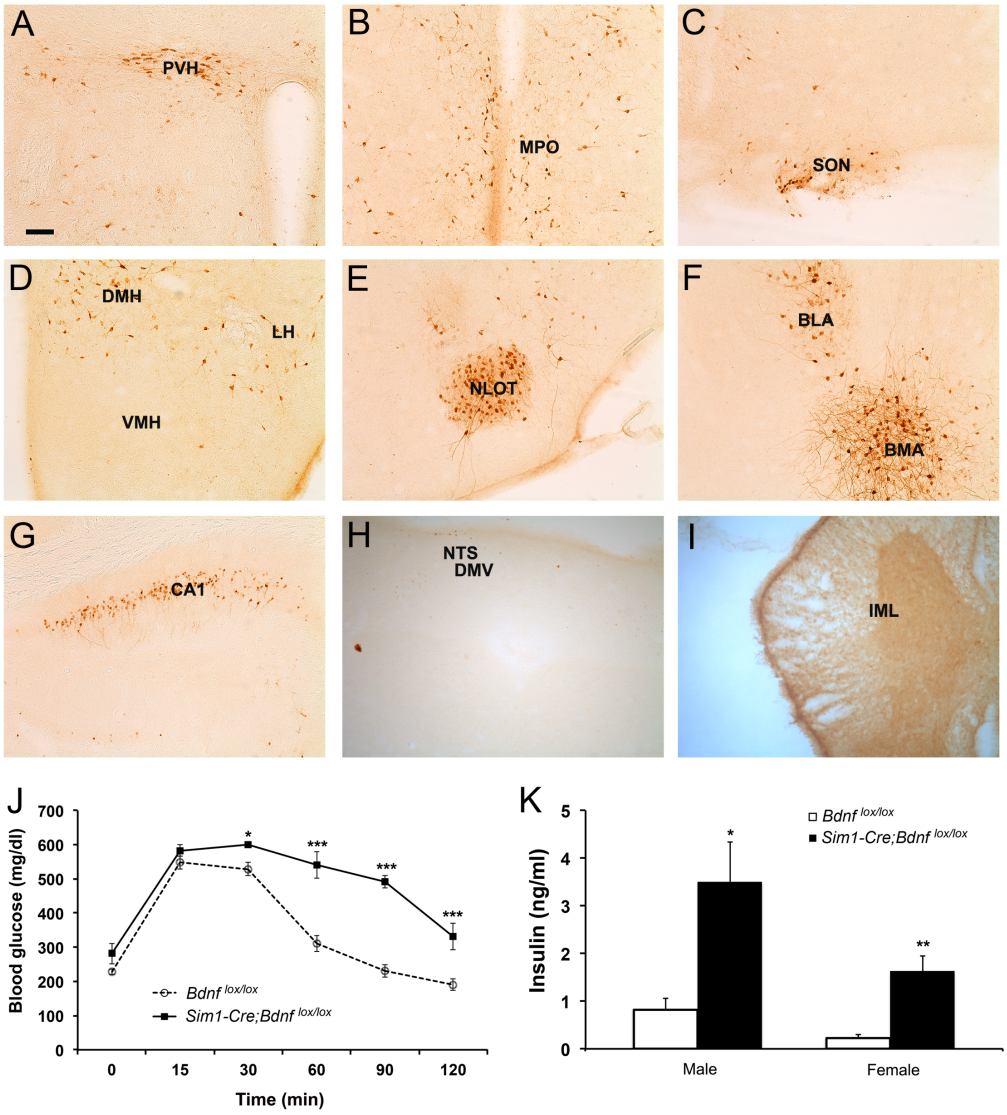
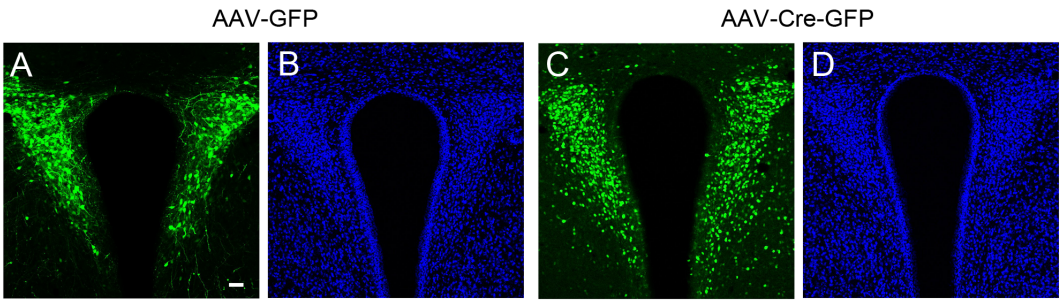


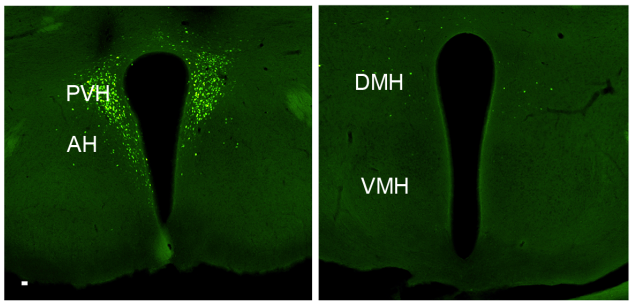
Figure S2, related to Figure 2. *Bdnf* deletion and impaired glucose tolerance in *Sim1-Cre;Bdnf*$^{lox/lox}$ mice

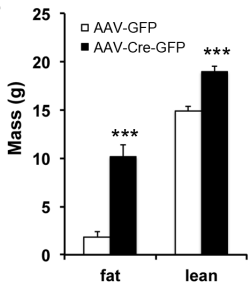
(A - I) Neurons expressing β-galactosidase were detected in the PVH (A), MPO (B), SON (C), DMH and LH (D), NLOT (E), amygdala (F), and CA1 (G), but not in the VMH (D), NTS (H), DMV (H), and spinal cord (I) of *Sim1-Cre;Bdnf*$^{klox/+}$ mice. The scale bar represents 50 μm. Abbreviations: BLA, basolateral amygdala; BMA, basomedial amygdala; CA1; hippocampal CA1 region; DMH, dorsomedial hypothalamus; DMV, dorsal motor nucleus; IML, intermediolateral column; LH, lateral hypothalamus; MPO, medial preoptic nucleus; NLOT, nucleus of the lateral olfactory tract; NTS, nucleus tractus solitarius; PVH, paraventricular hypothalamus; SON, supraoptic nucleus; VMH, ventromedial hypothalamus.

(J) Glucose tolerance test was performed in female *Bdnf*$^{lox/lox}$ (n=6) and *Sim1-Cre;Bdnf*$^{lox/lox}$ (n=3) mice at 16 weeks of age. Error bars indicate standard errors. * $P < 0.05$ and *** $P < 0.001$ by Student *t* test.

(K) Measurement of blood insulin were performed in *Bdnf*$^{lox/lox}$ and *Sim1-Cre;Bdnf*$^{lox/lox}$ mice at 20 weeks of age (n=4-5). Error bars indicate standard errors. * $P < 0.05$, and ** $P < 0.01$ by Student *t* test.

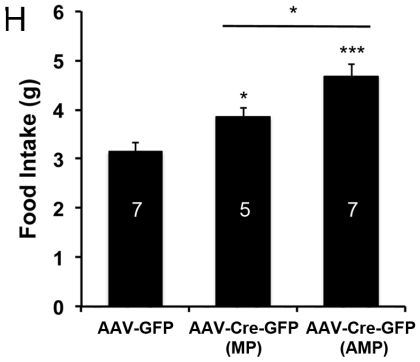


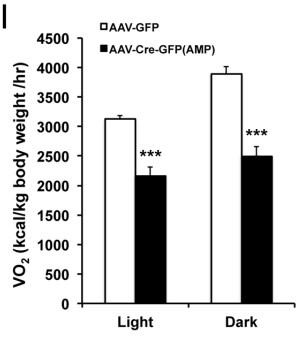


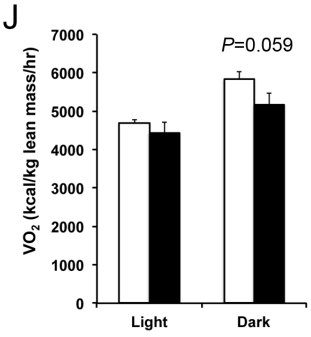


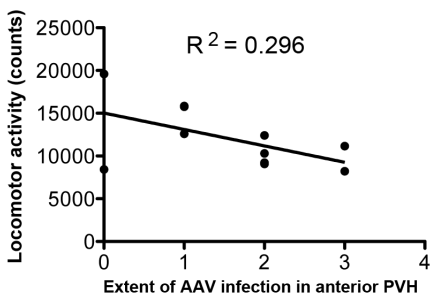
G

	Animal	PVH (anterior)	PVH (medial)	PVH (posterior)	AH	DMH	VMH	Food intake (% of AAV-GFP)	Body Weight (g)
AAV-Cre-GFP (AMP)	AAV-Cre-GFP1	++	++++	++++	++	+		143	36.4
	AAV-Cre-GFP3	++	++++	++++	+	+		128	42.6
	AAV-Cre-GFP5	++	++++	++++	+	+		134	38.6
	AAV-Cre-GFP7	+++	++++	++++	+	+		182	48.6
	AAV-Cre-GFP9	++	++++	++++	++	++		150	43.4
	AAV-Cre-GFP10	+++	++++	++++	++	++		159	45.6
	AAV-Cre-GFP13	+++	++++	++++	++	+		179	55.5
AAV-Cre-GFP (MP)	AAV-Cre-GFP4		++++	++++	+	+		104	27.4
	AAV-Cre-GFP8	+	++++	++++	++	+++	+	137	36.9
	AAV-Cre-GFP11	+	++++	++++	++	+++		134	32.5
	AAV-Cre-GFP12		++++	++++	++	+++		115	29.3
	AAV-Cre-GFP14	+	++++	++++	+	+		131	39.8
	AAV-Cre-GFP2		+	++	++	++		96	24.1









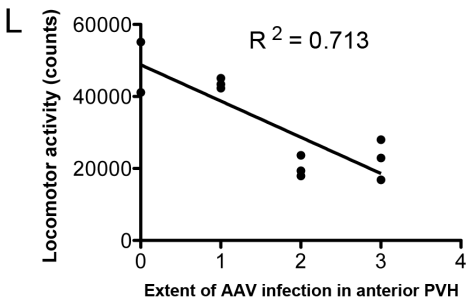


Figure S3, related to Figure 4. Injection of AAV-Cre-GFP into the PVH of *Bdnf*$^{lox/lox}$ mice led to obesity

(A and B) GFP fluorescence and DAPI staining of the same brain section from an AAV-GFP-injected mouse, showing the structure of the PVH. The scale bar represents 50 μm.

(C and D) GFP fluorescence and DAPI staining of the same brain section from an AAV-Cre-GFP-injected mouse, showing the structure of the PVH.

(E) GFP fluorescence in brain sections of a mouse injected with AAV-Cre-GFP, showing specificity of the injection. The scale bar represents 50 μm.

(F) Lean mass and fat mass of female *Bdnf*$^{lox/lox}$ mice 10 weeks after either AAV-GFP or AAV-Cre-GFP was bilaterally injected into the PVH. Error bars indicate standard errors. *** $P < 0.001$ by Student *t* test.

(G) List of the animals that received injection of AAV-Cre-GFP into the PVH. The number of "+" symbol indicates the extent of AAV infection in different hypothalamic regions. Food intake is listed as percentage increase over the average food intake of control mice that received injection of AAV-GFP into the PVH. Body weight and food intake were measured at week 8 and 9 of the post-injection period, respectively. AH, anterior hypothalamus; DMH, dorsomedial hypothalamus; VMH, ventromedial hypothalamus.

(H) Daily food intake during the 9th week of the post-injection period. The numbers inside columns indicate animal number. Error bars indicate standard errors. * $P < 0.05$ and *** $P < 0.001$ by Student *t* test.

(I and J) Oxygen consumption of *Bdnf*$^{lox/lox}$ mice injected with either AAV-GFP or AAV-Cre-GFP. The AAV-Cre-GFP mice had AAV infection throughout the PVH. Animal number was 8 and 6 for the AAV-GFP group and the AAV-Cre-GFP (AMP) group, respectively. The measurement was done during the 9th week of the post-injection period. VO_2 was normalized to either body weight (I) or lean mass (J). Error bars indicate standard errors. *** $P < 0.001$ by Student *t* test.

(K) There is no significant correlation between the extent of AAV infection in the anterior PVH and locomotor activity during light cycle. $P = 0.0838$.

(L) There is a good correlation between the extent of AAV infection in the anterior PVH and locomotor activity during dark cycle. $P < 0.01$.

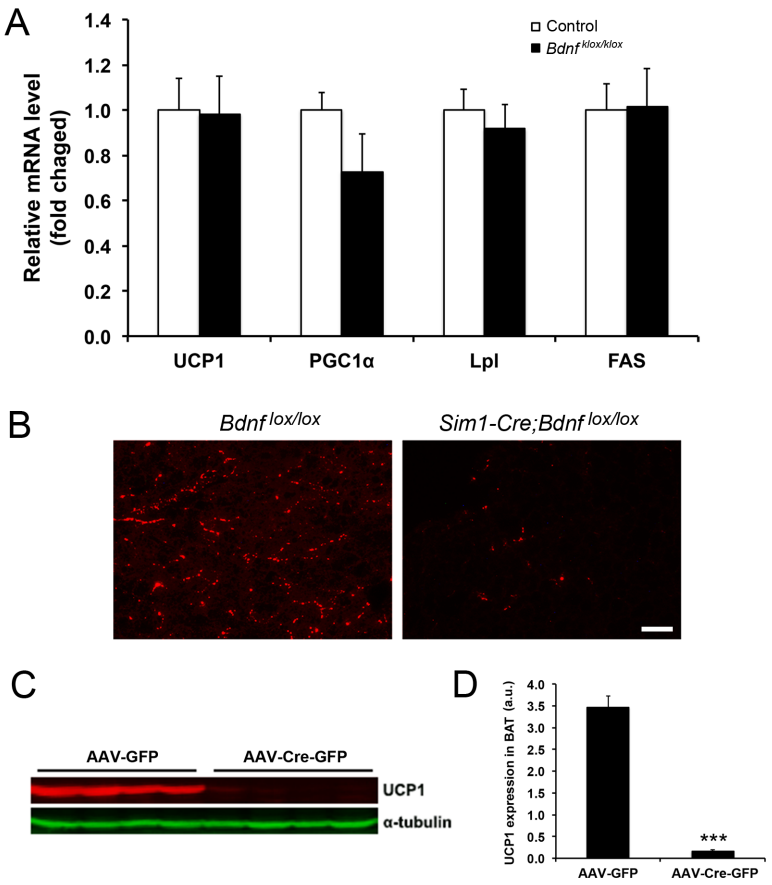


Figure S4, related to Figure 5. Gene expression in iBAT of *Bdnf* mutant mice

(A) Levels of iBAT mRNAs were analyzed with real-time PCR in 5 *Bdnf*$^{klox/klox}$ (k/k) mice and 5 control mice (2 WT mice and 3 *Bdnf*$^{klox/+}$ mice) at 6 weeks of age. Error bars indicate standard errors. Student's *t* test: no significant difference between genotypes.

(B) Paraffin sections of iBAT from 8-week-old mice were stained with an antibody against tyrosine hydroxylase. Scale bar, 50 μm.

(C) Immunoblots of UCP1 and α-tubulin. Protein extracts were prepared from iBATs of *Bdnf*$^{lox/lox}$ mice 5 weeks after either AAV-GFP or AAV-Cre-GFP was bilaterally injected into the PVH of these animals.

(D) Quantification of UCP1. Levels of UCP1 were normalized to α-tubulin in the same sample. *** $P < 0.001$ by Student's *t* test.

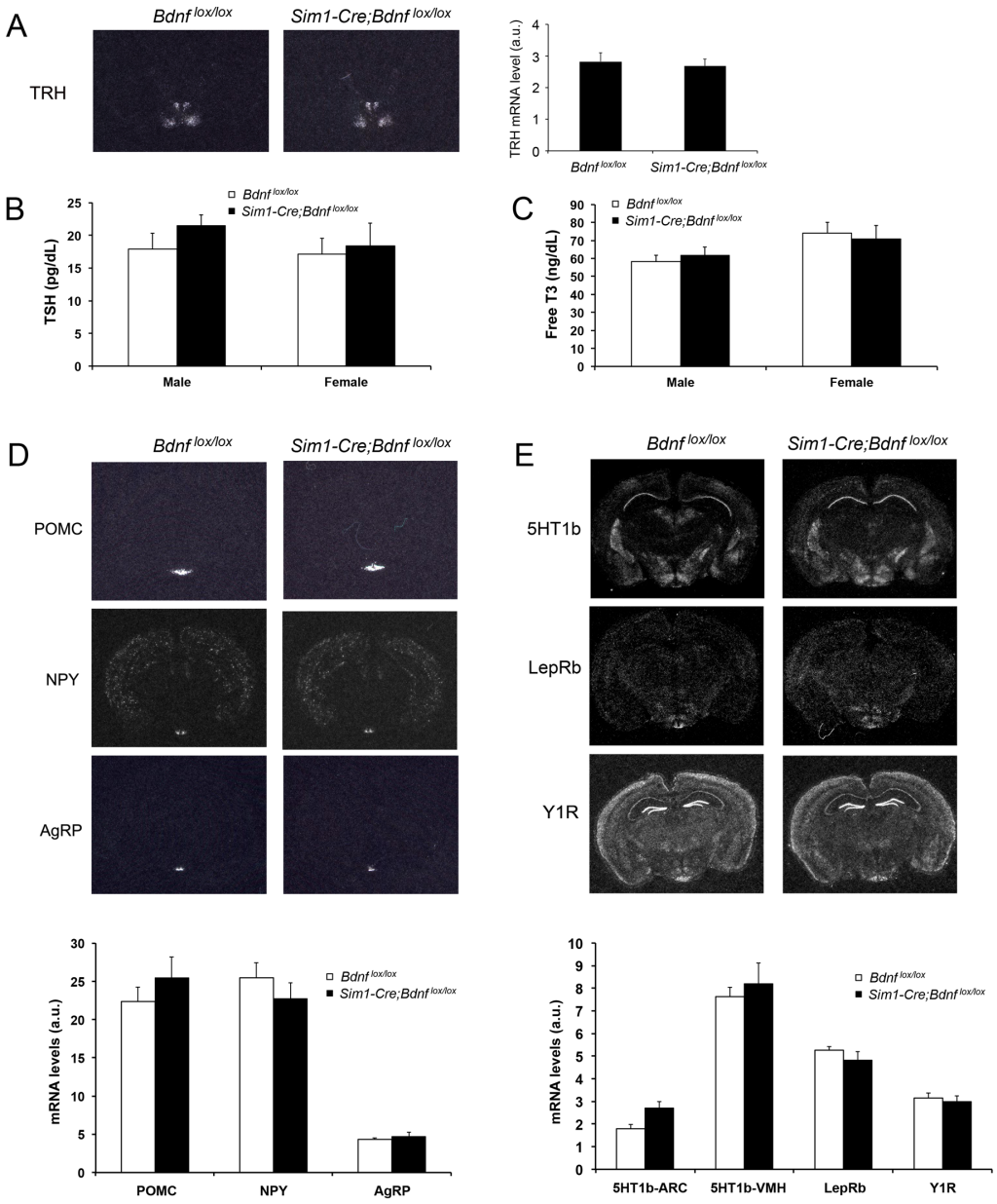


Figure S5, related to Figure 6. Gene expression in *Bdnf*$^{lox/lox}$ and *Sim1-Cre;Bdnf*$^{lox/lox}$ mice at 8 weeks of age

(A) Representative images of in situ hybridization for *Trh* mRNA and quantification of PVH *Trh* mRNA in mice. Error bars indicate standard errors.

(B) Levels of plasma TSH. Error bars indicate standard errors.

(C) Levels of plasma T3. Error bars indicate standard errors.

(D) Levels of mRNA for pro-opiomelanocortin (POMC), neuropeptide Y (NPY), and agouti-related protein (AgRP) in the ARH, as revealed by *in situ* hybridization. Error bars indicate standard errors.

(E) Levels of mRNA for serotonin receptor 1B (5HT1b), long-form leptin receptor (LepRb), and NPY Y1 receptor (Y1R) in the ARH, as revealed by *in situ* hybridization. Error bars indicate standard errors.

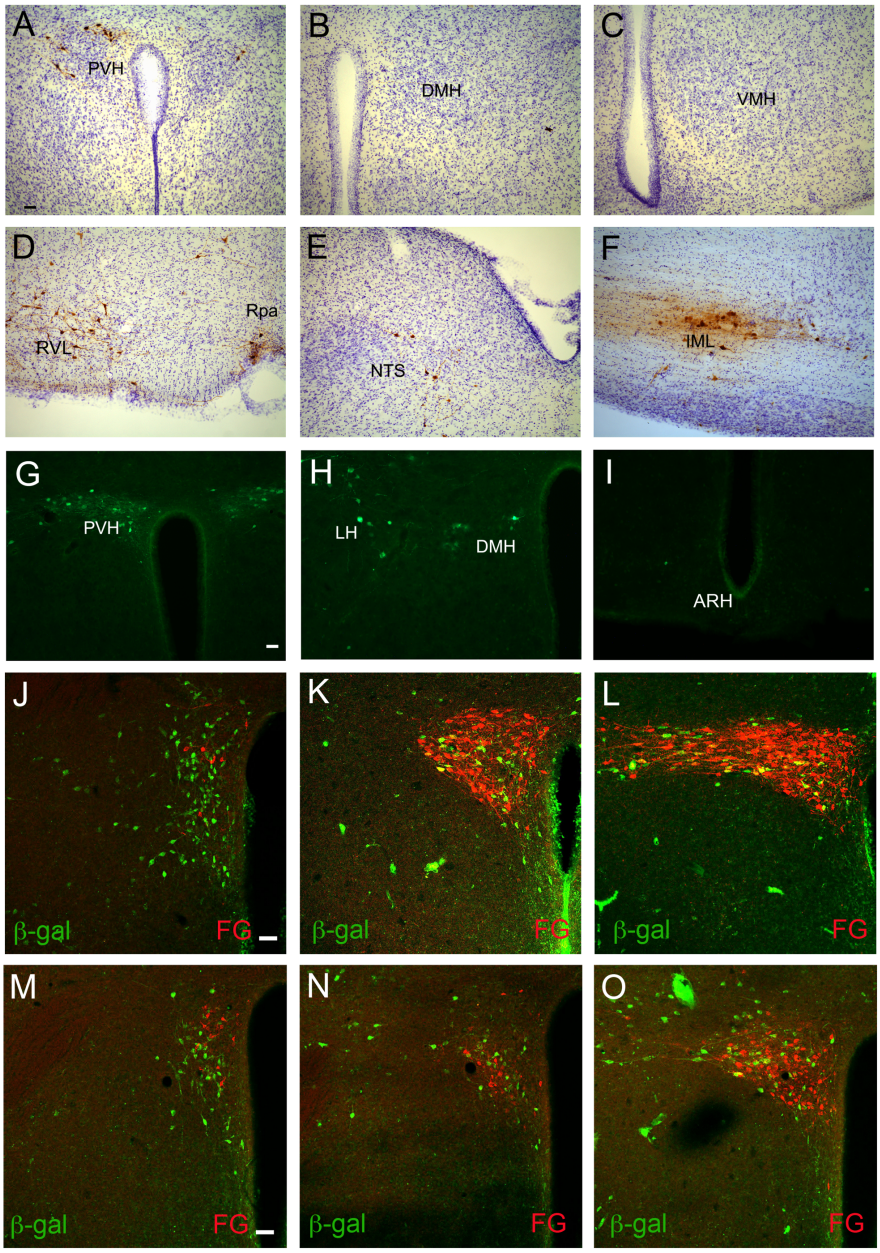


Figure S6, related to Figure 6. Projections of BDNF neurons in the PVH

(A-F) PRV labeled some neurons in the PVH, rostroventral lateral reticular nucleus (RVL), raphe pallidus (Rpa), NTS, and intermediolateral column of the spinal cord (IML), but not in the DMH and VMH, 3 days after it was injected into iBAT. The scale bar represents 50 μm.

(G-I) PRV-labeled neurons were detected in the PVH, DMH, lateral hypothalamus (LH), and ARH 4 days after PRV was injected into iBAT. The scale bar represents 50 μm.

(J-L) Colocalization of fluorogold injected into the spinal cord with β-galactosidase in the anterior (left), medial (middle), and posterior (right) PVH of *Bdnf*$^{LacZ/+}$ mice. The scale bar represents 50 μm.

(M-O) Colocalization of fluorogold injected into the NTS with β-galactosidase in the anterior (left), medial (middle), and posterior (right) PVH of *Bdnf*$^{LacZ/+}$ mice. The scale bar represents 50 μm.

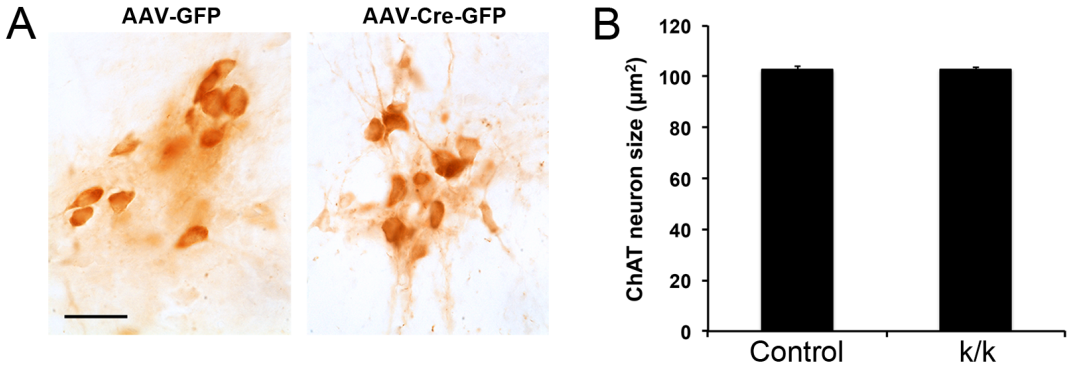


Figure S7, related to Figure 7. Size of cholinergic neurons in the IML of the spinal cord
(A) ChAT neurons in the thoracic IML of *Bdnf*$^{lox/lox}$ mice in which either AAV-GFP or AAV-Cre-GFP was bilaterally injected into the PVH. The scale bar represents 50 μm.
(B) Cell body size of ChAT neurons in the thoracic IML of *Bdnf*$^{klox/klox}$ mice (k/k, n=4) and control mice (2 WT mice and 2 k/+ mice) at 6 weeks of age. Error bars indicate standard errors. Student *t* tests: no significant difference between genotypes.

Supplemental Experimental Procedures

Acquisition and Analysis of Confocal Images

Brain sections were imaged using a Leica TCS SP8 MP multiphoton microscope (Leica, Buffalo Grove, IL) equipped with a 40x water immersion objective (NA1.10). Every other brain section throughout the PVH was examined, and four non-overlapping images from each section were randomly taken to cover the PVH. Quantification of colocalization was calculated from the captured images.

Probes for In Situ Hybridization

To generate antisense riboprobes, mouse cDNA sequences for TRH (GenBank accession number X59387, nucletides 308-537), POMC (GenBank accession number NM_008895, nucleotides 150-857), NPY (GenBank accession number NM_023456, nucleotides 74-367), AgRP (GenBank accession number NM_007427, nucleotides 208-603), LepRb (GenBank accession number U46135, nucleotides 3040-3608), Y1R (GenBank accession number AY413582, nucleotides 473-700), and 5HT1B (GenBank accession number NM_010482, nucleotides 672-933) were amplified by PCR and cloned into the pBluscript II KS (-) plasmid (Stratagene, Cedar Creek, TX, USA). The *Bdnf* probe was the mouse *Bdnf* coding region.

Brain Sections and Primary Antibodies for Immunohistochemistry

For neuropeptide immunohistochemistry, $Bdnf^{Lacz/+}$ mice received an ICV injection of colchicine (20 μg/animal, Tocris). Two days after colchicine injection, mice were deeply anaesthetized with avertin and transcardially perfused with phosphate-buffered saline (PBS) and 4% paraformaldehyde (PFA) sequentially. To activate Cre recombinase in mice harboring the $TrkB^{CreERT2/+}$ allele, we intraperitoneally injected the animals with 2 mg of tamoxifen (Sigma-Aldrich) dissolved in corn oil once a day for 5 consecutive days. The mice were perfused with PBS and 4% PFA one week after the injection regimen. Brains were removed, postfixed in 4% PFA overnight, and cryoprotected in 30% sucrose. Coronal brain sections (40 μm) were obtained using a sliding microtome.

The following primary antibodies were used for immunohistochemistry: rabbit anti-β-galactosidase (1:4,000; Cappel), mouse anti-β-galactosidase (1:300; Promega), mouse anti-GFP (1:1,000; Clontech), mouse anti-oxytocin (1:5,000), rabbit anti-CRH (1:500), rabbit anti-vasopressin (1:5,000), goat anti-ChAT (1:200) (Millipore), mouse anti-tyrosine hydroxylase (Sigma), rabbit anti-TRH (1:10,000; generous gift from Dr. M. Wessendorf, University of Minnesota), rabbit anti-somatostatin (1:500), rabbit anti-GHRH (1:500, Immunostar), and rabbit anti-Fluoro-Gold (1:10,000; Fluorochrome).

Primary Antibodies for Immunoblotting

The primary antibodies used for immunoblotting were anti-TH (1:1,000, Millipore), anti-UCP1 (1:1,000, Thermoscientific), and anti-α-tubulin (1:8,000, Sigma-Aldrich).

Quantitative Real Time PCR

Total RNA was isolated from iBAT using Ambion PureLink RNA mini kit combined with Trizol reagent (Life Technologies). First strand cDNA was reverse transcribed using total RNA and Super-Script™ II reverse transcriptase (Life Technologies) according to the manufacture's protocol. Quantitative PCR was performed with SYBR® Green PCR master mix (Roche) using a step-one thermal cycler (Applied Biosystems). Levels of mRNA for UCP1 (forward, tggaggtgtggcagtgttcat; reverse, tgacagtaaatggcaggggac), UCP2 (forward, gcattggcctctacgactctg; reverse, agcggacctttaccacatctg), UCP3 (forward, gacccacggccttctacaaa; reverse, attcccgcagtacctggactt), PGC1α (forward, ggagccgtgaccactgaca; reverse, tggtttgctgcatggttctg), PGC1β (forward, caagctctgacgctctgaagg; reverse, caagctctgacgctctgaagg), FAS (forward, tcgagacacatcgtttgagc; reverse, tcaaaaagtgcatccagcag)

and Lpl (forward, gtggccgagagcgagaac; reverse aagaaggagtaggttttatttgtggaa) were normalized to the 18S rRNA (forward, ccgcagctggaataatgga; reverse, ccctcttaatcatggcctca) in the same sample.

Measurement of Thyroid Hormones and BAT Norepinephrine

Serum free T_3 and TSH levels were measured using an ELISA kit according to the manufacturer's instruction (Nova TeinBio). BAT norepinephrine levels were measured using an ELISA kit (Eagle Biosciences) according to the manufacturer's instruction.

Pseudorabies Virus Injection and Histology

Recombinant pseudorabies virus (PRV-152; 8.5 x 10^8 pfu/ml) was obtained as gifts from Dr. Lynn Enquist, Princeton University). Virus inoculation was performed in a biosafety level 2 operating room. Mice were deeply anesthetized using isoflurane (MAC 4% induction, 2% maintenance) and the target area was shaved and wiped with 10% iodine. A small incision was made to expose iBAT pads. Two 2.5-μl injections of PRV-152 were made into the brown fat on one side using a Hamilton syringe with a 30-gauge needle. The needle was left in place for an additional minute. Injections were made under the dissecting microscope to check for spillage or efflux of virus solution. The tissue was gently swabbed and the incision was closed with 2-0 Vicryl suture. All animals were administered analgesia as needed (buprenorphine 0.01 mg/kg, SC, every 8-12h). Three or four days after injection, mice were deeply anesthetized, perfused and their brains removed for immunohistochemistry to detect PRV-labeled cells using rabbit anti-GFP (1:1,000; Clontech).

Measurement of ChAT Neuron Size

The T1-T3 segment of the spinal cord was removed from perfused animals, cut into 50 μm-thick sections, and collected in 96 well plates. Every 12th spinal cord sections from each animal were processed for ChAT immunohistochemistry. The cell body size of IML ChAT-positive neurons was measured using Neurolucida software (MicroBrightField) under Nikon Eclipse E800 microscope equipped with a motorized stage. All ChAT-positive neurons in spinal cord IML were outlined with the rubber band circle tool, and the area of an outlined region was considered as cell body size. All measurements were done blindly. Two to three spinal cord sections from each animal were used for measurement of cell body size and neuron counting.