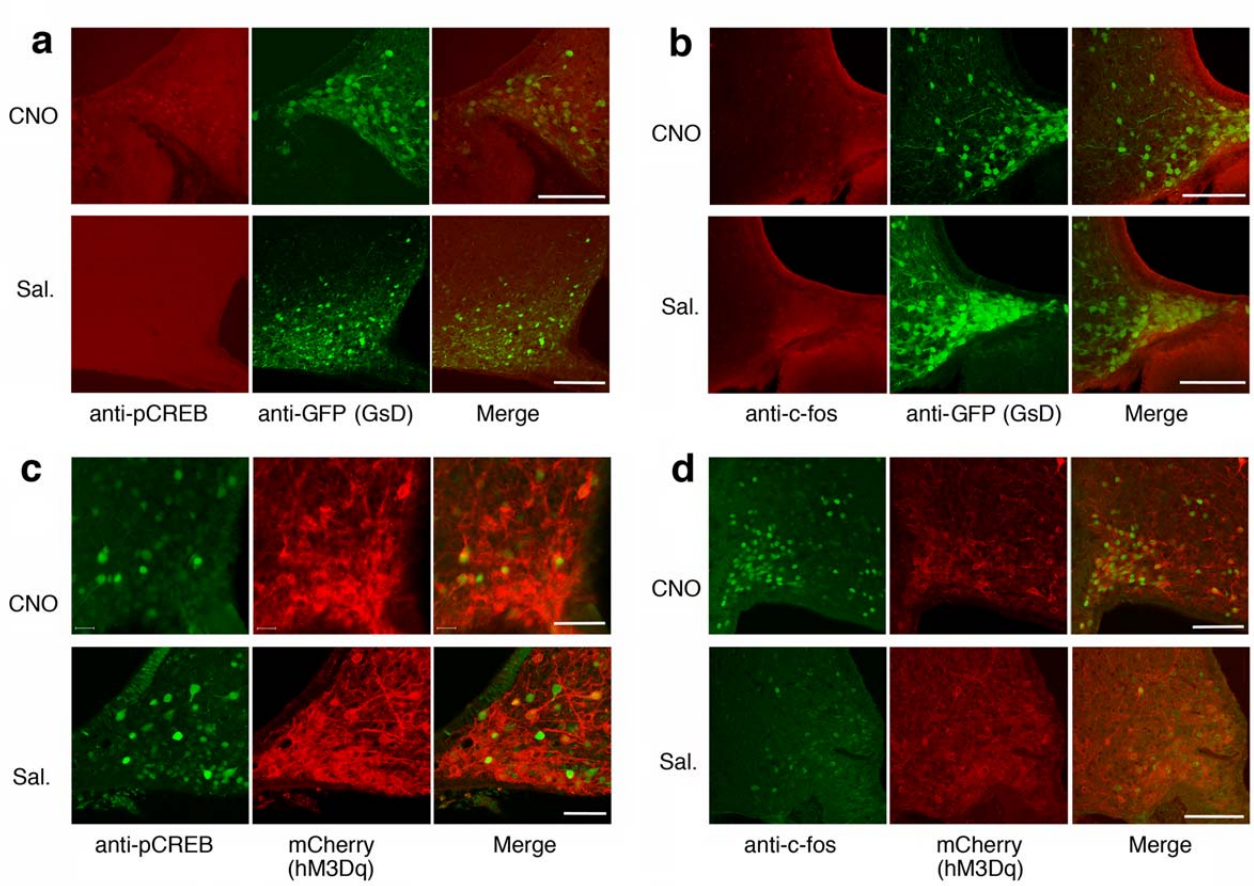
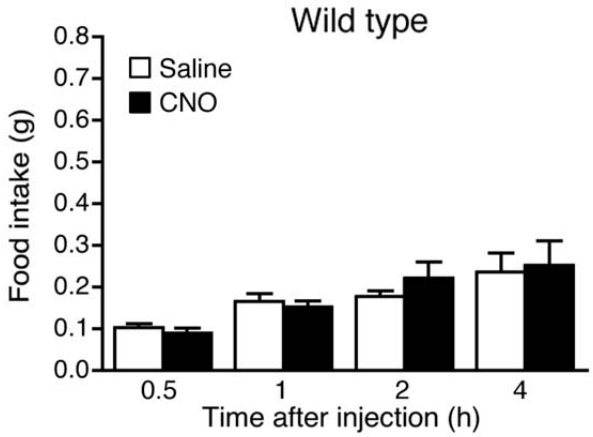
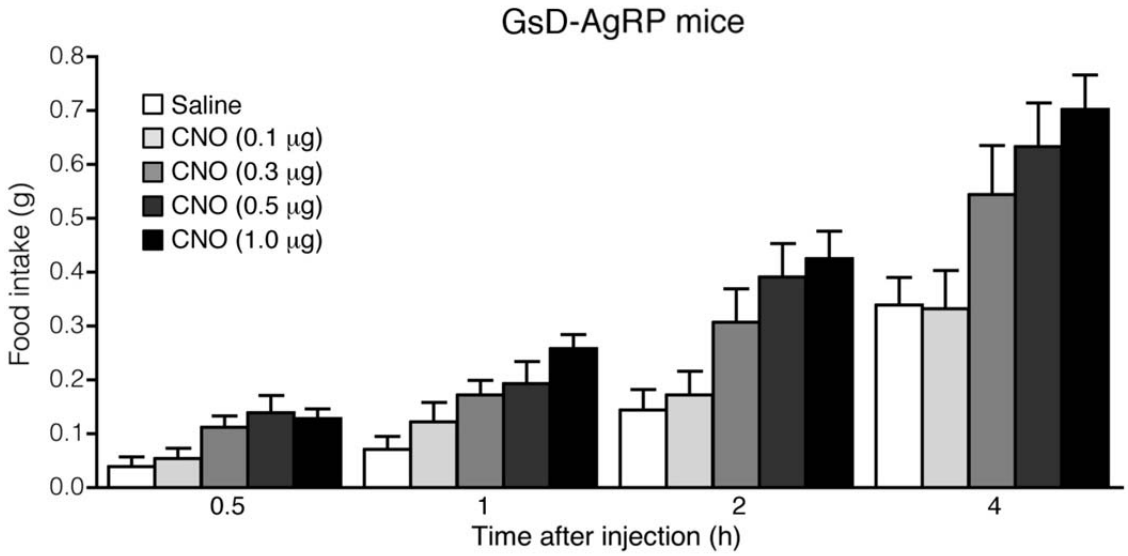


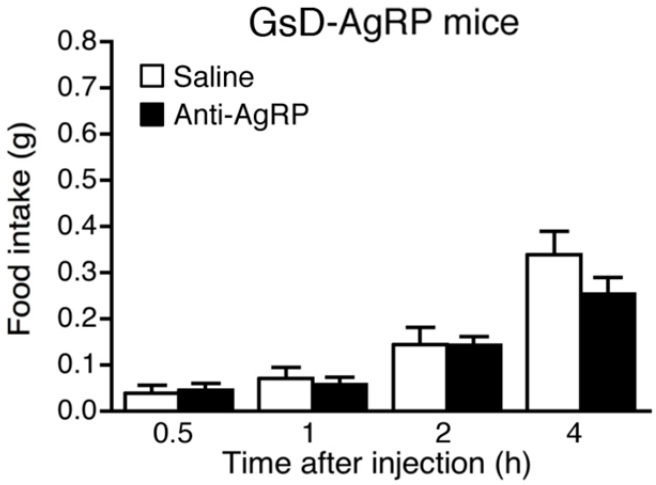
Supplementary Figure 1. CNO activation of the GsD designer receptor selectively promotes pCREB expression in AgRP neurons of the ARC. This figure shows representative low-magnification confocal images. (**a, b**) AAV-mediated functional expression of GsD (GsD-eGFP) in AgRP neurons. pCREB (a) or c-fos (b) expression in AgRP neurons of GsD-AgRP mice was studied after CNO (1 mg kg^{-1}) or saline (control) treatment. pCREB and c-fos expression were revealed by anti-pCREB- and anti-c-fos antibodies, respectively. The GsD DREADD was visualized by an anti-GFP antibody targeted at the eGFP tag fused to the C-terminus of GsD. The images show selective, CNO-mediated induction of pCREB expression in GsD-expressing AgRP neurons. (**c, d**) AAV-mediated functional expression of hM3Dq (hM3Dq-mCherry) in AgRP neurons. pCREB (c) or c-fos (d) expression in AgRP neurons of hM3Dq-AgRP mice was studied after CNO (1 mg kg^{-1}) or saline (control) treatment. pCREB and c-fos expression were revealed by anti-pCREB- and anti-c-fos antibodies, respectively. The hM3Dq DREADD was visualized by mCherry fluorescence (note that this DREADD contained a C-terminal mCherry tag). The images show selective, CNO-mediated induction of c-fos expression in hM3Dq-expressing AgRP neurons. Scale bars: (a, b, d), 100 μm; (c), 50 μm.



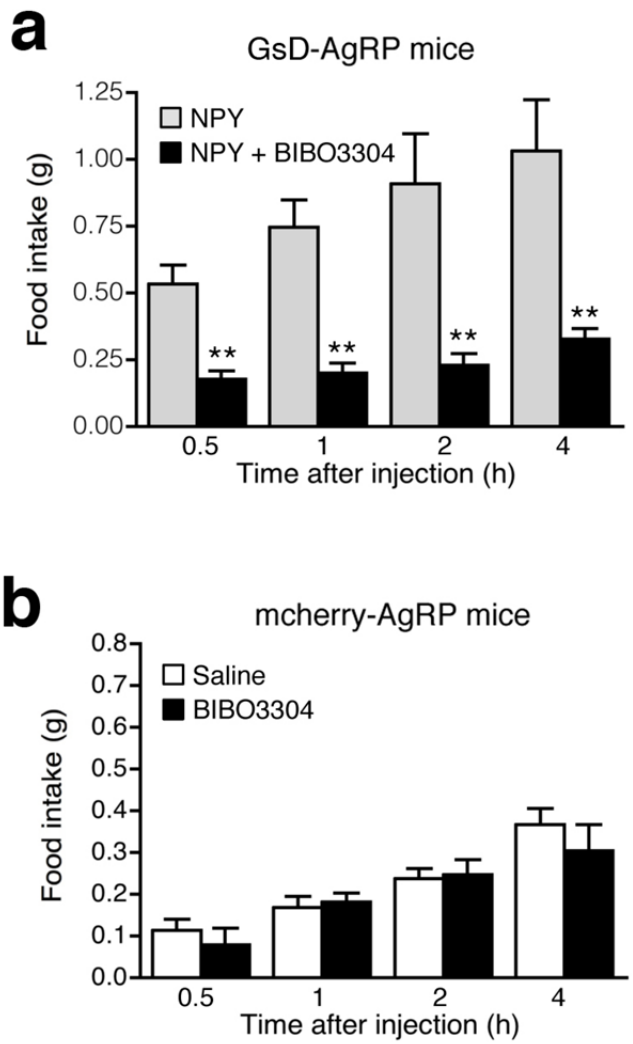
Supplementary Figure 2. CNO has no significant effect on food intake in WT mice. Food intake was measured after a single i.c.v. injection of either saline or CNO (1 μg) for the indicated period of time. Injections were performed between 9 to 10 a.m. Experiments were carried out with WT mice (17-week old males). Data are given as means ± s.e.m. (n=4 or 5 mice per group).



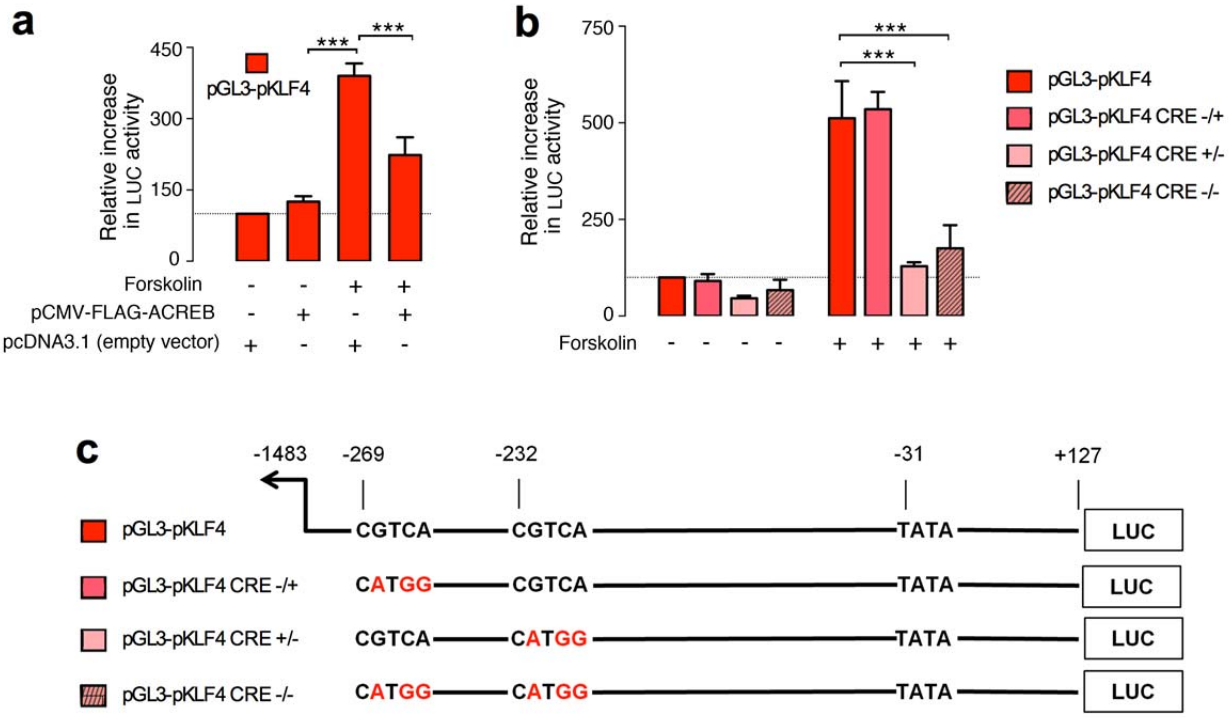
Supplementary Figure 3. CNO promotes food intake in GsD-AgRP mice in a dose-dependent manner. Food intake was measured after a single i.c.v. injection of either saline or CNO (0.1, 0.3, 0.5, or 1 μg) for the indicated time period. Injections were performed between 9 to 10 a.m. Experiments were carried out with male GsD-AgRP mice (10-16 weeks old). Data are given as means ± s.e.m. (n=4-7 mice per group).



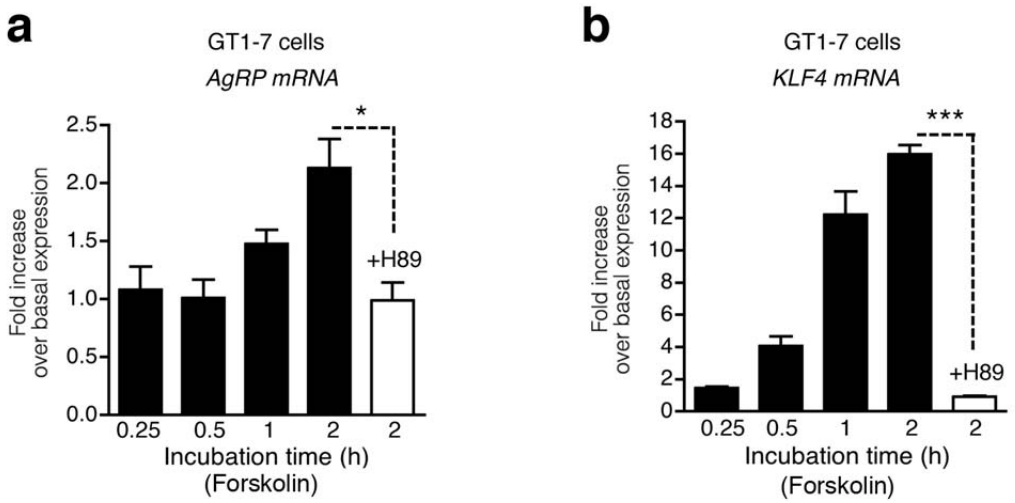
Supplementary Figure 4. In the absence of CNO, an anti-AgRP antibody has no significant effect on food intake in GsD-AgRP mice. Food intake was monitored after a single i.c.v. injection of either saline or an anti-AgRP antibody (1 μg) for the indicated period of time (injection time: 9 to 10 a.m.). Experiments were carried out with male GsD-AgRP mice (24 weeks old) in the absence of CNO. Data are given as means ± s.e.m. (n=6 or 7 mice per group).



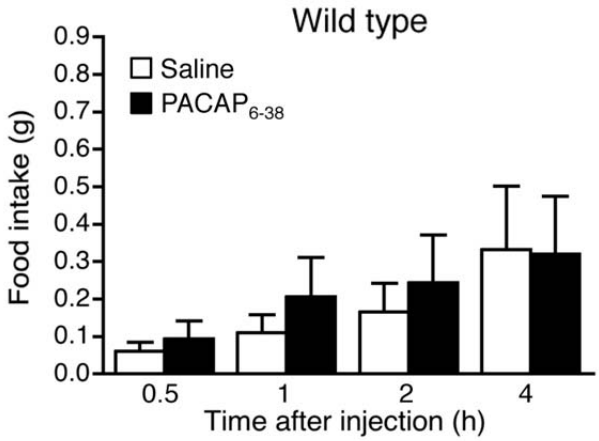
Supplementary Figure 5. An NPY Y_1 receptor blocker prevents NPY-induced increases in food intake. (**a**) GsD-AgRP mice were injected i.c.v. with NPY (0.85 µg) or NPY (0.85 µg) plus BIBO3304 (3.3 µg), a selective NPY Y_1 receptor antagonist (injection time: 9 to 10 a.m.). Food intake was monitored over the indicated period of time (15-week-old males; n=3 or 4 per group). (**b**) Using the same experimental protocol, control mice (mcherry-AgRP mice) were injected i.c.v. with either saline or BIBO3304 (3.3 µg) alone (13-15-week-old males; n=4 or 5 per group). Data are given as means ± s.e.m. **$p<0.01$ as compared to the group injected with NPY alone (Student's *t*-test).



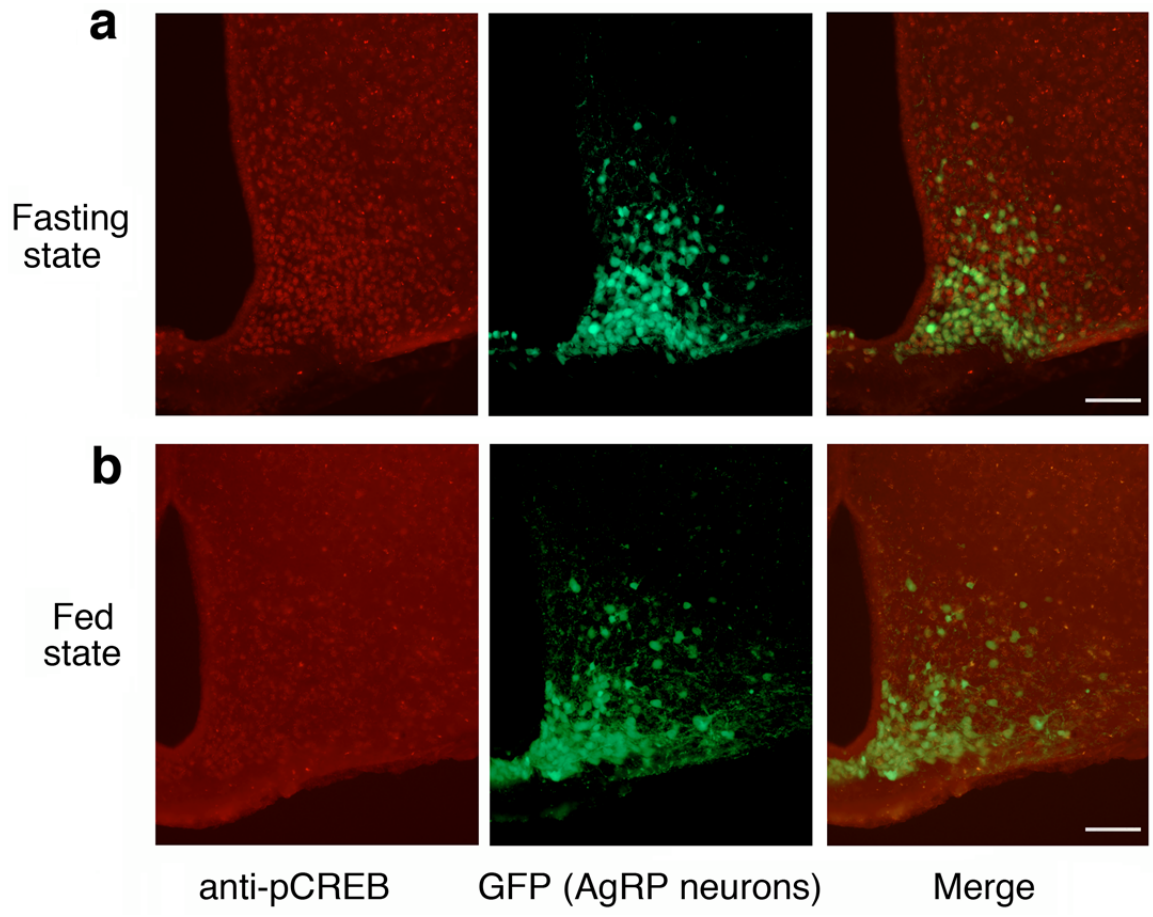
Supplementary Figure 6. The *KLF4* promoter is a direct target of activated CREB. (**a, c**) For *KLF4* promoter-luciferase reporter assays, the pGL3-pKLF4 luciferase reporter construct was expressed in HEK293T cells. Following incubation of transfected cells with forskolin (10 μM) or vehicle (DMSO), luciferase (LUC) activity was determined. Forskolin stimulation resulted in a robust increase of LUC activity in the pGL3-pKLF4-transfected cells. This response was greatly reduced after co-transfection of pGL3-pKLF4 with pCMV-FLAG-ACREB which codes for a dominant negative version of CREB. (**b, c**) The two half CRE sites contained within the *KLF4* promoter were mutated by site-directed mutagenesis. Mutation of the second half CRE site (at -232), but not of the first half CRE site (at -269), almost completely abolished forskolin-mediated stimulation of *KLF4* promoter activity. Data are presented as means ± s.e.m. of three independent experiments each performed in duplicate (***p <0.001; two-way ANOVA, Tukey's multiple comparisons test).



Supplementary Figure 7. Effect of forskolin on *AgRP* and *KLF4* mRNA expression in GT1-7 cells. **(a, b**) GT1-7 cells were incubated with forskolin (10 μM) for the indicated periods of time, followed by measurement of *AgRP* (a) and *KLF4* (b) transcript levels. Please note that addition of the PKA inhibitor, H89 (75 μM), completely suppressed the forskolin-induced increases in *AgRP* and *KLF4* expression. Data are given as means ± s.e.m. (n=4). $p<0.05$*, $p<0.001$***, as compared to the corresponding H89-treated group (Student's *t*-test).



Supplementary Figure 8. $PACAP_{6-38}$ has no significant effect on food intake in WT mice. WT mice (28-week-old males) received a single i.c.v. injection of either saline or $PACAP_{6-38}$ (1.2 μg), a potent PAC_1 receptor antagonist. Food intake was measured after the indicated periods of time. Data are given as means ± s.e.m. (n=4 per group).



Supplementary Figure 9. Fasting-induced increases in pCREB expression in AgRP neurons. This figure shows representative low-magnification confocal images. **(a)** Representative images of hypothalamic sections obtained from *Npy-GFP* mice after a 20 hr fast. pCREB-expressing cells are visualized by an anti-pCREB antibody (red stain, left panel). AgRP (NPY)-positive neurons are visualized by green GFP fluorescence (center panel). The right panel represents a merged image indicating that most AgRP neurons are pCREB-positive. (**b**) Images corresponding to the ones shows in (a) were obtained with freely fed *Npy-GFP* mice, indicating a striking reduction in pCREB-positive AgRP neurons. Scale bars, 100 μm.

Supplementary Table 1

Functional expression of the GsD designer receptor containing a C-terminal eGFP tag in COS-7 cells

DREADD	[^{3}H]NMS binding		CNO-induced cAMP accumulation		
	K_D	B_{max}	EC_{50}	Basal cAMP	E_{max} cAMP
	nM	*nmol/mg of protein*	*nM*	*pmol/well*	*pmol/well*
GsD	1.5 ± 0.1	11.7 ± 1.7	4.2 ± 1.4	2.2 ± 0.5	17. 1 ± 2.4
GsD-eGFP	2.1 ± 0.2	16. 2 ± 2.6	4.9 ± 1.6	1.1 ± 0.1	8.6 ± 1.3

[^{3}H]NMS saturation binding studies and cAMP assays were carried out with transiently transfected COS-7 cells, as described by Guettier et al. [16]. Data are given as means ± s.e.m. of three independent experiments carried out in duplicate. No significant [^{3}H]NMS binding or CNO-induced cAMP accumulation was observed with control cells transfected with empty vector DNA.

Supplementary Table 2

Summary of antibodies used for immunohistochemical studies

Antibody target	**Source of antibody**	**Catalog #**	**Dilution**
GFP	Abcam, Cambridge, MA	ab6662	1:400
AgRP	Phoenix Pharmaceuticals, Burlingame, CA	H-003-57	1:1,000
c-fos	Abcam, Cambridge, MA	ab99515	1:500
RFP (mcherry)	Abcam, Cambridge, MA	ab34764	1:400
pCREB	Cell Signaling Technology, Danvers, MA	9198	1:500
KLF4	R&D Systems, Minneapolis, MN	AF3640	1:200
HA	Cell Signaling Technology, Danvers, MA	3724	1:500
Anti-rabbit IgG, Alexa Fluor® 594-conjugated secondary antibody	Abcam, Cambridge, MA	ab150064	1:1,000
Anti-rabbit IgG, FITC conjugated secondary antibody	Life Technologies, Frederick, MD	A11008	1:1,000
Anti-goat IgG, Alexa Fluor® 488-conjugated secondary antibody	Abcam, Cambridge, MA	ab150137	1:1,000
Anti-goat IgG, Alexa Fluor® 594-conjugated secondary antibody	Life Technologies, Frederick, MD	A11058	1:1000

Supplementary Table 3

Summary of PCR primers used for qRT-PCR experiments

Mouse gene	**Primer sequence**	**Amplicon (bp)**
β-actin	Forward: 5' CTAAGGCCAACCGTGAAAAG Reverse: 5' ACCAGAGGCATACAGGGACA	104
AgRP	Forward: 5' TTTGTCCTCTGAAGCTGTATGC Reverse: 5' GCATGAGGTGCCTCCCTA	86
KLF4	Forward: 5' CACACAGGCGAGAAACCTTACC Reverse: 5' CGGAGCGGGCGAATTT	64
NPY	Forward: 5' CCGCTCTGCGACACTACAT Reverse: 5' TGTCTCAGGGCTGGATCTCT	68