SUPPLEMENTARY DATA

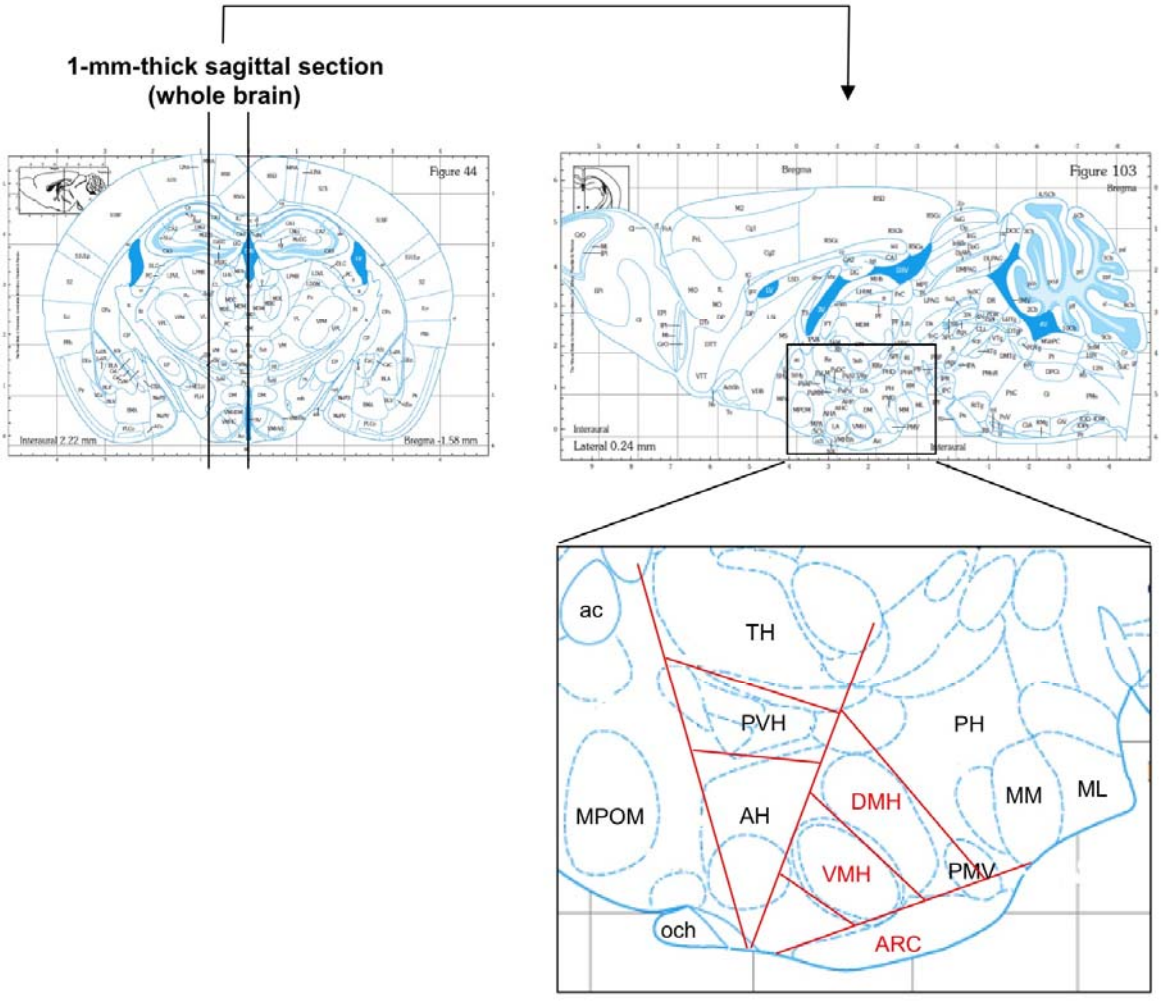
Supplementary Table 1. Body weight and plasma insulin concentration during basal and clamp periods for the mice studied in Figures 1 to 6.

	Body weight (g)	Plasma insulin (ng/ml)	
		Basal (t = 0 min)	Clamp (t = 105 min)
Figure 1			
Saline in VMH	29.9 ± 1.0	1.09 ± 0.12	1.74 ± 0.18*
Leptin in VMH	29.9 ± 0.6	0.94 ± 0.10	1.57 ± 0.16*
Figures 2 and 3			
(Saline + DMSO) in VMH	29.9 ± 0.5	1.00 ± 0.14	1.78 ± 0.16*
(Leptin + DMSO) in VMH	30.0 ± 0.5	0.95 ± 0.25	1.55 ± 0.11*
(Saline + MEK inhibitor) in VMH	29.9 ± 0.6	1.11 ± 0.10	1.81 ± 0.25*
(Leptin + MEK inhibitor) in VMH	30.6 ± 0.5	1.13 ± 0.19	1.94 ± 0.18*
(Saline + STAT3 inhibitor) in VMH	29.7 ± 0.4	0.86 ± 0.12	1.72 ± 0.19*
(Leptin + STAT3 inhibitor) in VMH	29.8 ± 0.7	1.04 ± 0.07	1.86 ± 0.31*
(Saline + PI3K inhibitor) in VMH	31.2 ± 0.6	1.07 ± 0.28	1.87 ± 0.28*
(Leptin + PI3K inhibitor) in VMH	30.6 ± 0.4	1.18 ± 0.21	1.76 ± 0.21*
Figure 4			
Saline i.p. + DMSO in VMH	30.0 ± 0.9	1.00 ± 0.14	1.78 ± 0.16*
Saline i.p. + MEK inhibitor in VMH	29.6 ± 0.8	0.90 ± 0.14	1.78 ± 0.16*
Leptin i.p. + DMSO in VMH	29.6 ± 0.8	0.72 ± 0.10	1.82 ± 0.78*
Leptin i.p. + MEK inhibitor in VMH	29.8 ± 0.4	0.71 ± 0.07	2.00 ± 0.57*
Figure 5			
(Saline + Saline) in VMH	29.9 ± 1.0	1.00 ± 0.14	1.78 ± 0.16*
(Saline + SHU9119) in VMH	29.9 ± 0.6	1.15 ± 0.17	2.66 ± 0.27*
(Leptin + Saline) in VMH	29.9 ± 0.6	0.95 ± 0.25	1.55 ± 0.11*
(Leptin + SHU9119) in VMH	30.1 ± 0.9	0.98 ± 0.37	2.28 ± 0.81*
Figure 6			
(Saline + DMSO) in VMH	30.0 ± 0.7	1.00 ± 0.14	1.78 ± 0.16*
(Saline + MEK inhibitor) in VMH	29.6 ± 0.5	1.00 ± 0.14	1.78 ± 0.16*
(MT-II + DMSO) in VMH	31.1 ± 1.0	0.90 ± 0.11	1.99 ± 0.21*
(MT-II + MEK inhibitor) in VMH	30.6 ± 0.6	0.80 ± 0.21	2.17 ± 0.42*

*P < 0.05 versus corresponding value for the basal period

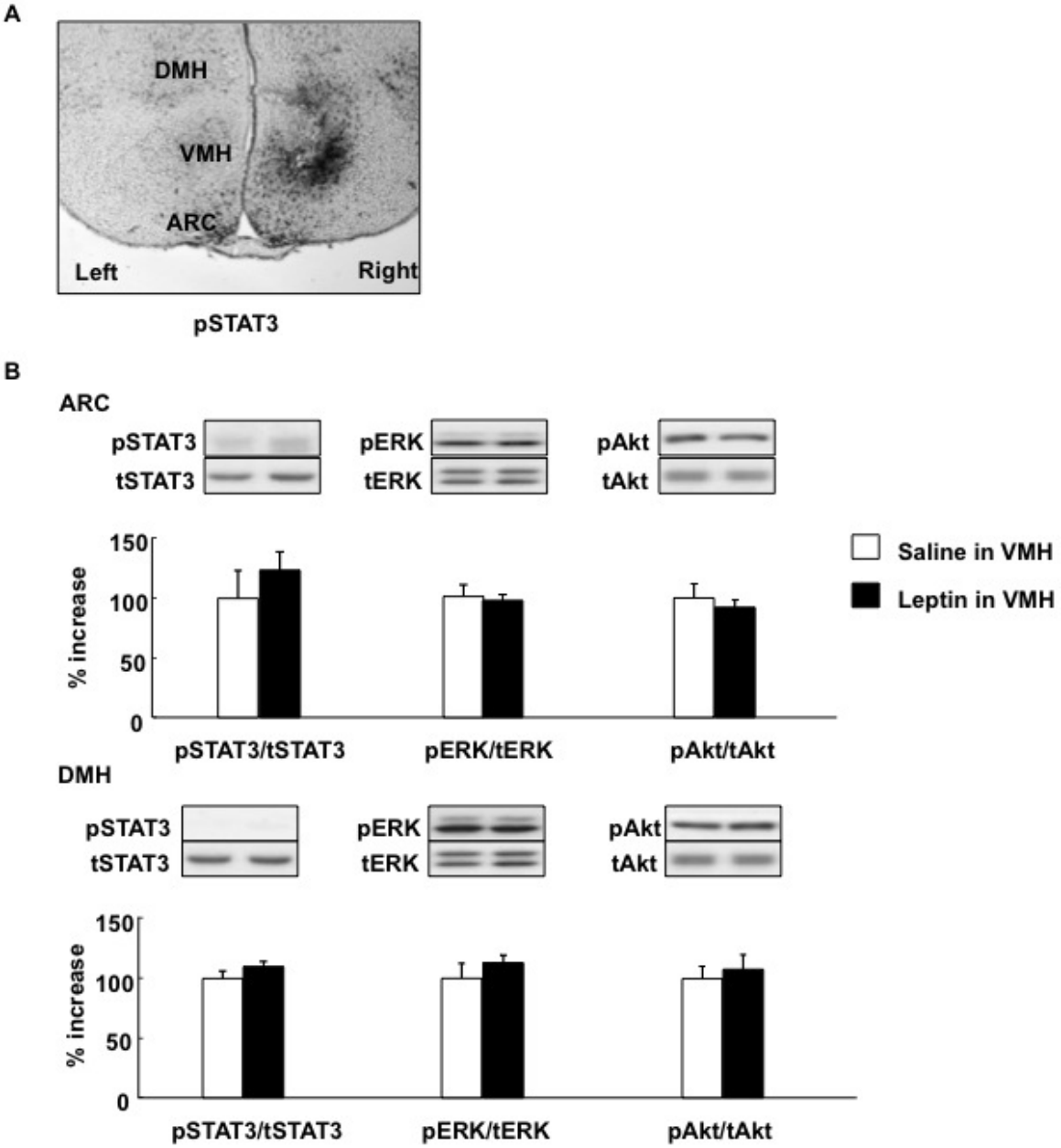
SUPPLEMENTARY DATA

Supplementary Figure 1. Sampling of medial hypothalamic nuclei. The hypothalamus present in 1-mm-thick sagittal sections of the entire mouse brain was dissected into the ARC, VMH, and DMH along the indicated red lines. The ARC was isolated as the ventral portion of the medial hypothalamus with a dorsal margin of the border with the ventral part of the VMH and DMH. The VMH and DMH were collected from the triangular area with an anterior-dorsal margin of the white matter separating the PVH and the anterior hypothalamus from the VMH-DMH, a ventral margin of the border with the ARC, and a posterior margin of the border with the mammillary body. ac, anterior commissure; MPOM, medial preoptic nucleus, medial part; TH, thalamus; AH, anterior hypothalamic area; och, optic chiasm; PH, posterior hypothalamic nucleus; PMV, premammillary nucleus, ventral part; MM, medial mammillary nucleus, medial part; ML, medial mammillary nucleus, lateral part. Modified with permission from: Franklin KBJ, Paxinos G. *The Mouse Brain in Stereotaxic Coordinates*. 3rd ed. San Diego, CA, Academic Press, 2007.



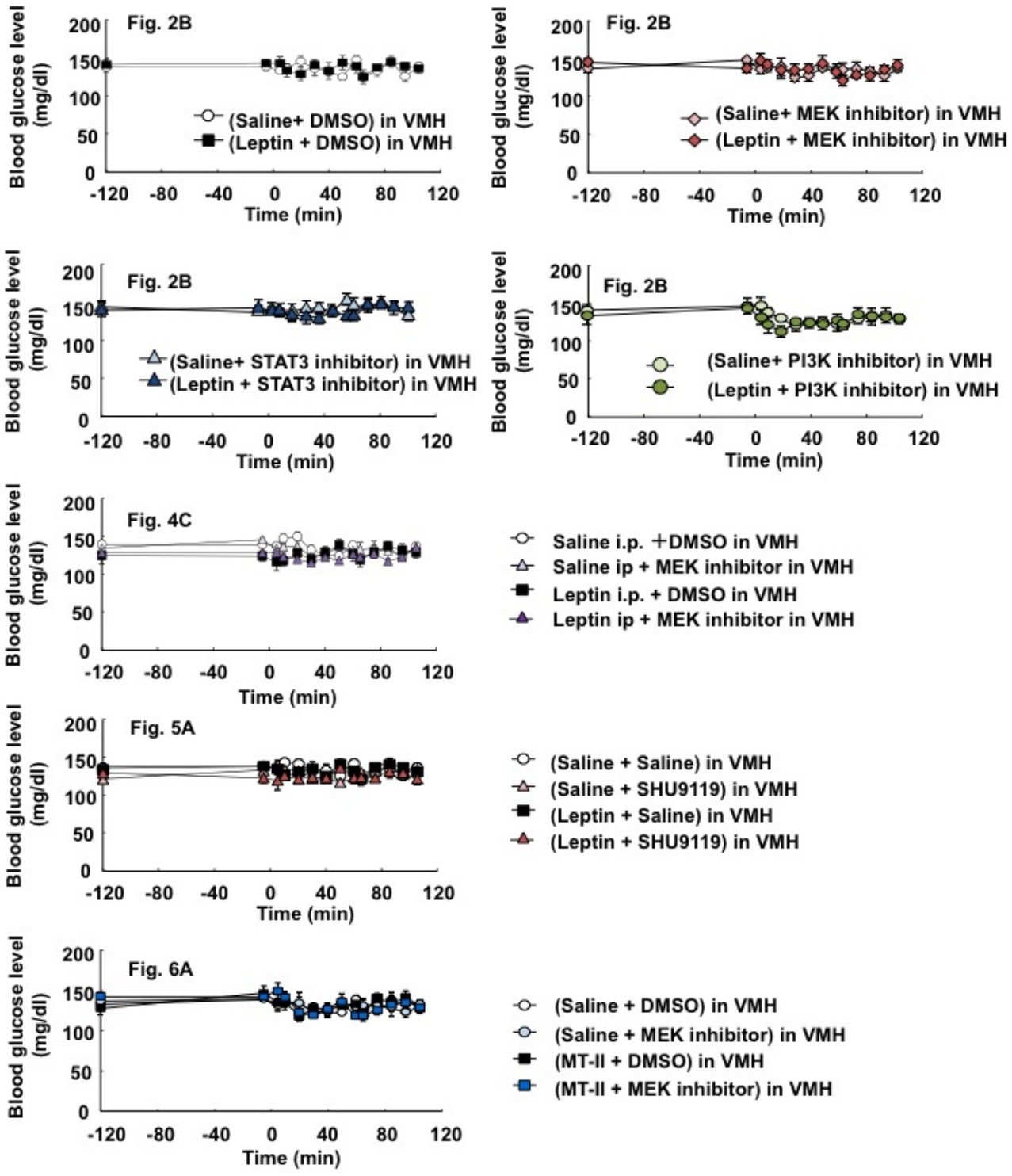
SUPPLEMENTARY DATA

Supplementary Figure 2. (A) Representative immunohistochemical analysis of the phosphorylation of STAT3 (on Tyr^{705}) in the hypothalamus at 30 min after injection of leptin into the right VMH. Phosphorylation of STAT3 was observed in the middle and dorsomedial regions of the right VMH. The left VMH remained intact without injection. The phosphorylation of STAT3 was visualized with rabbit polyclonal antibodies to Tyr^{705}-phosphorylated STAT3 (dilution of 1:1000) (Cell Signaling Technology) and with a Vectastain ABC kit (Vector Laboratories, Burlingame, CA). (B) Phosphorylation of STAT3 (Tyr^{705}), ERK (Thr^{202}/Tyr^{204}), and Akt (Ser^{473}) in the ARC and DMH at 30 min after unilateral injection of leptin or saline into the VMH. ARC and DMH samples were obtained from the mice studied in Figure 1B. The results were evaluated with the ratio of phosphorylated form to the total protein, and expressed as percent (%) increase of the ratio to that of saline-injected group. Representative immunoblots with antibodies to the phosphorylated (p) or total (t) forms of the proteins are shown above the quantitative data, which are presented as means ± SEM (n = 6 or 7 mice).



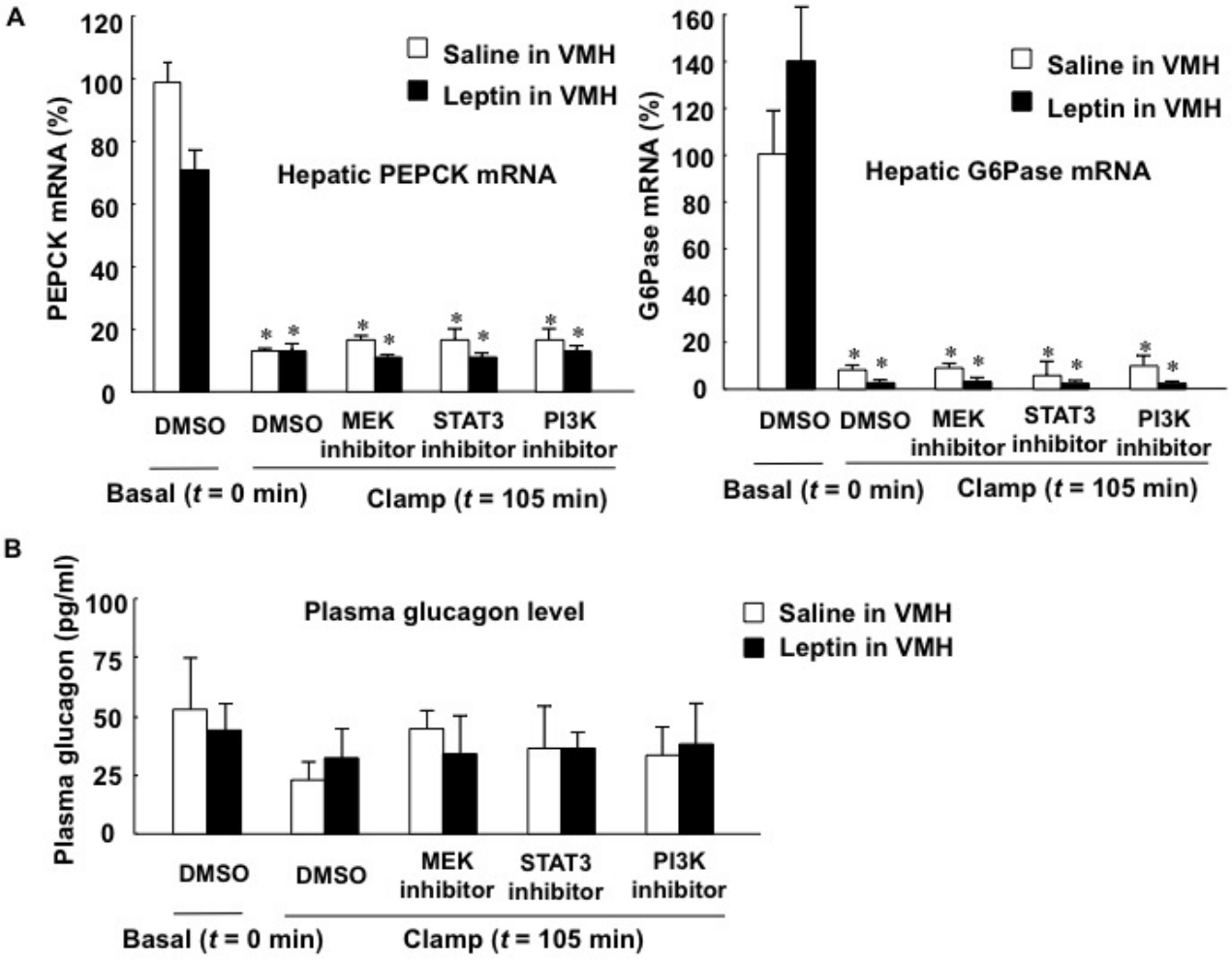
SUPPLEMENTARY DATA

Supplementary Figure 3. Blood glucose concentrations during basal and clamp periods for the mice studied in the indicated figures. The clamp period begins at time 0. Data are means ± SEM (*n* = 6 or 7 mice).



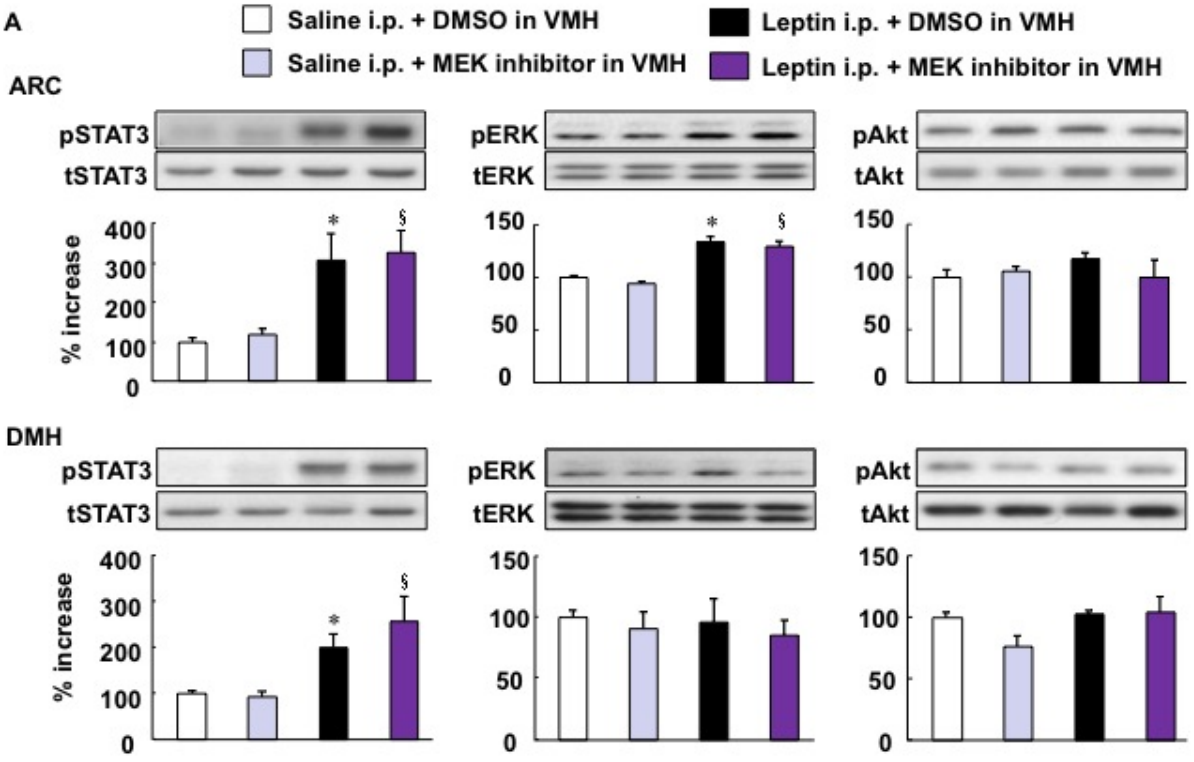
SUPPLEMENTARY DATA

Supplementary Figure 4. Effects of injection of leptin and various inhibitors on the hepatic abundance of PEPCK and G6Pase mRNAs and on plasma glucagon concentration. (A) RT and real-time PCR analysis of PEPCK and G6Pase mRNA abundance in the liver during the basal and clamp periods for mice studied in Figures 2 and 3. The normalized values are expressed relative to the corresponding value for the basal period in mice injected with DMSO followed by saline. *$P < 0.05$ versus the corresponding value for the basal period in DMSO- and saline-injected mice. (B) Plasma glucagon levels during the basal and clamp periods for mice studied in Figures 2 and 3. All data are means ± SEM (n = 6 or 7 mice).



SUPPLEMENTARY DATA

Supplementary Figure 5. Effects of i.p. injection of leptin and prior injection of the MEK inhibitor U0126 into the VMH on phosphorylation of STAT3, ERK, and Akt in the ARC and DMH, and immunohistofluorescence analysis of phosphorylated forms of STAT3 and ERK in the ARC. (A) The effects of bilateral injection of the MEK inhibitor U0126 or DMSO into the VMH at 1 h before i.p. injection of leptin or saline on phosphorylation of STAT3 (Tyr^{705}), ERK (Thr^{202}/Tyr^{204}), and Akt (Ser^{473}) in the ARC and DMH were determined. ARC and DMH samples were obtained 1 h after leptin injection from the mice studied in Figure 4A. The results were evaluated with the ratio of phosphorylated form to the total protein, and expressed as percent (%) increase of the ratio to that of saline-injected group. Representative immunoblots with antibodies to phosphorylated (p) or total (t) forms of the proteins are shown above the quantitative data, which are means ± SEM (n = 6 or 7 mice). *P < 0.05 versus Saline i.p. + DMSO in VMH. §P < 0.05 versus corresponding value for Saline i.p. + MEK inhibitor in VMH.
(B) Representative immunofluorescence analysis of phosphorylated forms of STAT3 (Tyr^{705}) and ERK (Thr^{202}/Tyr^{204}) in the ARC at 1 h after i.p. injection of saline (b, d, f) or leptin (c, e, g). The dashed line in panel a, which is modified with permission from Hof et al. (2000) (40), shows the ARC and corresponds to the area in panels b through g. Arrows indicate neurons that are positive for both pSTAT3 and pERK. 3V, third ventricle. Scale bars, 20 μm.



SUPPLEMENTARY DATA