hours before insulin (4.4 mU/2 µl) or saline injection. Values represent the mean ± SEM for 9-10 animals per group.

Fig. 4. Changes in hypothalamic mRNA levels for POMC (A), NT (B), NPY (C) and AgRP (D) following DMSO + insulin or cilostamide + insulin. Mice fasted for 24 hours were injected with cilostamide (10 µg/µl) or DMSO (control), followed 40 min later, with icv insulin (4.4 mU/2 µl) or saline. Two hours later the medial basal hypothalamus was harvested to measure *Pomc, NTs, Npy* and *Agrp* mRNA levels by qPCR. Values represent the mean ± SEM for 5 animals per group.

Supplementary Figures

Supplementary Fig. S1. Effects of peripheral injection of insulin or leptin on phosphorylation of Akt at Ser^{473} (A, B) and phosphorylation of STAT3 at Tyr^{705} (C, D) in the hypothalamus. A, Representative p-Akt and Akt Western blots obtained from the hypothalamic extract. B, Densitometric analysis of the immunoreactive bands for p-Akt. C, Representative p-STAT3 and STAT3 Western blots obtained from the hypothalamic extract. D, Densitometric analysis of the immunoreactive bands for p-STAT3. The values for p-Akt and p-STAT3 were first calculated as ratio of Akt and p-STAT3, respectively, and then expressed as relative to saline (control) group. Values represent the mean ± SEM for 6-8 animals per group. * $p < 0.05$, ** $p < 0.01$ vs saline control group. Sal

= saline, Ins = insulin, Lept = leptin.

Supplementary Fig. S2. Changes in insulin- or leptin-induced p-Akt^{ser473}- and p-STAT3^{tyr705}-positive cells in the hypothalamus following peripheral injection of insulin or leptin at 30 min post-injection. Upper Panel: Representative micrographs of p-Akt^{ser473} ICC of hypothalamic sections from saline, insulin or leptin–treated mice. Lower Panel: Representative micrographs of p-STAT3^{tyr705} ICC of hypothalamic sections from saline, insulin or leptin–treated mice. Mice were fasted overnight and injected with insulin (15 IU/kg, ip), leptin (3.5 mg/kg, ip) or saline and killed 30 min later. Images are representative sections at approximately bregma -1.82 to -1.9 in relation to the mouse brain atlas by Paxinos and Franklin [86]. Scale bars = 100μm. ME, Median eminence; ARC, arcuate nucleus; VMN, ventromedial nucleus; DMN, dorsomedial nucleus, 3v, third ventricle.

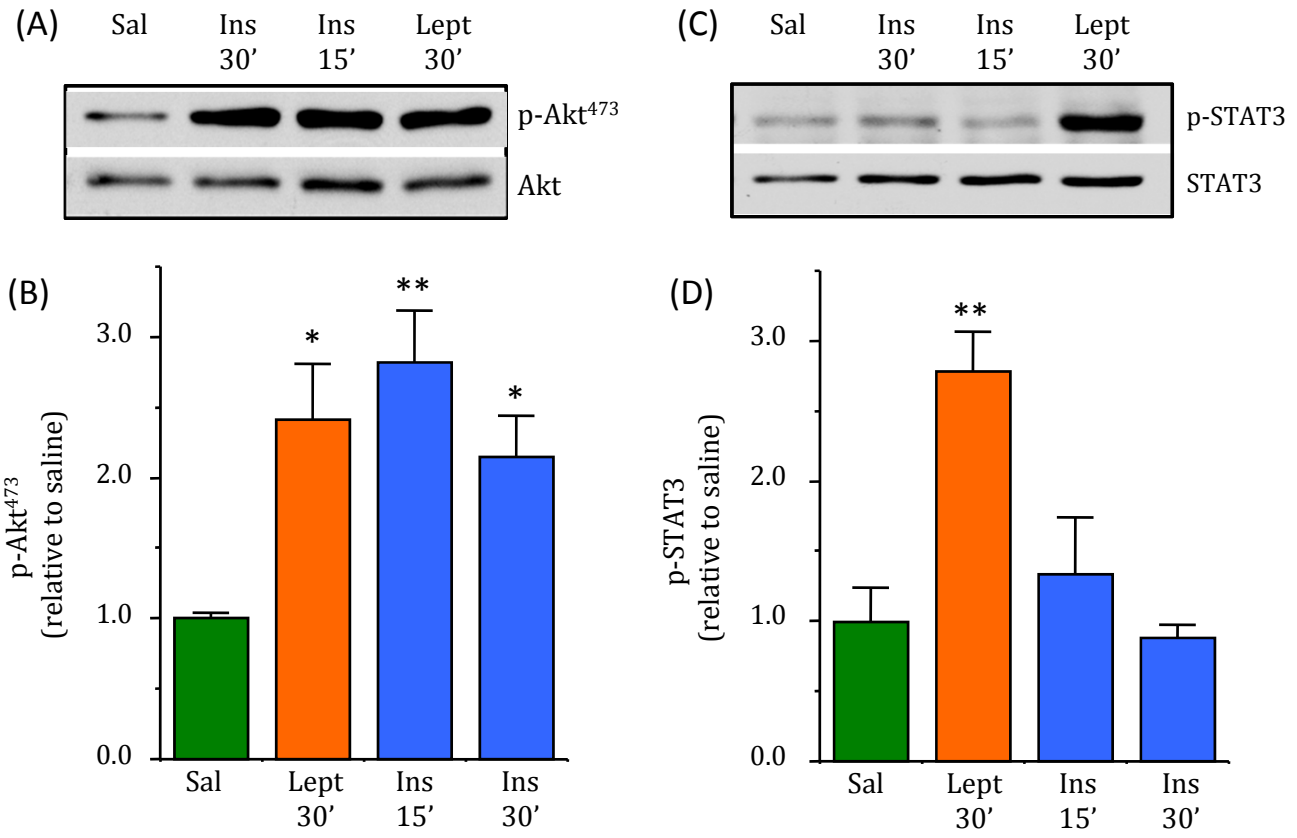
Supplementary Fig. S3. Effects of central injection of insulin or leptin on phosphorylation of Akt at Ser^{473} (A, B) and phosphorylation of STAT3 at Tyr^{705} (C, D) in the hypothalamus. A, Representative p-Akt and Akt Western blots obtained from the hypothalamic extract. B, Densitometric analysis of the immunoreactive bands for p-Akt. C, Representative p-STAT3 and STAT3 Western blots obtained from the hypothalamic extract. D, Densitometric analysis of the immunoreactive bands for p-STAT3. The values for p-Akt and p-STAT3 were first calculated as ratio of Akt and p-STAT3, respectively, and then

expressed as relative to saline (control) group. Values represent the mean ± SEM for 6-8 animals per group. * $p < 0.05$, ** $p < 0.01$ vs saline control group.

Supplementary Fig. S4. Changes in insulin- or leptin-induced p-Akt^{ser473}- and p-STAT3^{tyr705}-positive cells in the hypothalamus following central injection of insulin or leptin at 30 min post-injection. Upper Panel: Representative micrographs of p-Akt^{ser473} ICC of hypothalamic sections from saline, insulin or leptin–treated mice. Lower Panel: Representative micrographs of p-STAT3^{tyr705} ICC of hypothalamic sections from saline, insulin or leptin–treated mice. Mice were fasted overnight and injected with insulin (4.4 mU), leptin (4 µg/2µl, ip) or saline (2µl) and killed 30 min later. Images are representative sections at approximately bregma -1.82 to -2.00 in relation to the mouse brain atlas by Paxinos and Franklin [84]. Scale bars = 100µm. ME, Median eminence; ARC, arcuate nucleus; VMN, ventromedial nucleus; DMN, dorsomedial nucleus, 3v, third ventricle.

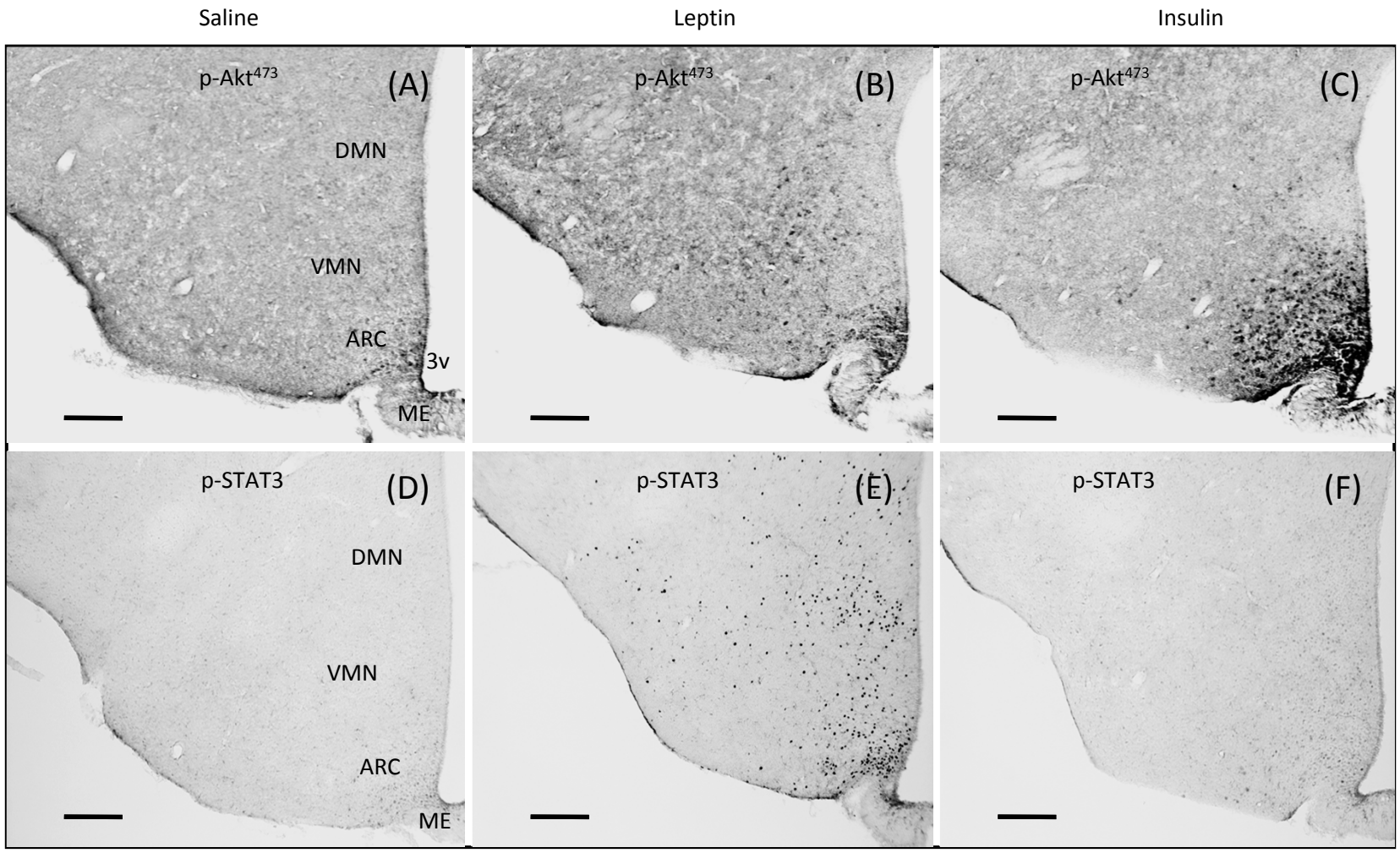
Supplementary Fig. S5. Changes in the number of p-Akt^{ser473}–positive cells in the arcuate (ARC), ventromedial nucleus (VMN) and dorsomedial nucleus (DMN) in the hypothalamus of mice following intra-peritoneal (ip) or intracerebroventricular (icv) injection of leptin, insulin or saline (control). One of five series from each animal was analyzed. All p-Akt^{ser473}–positive cells were counted in three or four evenly-matched sections through the ARC regions (Bregma approximately -1.7 to -2.06) of individual animals and expressed as the number per section. Values represent the mean ± SEM for 3 animals per group. Note that the vertical scales

in panels A and B are different. * $p < 0.05$, ** $p < 0.01$ vs saline control group; a $p < 0.01$ vs leptin group.



Supplementary Figure 1
M. Sahu et al

Effect of peripheral insulin or leptin injection on p-Akt^{473} and p-STAT3 in the hypothalamus



Supplementary Figure 2
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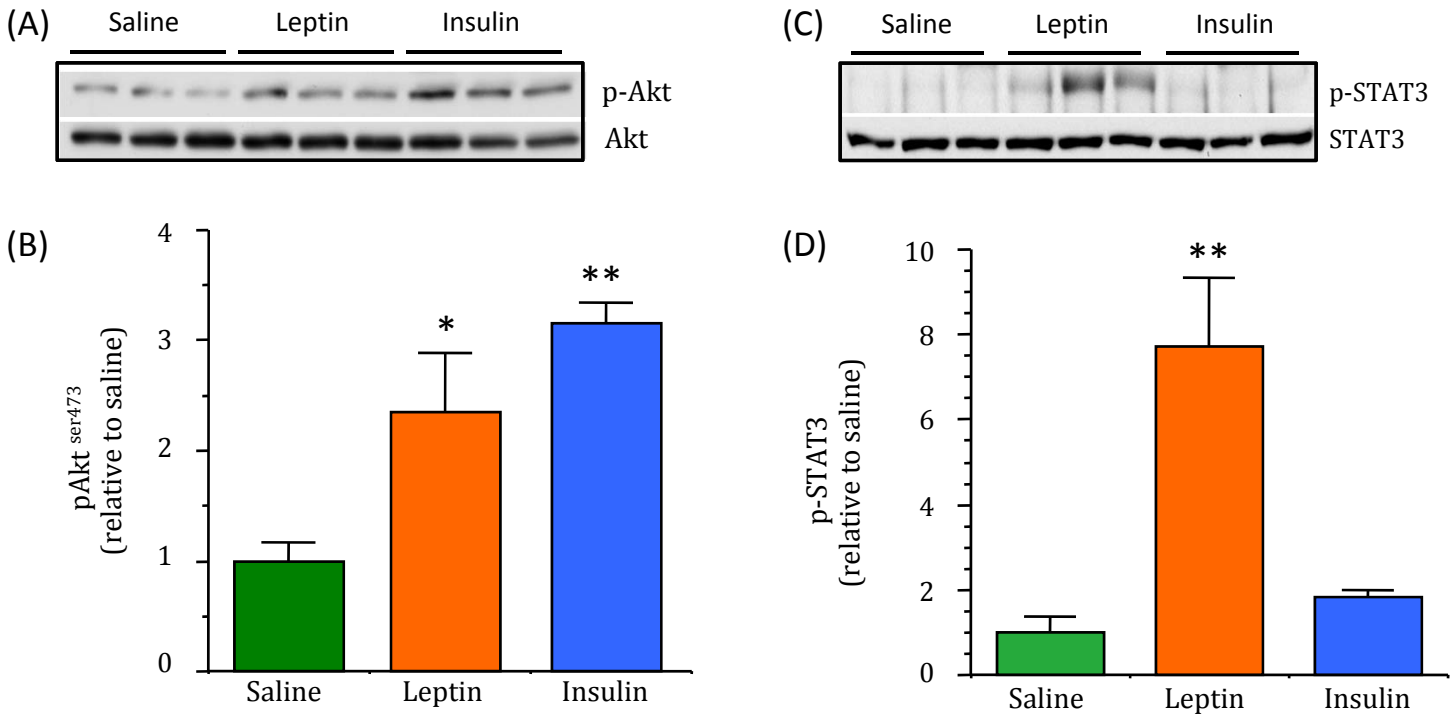
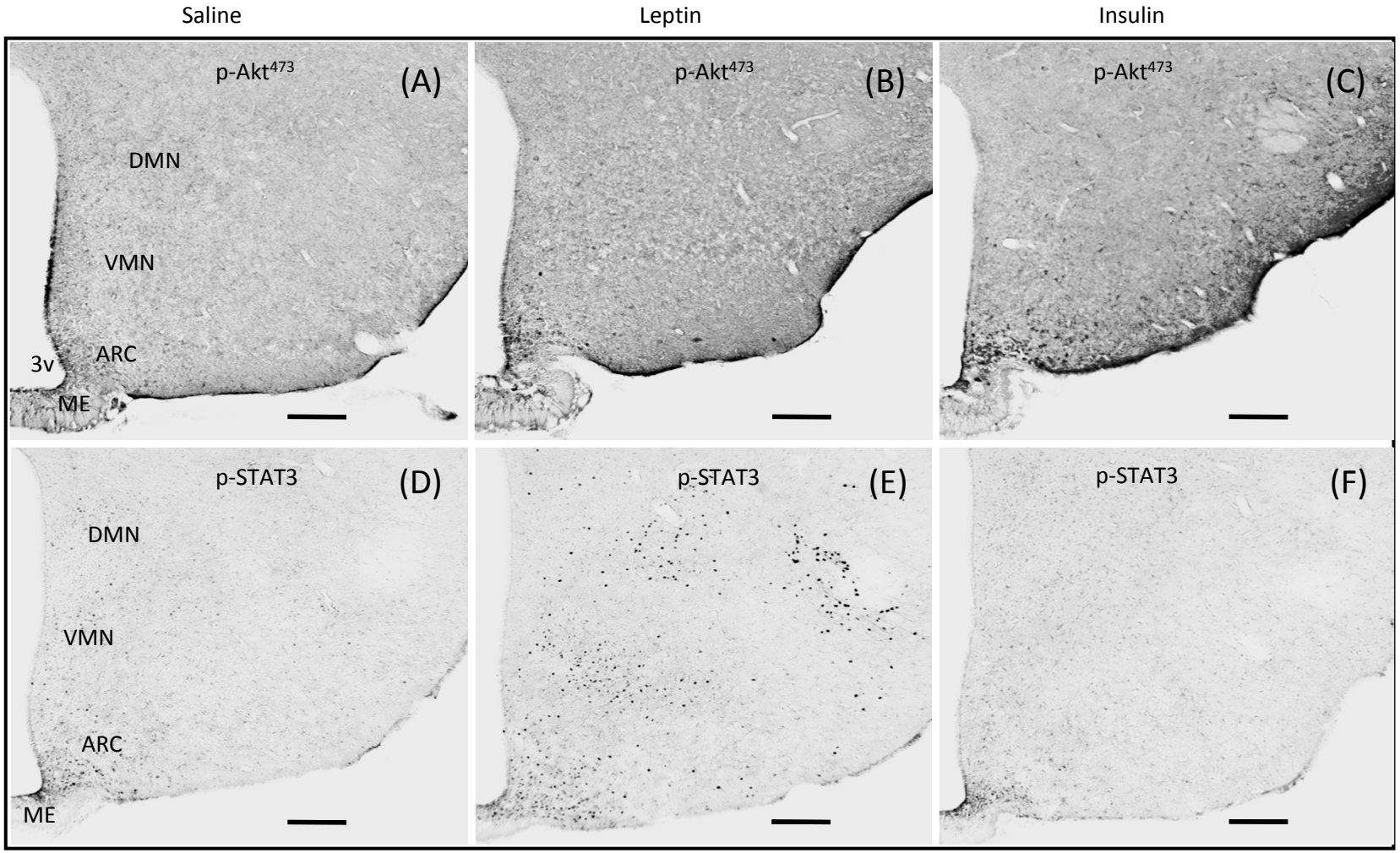


Supplementary Figure 3
M. Sahu et al

Effect of central insulin or leptin injection on p-Akt^{473} and p-STAT3 in the hypothalamus



Supplementary Figure 4
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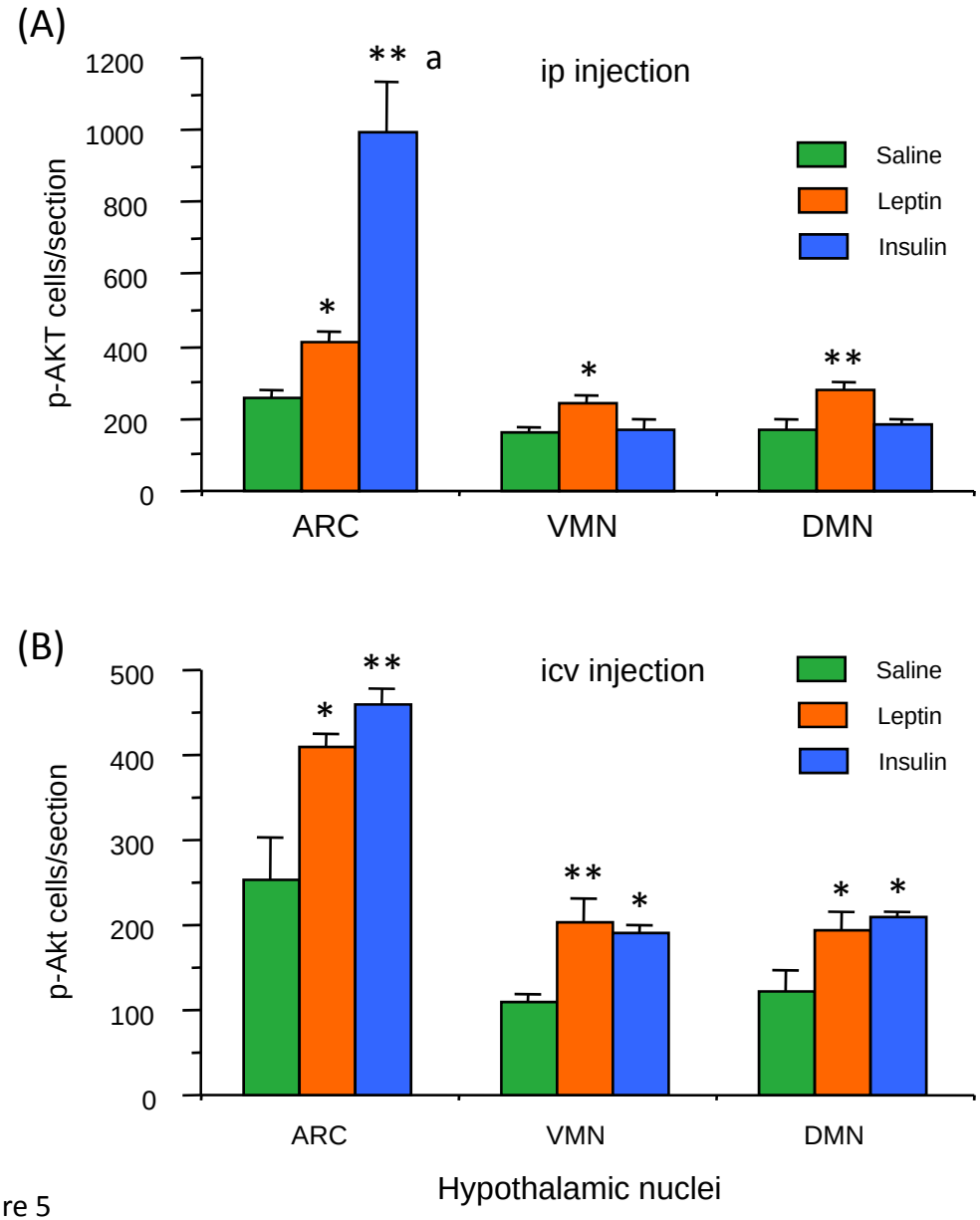

Supplementary Figure 5
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