Supplementary Fig. 1. Leptin activates PI3K in rat hypothalamus. Rats fasted for 24 hr were injected ICV with 4 μg of leptin or vehicle (CSF) and 45 min later, the mediobasal hypothalamus (MBH) were processed for the assay of PI3-Kinase activity. An aliquot of the hypothalamic homogenate (~1 mg) was pre-cleared with 40 μl of 50% protein-G-agarose conjugate for 30 min before incubated under shaking with an antibody against IRS-1 (Upstate Biotechnology, NY) overnight at 4°C. The assay of PI3-kinase activity associated with the IRS-1 immunoprecipitate and subsequent thin-layer chromatograph were carried out according to a previously described protocol (Avanti Polar Lipids, AL) with phosphatidylinositol as a substrate in the presence of [γ-32 P] ATP. The phosphatidylinositol 3-phosphate (PI-P₃) and PI-P₂ products were quantitated using densitometer and NIH Image 1.6 software (lower panel). The results in lower panel are shown as mean ± s.e.m. for three animals in each group. *P < 0.05 versus vehicle treated group.