## **Supplementary Information**

Leptin induced GRP78 expression through the PI3K-mTOR pathway

in neuronal cells

Mina Thon<sup>1</sup>, Toru Hosoi<sup>1</sup>\*, Michiko Yoshii<sup>1</sup>, Koichiro Ozawa<sup>1</sup>\*

<sup>1</sup>Department of Pharmacotherapy, Graduate School of Biomedical and Health

Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551,

Japan

\*Correspondence to: Koichiro Ozawa (e-mail: ozawak@hiroshima-u.ac.jp) or

Toru Hosoi (e-mail: toruh@hiroshima-u.ac.jp), Department of Pharmacotherapy,

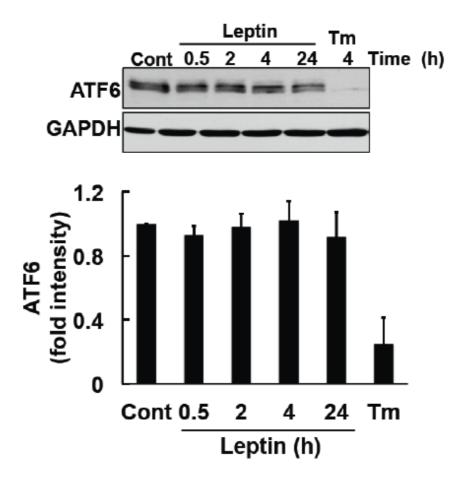
Graduate School of Biomedical and Health Sciences, Hiroshima University, 1-2-3

Kasumi, Minami-ku, Hiroshima 734-8551, Japan. TEL: +81-82-257-5338

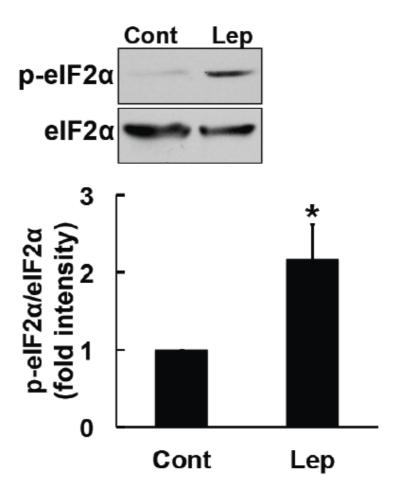
Supplementary information includes:

Supplementary Figures S1 to S4

## **Supplementary Figure 1.**

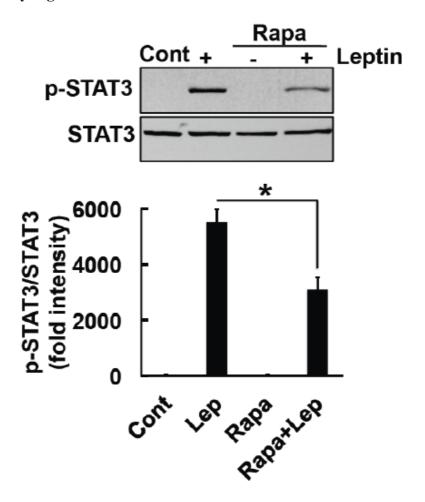


Supplementary Figure 1. Leptin did not induce ATF6 processing. SH-SY5Y-ObRb cells were treated with leptin (0.5  $\mu$ g/ml) for 0.5, 2, 4, and 24 h. Tunicamycin (Tm) (1  $\mu$ g/ml, 4 h) was used as the positive control. Densitometric analysis of ATF6 levels using image analyzing software. Typical data of 3 independent experiments were shown.



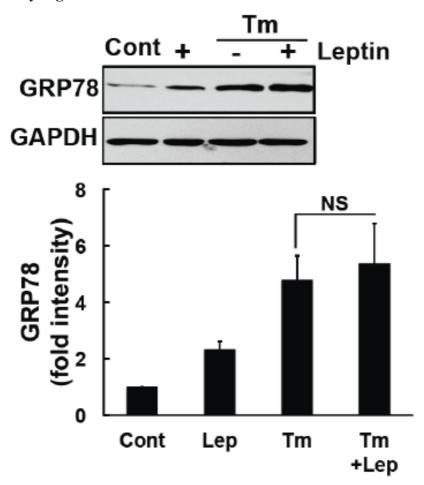
Supplementary Figure 2. Leptin increased the phosphorylation of eIF2 $\alpha$ . SH-SY5Y-ObRb cells were treated with leptin (0.5 µg/ml) for 4 h. Densitometric analysis of p-eIF2 $\alpha$  levels using image analyzing software. Data are expressed as the mean  $\pm$  S.E. of 4 independent experiments (n = 4). \*P < 0.05.

## **Supplementary Figure 3.**



Supplementary Figure 3. Rapamycin inhibited leptin signal in SH-SY5Y-ObRb cells. SH-SY5Y-ObRb cells were pretreated with rapamycin (10 nM) for 24 h followed by leptin (0.5  $\mu$ g/ml, 30 min). Western blotting analysis was performed using specific antibodies for phospho-STAT3 (Tyr705) and STAT3. Densitometric analysis of phospho-STAT3 (Tyr705) levels using image analyzing software. The leptin-induced phosphorylation of STAT3 was inhibited by rapamycin. Data are expressed as the mean  $\pm$  S.E. of 4 independent experiments (n = 4). \* P < 0.05.

## **Supplementary Figure 4.**



Supplementary Figure 4. GRP78 levels would not be increased in ER stressed model. SH-SY5Y-ObRb cells were pretreated with Tm (0.05  $\mu$ g/ml) for 4 h followed by leptin (0.5  $\mu$ g/ml, 24 h). Western blotting analysis was performed using antibodies for GRP78 and GAPDH. Densitometric analysis of GRP78 using image analyzing software. Data are expressed as the mean  $\pm$  S.E. of 3 independent experiments (n = 3).