Supplementary File

Western Blot Analysis

12.5%. SDS-PAGE was performed according to the method described by Laemmli (1970) [1]. In brief, sample buffer for reducing conditions was 60 mM Tris-HCl pH 6.8, 2% SDS, 5% β-mercaptoethanol (β-ME), 10% glycerol, and 0.01% bromophenol blue. For non-reducing conditions, β-ME was excluded from the sample buffer described above. The protein samples were mixed with 5×sample buffer and incubated for 1 h at room temperature. For heat-denaturation, samples were heated at 95 °C for 10 min unless indicated. For Western blot analysis, proteins separated by SDS-PAGE were electroblotted onto a PVDF (polyvinylidene difluoride) membrane (Millipore, Massachusetts, USA). The resulting membrane was blocked with 3% skim milk in Tris-buffered saline (TBS-T; 100 mM Tris, 150 mM NaCl, pH 7.4, and 0.1% Tween 20) at room temperature for 1 h and then incubated with 1:1000 diluted adiponectin antibodies in TBS-T containing 3% skim milk at 4 °C overnight. Unbound antibodies were removed by three 5-min washes in TBS-T at room temperature with gentle shaking. After washing, the membrane was incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (1:2000) for 1 h at room temperature and then washed thoroughly. Detections used the chemiluminescent method with ECL Western blotting detection reagent (Amersham Biosciences, Connecticut, USA) or the colorimetric method with the CN/DAB Substrate Kit (Thermo Scientific) following the protocol provided by the manufacturer. For quantification purposes, the stained membranes were scanned with a flatbed scanner (Epson Perfection V750 Pro), which converted the image to a graphic picture file. The digitized image stored in the file was then analyzed by image analysis software (VisionWorksLS software,Ultra-Violet Products Ltd.).

Preparation of anti-ADN Antibody

The procedure of anti-ADN antibody was modified by Hu *et. al.* (2003) [2]. In brief, the expression vector was pET30 vector (Novagen, Darmstadt, Germany). Porcine ADN (18-244, without the secretory leader peptide sequence) encoding residues DNA constructs were transformed into BL21 (DE3) *E. coli* (Novagen, Darmstadt, Germany). After optimization of the expression conditions, we could not make the ADN recombinant protein to be expressed in a soluble form. Inclusion bodies were resuspended in lysis buffer (20 mM Tris, 500 mM NaCl, 8 M urea, 5 mM β-mercaptoethanol, pH 8), incubated for 1hr at RT and centrifuged at 18,000 rpm. The solubilized protein was refolded in the presence of 200 volumes of 2 mol l^{-1} urea, 20 mmol l^{-1} Tris-HCl (pH 8.0) for 3 days at 4℃. To produce porcine ADN monoclonal antibody, male BALB/cJ mice were purchased from the Animal Center of National Taiwan University. Animals were housed with an inverse 12 hours day-night cycle with lights on at 6:00 am in a temperature (25 ± 1 °C) and humidity (60 ± 5%) controlled room with food and water provided *ad libitum*. In brief, we injected the mice with total 350 μg porcine ADN for 4 times in 2 months to immunization of mice, according to the protocol of the reference, and then the antiserum was separated [2]. All animal experiments were performed according to regulations approved by the Animal Ethical Committee of National Taiwan University.

References

1. Laemmli, U.K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **1970**, *227*, 680–685.
2. Hu, X.B.; Zhang, H.T.; Yanf S.L.; Gong Y. Cloning and expression of adiponectin and its globular domain, and measurement of the biological activity in vivo. **2003**, *35*, 1023–1028.