Supplementary Methods

Genetic background.

In Fig. 5, Fig. 6, Supplementary Fig. 2, Supplementary Fig. 3, Supplementary Fig. 4d-g, and Supplementary Fig. 5, although the knockout animals have a C57Bl/6 × 129/sv genetic background, all experiments were performed using littermates. We then backcrossed the original *AdipoR1*$^{-/-}$ or *AdipoR2*$^{-/-}$ mice (C57Bl/6 and 129/sv background) with C57Bl/6 mice more than seven times. These *AdipoR1*$^{-/-}$ or *AdipoR2*$^{-/-}$ (C57Bl/6 background) and the original *AdipoR1*$^{-/-}$ or *AdipoR2*$^{-/-}$ mice (C57Bl/6 and 129/sv background) appeared to tend to show almost the same phenotypes (**Fig. 4** and **Supplementary Fig. 4a-c**), therefore Fig. 4 and Supplementary Fig. 4a-c present the data obtained from the experiments using these *AdipoR1*$^{-/-}$ or *AdipoR2*$^{-/-}$ mice (C57Bl/6 background). AdipoR1・R2 double knockout (*AdipoR1*$^{-/-}$・*AdipoR2*$^{-/-}$) mice were prepared by *AdipoR1*$^{+/-}$・*AdipoR2*$^{+/-}$ mouse intercrosses (**Fig. 5**, **Fig. 6**, **Supplementary Fig. 2**, **Supplementary Fig. 3**, **Supplementary Fig. 4d-g**, and **Supplementary Fig. 5**).

Generation of adiponectin deficient *db/db* mice. Adiponectin deficient[16, 18] *db/db* mice (*adipo*$^{-/-}$*db/db* mice) were prepared by *adipo*$^{+/-}$*db/*+ mouse intercrosses (**Supplementary Fig. 1d**,**e**).

Genotyping. Genotyping of the mice was performed using PCR. Three primers were used for *AdipoR1* genotyping: the forward primer 1 (5'- GCA GGG TAA GCT GAT TAG CTA TG -3'), the forward primer 2 (5'- ATA GAT CTC TCG TGG GAT CAT TG -3'), and the reverse primer (5'- TTA CTG CAC TTC TTC TGC TGG A -3'). Three primers were used for *AdipoR2* genotyping: the forward primer 1 (5'- AGC CTA CTG CCT ACT GTA TTG T -3'), the forward primer 2 (5'- ATA GAT CTC TCG

TGG GAT CAT TG -3'), and the reverse primer (5'- ACT CTT CTA ACC TTC ATC AGG AG -3').

Immunoprecipitation, immunoblotting, quantitative analysis by real-time PCR. A real-time PCR method was used to quantify the *AdipoRs* mRNAs[12]. The primer sets and the probes for m*AdipoR1* and *R2* were as follows or as indicated;

the forward primer for m*AdipoR1* was ACGTTGGAGAGTCATCCCGTAT,

the reverse primer for m*AdipoR1*, CTCTGTGTGGATGCGGAAGAT,

and the probe for m*AdipoR1*, CCTGCTACATGGCCACAGACCACCT;

the forward primer for m*AdipoR2* (Exon7-8) was GGCAGATAGGCTGGCTAATGC, the reverse primer for m*AdipoR2* (Exon7-8), GGAAGAGCTGATGAGAGTGAAACC,

and the probe for m*AdipoR2* (Exon7-8), TAGCCTCTATATCACCGGAGC.

For quantification of the other genes including m*AdipoR2*, a set of predesigned primers and a probe for each gene (Assay on demand, Applied Biosystems, Foster City, CA) was used.

m*AdipoR2* pre-mixed probe;Exon1-2: Mm00815950_m1,

Exon2-3: Mm01184028_m1, Exon3-4: Mm01184029_m1

Exon4-5: Mm01184030_m1, Exon5-6: Mm01184031_m1

Exon6-7: Mm01184032_m1.

The relative amount of each transcript was normalized to the amount of *beta-actin* transcript in the same cDNA. The procedures used for immunoprecipitation and immunoblotting have been described previously[8,18,30]. Phosphorylation and the

protein levels of IRbeta, IRS-2, Akt and AMPKα were determined as described elsewhere[8,18,30]. Representative data from one of 2-3 independent experiments are shown. Rabbit polyclonal antibodies specific for AdipoR1 and AdipoR2 were from Immuno-Biological Laboratories Co., Ltd.

Hyperinsulinemic-euglycemic clamp study. Clamp studies were carried out as described previously[18,30], with slight modifications. In brief, 2-3 days before the study, an infusion catheter was inserted into the right jugular vein under general anesthesia with sodium pentobarbital. Studies were performed on mice under conscious and unstressed conditions after a 6-h fast. A primed continuous infusion of insulin (Humulin R, Lilly) was given (5.0 milliunits/kg/min), and the blood glucose concentration, monitored every 5 min, was maintained at 120 mg/dL by administration of glucose (5 g of glucose per 10 mL enriched to 20% with [6,6-$^2H_2$] glucose (Sigma)) for 120 min. Blood was sampled via tail tip bleeds at 90, 105, and 120 min for determination of the rate of glucose disappearance (Rd). Rd was calculated according to nonsteady-state equations[18,30], and endogenous glucose production (EGP) was calculated as the difference between Rd and exogenous glucose infusion rates [18,30].

Mice primary hepatocytes. Hepatocytes were isolated from 8-week old male mice by the collagenase perfusion method[13], with slight modifications. Aliquots of 3.75×10^5 cells were plated onto collagen I-coated 12-well dishes (Iwaki, Chiba, Japan) in Williams medium E supplemented with 10% (vol/vol) fetal calf serum, 10 nM dexamethasone, 1 nM insulin, 100 units/ml of penicillin, and 100 μg/ml of streptomycin. After incubation at 37 °C in 5% CO_2 for 48 h, the cells were washed twice with PBS and incubated with Williams medium E without bovine serum albumin for 6 h.

Binding assay. Binding assay was carried out as described previously[8,12], with slight modifications. Cells were seeded at a density of 3.75×10^5 cells per 12 well.

After 48 h, the cells were incubated at 4 °C overnight with binding buffer (ice-cold phosphate buffered saline (PBS), 0.1% bovine serum albumin) containing designated concentrations of ^{125}I-labelled adiponectin (5,000 counts per min per ng protein) plus unlabelled competitors. The cells were then washed twenty times with 1 ml of ice-cold PBS per well and trypsinized in 800 μl of PBS. The ^{125}I-labelled cell suspension was counted. The cell-bound radioactivity was determined using a γ-counter[8, 12]. Nonspecific binding was determined using a 200-fold excess of unlabelled adiponectin. Specific binding was calculated by subtracting nonspecific binding from the total binding. The values presented represent an average of triplicate determinations of 3 experiments.

Insulin resistance index. The areas of the glucose and insulin curves were calculated by multiplying the cumulative mean height of the glucose values (1mg/mL = 1 cm) and insulin values (1 ng/mL = 1 cm), respectively, by time (60 min = 1 cm)[6]. The insulin resistance index was calculated from the product of the areas of glucose and insulin × 10^{-2} in the glucose tolerance test[6]. The results are expressed as the percentage of the value of control wild-type littermates.

Blood sample assays. Plasma NEFAs (Wako Pure Chemical Industries Ltd., Osaka, Japan) were assayed by enzymatic methods[30].