Supplementary Notes

Adiponectin resistance in *db/db* mice, in which *AdipoRs* are decreased

The % decrease in plasma glucose levels in response to adiponectin was significantly reduced in *db/db* mice as compared with wild-type mice (**Supplementary Fig. 1a**). Moreover, we have previously shown that in *ob/ob* mice the effect of adiponectin on the activation of AMP kinase was indeed decreased as compared with the wild-type control mice[13].

AdipoRs protein expression in the lysates and membrane fractions of liver of *db/db* mice

Injection with adenoviruses expressing AdipoR1[12] resulted in increased AdipoR1 protein expression in a dose-dependent manner (**Supplementary Fig. 1b** top) and also increased AdipoR2 protein expression in the lysates (**Supplementary Fig. 1b** bottom**)** and membrane fractions of liver of *db/db* mice (**Supplementary Fig. 1c**).

FFA levels in *db/db* mice expressing AdipoR1 or AdipoR2 in the liver

Adenovirus-mediated expression of AdipoR1 or AdipoR2 in the liver of *db/db* mice had no significant effect on circulating FFA levels under the fed state or fasted state (**Supplementary Fig. 1f**,**g**). However, Adenovirus-mediated restoration of AdipoR1 in the liver significantly decreased the gluconeogenic enzymes in the liver of *db/db* mice (**Fig. 2e**,**f**), thereby decreasing the amount of insulin required for reducing plasma glucose levels, which led to the reduced EGP, apparently increased GIR (**Fig. 2a**) and amelioration of diabetes in *db/db* mice (**Fig. 1c**). These data were consistent with that AdipoR1 reduced gluconeogenesis independently of FFA reduction.

Increased AdipoR2 expression in AdipoR1 knockout mice

Reduced insulin action in the liver of AdipoR1 knockout mice (**Fig. 4b**) could be associated with activation of FoxO1, thereby upregulating AdipoR2, as we reported previously[13]. In fact, hepatic *AdipoR2* as well as *Acox1* expression levels (**Supplementary Fig. 4b**) were significantly higher in AdipoR1 knockout mice than in wild-type mice, which is consistent with the findings that AdipoR2 appeared to be more involved in PPARα activation than AdipoR1 (**Fig. 3**).

Generation of AdipoR2 knockout mice

To clarify the physiological roles of AdipoR2, we generated AdipoR2 knockout mice. Two distinct AdipoR2 mutant mice were generated from distinct ES cell clones in which the *AdipoR2* gene was disrupted by homologous recombination. Both mice lines of mice showed identical phenotypes in all the experiments carried out in this study.

Real time PCR analysis using the primer sets and the probes for detecting coding sequence derived from exon 3 revealed the abrogation of *AdipoR2* mRNA expression in the liver, skeletal muscle and white adipose tissue from homozygous *AdipoR2* knockout mice (**Supplementary Fig. 3b**,**d**,**f**). However, when we performed Northern blot analysis using probes for detecting the coding sequence derived from exon 4, we found that the expression of *AdipoR2* mRNA was not absent but rather markedly reduced in the homozygous *AdipoR2* knockout mice compared to normal wild-type littermates in all of the tissues we studied including liver, muscle and adipose tissues (**Supplementary Fig. 2e** and data not shown). We then attempted to clone the cDNA from the liver by RT-PCR. Four different pairs of PCR primers covering the entire *AdipoR2* coding region successfully amplified the homozygous *AdipoR2* knockout and wild-type *AdipoR2* mRNA. RT-PCR from the AdipoR2 knockout mice using several

different primer pairs consistently yielded amplified cDNA products that were approximately 0.1 kb smaller than the corresponding products from the wild-type mice. We then sequenced the RT-PCR products from two different primer pairs, focusing on regions spanning exon 3. Sequence analysis showed that the cDNA from homozygous *AdipoR2* knockout mice had a 90-bp deletion. Exon 3 was deleted in its entirety, but the exon 1, 2, 4-8 sequence was intact (**Supplementary Fig. 2d**). The deletion encompassed the coding sequence for the first predicted intracellular domain of the AdipoR2 protein.

We next examined whether this aberrantly spliced *AdipoR2* mRNA could make a protein. Even when we carried out forced-overexpression of almost the same amount of wild-type or mutant *AdipoR2* mRNA in COS cells (**Supplementary Fig. 2g**) (although the expression of *AdipoR2* mRNA in liver was markedly reduced in the homozygous *AdipoR2* knockout mice compared to normal wild-type littermates (**Supplementary Fig. 2e**)), we could not detect AdipoR2 protein in cells transfected with mutant *AdipoR2*, whereas we could detect AdipoR2 protein in cells transfected with wild-type *AdipoR2* (**Supplementary Fig. 2h**), indicating that this aberrantly spliced *AdipoR2* mRNA could not make a protein. These data also indicated AdipoR2 protein is essentially abrogated in AdipoR2 knockout mice and in AdipoR1･R2 double knockout mice.

Expression levels of AdipoR1 and AdipoR2, and plasma adiponectin levels

There were no significant differences in expression levels of AdipoR1 in liver, skeletal muscle and white adipose tissue from AdipoR2 knockout mice as compared with wild-type mice under basal state (**Supplementary Fig. 3a**,**c**,**e**). There were also no significant differences in expression levels of *AdipoR2* in liver, skeletal muscle and

white adipose tissue from AdipoR1 knockout mice as compared with wild-type mice under basal state (**Supplementary Fig. 3b**,**d**,**f**).

There were also no significant differences in plasma adiponectin levels among wild-type, AdipoR1 knockout, AdipoR2 knockout and AdipoR1・R2 double knockout mice (**Supplementary Fig. 3g**,**h**). Our observations that disruption of AdipoRs was not associated with increased adiponectin raises the possibility that AdipoRs may not be involved in the degradation or internalization of adiponectin, although AdipoR1 and AdipoR2 are the major adiponectin receptors, which mediate the major, if not the entire, part of adiponectin binding and adiponectin actions *in vivo*.

Adiponectin sensitivity test in AdipoR1 knockout, AdipoR2 knockout and AdipoR1・R2 double knockout mice

The glucose-lowering effect of adiponectin tended to be reduced in AdipoR1 knockout mice as compared with wild-type littermates, but it was not reduced in AdipoR2 knockout mice (**Supplementary Fig. 4c**), whereas the glucose-lowering effect of adiponectin was completely abolished in AdipoR1・R2 double knockout mice (**Fig. 5c**).

Adiponectin-stimulated AMPK activation in AdipoR1 knockout, AdipoR2 knockout and AdipoR1・R2 double knockout mice

In skeletal muscle, AMPK phosphorylation stimulated with adiponectin appeared to be decreased in AdipoR1 knockout and AdipoR1・R2 double knockout as compared with wild-type and AdipoR2 knockout mice (**Supplementary Fig. 5a**), suggesting that AdipoR1 may also play an important role in the activation of AMPK in skeletal muscle.

The observations that adiponectin increased the phosphorylation of AMPK in the liver and skeletal muscle *in vivo* and also hepatocytes *ex vivo* isolated from wild-type littermates and AdipoR2 knockout mice, but failed to increase the phosphorylation of AMPK in the liver and skeletal muscle *in vivo* and also hepatocytes *ex vivo* isolated from AdipoR1 knockout mice and AdipoR1・R2 double knockout mice, indicated that AdipoR1 was required for adiponectin-mediated activation of AMPK. We previously reported that adiponectin increased alpha2 AMPK activity in skeletal muscle, and that adiponectin was capable of stimulating phosphorylation and activation of alpha1 and alpha2 AMPK in the liver[8]. These observations raised the possibility that AdipoR1 may be required for adiponectin-mediated activation of alpha2 AMPK in skeletal muscle. How AdipoR1 might be related to adiponectin-mediated activation of alpha1 and alpha2 AMPK in the liver needs to be clarified.

Insulin-stimulated Akt phosphorylation in skeletal muscle of AdipoR1・R2 double knockout mice

Akt phosphorylation stimulated with insulin appeared to be decreased in skeletal muscle of AdipoR1・R2 double knockout as compared with wild-type littermates (**Supplementary Fig. 5b**).

Biochemical measurements in skeletal muscle and WAT of AdipoR1 knockout, AdipoR2 knockout and AdipoR1・R2 double knockout mice

The expression of genes encoding enzymes involved in fatty acid oxidation such as *Acox1* appeared to be decreased in skeletal muscle of AdipoR2 knockout and AdipoR1・R2 double knockout as compared with wild-type and AdipoR1 knockout mice

(**Supplementary Fig. 5c**), suggesting that AdipoR2 may also play an important role in the proper expression of enzymes involved in fatty acid oxidation in skeletal muscle.

The expression levels of *Acox1* in skeletal muscle (**Supplementary Fig. 5c**) and *Cat* in white adipose tissue (WAT) (**Supplementary Fig. 5f**) seemed to be decreased in AdipoR2 knockout as compared with AdipoR1 knockout mice, raising the possibility that the reduction of PPARα target genes such as *Acox1* in skeletal muscle and *Cat* in WAT in AdipoR1･R2 double knockout mice may be predominantly due to R2 depletion. Moreover, in AdipoR2 knockout mice, *Ucp2* expression in skeletal muscle was decreased (**Supplemental Fig. 5d**), *Ccl2* expression in WAT was increased (**Supplemental Fig. 5e**), and *Sod1* expression in WAT was decreased (**Supplemental Fig. 5g**), all of which showed alterations similar to those in AdipoR1･AdipoR2 knockout mice, albeit not significantly.