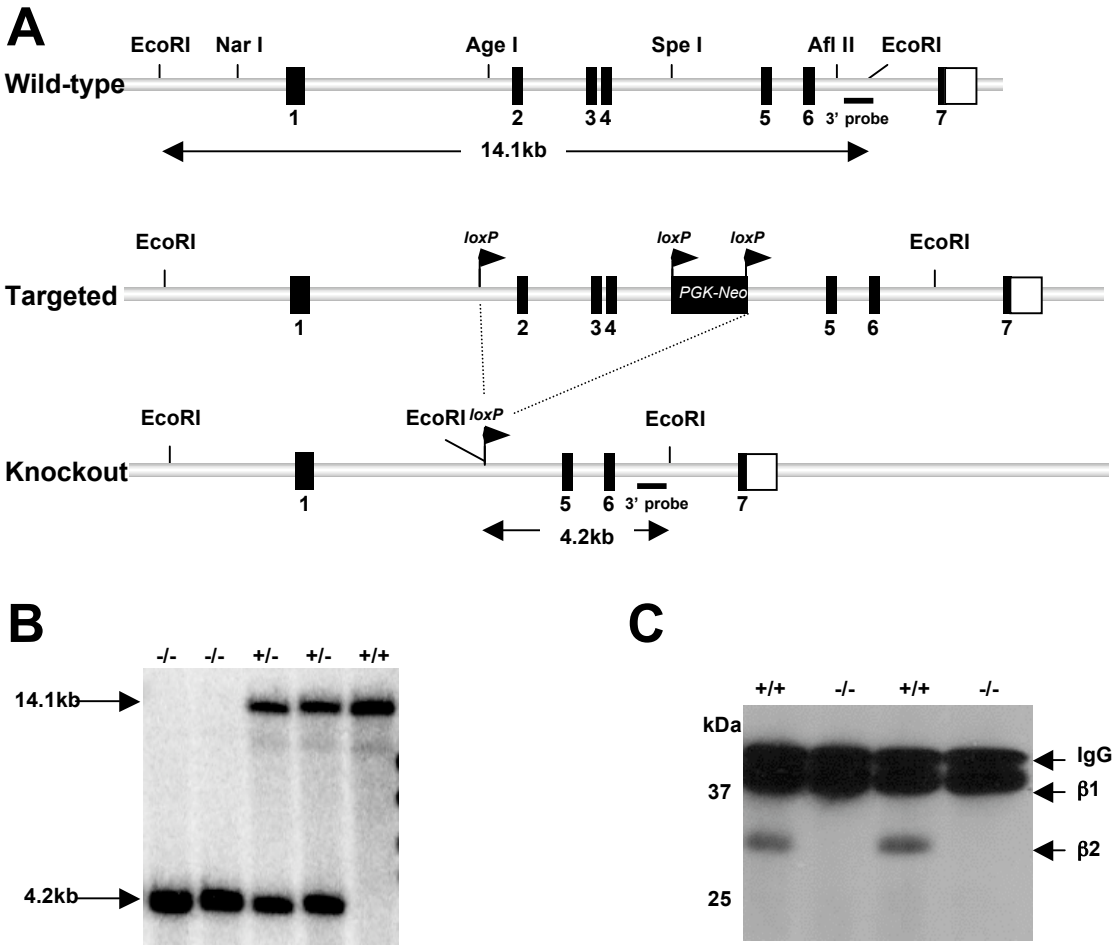

Supplementary Figure 1. Generation of AMPKβ2 knockout mouse.
(A) Structural representation of the mouse AMPKβ2 gene (Prkab2) of wild-type, targeted and germline knockout mice. (B) Genotyping was performed by Southern blotting of EcoRI digested DNA using a 3' probe. Representative wild-type (+/+), knockout (-/-) and hemizygous (-/+) samples are shown. (C) AMPK β-subunit expression in cardiac muscle lysates from AMPK β2 wild-type (+/+) and knockout (-/-) mice. Lysates were immunoabsorbed using α1/α2-specific antibodies and Western blots probed with a monoclonal antibody against both β1 and β2 subunits (Epitomics).

	Genotype									Total
AMPK β1 allele	+/+	+/+	+/+	+/-	+/-	+/-	-/-	-/-	-/-	
AMPK β2 allele	+/+	+/-	-/-	+/+	+/-	-/-	+/+	+/-	-/-	
Expected Freq (%)	6.25	12.50	6.25	12.50	25.00	12.50	6.25	12.50	6.25	100.00
Observed Freq (%)	8.33	18.75	5.56	11.81	27.08	13.19	3.47	11.81	0.00	100.00
Observed no. (O)	11	27	8	17	40	19	5	17	0	144
Expected no. (E)	9	18	9	18	36	18	9	18	9	144
O-E	2	9	-1	-1	4	1	-4	-1	-9	0
$(O-E)^2$	4	81	1	1	16	1	16	1	81	
$(O-E)^2/E$	0.44	4.50	0.11	0.06	0.44	0.06	1.78	0.06	9.00	16.44

Supplementary Table 1. **Chi-squared statistical analysis of lethality in AMPK β1/β2 double knockout mice.** Compound heterozygous β1/β2 knockout mice ($\beta1^{+/-}\beta2^{+/-}$) were intercrossed and a total of 144 progeny were genotyped by PCR. The nine possible genotypes are shown along the top of the table, along with the expected frequencies. No β1β2 double knockout mice ($\beta1^{-/-}\beta2^{-/-}$) were observed. A Chi-squared statistical test was performed to determine whether the observed frequencies agreed with the null hypothesis. The χ^2 value obtained was 16.44, which corresponds to a p value <0.05 (9 groups = 8 degrees of freedom). Therefore, the data is not consistent with the expected ratio and there is a significant departure from the null hypothesis. Therefore we conclude that AMPK β1β2 double knockout mice are embryonically lethal.

Supplementary Results

Generation of AMPK β-subunit knockout mice.

The generation of AMPK β1 germ-line knockout mice has recently been reported (30). These animals display enhanced hepatic insulin sensitivity, and are resistant to the detrimental effects of feeding on a high fat diet compared to wild-type littermate controls (Dzamko et al, manuscript in preparation).

We generated germ-line AMPK β2 knockout mice ($\beta2^{-/-}$) on a C57Bl/6 genetic background by standard homologous recombination techniques, using a targeting approach similar to that used for generating our $\beta1^{-/-}$ mice (see supplementary figure 1A and (30)). We deleted coding exons 2, 3, and 4 of the *Prkab2* locus, to affect minimal genetic disruption, and allow for the generation of floxed mice for future conditional gene deletion studies. The germline deletion results in a transcript with a frameshift mutation and premature translation termination in exon 6. Like our $\beta1^{-/-}$ mice, $\beta2^{-/-}$ mice showed no overt behavioural or externally visible phenotype. Moreover, the mice were fertile and heterozygous intercrosses generated wild-type, knockout and heterozygous progeny at the expected Mendelian frequency (1:1:2). Using Western blot analysis, we confirmed that genetic deletion resulted in complete loss of β2 protein in cardiac muscle (supplementary Fig 1C), as well as other tissues including liver and skeletal muscle (data not shown). The loss of the β2 subunit also resulted in lower protein levels of the catalytic α subunits, and lower AMPK activity (Dzamko et al, manuscript in preparation), a situation analogous to loss of α subunits in $\beta1^{-/-}$ mice. When mice heterozygous for AMPK β1 and β2 ($\beta1^{+/-}\beta2^{+/-}$) were intercrossed, no double knockout ($\beta1^{-/-}\ \beta2^{-/-}$) pups were obtained from 144 progeny (supplementary table 1). This indicates embryonic lethality; a similar result has been shown for attempts to generate AMPK α1/α2 double knockout mice (37).

Supplementary Results

Generation of AMPK β-subunit knockout mice.

The generation of AMPK β1 germ-line knockout mice has recently been reported (1). These animals display enhanced hepatic insulin sensitivity, and are resistant to the detrimental effects of feeding on a high fat diet compared to wild-type littermate controls (Dzamko et al, manuscript in preparation).

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1. Scott, J.W., van Denderen, B.J., Jorgensen, S.B., Honeyman, J.E., Steinberg, G.R., Oakhill, J.S., Iseli, T.J., Koay, A., Gooley, P.R., Stapleton, D., et al. 2008. Thienopyridone drugs are selective activators of AMP-activated protein kinase beta1-containing complexes. *Chem Biol* 15:1220-1230.
2. Viollet, B., Athea, Y., Mounier, R., Guigas, B., Zarrinpashneh, E., Horman, S., Devin-Leclerc, J., Beauloye, C., Foretz, M., Andreelli, F., et al. 2008. AMPK: Lessons from animal models *Frontiers in Bioscience* in press.