SUPPLEMENTARY ONLINE DATA

AS160 deficiency causes whole-body insulin resistance via composite effects in multiple tissues

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Figure S1 Generation and basic characterization of the AS160-knockout mouse

(A) Strategy for generating the AS160-knockout mouse. The diagram shows the conditional and conventional allele of AS160. The tenth exon harbouring the T649A mutation is flanked by loxP sites. The AS160-knockout mouse was generated by mating the AS160T649A knockin mouse with the Bal1 mouse line that expresses Cre recombinase in all tissues. The tenth exon harbouring the T649A mutation was excised through loxP-Cre recombination. (B–D) Immunoprecipitation of full-length AS160 and truncated AS1601–609 from tissue lysates. Tissues were removed from 8-week-old male mice. Full-length AS160 proteins and truncated AS1601–609 fragment (if the latter had been present) were immunoprecipitated from 60 μg of soleus lysates, 400 μg of adipose lysates or 400 μg of heart lysates using the anti-AS160(N) antibody. The immunoprecipitates were analysed via Western blotting with the anti-AS160(N) antibody (used at 1 μg/ml at 4 °C overnight). The molecular
Figure S2  Body weight of the AS160-knockout mice

Wild-type (+/+), heterozygous (+/−) and homozygous (−/−) AS160-knockout male mice were weighed once per week from 7 to 23 weeks of age.

Figure S3  Plasma insulin levels during a glucose tolerance test

The data are given as the mean (±S.E.M.) from seven to eight male mice (12–13 weeks old). KO, knockout; WT, wild-type.

Figure S4  Blood glucose and plasma insulin levels during hyperinsulinaemic–euglycaemic clamp

(A) Blood glucose levels during euglycaemic clamp in the AS160-knockout and wild-type male mice (16 weeks old). Data are given as means ± S.E.M. for six (wild-type) or ten (knockout) mice. (B) Plasma insulin levels before and after hyperinsulinaemic–euglycaemic clamps in the AS160-knockout mice and wild-type littermates. Data are given as means ± S.E.M. for six (wild-type) or ten (knockout) mice. (C) Plasma glucose specific activity during t = 80 to 120 min of the hyperinsulinaemic–euglycaemic clamp period. The slope of the relationship between glucose specific activity and time was not significantly different from 0, demonstrating that this variable was in a steady state. Data are given as means ± S.E.M. for six (wild-type) or ten (knockout) mice. KO, knockout; WT, wild-type.
Figure S5  GLUT1 protein levels in adipose tissue and skeletal muscle

GLUT1 proteins were detected in 40 μg of total lysates in adipose tissue and skeletal muscle (gastrocnemius (GAS)) from the AS160-knockout and wild-type mice (8 weeks old) using an anti-GLUT1 antibody.

Figure S6  The expression pattern of AS160 in mouse tissues

The expression of AS160 was determined in tissues from 8-week-old male wild-type mice. Tissue lysates (40 μg) were subjected to Western blotting analysis, and AS160 was detected using the anti-AS160(C) antibody. GAS, gastrocnemius.