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online data

SUPPLEMENTARY ONLINE DATA

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

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Supplementary Figures S1–S6 are on the following pages

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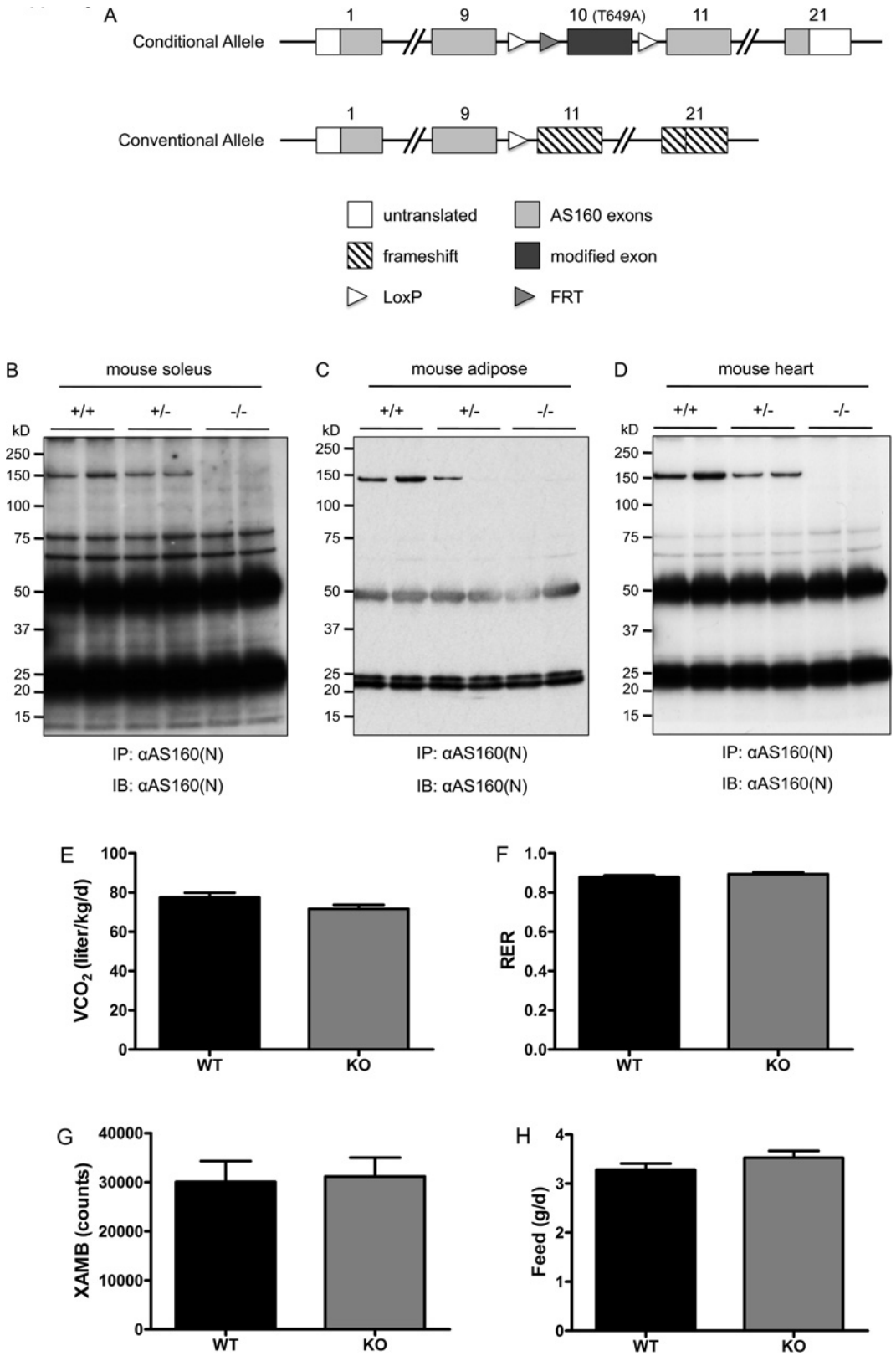

Figure S1 Generation and basic characterization of the AS160-knockout mouse

(**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 AS160^{T649A} 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 AS160$^{1–609}$ from tissue lysates. Tissues were removed from 8-week-old male mice. Full-length AS160 proteins and truncated AS160$^{1–609}$ 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

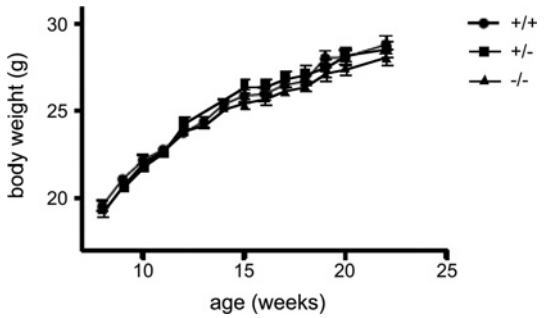

Figure S2 Body weight of the AS160-knockout mice

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

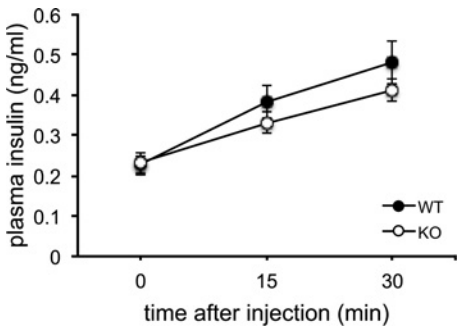

Figure S3 Plasma insulin levels during a glucose tolerance test

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.

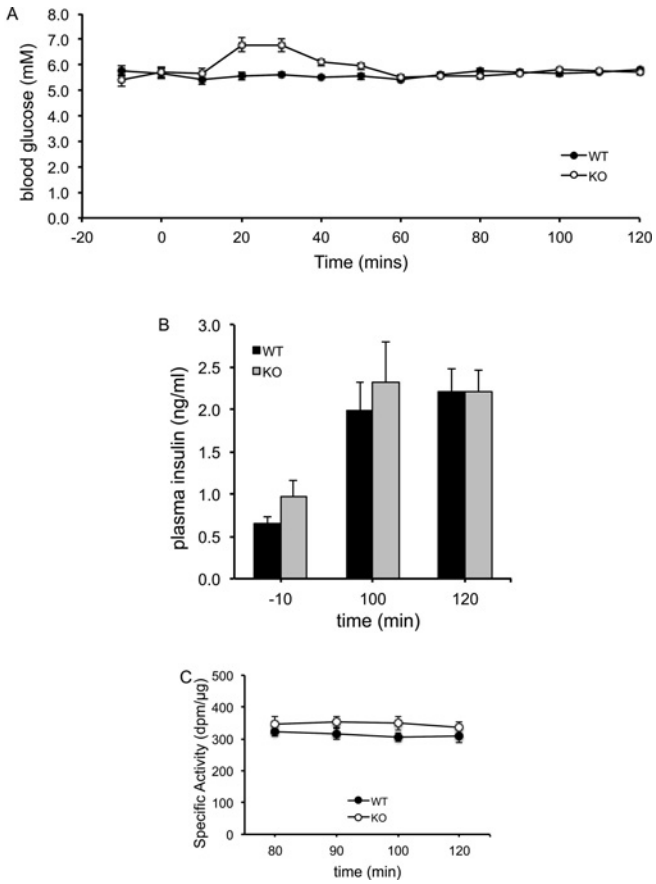

Figure S4 Blood glucose and plasma insulin levels during hyperinsulinaemic–euglycaemic clamp

(**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.

mass in kDa is indicated on the left-hand side. (**E**–**H**) Indirect calorimetry measurement. The AS160-knockout and wild-type male mice (8-week-old) were monitored using an Oxymax/CLAMS system (Columbus Instruments). The data are given as the means ± S.E.M. ($n = 6$–7) for $\dot{V}_{CO_2}$ (CO_2 production) RER, X_{AMB} (ambulatory activity) and feed intake, as indicated. IB, immunoblot; IP, immunoprecipitate; KO, knockout; WT, wild-type.

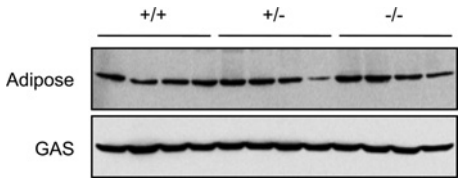

Figure S5 GLUT1 protein levels in adipose tissue and skeletal muscle

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.

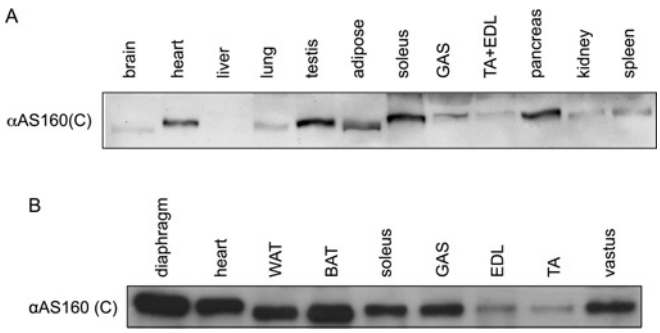

Figure S6 The expression pattern of AS160 in mouse tissues

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.

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