Supplementary data 24445-RG-1

Figure legend for Figure 1 (supplementary data).

Body length was measured in male mice of the indicated genotypes at 12 weeks

of age. Data represent the mean ± SEM for 8-10 animals of each genotype. ** P

< 0.01.

Table 1, Supplementary data.

Resting metabolic rate (RMR) and body temperature (Tb) characteristics

	RMR (ml O ₂ min ⁻¹) 30°C	22°C	Tb(C°)
<i>RIPCreIrs2KO</i>	0.68±0.03 ^A	1.22±0.07 ^A	37.7±0.1 ^A
Control	0.58±0.05	1.06±0.10	37.9±0.2
<i>NesCreIrs2KO</i>	0.62±0.07 ^A	0.91±0.09 ^A	37.6±0.2 ^A
Control	0.52±0.02	0.90±0.07	37.6±0.3

Data represent the mean \pm SEM for 8 animals of each genotype. ^AN.S.

Methods and references for Table 1, supplementary data.

Measurement of resting metabolic rate and activity

Resting metabolic rate (RMR) was estimated in 12 week old male mice at both 30±0.1°C (thermoneutral) and 22±0.1°C (housing temperature) by openflow respirometry using a paramagnetic oxygen analyser (Series 1100, Servomex Group Ltd, Crowborough, Sussex, U.K.) as previously described (1), with access to food and water prior to metabolic measurements. In brief, mice were weighed (0.01g, Sartorius) and rectal body temperature measured (2751-K, Digitron Instrumentation Ltd, U.K.) in advance of being placed individually within an airtight Perspex chamber inside a temperature-controlled incubator (INL-401N-010, Gallenkamp, U.K.). Silica gel dried air was pumped through the system (Charles Austin Pump Ltd, U.K) at 600-800mls min⁻¹ (DM3A, Alexander Wright Flow Meter, U.K.), re-dried and a sub-sample (~150mls min⁻¹) passed through the oxygen analyzer. Energy expenditure was calculated using the Weir equation (2) with all samples corrected for standard temperature and pressure (3) and downloaded directly on to a microcomputer. The lowest 10 consecutive readings (equivalent to 5 minutes in the chamber) in oxygen concentration were taken as an estimate of the resting metabolic rate (mls O₂ min⁻¹). A general-linear model was used to control for observed body mass differences between groups.

Activity was calculated in individual mice over a 24h period using a TSE InfraMot infra-red activity sensor (Technical and Scientific Equipment GmbH, Germany). This system determined activity of an individual mouse through the use of passive infrared sensors which registered activity by sensing infrared radiation and its spatial displacement over time. The sample interval was 1 min and the data collected over a 24h period was downloaded directly to a Microsoft Excel spreadsheet for analysis.

1. Selman, C., Lumsden, S., Bunger, L., Hill, W.G. and Speakman, J.R. (2001) Resting metabolic rate and morphology in mice (Mus musculus) selected for high and low food intake. *J Exp Biol*, **204**, 777-784.

 Weir, J.B.d.V. (1949) New methods for calculating metabolic rate with special reference to protein metabolism. *Journal of Physiology*, **109**, 1-9.
Speakman, J.R. (2000) The cost of living: Field metabolic rates of small mammals. *Advances in Ecological Research*, **30**, 177-297.



Figure 1, Supplementary data