Inventory of Supplementary Material

**Supplementary Materials and Methods** – Provides a more detailed explanation of the methods utilized in this manuscript.

**Supplementary References** – Provides a list of references supporting the supplemental material.

**Supplementary Table** – one table that contain the sequences of the oligonucleotide primers used in this study.

**Supplementary Figures** – four figures with legends that augment and extrapolate upon the information and figures provided in the main text and figures.
Supplementary Materials and Methods

Generation of Alkbh7−/− Mice

Alkbh7−/− mice were generated by GenOway (Lyon, France) by deletion of the coding sequence, exons 2 to 4, of the Alkbh7 gene. For a general methodological overview of the gene knockout technology see the review by (Galli-Taliadoros et al., 1995). Briefly, a targeting vector containing two loxP sites flanking exons 2 to 4 of the Alkbh7 gene, a neomycin positive selection cassette, and a diphtheria toxin A (DTA) negative selection cassette was constructed (Figure S1A, DTA cassette not shown) and electroporated into 129/SvPas mouse embryonic stem (ES) cell lines in vitro. The targeting vector was integrated into the ES cell genome by homologous recombination and the neomycin cassette located between exons 1 and 2 of the Alkbh7 gene allowed for selection of ES cells that had successfully integrated the vector by the appropriate homologous recombination. The negative selection DTA cassette located outside the region of homology to the Alkbh7 gene ensured that the ES cells in which random integration of the Alkbh7 gene had occurred died. The DNA from the targeted ES cells was then analyzed by Southern blot analysis of BglII- and AvrII digested genomic DNA for the 5´- and 3´-probe, respectively, to confirm correct recombination of the modified Alkbh7 gene into the DNA of the ES cells (Figure S1A). The targeted ES cells were microinjected into host blastocysts and implanted into a C57BL/6 mouse strain foster mother to produce ES cell mouse chimeras. The chimeras were then bred with wild-type C57BL/6 mice to obtain offspring with the deletion introduced into the germline called the F1-generation. Further breeding of the F1 generation mice with Cre expressing mice allows for loxP-site mediated Cre excision of Alkbh7 exons 2 to 4 and the neomycin cassette, which generated initially heterozygous and subsequently homozygous Alkbh7 knockout mice (Figure S1B and C). Further details regarding the cloning of the targeting vector and the Southern blots analysis are available upon request.

Genotyping of Alkbh7 Mice

Genotyping was carried out by PCR on mouse ear DNA extracted by the Hot Shot DNA preparation method (Truett et al., 2000). The DNA was amplified using the Expand Long Template PCR System (Roche Diagnostics) and the following cycling procedure: 94°C for 2 minutes, 15 cycle of 30 sec at 94°C, 30 sec at 63°C, 4 min at 68°C and a final extension step of 68°C. Standard reactions (25 µl) contained buffer 1
Protein Extracts
MEF cells grown to 80% confluence in a T-75 cell culture flask (Sarstedt) were suspended in 5 ml lysis-buffer (50 mM MOPS pH 7.5, 1 mM EDTA pH 8.0, 100 mM KCl, 0.3% (vol/vol) Triton X-100, 1 mM PMSF, 1 mM DTT) and incubated on ice 30 min. The proteins were centrifuged at 16,000 g at 4°C for 20 min. When necessary, extracts were stored at -80 °C. Proteins from mouse whole testis and heart tissues were extracted in homogenizing Lysing matrix D tubes (MP Biomedicals) containing 1 ml cell lysis buffer as described by the manufacturer. Protein concentrations were determined by Bradford assay (Bradford, 1976).

Western Blot Analysis
Proteins (60 µg) from heart, testis and MEFs were heated to 95°C for 5 min in NuPage LDS loading buffer (Life Technologies). Proteins were resolved on a 12% SDS-PAGE gel using the NuPage SDS-PAGE system (Life Technologies). The proteins were blotted onto polyvinylidene fluoride membranes using the iBlot Dry system from Life Technologies according to the manufacturer’s instructions. Membranes were incubated with 1% (wt/vol) blocking reagent (Roche) before the incubation with the appropriate primary antibodies diluted in 0.5% (wt/vol) blocking solution overnight at 4°C. Membranes were washed three times in PBS + 0.5% (vol/vol) Tween20 prior to incubation with the appropriate secondary antibodies conjugated to horse radish peroxidase diluted in 0.5% (vol/vol) blocking solution (Roche) for one hour at room-temperature. The signals were visualized using the Immune Star Western C Chemiluminescence kit from Bio-Rad (Hercules) using a ChemiDoc MP imaging system (Bio-Rad).

Generation of Mouse Embryonic Cell Lines
Mouse embryonic fibroblast (MEF) cell lines were established as previously described (TODARO and GREEN, 1963). Cells were handled according to standard procedures developed previously (Phelan, 2001).
**Immunocytochemistry**

Mouse embryonic fibroblast cells (MEFs) were grown to 80% confluence. The cells were then fixed with 100% (vol/vol) ice-cold acetone for 10 min at -20 °C and blocked with 3% (wt/vol) bovine serum albumin (BSA) in PBST (137 mM NaCl, 2.7 mM KCl, 10 mM Sodium Phosphate dibasic, 2mM Potassium Phosphate monobasic, 0.1% Tween-20, pH 7.4) for 45 min at room temperature. 4’, 6-diamidino-2-phenylindole (DAPI) at 1 µg/ml was used to counterstain MEF cell nuclei. The cells were visualized using an Axio Observer.A1 microscope (Carl Zeiss) equipped with an AxioCam MR Rev.3 fluorescence camera (Carl Zeiss), and the images were analyzed using AxioVision 4.8 software (Carl Zeiss) and ImageJ software (Abramoff et al., 2004).

**Supplementary References**


Supplementary Table

Table S1. qRT-PCR Primer List

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Forward Primer (5’-3’)</th>
<th>Reverse Primer (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkbh7</td>
<td>CTGATGCAAGCCAGGTGAT</td>
<td>TGGTAGATCCACAGAATGACA</td>
</tr>
<tr>
<td>Gapdh</td>
<td>TCGTCCCCTAGACAAAAATGGT</td>
<td>CGCCCAATACGGCCAAA</td>
</tr>
</tbody>
</table>
Supplementary Figures

A  Targeting vector design and homologous recombination in ES cells

5' probe

\[ \text{BgII} \]
\[ \text{AvrII} \]

E1 \[ \text{Neo} \] E2 \[ \text{loxP} \] E3 \[ \text{loxP} \] E4

3' probe

\[ \text{BgII} \]
\[ \text{AvrII} \]

Recombined Alkbh7 locus

\[ \text{AvrII} \]

5' targeting

\[ \text{BgII} \] 5.8kb

Wild-type Alkbh7 allele

\[ \text{BgII} \] 6.7kb

Recombined Alkbh7 allele

\[ \text{AvrII} \] 10.5kb

B  Validation of Cre excision in the mouse F1 generation

\[ \text{E1} \] \[ \text{E2} \] \[ \text{E3} \] \[ \text{E4} \]

Wild-type Alkbh7 allele

\[ \text{E1} \] \[ \text{Neo} \] \[ \text{E2} \] \[ \text{E3} \] \[ \text{E4} \]

Recombined Alkbh7 allele in F1 chimeras

\[ \text{E1} \]

Alkbh7 knockout allele

\[ \text{Mouse no.} \]

14745

14746

14747

Wild-type

3.6kb

2.5kb

5.5kb

In vivo Cre excision

B  Validation of homozygous Alkbh7 knockout mice

Wild-type

Alkbh7\(^{-/-}\)

Alkbh7\(^{+/-}\)

Alkbh7\(^{+/-}\)

Alkbh7\(^{-/-}\)

Wild-type

Alkbh7\(^{+/-}\)

Testis

Heart

MEFs

Alkbh7\(^{-/-}\) genotype

2151bp

1030bp

Alkbh7\(^{-/-}\) genotype

Alkbh7\(^{+/-}\) genotype

Alkbh7\(^{+/-}\) genotype

Alkbh7\(^{-/-}\) genotype
Figure S1. Strategy and Validation of the Alkbh7 Deletion in Mice. (A) Southern blot screening of embryonic stem (ES) cells. Genomic DNA from ES cells was digested with BglII and AvrII and the 5’ and 3’ regions were probed to validate presence of the wild-type and Alkbh7 recombined allele. (B) Validation of Cre mediated excision of Alkbh7 exons 2 to 4 in the mouse F1 generation by PCR. The PCR products of the wild-type allele (3.6kb) and the Alkbh7 knockout allele (2.5kb) in the heterozygous mice is depicted to the right. (C) Left; PCR amplification of DNA extracted from mouse ears from heterozygous breedings: wild-type allele (+/+)) is 2151bp and targeted Alkbh7 knockout allele (-/-) is 1030 bp long. Both alleles are present in mice that are heterozygous (+/-). Right; Protein extracts from wild-type (+/+) and Alkbh7/-(--/--) testis, heart, and mouse embryonic fibroblasts (MEFs). PCR, polymerase chain reaction; Neo, neomycin resistance cassette; loxP, Cre specific excision sites; E1-E4, exons 1-4; bp, base pairs; kb, kilobases; no., number.

Figure S2. Body Composition Changes and Food Intake in Alkbh7-/ Mice Fed a HFD. (A) Body composition change in Alkbh7-/ and wild-type mice after eight weeks on a HFD. (B) Food intake in Alkbh7-/ and wild-type mice fed a SD or a HFD. n = 10. Data are expressed as mean ± SEM. *, p < 0.05; **, p < 0.01.
Figure S3. Amino Acid Profile in Plasma. (A and B) Fed and fasted plasma amino acid levels in Alkbh7−/− and wild-type mice (n = 6). Data are expressed as mean ± SEM. *, p < 0.05.
Figure S4. Glucose and Insulin Tolerance in Male Alkbh7<sup>−/−</sup> Mice. (A and B) Glucose tolerance test (GTT) and insulin tolerance test (ITT) in male Alkbh7<sup>−/−</sup> and wild-type mice (n = 9). Data are expressed as mean ± SEM. *, p < 0.05.