Supplementary Methods:

Plasmid constructs for homologous recombination at the *Hnf4α* locus

Two mouse 129/Sv genomic DNA bacterial artificial chromosome (BAC) clones (kindly provided by S.A. Duncan and in Briancon et al., 2004), were used as templates for constructs and probe syntheses (fig. S1 A, B). To replace the coding sequence of the *a7*-specific first exon (exon 1D) by that of the *a1*-specific first exon (exon 1A), a PCR fragment containing the 88 bp exon 1A CDS, preceded by 15 bp of the *a7* 5’ UTR was obtained by using an *a7/a1* mosaic forward primer. The *a7* sequences abut the ATG and carry a KpnI site present in the endogenous *a7* 5’ UTR. The fragment was cloned into the Bluescript ® II KS plasmid (Stratagene) within the KpnI-SmaI sites. A NcoI-NotI 152 bp PCR fragment, containing the exon 1A 3’ end grafted to the 5’ end of the *a7*-specific first intron, was then ligated between the NcoI site, present in the previously subcloned exon 1A, and the NotI site present in the plasmid. Thus the exon 1A splice donor site and the 5’ end of the *a7* first intron were accurately re-created. PCR assays were performed with the Expand™ High Fidelity PCR system (Boehringer Mannheim). A 4.1 kb HinDIII-KpnI restriction fragment containing the sequences located upstream of the endogenous *a7* ATG was then subcloned in front of the exon 1A using the 5’UTR KpnI site (see above). The whole mosaic construct was ligated into the pPGKNEOLox2PPGKDTA plasmid (from P. Soriano), upstream of the neomycin-resistance cassette flanked by loxP sites (‘floxed’). A blunted 2.9 kb AlwNI-AlwNI restriction fragment present in the endogenous *a7* first intron was then inserted 3’ of the *neo* cassette and 5’ of the *DT-A* cassette (diphtheria toxin-A). This *DT-A* cassette was used to eliminate potential non-homologous recombinant ES clones.

For the reciprocal construct, a PCR fragment containing the 49 bp exon 1D coding sequence preceded by 12 bp from the *a1* 5’ UTR was obtained. These extra 12 nucleotides carry a NgoMI site, present in the endogenous *a1* 5’ UTR, and used for subsequent cloning. Another PCR fragment containing the 5’ most 232 bp from the *a1*-specific first intron (ending in 3’ with the endogenous *a1* SmaI site) was cloned downstream of the exon 1D splice donor site. Care was taken to retain the *a1* 5’ UTR, the exon 1D splice donor site and the 5’ end of the *a1*-specific intron. A 1.4 kb fragment contiguous to and upstream of the endogenous *a1* ATG was then cloned upstream of the exon 1D. This construct was integrated in front of the floxed *neo* cassette into the pPGKNEOLox2PPGKDTA plasmid, as for the exon 1D replacement.
blunted Smal-XhoI restriction fragment (4 kb), subcloned from the a1 first intron, was then inserted between the neo and the DT-A cassettes. Each DNA construct was linearized and purified prior to ES cell electroporation. The sequences of the exons 1A/1D and all the construct junctions were verified by sequencing.

**PCR genotyping of mice and ratios of live births**

Genomic DNA was extracted from mouse tails and subjected to PCR analysis using primers framing exon 1D (forward, 5'-TCACTGCCTCCTGGTGACTGGCTCCCGG-3'; reverse, 5'-CCAGCCGTCTCCCAGCCCCAGATATTGGCC-3') or exon 1A (forward, 5'-GGAGAATGCGACTCTCTAAAACCCT-3'; reverse, 5'-TCTGGCCACAGTACGACGAAGGC-3'). In PCR assays, the amplified band differs by 39 bp because exon 1A is 39 bp longer than exon 1D. Primers specific for the neo cassette confirmed deletion of the selection marker after crosses with pgk-cre mice (not shown). Cre-recombinase specific primers have been described previously (Hayhurst et al., 2001). a1-only and a7-only mice were born in agreement with mendelian inheritance from heterozygous crosses: 49 a1-only and 31 wild-type out of 165 births (\( \chi^2 = 0.13 \)), and 33 a7-only and 47 wild-type out of 170 births (\( \chi^2 = 0.24 \)).

**RNA extraction and Northern blot analysis**

Total RNA was extracted from mouse tissues with TRIzol reagent (Invitrogen™ Life Technologies), or isolated through a CsCl cushion (pancreas) or with the Ambion RNAqueous®-Micro kit (islets). Pancreatic islets were hand-picked after standard digestion with collagenase P (Roche). For Northern blots, \(^{32}\)P-labeled probes were obtained by random priming (Amersham). Probe templates were synthesized by PCR from liver RT products or extracted from plasmids provided by G. Hayhurst. Signals were analyzed with a Storm 860 apparatus (Molecular Dynamics) and the ImageQuant software.

**Western blot analysis**

Nuclear protein extracts were prepared from adult mouse tissues following Dounce homogenization in 15 mM HEPES, 15 mM KCl, 2 M sucrose, 1 mM EDTA, 0.5 mM DTT, 0.5 mM spermine, 0.5 mM spermidine, 0.5 mM pefabloc (Roche), and a cocktail of protease inhibitors (Complete, Roche). Proteins were measured with the Bradford assay (Bio-Rad),
separated (20 μg) on a 4-12% polyacrylamide Bis-Tris NuPAGE™ gel (Invitrogen) and transferred to a nitrocellulose membrane, which was probed with α-HNF4α.

**Serum and bile chemistry, glucose tolerance and insulin sensitivity tests**

Blood was collected retro-orbitally for biochemical and endocrinological assays and by intra-cardiac puncture for coagulation tests. Cholesterol in lipoprotein fractions was determined by FPLC. Glucose tolerance tests were performed by injecting a solution of 20% glucose in 0.9% NaCl intraperitoneally at a dose of 2g/kg body weight, and blood was collected from tails prior to and 15, 30, 45, 60, 90, 120, 150 and 180 minutes after injection. Glucose concentrations were measured by an Accu-Chek Active blood glucose sensor, using Accu-Chek active sticks (Roche Diagnostics). For the insulin sensitivity test, mice were injected intraperitoneally with regular pork insulin (0.2-0.5 IU/kg body weight) and blood glucose concentrations were measured as above until 90 minutes after injection. The presence of glucose in urine was investigated with Clinistix ® strips (Bayer Diagnostics).

**Oil Red O staining**

Liver cryosections from mice fed ad libitum or fasted for 24h were fixed in 4% paraformaldehyde and stained for 10 minutes in a solution of oil red O (Sigma) at 3 g/L in 60% isopropanol.

**Electrophoretic mobility shift assays**

Cos7 cells were grown in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum under humidified 7% CO₂, and transfected by phosphate calcium co-precipitation with pCB6 HNF4α1tag (Spath and Weiss, 1997), CMV-HNF4α7-VSV (Torres-Padilla et al., 2001) or the empty vector pCB6. Whole cell extracts were prepared (Jacquemin et al., 1999) and EMSA were adapted from reference (Cereghini et al., 1988). The P³²-labeled double-stranded oligonucleotides (37 bp long) were designed in the 10 kb region upstream of the mouse CAR start codon (GenBank contig NT_078306.1) and carry the HNF4α binding sites determined in silico using the MatInspector program (Quandt et al., 1995). ApoCIII is a well-known HNF4α binding oligonucleotide (Mietus-Snyder et al., 1992), used as a control. The C-terminal HNF4α antibody was used to confirm that the complexes observed were due to HNF4α proteins. Dried gels (6% polyacrylamide) were exposed in a PhosphorImager.
cassette.

References:


