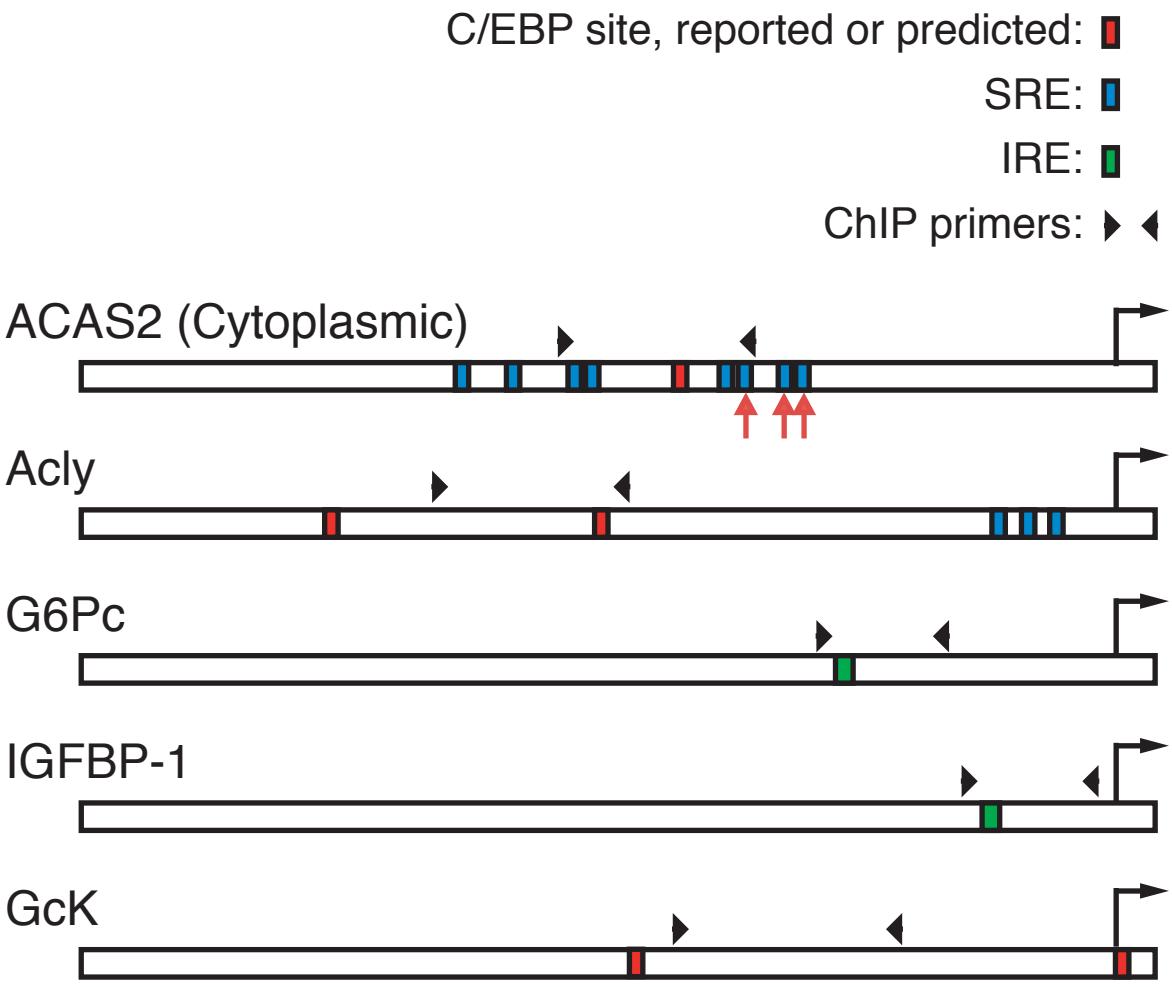
Supplemental Figure S1



C/EBPα target promoters bp-1000-+50

Suplementary figure S1 legend:

Schematic presentation of the C/EBPα target promoters analysed in this work. The proximal promoters (bp 1000-+50) of ACAS2, ACL, IGFBP-1, G6Pc and GcK are shown. The position of C/EBP sites (red boxes), SREs (blue boxes) and IREs (green boxes) are indicated. To analyse for the presence of consensus C/EBP binding sites the sequence of the proximal promoters of ACAS2, ACL and GcK (Ikeda et al., 2001; Moon et al., 2000 and Magnuson et al., 1989 respectively) were retrieved from the ENSEMBL genome database and analyzed using Matinspector software (Genomatix). This analysis revealed the presence of several C/EBP consensus binding sites. The indicated SREs, IREs and some C/EBP sites were previously reported (Ikeda et al., 2001; Moon et al., 2000 and 2002; O'Brien et al., 2001). Red arrows denote the SREs mutated in the pACAS2mSRE-LUC construct used in figure **3H**. ChIP primers (Arrowheads) were constructed as described in the supplementary methods. To ensure recovery of both chromatin bound SREBP1 and C/EBP, primer pairs located within the vicinity of both C/EBP sites and SREs were selected. In addition, sonication conditions for preparation of the sheared x-linked chromatin were optimized to yield DNA pieces with a length of approximately 500-1200 bp.

Additional references:

Magnuson M.A., Andreone T.L., Printz R.L., Koch S and Granner D.K. (1989) Rat glucokinase gene: Structure and regulation by insulin. *P.N.A.S*, **86**, 4838-4842

Moon Y-A, Lee J-J., Park S-W., Ahn Y-H. and Kim K-S. (2000) The roles of sterol regulatory element-binding proteins in the transactivation of the rat ATP citrate-Lyase promoter. *J Biol Chem* , **275**, 30280-30286

Moon Y-A, Lee J-J., Park S-W., and Kim K-S. (2002) Characterization of cis-acting elements in the ATP citrate lyase gene promoter. *Exp Mol Med,* **1**, 60-68

Supplementary Methods:

Protein extraction, Co-IP, TAP pull down and Western blotting.

For protein analysis by Western blotting, nuclei were prepared {Timchenko, 1997 #27} and lysed in 5X SDS loading buffer. Nuclear lysate from approximately 2mg liver or 40mg epididymal fat pad was loaded in each lane and proteins were resolved on 15% SDS-PAGE gels and blotted onto nitrocellulose membranes (protran, Schleicher and Schuell Bioscience). For analysis of C/EBPα phosphorylation by Anderson PAGE, extracts were prepared as described {Michael, 2000 #28} with the following modification: After centrifugation (1h at 42000rpm) C/EBPα was extracted from pellets using SDS lysis buffer [0,4M Tris (pH 6,8), 20% sucrose, 6% SDS, 3% mercaptoethanol]. Proteins extracted from approximately 10mg liver, 200mg epididymal fat pad and 20 mg lung were resolved by 15% Anderson PAGE {Nebreda, 1995 #29} and blotted onto polyvinyl diflouride membranes (Hybond-P, Amersham Biosciences). Western blots were blocked in 5% milk 1 hour at room temperature and incubated 1 hour with the indicated primary antibodies followed by 3x5 minutes wash. After washing, the blots where incubated 1 hour with secondary antibodies against rabbit or mouse IgG (Jackson Immunolaboratories, 1:10.000) and washed as above before they were developed using ECL (Amersham Biosciences). The following primary antibodies were used: Polyclonal anti-SREBP-1 (C18, 1:1000; 2A4, 1:1500), anti-C/EBPα (14AA, 1:1500), Sp1 (PEP 2, 1:1000), (Santa Cruz Biotechnology), anti-MAPK p42/44 (Cell Signaling), monoclonal anti-Flag (Sigma, 1:2500) and anti-C/EBPα Phospho Thr222,226 (2C6) (supernatant from hybridoma culture; 1:5). All washes and dilutions was carried out using PBS supplied with 0,1% Tween 20 (Sigma).

For TAP pull down experiements 2mg of nuclear extracts were incubated in immunoprecipitation buffer [50 mM Tris HCl pH 8, 100mM NaCl, 1,5 mM $MgCl_2$, 10% glycerol, 0.5 mM DTT and protease inhibitors (Roche)] with or without 1μg of rabbit IgG for at 4°C. After 1h, 20 μl of protein A beads were added and extracts were incubated for another 1h. Beads were washed three times in immunoprecipitation buffer and boiled for 10 min in SDS loading buffer before electrophoresis. Western blots were probed with the following antibodies: C/EBPα (14AA, sc-61) and SREBP-1 (C-20, sc-366)

For co-immunoprecipitation, whole cell extracts were prepared in CoIP lysis buffer [50mM Tris, 150mM NaCl, 0.1%NP-40, 10% glycerol, 5mM $MgCl_2$], supplemented with 1 mM DTT, 1 mM sodium orthovanadate, and protease inhibitor cocktail (Roche). Immunoprecipitations were performed using the M2 beads (Sigma) for 3 hours at 4°C. Immobilized complexes were washed with CoIP lysis buffer five times, bound proteins were then eluted from the beads using the FLAG peptide (Sigma) and eluates were analyzed by Western blotting as described above.

Mouse Physiology: Blood Glucose was measured on 5μl tail vein blood using a HemoCue Glucose 201 analyzer (HemoCue). Serum was prepared by centrifugation of blood collected by cardiac puncture in tubes containing EDTA and free fatty acid and insulin contend where determined using Free fatty acids, Half-micro test (Roche) and Ultrasensitive Mouse Insulin Elisa (Mercordia), respectively. Glucose tolerance tests were performed after an overnight fast (16 hours) by injecting a 12% glucose solution (2g glucose/kg mouse bodyweight) into the peritoneal cavity and measuring glucose in tail vein blood as described above before and 15, 30, 60 and 120 minutes after injection. 2-deoxy glucose uptake was determined after intraperitoneal injection of overnight (16 hours) fasted mice with a 12% glucose solution supplied with ^3H labeled 2-deoxy-glucose (30nCi/μl). As above, 2g glucose/kg mouse bodyweight of glucose was injected. After one hour, mice were sacrificed and skeletal muscle (thigh) and fat pats (epididymal) were collected. Tissue was weighed, dissolved in Solvable (Perkin Elmer) according to the manufacturer and the relative glucose uptake was determined by scintillation counting. Liver lipids were extracted as described {Inoue, 2004 #9}. Total triglyceride content was determined using serum triglyceride determination kit (sigma). Cholesterol content was determined using CHOL enzymatic colorimetric test (Roche). For glycogen determination, liver pieces (100mg) was boiled in 30% KOH. Glycogen was precipitated with ethanol. The glycogen containing pellets were dissolved in 2M HCl and hydrolyzed by boiling the samples for 30 minutes. The glucose content was determined using O-toluidine reagent kit (Sigma diagnostics).

Chromatin immunoprecipitation:

Livers where collected from mice and snap frozen in liquid nitrogen. Frozen liver pieces (200 mg) where homogenized in 20 ml DMEM supplied with 10% foetal calf serum (Invitrogen) and 1% formaldehyde (Sigma) and incubated 5 min at room

temperature. Glycine was added to 125 mM and incubation was continued for 10 min. After incubation the homogenized tissue were collected by centrifugation, washed twice with ice cold PBS and incubated in 1ml of lysis buffer [1% SDS, 10 mM EDTA, 50 mM Tris (pH8)] for 10min on ice. After lysis, chromatin was sheared by sonication (10x10s using a Sonoplus GM 200 sonicator (Bandelin Electronics), probe MS 72 at maximum output). After sonication, lysates were cleared by centrifugation (10 min, 14000 rpm). 50-200µl of the lysate (corresponding to 20-40mg liver) was added to 1600µl dilution buffer [0,01% SDS, 1,1% triton X-100, 1,2 mM EDTA, 16,7 mM Tris (pH 8), 167 mM NaCl] and cleared by incubation with 50µl protein A beads (Amersham, 50% in dilution buffer) for 2 hours at 4°C. 4µg of the indicated antibody (anti-SREBP-1 (C18), anti-C/EBPα (14AA) or unspecific rabbit IgG, Santa Cruz Biotechnology), was added and lysates were incubated 16 hours at 4°C. 50µl 50% protein A bead solution was added and the lysates were incubated 2 hours at 4°C. Beads were collected by centrifugation and washed in dilution buffer, dilution buffer with 500 mM NaCl, and twice in TE. For Re-ChIP experiment, beads from 5 ChIP experiments were pooled and the precipitated chromatin was eluted with 100mM DTE at 37°C for 30min. The eluate was diluted 20 times in dilution buffer and a second round of ChIP was performed as described above. After washing 200µl TE and 2ng RNAse A was added and precipitated chromatin was de-cross linked at 65°C for 2h. 50 ng Proteinase K and SDS to 0,5% was added and samples were incubated 1 hour at 42°C. DNA was collected using Qiaquick gel extraction kit (Qiagen) according to the instructions of the manufacturer. Precipitated DNA was analyzed by PCR. The following PCR conditions where applied: 2 min, 96° C; 30x[10 sec, 96°C; 10 sec, 60°C; 45 sec, 72°C]. After the PCR reaction, the ChIP was evaluated on a 2% agarose gel.

ChIP Primers:

ChIP and real time PCR primers were constructed using primer 3 software (MIT, http://frodo.wi.mit.edu/primer3/primer3_code.html) based on sequence information retrieved from the ENSEMBL data-base (www.ensembl.org).

Primer list for ChIP PCR:

Name	Left	Right
G6Pc	TTTTTGTGTGCCTGTTTTGC	CAGCCCTGATCTTTGGACTC
IGFBP1	GCCGGCTAGGTCTTTGATTT	CTTATGAAGGGCTGGCTGTG
ACAS	GGAGTGTCACCCAGAAGGAA	TGGATGTGACACGAGAGGAG
ACLY	CTCTAGGCTCCTCCCCATTC	GCCTCGTAGGTTCTCCTCCT

β-globin	CCTGCCCTCTCTATCCTGTG	GCAAATGTGTTGCCAAAAAG
GcK	ACTGGTTGGAGCTGCTGTG	CTTGGCTTCCTTCCTTCCTT

Quantitative real-time PCR:

For real time PCR, first strand cDNA was synthesized from 5mg total RNA using Ready-To-Go T-primed first strand kit (Amersham Bioscience). Relative mRNA levels were determined by real time PCR on a DNA Engine (MJ Research) using DyNAmo SYBR green qPCR kit (Finnzymes). The following PCR conditions where applied: 2 min, 96° C, 40x[10 sec, 96°C; 10 sec, 60°C; 10 sec, 72°C]. After each elongation step, the reaction was quantified in a reading step. After each reaction, the product quality was tested by melting curve analysis. All cDNA levels where normalized to the level of ubiquitin cDNA.

Primers:

Name	Left	Right
G6Pc	TGGTAGCCCTGTCTTTCTTTG	TTCCAGCATTCACACTTTCCT
IGFBP1	GGGTTAGGGGAGGGAGGTT	CCAGCAAATGTGCATCAGAC
TAT	ATGGTGGGAATTGAGATGGA	ACCCGGAAGAAATTTGGGTA
Apoc3	CGCTAAGTAGCGTGCAGGAG	GGTTGGTCCTCAGGGTTAGA
PEPCK	ACACACACACATGCTCACAC	ATCACCGCATAGTCTCTGAA
ME	GTCGTCAAGGCTATTGTGGTAA	GCCGTGTAAAGGGCCAGTT
ACAS	ACCTGGCTTGCCTAAAACAC	ACCGTAGATGTATCCCCCAGG
ACLY	AGGAACTGTGGCTCCTTCAC	GACGATACAGCCCTTGCTTC
GOT1	AACGTGAAGACAATGGCTGA	ACCACTCGGCAGGAGATAGA
SCD1	TATGGATATCGCCCCTACGA	TCGATGAAGAACGTGGTGAA
GK	GATCCGGGAAGAGAAGCAAG	GACAGGGATGAGGGACAGAG
GS	TTCCACCTAGAGCCCACATC	TCTTCTTCGTCTTCCGCATC
Glut2	GCAACTGGGTCTGCAATTTT	CCAGCGAAGAGGAAGAACAC
IR	CATGGATGGAGGCTATCTGG	GAAAGCTGGGGTGGAGGT
Allbumin	ACAGACCGGAGGGCTTATCT	TGGTGTAGACAGGTCAGGATGT
CPS1	GAGCAGTTGCACAATGAAGG	CCTGGAAATTGGTGAGGAGA
FBP1	CTTTTTATACCCCGCCAACAAG	CATGACGACTGGTGCCTTC
HMG-COAS	TGGTACCTTGAACGAGTGGA	CAGATGCTGTTTGGGTAGCA
CPT-I	ATGACGGCTATGGTGTTTCC	TGTCCATCATGGCTTGTCTC
CPT-II	TGGATAGGCTGCAAT	AAACTCTCGGGCATT
FAS	GGGTGACTCATTCCAGAACC	ACCAGCCCTCTCCATATCCT
PPARγ	GCCCTTTGGTGACTTTATGG	CAGCAGGTTGTCTTGGATGT
C/EBPα	CAAGAACAGCAACGAGTACCG	GTCACTGGTCAACTCCAGCAC
C/EBPβ	GGAGACGCAGCACAAGGT	AGCTGCTTGAACAAGTTCCG
PGC-1α	GTAAATCTGCGGGATGATGG	GGTGGAAGCAGGGTCAAAA
SREBP1	GATGTGCGAACTGGACACAG	CATAGGGGGCGTCAAACAG
AP2	GGATGGAAAGTCGACCACAA	GCTCATGCCCTTTCATAAACTC
GLUT4	CTGTCGCTGGTTTCTCCAAC	CAGGAGGACGGCAAATAGAA
Adipsin	GATGTGTGCAGAGAGCAACC	TCAATCATGAACCGGACAAC

IR	CATGGATGGAGGCTATCTGG	CCTTGAGCAGGTTGACGATT
Thiolase B	TCCAGGACGTGAAGCTAAAGC	CATTGCCCACGGAGATGTC
LDL-R	TGCAAGACCAACGAGTGTTTG	GCAGATGTGGGAACAGCCA
Ubiquitin	TGGCTATTAATTATTCGGTCTGCAT	GCAAGTGGCTAGAGTGCAGAGTAA
LCAD	TCACCAACCGTGAAGCTCGA	CCAAAAAGAGGCTAATGCCATG
MCAD	AGCTGCTAGTGGAGCACCAAG	TCGCCATTTCTGCGAGC
CYP4A10	CCACTCATTCCTGCCCTTCTC	TGTTTCCCAATGCAGTTCCTG