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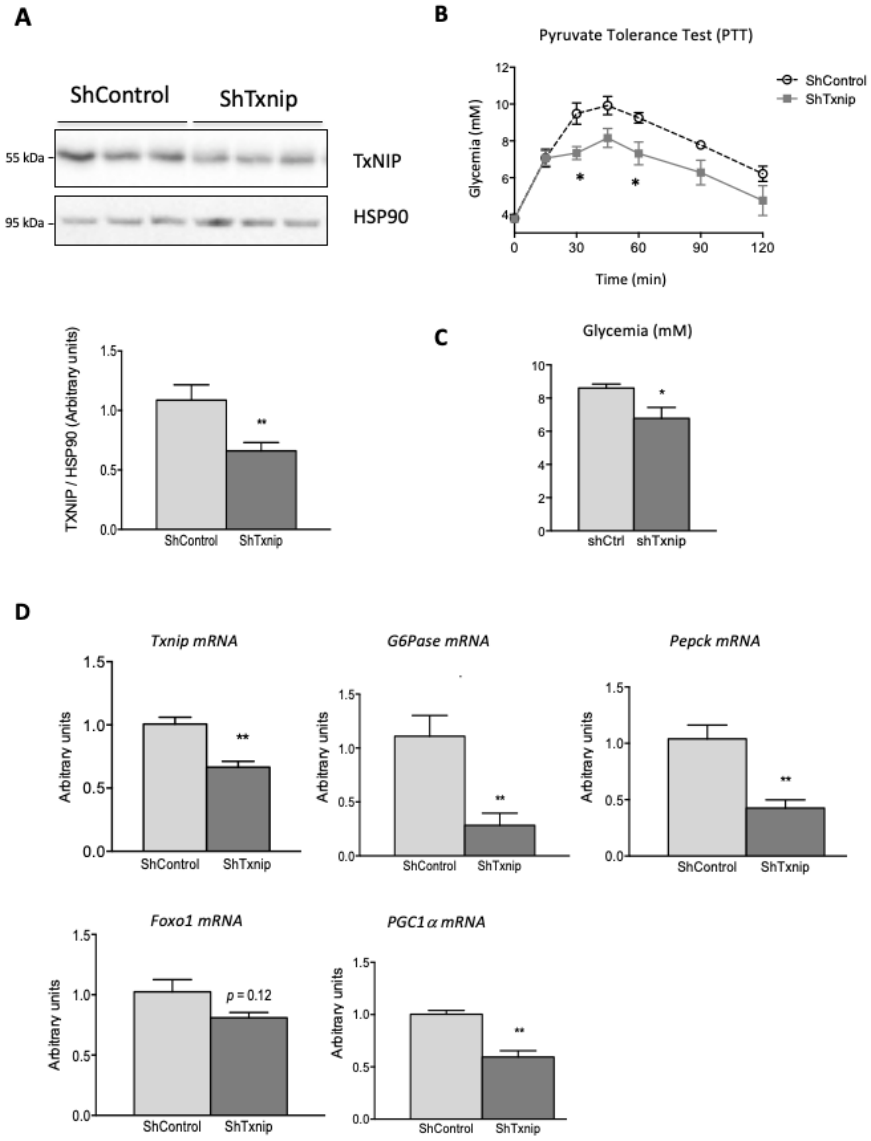
Supplemental information

Dual regulation of TxNIP

by ChREBP and FoxO1 in liver

Benedicte Noblet, Fadila Benhamed, InSug O-Sullivan, Wenwei Zhang, Gaëlle Filhoulaud, Alexandra Montagner, Arnaud Polizzi, Solenne Marmier, Anne-Françoise Burnol, Sandra Guilmeau, Tarik Issad, Hervé Guillou, Catherine Bernard, Terry Unterman, and Catherine Postic

Figure S1. *Txnip* silencing in liver reduces blood glucose concentrations in C57BL/6J mice. **Related to Figure** 8.



Adult C57BL/6J male mice were injected intravenously with a single dose of 5.10^9 pfu of ShControl or ShTxnip adenovirus (GeneCust) at Day1. Seven days later, mice were challenged to a pyruvate tolerance test (PTT). Data are expressed as means ± SEM, n=6 to 8 individual mice/group. Significance is based on two-way ANOVA followed by Bonferroni post hoc test, *p<0.05, **p<0.01, when compared to ShControl (**A**) Pyruvate tolerance test (PTT) (**C**). Blood glucose concentrations at sacrifice. (**D**). qPCR analysis of *Txnip*, *G6Pase, Pepck, Foxo and PGC1α.*

Table S1. Sequences of PCR primers used for mutagenesis. Related to Figure 2.

	Forward Primer	Reverse Primer
ChoRE$_a$ WT	CTG TGC ACG AGG GCT GCA CGA GCC TCC	GGA GGC TCG TGC AGC CCT CGT GCA CAG
ChoRE$_a$ mutated	CTG TGC ACC ATG GCT GGA CGA GCC TCC	GGA GGC TCG TCC AGC CAT GGT GCA CAG

IRE WT	AGG CCT GGT AAA CAA GGG CCA AGT A	TAC TTG GCC CTT GTT TAC CAG GCC T
IRE partial mutant 1	AGG CCT AGT AAC CAA GGG CCA AGT A	TAC TTG GCC CTT GGT TAC TAG GCC T
IRE partial muant 2	AGG TCT AGC AAC CAA GGG CCA AGT A	TAC TTG GCC CTT GGT TGC TAG ACC T
IRE mutated	AGG TCT AGC AAC CAA TGG CCA AGT A	TAC TTG GCC ATT GGT TGC TAG ACC T

The consensus sequences for recognition and binding of the transcription factors ChREBP and FoxO1 are underlined. The sequences of the endogenous promoter (murine-WT) are indicated for comparison. Mutations are indicated in red. The 'IRE partial mutant 1' mutant serves as a template for obtaining the 'IRE mutated' (last lane). Abbreviations: ChoREa, Carbohydrate Response Element proximal to the *Txnip* promoter; IRE, Insulin Response Element; WT, unmutated wild type promoter.

Table S2. Sequences of the primers used for qPCR and ChIP-qPCR. Related to Figures 1, 3, 4, 5, 6, 7, 8 and S1.

	Forward	Reverse
Acc	GAG GTG GCT AAG AGG AGG CTC T	CAG CAC CGA GAC TGA ACT GTA AGG
Chrebp total	ATG ACC CCT CAC TCA GGG AAT A	GAT CCA AGG GTC CAG AGC AG
Chrebp α	CGA CAC TCA CCC ACC TCT TC	TTG TTC AGC CGG ATC TTG TC
Chrebp β	TCT GCA GAT CGC GTG GAG	CTT GTC CCG GCA TAG CAA C
Foxo1	TGT TAC TTA GCT CTC TCC CCT CG	AGA CGA GCA GTG GCT CAA T
Foxo1 (TGN)	GGA TGG TGA AGA GCG TGC CC	CGC TCT TGC CTC CCT CTG GA
Foxo3	CTCTCAGGCTCCTCACTGTA	ATGAGTTCACTACGGATGAT
G6Pase	TTA CCA GCC TCC TGT CGG	GAC ACA ACT GAA GCC GGT TAG
Lpk	CTT GCT CTA CCG TGA GCC TC	ACC ACA ATC ACC AGA TCA CC
Txnip	GAC TAG AGA GCC CCA CCA CC	GGA CGC ACG GAT CCA CCT CA
Igfbp1	CCT GCC AAC GAG AAC TCT AT	AGG GAT TTT CTT TCC ACT CC
Pepck	TGG CTA CGT CCC TAA GGA A	GGT CCT CCA GAT ACT TGT CGA
Cyclophilin	ATG GCA CTG GTG GCA AGT CC	TTG CCA TTC CTG GAC CCA AA
Tbp	CCC CAC AAC TCT TCC ATT CT	GCA GGA GTG ATA GGG GTC AT
Chore *Lpk*	GTC CCA CAC TTT GGA AGC AT	CCC CAA CAC TGA TTC TAC CC
Chore *Txnip*	AAG GGC CAA GTA GCC AAT GGG	GTG CTG GCC TGG AGG
IRE *Pepck*	TAC AGA CAT TAT CTA GAA GTC TCA	CAA GGG CAG GCC TAG CCG AGA
IRE *Txnip*	AAC AAC AAC CAT TTT CCC CGC TAG	ATA GCC GCC TGG CTT GGC GCT

Table S3. Antibodies and dilutions used for Western Blot analysis. Related to Figures 1, 3, 4, 5, 7, 8 and S1.

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Protein	Size (kDa)	Dilution (solution)	Secondary	Reference
ACC	265	1/5000 (BSA 5%)	Rabbit	Cell Signaling (#3662)
ChREBP	93	1/3000 (BSA 5%)	Rabbit	Nouvs (Nb400-135)
FoxO1	70	1/1000 (BSA 5%)	Rabbit	Cell Signaling (#2880)
GAPDH	36	1/2000 (lait 5%)	Rabbit	Santa Cruz (sc-25778)
HSP90	85	1/1000 (lait 5%)	Rabbit	Cell Signaling (#4874)
L-PK	62	1/300 (BSA 2%)	Rabbit	Dr A. Kahn (Cochin)
PEPCK	69	1/2000 (lait 5%)	Rabbit	Santa Cruz (sc-32879)
℗FoxO1	70	1/800 (BSA 5%)	Rabbit	Cell Signaling (#9461)
TxNIP	44	1/2000 (lait 5%)	Mice	MBL (K0205-3)

Transparent Methods

Animals

Ten to twelve week-old adult male C57BL/6J, *db/db*, *Chrebp*$^{+/+}$ and *Chrebp*$^{-/-}$ (Iroz et al., 2017), transgenic mice overexpressing a constitutively active form of FoxO1 in liver (FoxO1^{TGN}) (Zhang et al., 2006), liver specific triple FoxO1, 3, 4 knockout (LFoxO^{TKO}) (Zhang et al., 2016), liver specific insulin receptor knockout (LIR^{KO}) and liver specific insulin receptor and FoxO1 double knockout (LIRFox01^{KO}) (O-Sullivan et al., 2015) mice were used for *in vitro* and *in vivo* experiments as indicated. Procedures were carried out according to the French guidelines for the care and use of experimental animals (Animal authorization agreement n° CEEA34.AFB/CP.082.12, Paris Descartes Ethical Committee). Mice were maintained in a 12-hour light/dark cycle with water and standard diet (65% carbohydrate, 11% fat, and 24% protein) unless specified.

Nutritional and circadian challenges

Mice were studied in the fasted, fed or refed state. ZT stands for Zeitgeber time: ZT0 is defined as the time when the lights are turned on and ZT12 as the time when lights are turned off (7 pm). The fed group was fed ad libitum. The fasted group was fasted from ZT0 until ZT12. The refed group was fasted from ZT0 to ZT12 (included) and refed from ZT12 to ZT24. For circadian rhythms experiments, mice were killed by cervical dislocation at several time points in a pair-wise manner: ZT0, ZT4, ZT8, ZT12, ZT14, ZT16, ZT20, ZT24 as indicated. Liver was removed, snap-frozen in liquid nitrogen and stored at −80 °C until use.

Primary cultures of mouse hepatocytes

Mouse hepatocytes were isolated as described (Dentin et al., 2004). Briefly, hepatocytes were isolated from the livers of fed male mice by a modification of the collagenase method (Berry and Friend, 1969). Briefly, livers from mice were perfused with Hank's balanced salt solution (HBSS, KCl, 5.4 mm; KH_2PO_4, 0.45 mm; NaCl, 138 mm; $NaHCO_3$, 4.2 mm; Na_2HPO_4, 0.34 mm; glucose, 5.5 mm; HEPES, 1 m; EGTA, 50 mm; $CaCl_2$, 50 mm; pH 7.4). Livers were washed at a rate of 5 ml/min using the portal vein before collagenase (0.025%) was added. Cell viability was assessed by the trypan blue exclusion test and was always higher than 60%. Hepatocytes were seeded (in 60-mm Petri dishes at a density of 2×10^6 cells for RNA extraction or 6-well plates at a density of 4×10^5 cells per well for luciferase assays) in medium M199 with Earle salts (Invitrogen), supplemented with 10 μg/ml of streptomycin, 100 units/ml of

penicillin, 2.4 mm of glutamine, 0.1% (w/v) bovine serum albumin, 2% (v/v) Ultrocer G (Invitrogen), 100 nm dexamethasone (Soludecadron, Merck Sharp), and 100 nm insulin (Actrapid, Novo-Nordisk). After cell attachment (6 h), the medium was replaced by fresh M199 medium for 24 h. For adenoviral infections, hepatocytes from C57BL/6J male mice were incubated under low glucose concentration (5 mM) with specific adenovirus (from 0.1 to 3 pfu/cell) for 24 h. For glucose stimulation experiments, hepatocytes from *Chrebp*$^{+/+}$ and *Chrebp*$^{-/-}$ mice (Iroz et al., 2017) were incubated in the presence of low (5mM) or high glucose concentrations (25 mM) for 24 hours.

Adenoviral injection *in vivo*

To silence TxNIP expression, adult C57BL/6J (+/+) and *db/db* male mice were injected intravenously with a single dose of $5.0x10^9$ pfu of ShControl or ShTxnip adenovirus (GeneCust) at Day1. At Day 4, fasting blood glucose was measured. At Day 7, mice were challenged with a pyruvate tolerance test (PTT). At Day 8, fed blood glucose concentrations were measured. Mice were sacrificed under fasting conditions. To silence ChREBP, adult C57BL/6J male mice were injected with a single dose of $5.0x10^9$ pfu of ShControl or ShChREBP adenovirus (GeneCust) at Day1. Seven days later, mice were challenged to nutritional manipulations as indicated (Fasted or Refed) before sacrifice.

Pyruvate tolerance test

Intraperitoneal pyruvate tolerance test (PTT, 2g/kg body weight) was performed in overnight fasted awake adult C57BL/6J (+/+) and *db/db* mice 7 days post adenovirus injection.

Mutagenesis and Luciferase assays

A TxNIP promoter-luciferase reporter plasmid (pGL3B-1081) was obtained from Addgene (cat #18758). Hepatocytes from C57BL/6J male mice were plated in 6-well plates (4×10^5 cells per well) and transfected with TxNIP luciferase reporter constructs (0.2 µg DNA per well) including either Wild type or mutated on the ChoRE (ChoREa mutated) or on the IRE (IRE mutated) (Yu et al., 2009) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA). Mutants were generated using oligonucleotides described in Table S1. The luciferase assay was conducted using the dual luciferase substrate system (E1501; Promega, Madison, WI), and the result was normalized with the internal control Beta galactosidase. Each experiment was performed in triplicate and repeated 5 to 8 times as indicated.

ChIP analysis

In vivo ChiP assays from mouse livers were performed as described (Marmier et al., 2015). Briefly, genomic DNA regions of interest were isolated using antibodies against ChREBP (Novus) or Fox01 (Cell Signaling) or non-immune IgG as a control (Cell Signaling). QPCR reactions were carried out in triplicate using SYBR Green Supermix (Bio-Rad) on a CFX Connect™ Real Time PCR system. Positive and negative control sites were tested for each factor as well as the sites of interest. The resulting signals were normalized for primer efficiency by carrying out qPCR for each primer pair using input DNA (pooled unprecipitated genomic DNA from each sample). DNA fragments were quantified by qPCR, using primers described in Table S2. Results are expressed as fold enrichment.

Gene expression analysis

Total cellular RNA was extracted using the SV total RNA isolation system (Promega). For qPCR analysis, total RNA samples (2 µg) were reversed transcribed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Primers for SYBR Green assays are presented in Table S2. Amplifications were performed on an ABI Prism 7300 Real Time PCR System (Applied Biosystems). qPCR data were normalized by TATA-box binding protein (TBP) mRNA levels and analyzed with LinRegPCR.22.

Western blotting analysis

Proteins from liver lysates were subjected to 10% SDS-PAGE gel electrophoresis and transferred to nitrocellulose membranes. Antibodies and dilutions used are indicated in Table S3.

Wheat germ agglutinin purification

For ChREBP immunoprecipitation, cells were lysed on IPH buffer (20 mmol/L Tris/HCl, 150 mmol/L NaCl, 0.5% NP-40 [v/v], and protease inhibitors) as described (Guinez et al., 2011). Briefly, proteins were incubated with 2 µg of anti-ChREBP antibody (Novus) and placed at 4°C overnight. Bound proteins were recovered after addition of 30 µl of Sepharose-labeled protein G (Sigma) for 1 h at 4°C. Beads were gently centrifuged for 1 min and washed four times for 5 min each. For wheat germ agglutinin ([WGA] a GlcNAc-binding lectin) precipitation, 1 mg of proteins was incubated with 30 µl of WGA agarose beads (Sigma). Then, proteins were eluted from the beads in a Laemmli buffer and separated by SDS-PAGE.

Biochemical analysis

Blood glucose was measured in total blood using an Accu-Check glucometer (Roche).

Statistical Analysis

Data representing at least three independent experiments are reported as means ± S.E.M, and were analyzed using Prism 5.0, GraphPad) software. A student's T-test was used when comparing two groups (followed by Mann-Whitney post hoc test) or two-way ANOVA when comparing three or more groups followed by a Bonferroni post hoc test. Statistical significance was defined as $p<0.05$.

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