Table S1:

Sequences (5'-3')	Use
CGAGTCAAAGCCGTCAGGAT	Rat TXNIP 5’ qPCR primer
TTCATAGCGCAAGTAGTCCAAGGT	Rat TXNIP 3’ qPCR primer
ACTCGTGTCAAAGCCGTTAGG	Human TXNIP 5’ qPCR primer
TCCCTGCATCCAAAGCACTT	Human TXNIP 3’ qPCR primer
CTCGCGTGGCTCTTCTG	Human TXNIP-ChoRE 5’ ChIP primer
GCAGGAGGCGGAAACGT	Human TXNIP-ChoRE 3’ ChIP primer
CGGGATTGTCTGCCCTAATTAT	Human GAPDH 5’ ChIP control primer
GCACGGAAGGTCACGATGT	Human GAPDH 3’ ChIP control primer
TCCCTGGCCCTACCTGCTCTT	Rat TXNIP (a) 5' ChIP primer
TTTGGAGGCTGGGGTAGGGGA	Rat TXNIP (a) 3' ChIP primer
AATGACAAGGCTCTGGCGGGGT	Rat TXNIP (b) 5' ChIP primer
TATTCCTCACCCACCTCCTCCCAC	Rat TXNIP (b) 3' ChIP primer
CCAATACAGCTTCAGCCCTGGGGA	Rat TXNIP (c) 5' ChIP primer
TGCATACCCCTCAGCCTGTTTCAGT	Rat TXNIP (c) 3' ChIP primer
ATCTGACAAGTCCCCCGCCC	Rat TXNIP (d) 5' ChIP primer
GCCTACCCGATGTGCTCCCA	Rat TXNIP (d) 3' ChIP primer
TCTGCGGCCTCGCTGATTGG	Rat TXNIP (e) 5' ChIP primer
CCCTCGTGCACAGTTCTCCCA	Rat TXNIP (e) 3' ChIP primer
AAGGACCAAGTAGCCAATGGG	Rat TXNIP (f) 5' ChIP primer
GTGCTGGCCCGGAGG	Rat TXNIP (f) 3' ChIP primer
TGGGAGAACTGTGCACGAGGGA	Rat TXNIP (g) 5' ChIP primer
CGGGAGCCGGAAACGGCATT	Rat TXNIP (g) 3' ChIP primer
TAAGCCCTCTCTGCCTCACGGA	Rat TXNIP (h) 5' ChIP primer
GGGTTCAAGAAAAACGGAAGCCGGA	Rat TXNIP (h) 3' ChIP primer
TGACTTATGGGGCTGGGGGTGG	Rat TXNIP (i) 5' ChIP primer
GGGGCCAACAGCTCAAACCATTC	Rat TXNIP (i) 3' ChIP primer
ACCATGCTTCACTGACATTCTGA	Rat GAPDH 5’ ChIP control primer
GGTCTGCCTCCCTGCTAACC	Rat GAPDH 3’ ChIP control primer
GGGAAGCCACTGAAGAGAGA	Rat LPK-ChoRE 5’ ChIP primer
TGTATTTAGCCGAGGTGAGG	Rat LPK-ChoRE 3’ ChIP primer

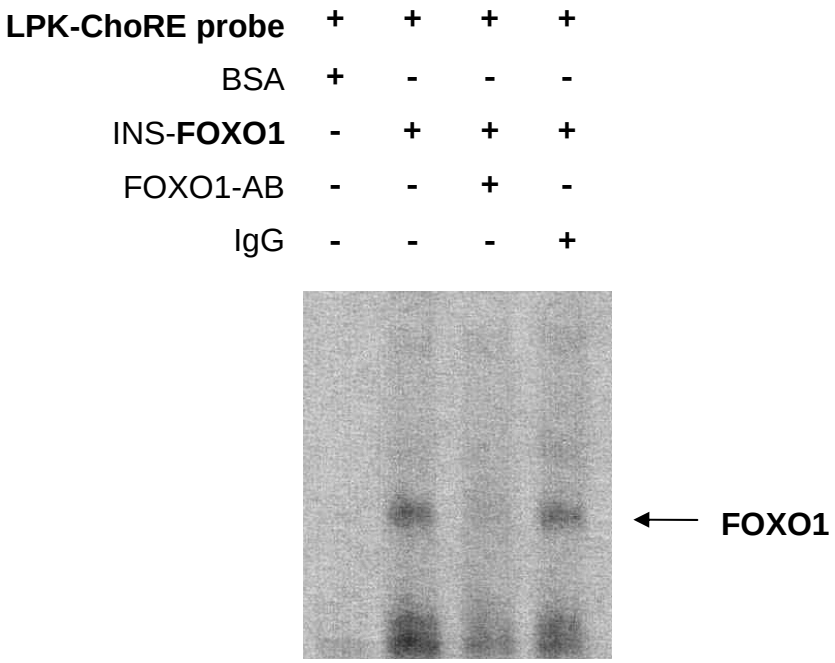

Fig. S1

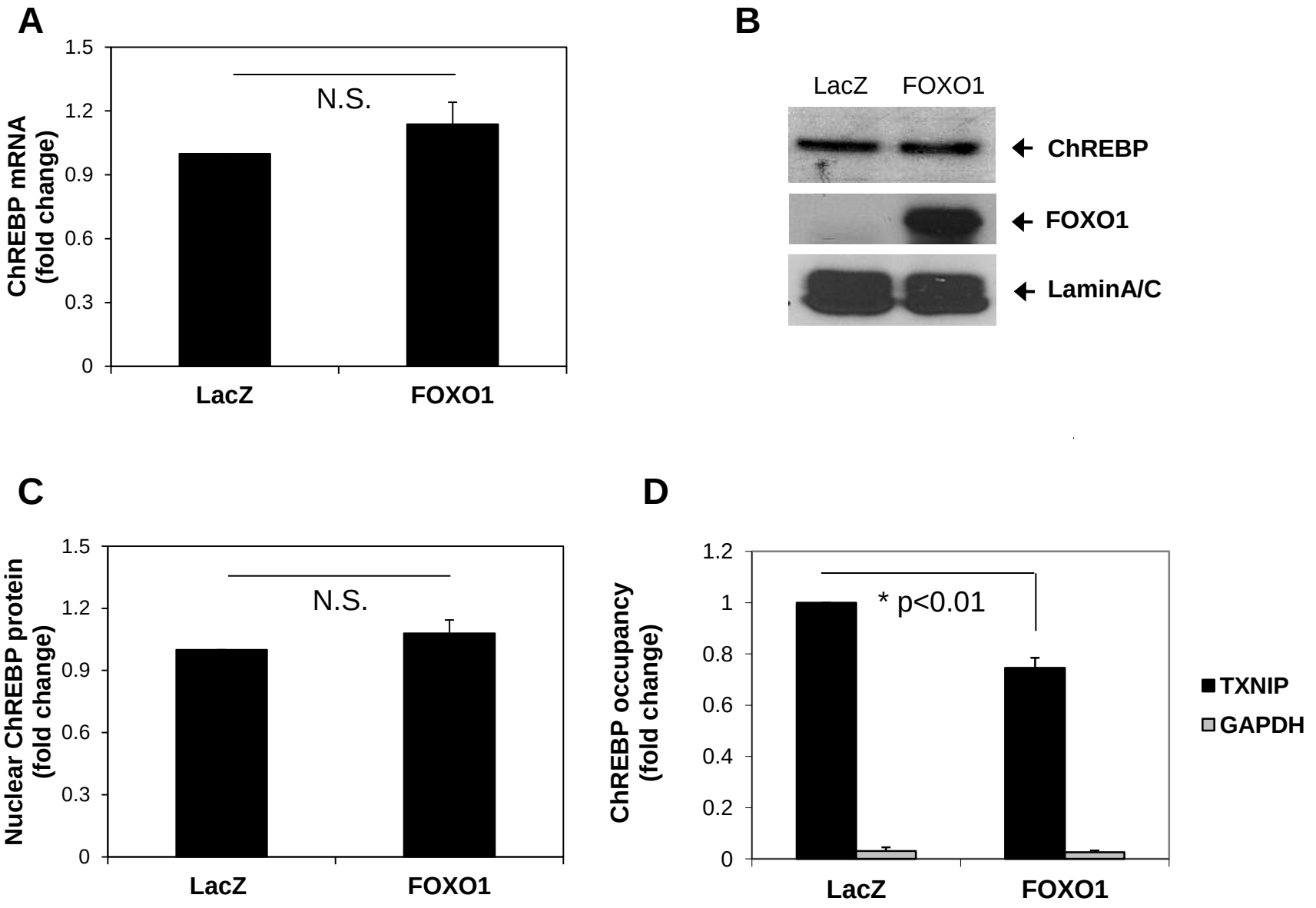
A
ChREBP mRNA (fold change)
1.5
1.2
0.9
0.6
0.3
0
N.S.
LacZ
FOXO1
B
LacZ
FOXO1
ChREBP
FOXO1
LaminA/C
C
Nuclear ChREBP protein (fold change)
1.5
1.2
0.9
0.6
0.3
0
N.S.
LacZ
FOXO1
D
ChREBP occupancy (fold change)
1.2
1
0.8
0.6
0.4
0.2
0
* p<0.01
LacZ
FOXO1
TXNIP
GAPDH

Fig. S2

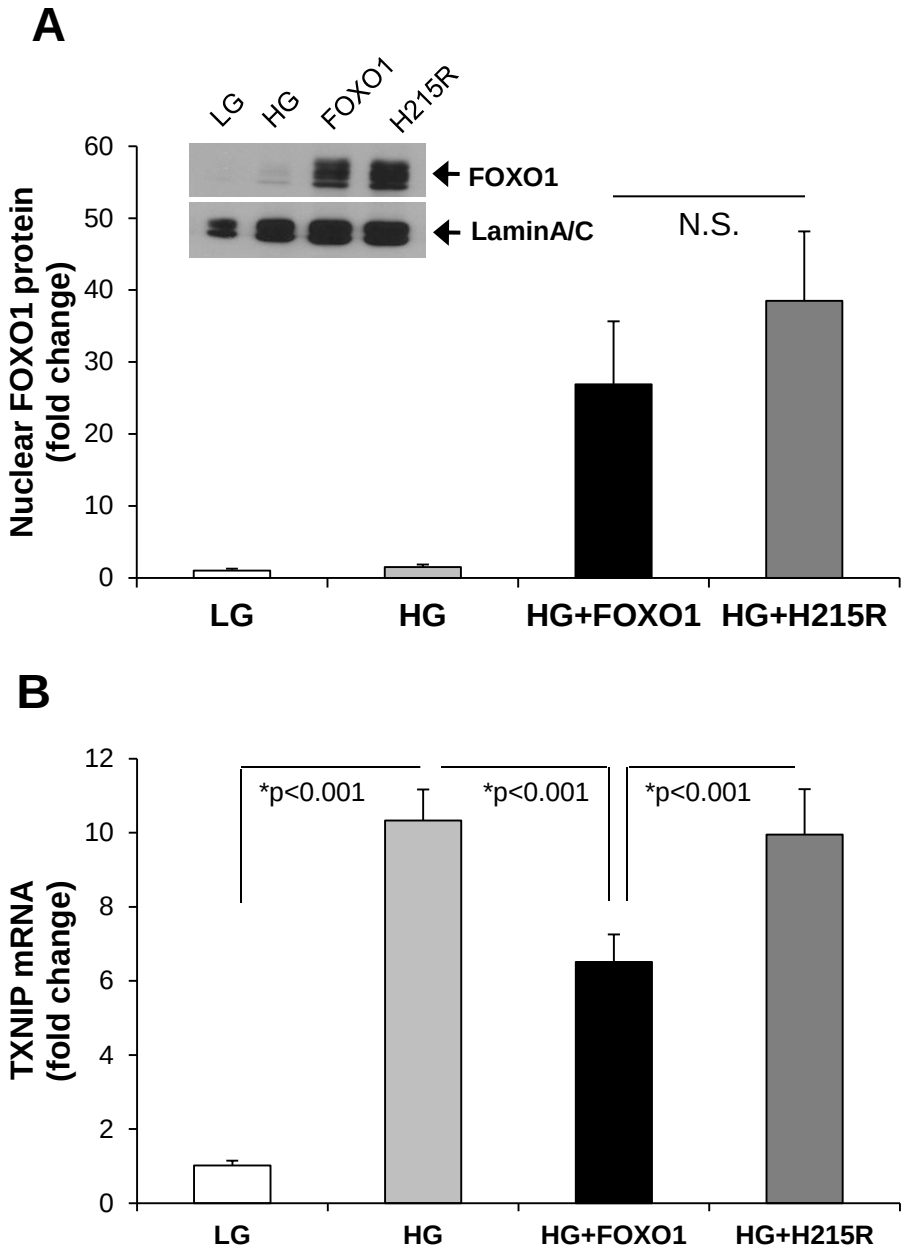
A
LG
HG
FOXO1
H215R
FOXO1
LaminA/C
N.S.
Nuclear FOXO1 protein (fold change)
60
50
40
30
20
10
0
LG
HG
HG+FOXO1
HG+H215R
B
*p<0.001
*p<0.001
*p<0.001
TXNIP mRNA (fold change)
12
10
8
6
4
2
0
LG
HG
HG+FOXO1
HG+H215R

Fig. S3

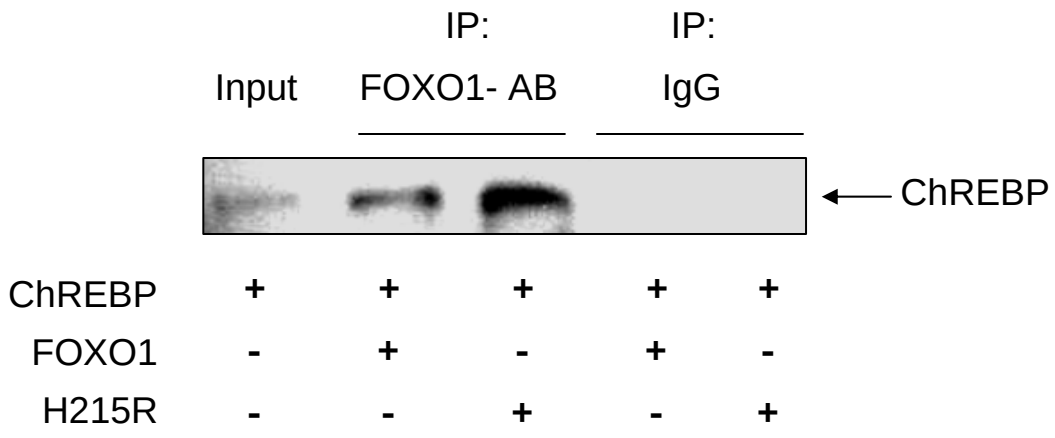

Fig. S4

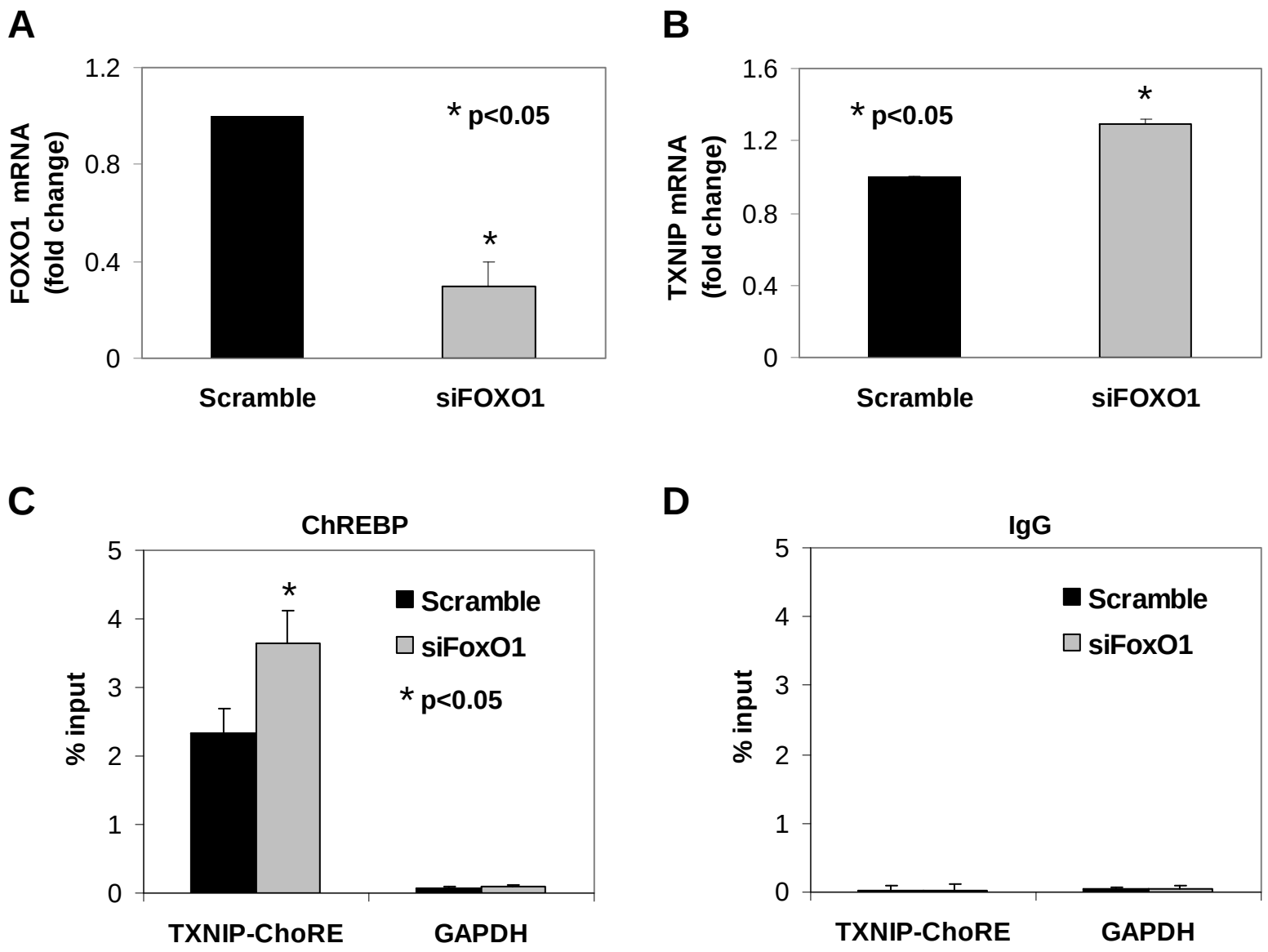
A
FOXO1 mRNA (fold change)
1.2
0.8
0.4
0
* p<0.05
*
Scramble
siFOXO1
B
TXNIP mRNA (fold change)
1.6
1.2
0.8
0.4
0
* p<0.05
*
Scramble
siFOXO1
C
ChREBP
% input
5
4
3
2
1
0
*
Scramble
siFoxO1
* p<0.05
TXNIP-ChoRE
GAPDH
D
IgG
% input
5
4
3
2
1
0
Scramble
siFoxO1
TXNIP-ChoRE
GAPDH

Fig. S5

SUPPLEMENTAL FIGURE LEGENDS

Figure S1: **FOXO1 binding to LPK promoter ChoRE sequence.**
Electromobility shift assays were performed as described in the methods section using whole cell extract of INS-1 cells transfected with FOXO1 expression plasmid (INSFOXO1) and a DIG-labeled LPK-ChoRE oligonucleotide probe (5'-gtaagccacggggcactcccgtggttcctgg-3' and 3'-ccaggaaccacgggagtgccccgtggcttac-5'). Inhibition assays were performed using anti-FOXO1 antibodies and compared to control IgG.

Figure S2: **FOXO1 effects on ChREBP expression and promoter occupancy.**
INS-1 cells were transiently transfected with FOXO1 expression plasmid or LacZ control plasmid. **(A)** Cells were harvested 48h post-transfection and TXNIP mRNA levels were measured by quantitative RT-PCR. **(B)** Cells were harvested 72h post-transfection, cell fractionation was performed, and nuclear ChREBP protein levels were measured by Western bloting. **(C)** Quantification of nuclear ChREBP corrected for Lamin A/C in three independent experiments. **(D)** Cells were harvested 24h post-transfections and ChIP assays were performed using ChREBP antibodies or rabbit IgG. The TXNIP promoter region and GAPDH coding region were amplified by quantitative real-time PCR, and the fold change in bound promoter was calculated. Bars represent means ±SEM; n=3-5.

Figure S3: **FOXO1 and FOXO1-H215R effects on endogenous TXNIP mRNA expression. (A)** Confirmation of comparable FOXO1 and FOXO1-H215R overexpression and nuclear localization. INS-1 cells transiently transfected with FOXO1 expression plasmid, FOXO1 DNA-binding mutant (H215R) or LacZ control plasmid were incubated in 5mM (LG) or 25mM (HG) glucose medium 24h post-transfection. Cells were harvested 72h post-transfection, cell fractionation was performed and nuclear FOXO1 levels were measured by immunoblotting. Bars represent quantification of three independent experiments corrected for LaminA/C. Insert: Representative blot. **(B)** FOXO1 and FOXO1-H215R effects on glucose-induced TXNIP mRNA expression. Cells were harvested 48h post-transfection and endogenous TXNIP mRNA levels were measured by quantitative real-time PCR. Bars represent mean fold change ±SEM; n=3.

Figure S4: **FOXO1-ChREBP interaction.**
ChREBP, wild-type FOXO1 and the FOXO1 DNA-binding mutant H215R were in vitro translated using TNT Quick Coupled Transcription/Translation System (L1171 Promega). For co-immunoprecipitation, input loaded was 12.5% (lane 1) and 4µl of ChREBP and FOXO1 or H215R were incubated with 2µg of anti-FOXO1 antibodies (lane 2 and 3) or control IgG (lane 4 and 5) for 4h at 4 °C in 200µl immunoprecipitation buffer, followed by addition of protein A-Sepharose beads (50µl slurry) and overnight incubation at 4°C. Beads were washed 6 times, resuspended in 20µl of SDS electrophoresis sample buffer, boiled for 5 min at 95 °C and centrifuged for 5 min at 10000 rpm. Samples were analyzed by SDS-PAGE and immunoblotting with rabbit anti-ChREBP followed by horseradish peroxidase-conjugated secondary anti-rabbit antibodies. Bands were visualized by ECL Plus detection reagent.

Figure S5: **Effects of FOXO1 knock down on TXNIP expression and ChREBP DNA-binding.** INS-1 cells were transfected with siFOXO1 or scrambled control (Dharmacon), incubated in 5mM glucose media and harvested for mRNA and ChIP analysis. **(A)** FOXO1 knock down efficiency. **(B)** TXNP mRNA expression as assessed

by quantitative RT-PCR 96h post-transfection. **(C-D)** ChIP assays were performed 72h post-transfection using ChREBP antibodies or rabbit IgG. The GAPDH coding region and TXNIP ChoRE region were amplified by quantitative real-time PCR and ChREBP occupancy of the TXNIP promoter was calculated. Bars represent means ±SEM; n=4.