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Supplemental Experimental Procedures

SUPPLEMENTAL DATA

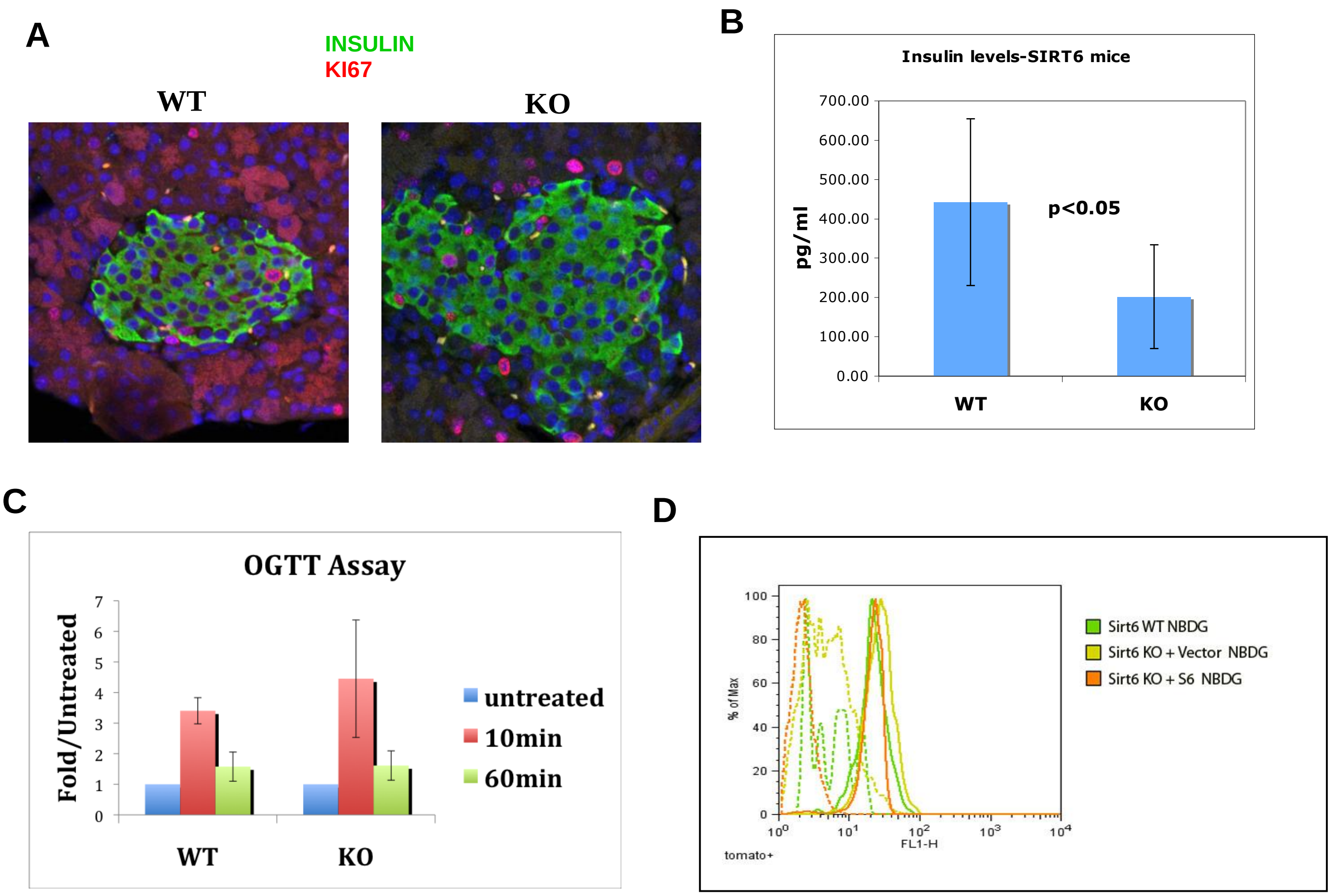


Figure S1 related to Figure 1. Normal beta islets, hypoinsulinemia and normal glucose absorption in SIRT6 deficient mice.

A- Pancreas from SIRT6 wild type (WT) and KO mice were stained with the proliferation marker KI67 (red), and insulin (green) to distinguish beta islets, as published (Nir et al., 2007).

B- Insulin was measured in blood of SIRT6 wild type (WT) and KO mice, using 20 days old animals. n = 6 each genotype. Error bars indicate the standard error of the mean.

C- Oral Glucose Tolerance Test (OGTT). 2g/kg weight of glucose was given orally to SIRT6 wild type (WT) and KO mice. Blood was collected at the indicated times, and glucose level measured.

D- Re-expression of SIRT6 rescues the glucose uptake defect in SIRT6 deficient MEFs. SIRT6 KO cells were infected with a SIRT6 expressing-lentivirus. and glucose uptake was measured following 1 hr. incubation with NBDG ($p< 0.005$).

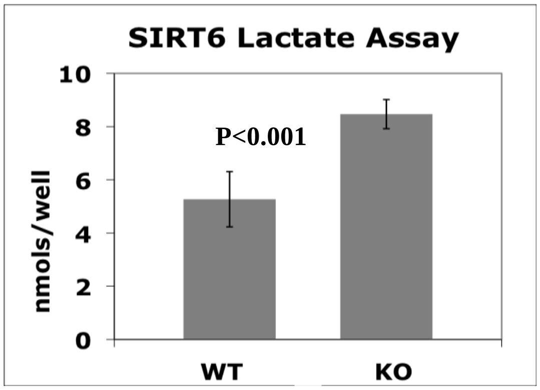
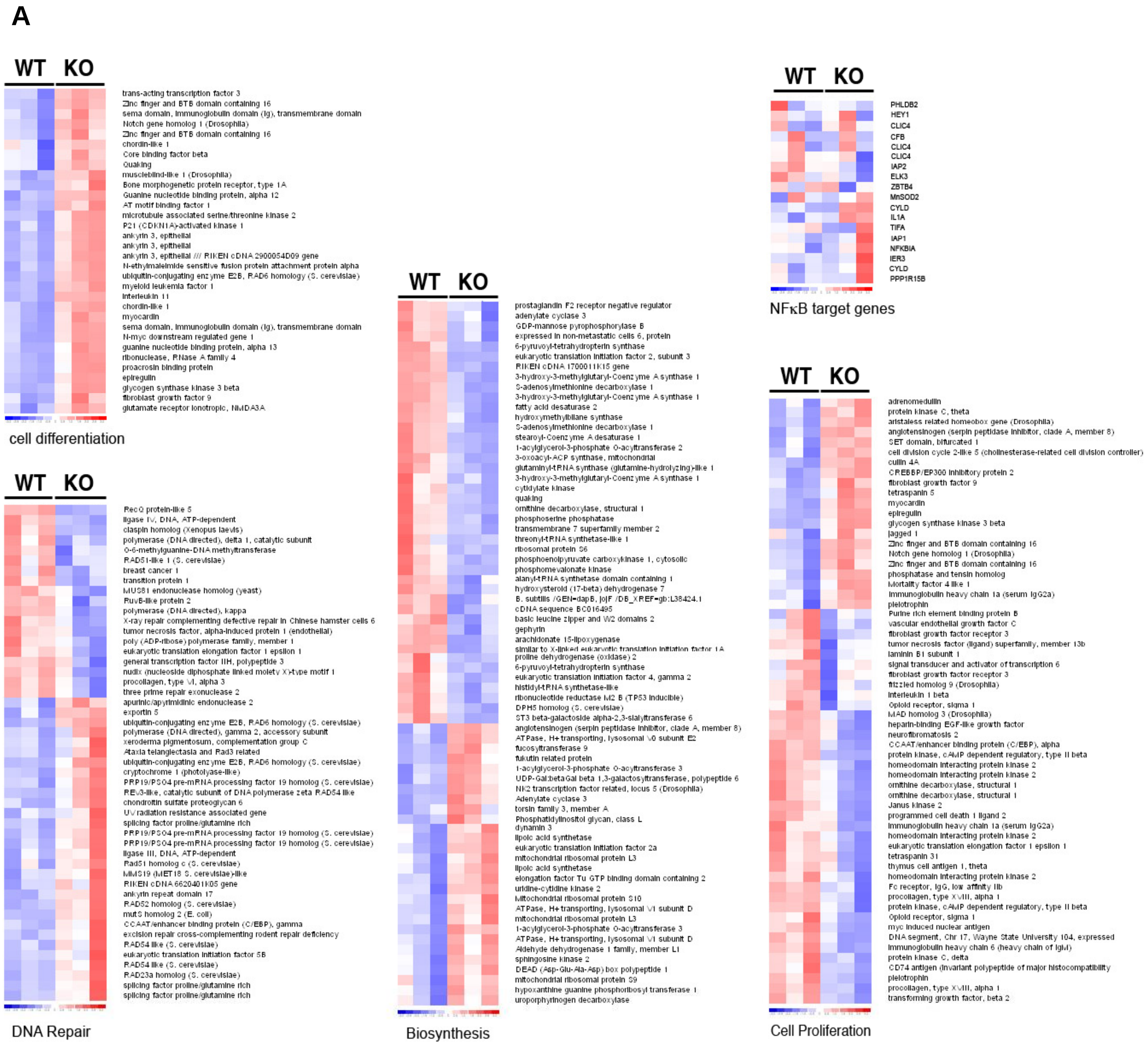


Figure S2 related to Figure 2.

Increased lactate in SIRT6 deficient MEFs

Supernatant was collected 24hr. after seeding $1x10^6$ cells of each genotype, and used in the colorimetric Lactate Assay Kit (BioVision). O.D. was measured at 570nm, 30 min. after addition of substrate.


A
WT
KO
trans-acting transcription factor 3
Zinc finger and BTB domain containing 16
sema domain, immunoglobulin domain (Ig), transmembrane domain
Notch gene homolog 1 (Drosophila)
Zinc finger and BTB domain containing 16
chordin-like 1
Core binding factor beta
Quaking
muscleblind-like 1 (Drosophila)
Bone morphogenetic protein receptor, type 1A
Guanine nucleotide binding protein, alpha 12
AT motif binding factor 1
microtubule associated serine/threonine kinase 2
P21 (CDKN1A)-activated kinase 1
ankyrin 3, epithelial
ankyrin 3, epithelial
ankyrin 3, epithelial /// RIKEN cDNA 2900054D09 gene
N-ethylmaleimide sensitive fusion protein attachment protein alpha
ubiquitin-conjugating enzyme E2B, RAD6 homology (S. cerevisiae)
myeloid leukemia factor 1
interleukin 11
chordin-like 1
myocardin
sema domain, immunoglobulin domain (Ig), transmembrane domain
N-myc downstream regulated gene 1
guanine nucleotide binding protein, alpha 13
ribonuclease, RNase A family 4
proacrosin binding protein
epiregulin
glycogen synthase kinase 3 beta
fibroblast growth factor 9
glutamate receptor ionotropic, NMDA3A
cell differentiation
WT
KO
RecQ protein-like 5
ligase IV, DNA, ATP-dependent
claspin homolog (Xenopus laevis)
polymerase (DNA directed), delta 1, catalytic subunit
O-6-methylguanine-DNA methyltransferase
RAD51-like 1 (S. cerevisiae)
breast cancer 1
transition protein 1
MUS81 endonuclease homolog (yeast)
RuvB-like protein 2
polymerase (DNA directed), kappa
X-ray repair complementing defective repair in Chinese hamster cells 6
tumor necrosis factor, alpha-induced protein 1 (endothelial)
poly (ADP-ribose) polymerase family, member 1
eukaryotic translation elongation factor 1 epsilon 1
general transcription factor IIH, polypeptide 3
nudix (nucleoside diphosphate linked moiety X)-type motif 1
procollagen, type VI, alpha 3
three prime repair exonuclease 2
apurinic/apyrimidinic endonuclease 2
exportin 5
ubiquitin-conjugating enzyme E2B, RAD6 homology (S. cerevisiae)
polymerase (DNA directed), gamma 2, accessory subunit
xeroderma pigmentosum, complementation group C
Ataxia telangiectasia and Rad3 related
ubiquitin-conjugating enzyme E2B, RAD6 homology (S. cerevisiae)
cryptochrome 1 (photolyase-like)
PRP19/PSO4 pre-mRNA processing factor 19 homolog (S. cerevisiae)
REV3-like, catalytic subunit of DNA polymerase zeta RAD54 like
chondroitin sulfate proteoglycan 6
UV radiation resistance associated gene
splicing factor proline/glutamine rich
PRP19/PSO4 pre-mRNA processing factor 19 homolog (S. cerevisiae)
PRP19/PSO4 pre-mRNA processing factor 19 homolog (S. cerevisiae)
ligase III, DNA, ATP-dependent
Rad51 homolog c (S. cerevisiae)
MMS19 (MET18 S. cerevisiae)-like
RIKEN cDNA 6620401K05 gene
ankyrin repeat domain 17
RAD52 homolog (S. cerevisiae)
mutS homolog 2 (E. coli)
CCAAT/enhancer binding protein (C/EBP), gamma
excision repair cross-complementing rodent repair deficiency
RAD54 like (S. cerevisiae)
eukaryotic translation initiation factor 5B
RAD54 like (S. cerevisiae)
RAD23a homolog (S. cerevisiae)
splicing factor proline/glutamine rich
splicing factor proline/glutamine rich
DNA Repair
WT
KO
prostaglandin F2 receptor negative regulator
adenylate cyclase 3
GDP-mannose pyrophosphorylase B
expressed in non-metastatic cells 6, protein
6-pyruvoyl-tetrahydropterin synthase
eukaryotic translation initiation factor 2, subunit 3
RIKEN cDNA 1700011K15 gene
3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1
S-adenosylmethionine decarboxylase 1
3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1
fatty acid desaturase 2
hydroxymethylbilane synthase
S-adenosylmethionine decarboxylase 1
stearoyl-Coenzyme A desaturase 1
1-acylglycerol-3-phosphate O-acyltransferase 2
3-oxoacyl-ACP synthase, mitochondrial
glutaminyl-tRNA synthase (glutamine-hydrolyzing)-like 1
3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1
cytidylate kinase
quaking
ornithine decarboxylase, structural 1
phosphoserine phosphatase
transmembrane 7 superfamily member 2
threonyl-tRNA synthetase-like 1
ribosomal protein S6
phosphoenolpyruvate carboxykinase 1, cytosolic
phosphomevalonate kinase
alanyl-tRNA synthetase domain containing 1
hydroxysteroid (17-beta) dehydrogenase 7
B. subtilis /GEN=dapB, jojF /DB_XREF=gb:L38424.1
cDNA sequence BC016495
basic leucine zipper and W2 domains 2
gephyrin
arachidonate 15-lipoxygenase
similar to X-linked eukaryotic translation initiation factor 1A
proline dehydrogenase (oxidase) 2
6-pyruvoyl-tetrahydropterin synthase
eukaryotic translation initiation factor 4, gamma 2
histidyl-tRNA synthetase-like
ribonucleotide reductase M2 B (TP53 inducible)
DPH5 homolog (S. cerevisiae)
ST3 beta-galactoside alpha-2,3-sialyltransferase 6
angiotensinogen (serpin peptidase inhibitor, clade A, member 8)
ATPase, H+ transporting, lysosomal V0 subunit E2
fucosyltransferase 9
fukutin related protein
1-acylglycerol-3-phosphate O-acyltransferase 3
UDP-Gal:betaGal beta 1,3-galactosyltransferase, polypeptide 6
NK2 transcription factor related, locus 5 (Drosophila)
Adenylate cyclase 3
torsin family 3, member A
Phosphatidylinositol glycan, class L
dynamin 3
lipoic acid synthetase
eukaryotic translation initiation factor 2a
mitochondrial ribosomal protein L3
lipoic acid synthetase
elongation factor Tu GTP binding domain containing 2
uridine-cytidine kinase 2
Mitochondrial ribosomal protein S10
ATPase, H+ transporting, lysosomal V1 subunit D
mitochondrial ribosomal protein L3
1-acylglycerol-3-phosphate O-acyltransferase 3
ATPase, H+ transporting, lysosomal V1 subunit D
Aldehyde dehydrogenase 1 family, member L1
sphingosine kinase 2
DEAD (Asp-Glu-Ala-Asp) box polypeptide 1
mitochondrial ribosomal protein S9
hypoxanthine guanine phosphoribosyl transferase 1
uroporphyrinogen decarboxylase
Biosynthesis
WT
KO
PHLDB2
HEY1
CLIC4
CFB
CLIC4
CLIC4
IAP2
ELK3
ZBTB4
MnSOD2
CYLD
IL1A
TIFA
IAP1
NFKBIA
IER3
CYLD
PPP1R15B
NFκB target genes
WT
KO
adrenomedullin
protein kinase C, theta
aristaless related homeobox gene (Drosophila)
angiotensinogen (serpin peptidase inhibitor, clade A, member 8)
SET domain, bifurcated 1
cell division cycle 2-like 5 (cholinesterase-related cell division controller)
cullin 4A
CREBBP/EP300 inhibitory protein 2
fibroblast growth factor 9
tetraspanin 5
myocardin
epiregulin
glycogen synthase kinase 3 beta
jagged 1
Zinc finger and BTB domain containing 16
Notch gene homolog 1 (Drosophila)
Zinc finger and BTB domain containing 16
phosphatase and tensin homolog
Mortality factor 4 like 1
Immunoglobulin heavy chain 1a (serum IgG2a)
pleiotrophin
Purine rich element binding protein B
vascular endothelial growth factor C
fibroblast growth factor receptor 3
tumor necrosis factor (ligand) superfamily, member 13b
laminin B1 subunit 1
signal transducer and activator of transcription 6
fibroblast growth factor receptor 3
frizzled homolog 9 (Drosophila)
interleukin 1 beta
Opioid receptor, sigma 1
MAD homolog 3 (Drosophila)
heparin-binding EGF-like growth factor
neurofibromatosis 2
CCAAT/enhancer binding protein (C/EBP), alpha
protein kinase, cAMP dependent regulatory, type II beta
homeodomain interacting protein kinase 2
homeodomain interacting protein kinase 2
ornithine decarboxylase, structural 1
ornithine decarboxylase, structural 1
Janus kinase 2
programmed cell death 1 ligand 2
immunoglobulin heavy chain 1a (serum IgG2a)
homeodomain interacting protein kinase 2
eukaryotic translation elongation factor 1 epsilon 1
tetraspanin 31
thymus cell antigen 1, theta
homeodomain interacting protein kinase 2
Fc receptor, IgG, low affinity IIb
procollagen, type XVIII, alpha 1
protein kinase, cAMP dependent regulatory, type II beta
Opioid receptor, sigma 1
myc induced nuclear antigen
DNA segment, Chr 17, Wayne State University 104, expressed
immunoglobulin heavy chain 6 (heavy chain of IgM)
protein kinase C, delta
CD74 antigen (invariant polypeptide of major histocompatibility
pleiotrophin
procollagen, type XVIII, alpha 1
transforming growth factor, beta 2
Cell Proliferation

B

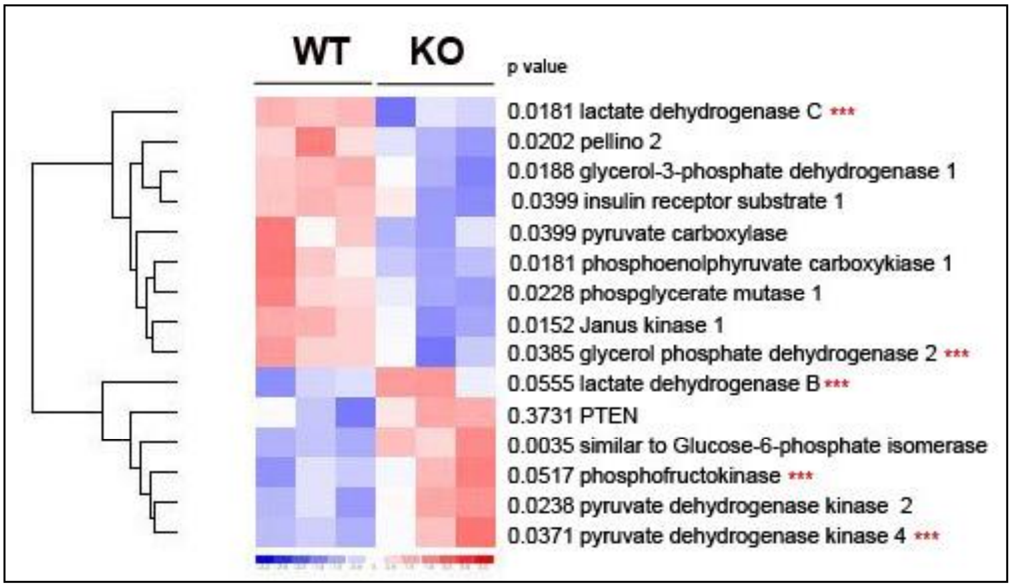


Figure S3 related to Figure 3. Expression array analysis in SIRT6 muscle

A. RNA was prepared from muscle of three SIRT6 wild type and KO mice, and hybridized onto an Affymetrix GeneChip Mouse Genome 430 2.0 Array. Fold-change threshold was set to 2. The clustering figures were generated using dChip2006 Hierarchical clustering feature, and genes in each of these pathways were filtered using the dChip Software Tool Analysis. Note lack of statistical differences in expression of NF-κB targets between WT and KO ES cells.

B. Glucose metabolism array analysis in SIRT6 muscle**.** RNA was prepared as described in Supplemental Figure 3A. Genes were filtered using a custom-prepared filter for glucose metabolism (196 genes analyzed). Shown in here are the genes with the highest p-values (main glycolytic genes are highlighted with red asterisks).

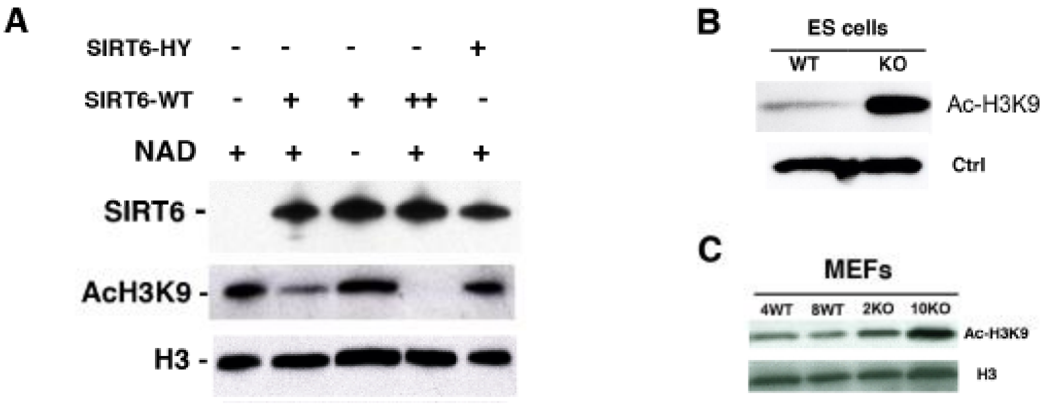


Figure S4 related to Figure 3. SIRT6 functions as a histone H3K9 deacetylase *in vitro* and *in vivo.*

A. In vitro deacetylation assay. Flag-tagged purified SIRT6 was incubated with 10μg Hela-purified histones (Millipore) for 1hr. at 37^0C, in the presence or absence of NAD. Western blots were developed with the indicated antibodies.

B-C. Whole cell extracts from SIRT6 wild type (WT) and KO ES cells (B) and MEFs (C) were blot with the indicated antibodies.

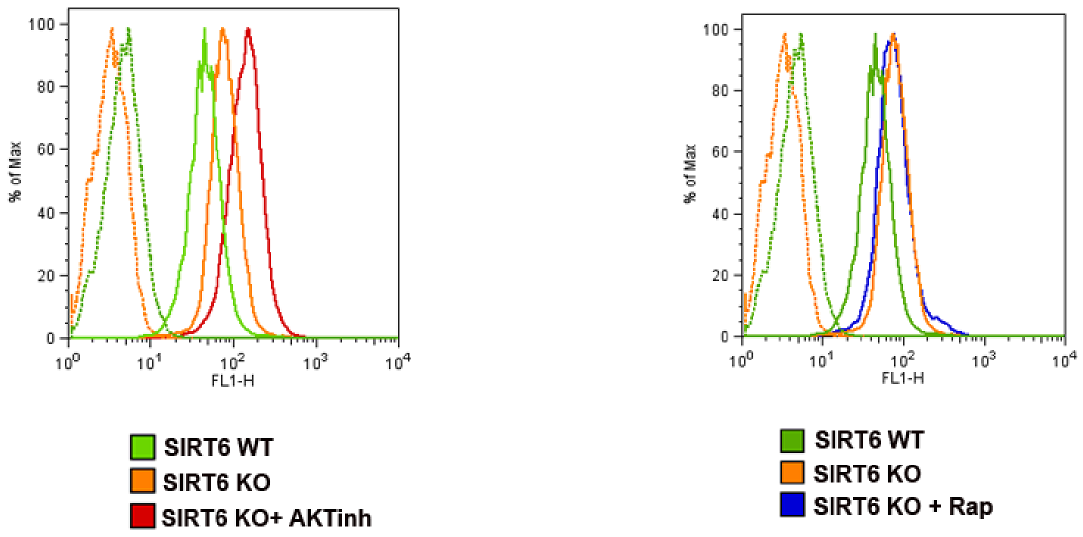


Figure S5 related to Figure 4. Treatment with AKT inhibitor or Rapamycin (mTOR inhibitor) do not rescue the glucose uptake defect in SIRT6 deficient cells.

SIRT6 wild type (WT) and KO ES cells were grown in the presence of the fluorescent glucose analog NBDG for 1 hr., and KO cells were either left untreated or pre-treated for 24hr. with 20µM AKT inhibitor XI (Calbiochem) (left panel) or 20 nM Rapamycin (right panel). Glucose uptake was then quantified using flow cytometry (FACs). Dotted lines are controls without the fluorescent NBDG glucose analog. Note lack of statistical difference following treatment (p=0.54 for AKT inh. and p=0.29 for Rap.).

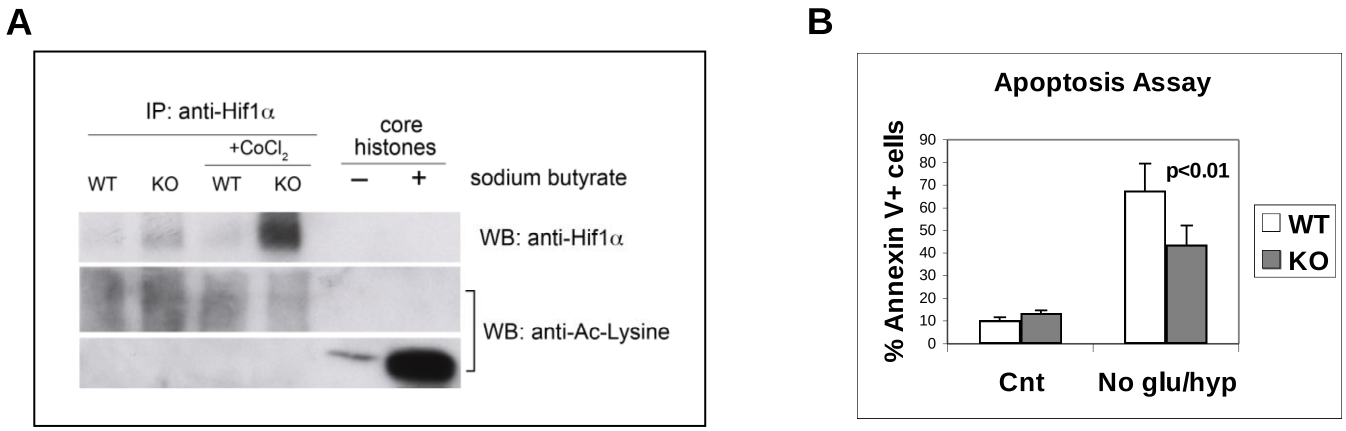


Figure S6 related to Figure 6.

A. Lack of acetylation in Hif1α

Hif1α was immunoprecipitated from SIRT6 wild type (WT) and KO cells, and the samples were analyzed by western blots with antibodies against Hif1α (Novus) and a pan-Acetyl Lysine (Cell Signaling). Note that even in the SIRT6 KO cells, where total Hif1α levels were significantly increased, we failed to detect acetylation. Histones (untreated or treated with the deacetylase inhibitor butyrate) were used as controls for specificity of the pan-Acetyl Lysine antibody.

B. SIRT6 KO cells are more resistant to Hypoxia/No Glucose-induced apoptosis.

SIRT6 wild type (WT) and KO ES cells were cultured either in control ES medium (Cnt) or in medium without glucose in 1% oxygen for 24 hr. Apoptosis was measured by Annexin V staining (BD Pharmingen), as indicated by the manufacturer. Results are the average of three independent experiments. Error bars indicate the standard error of the mean.

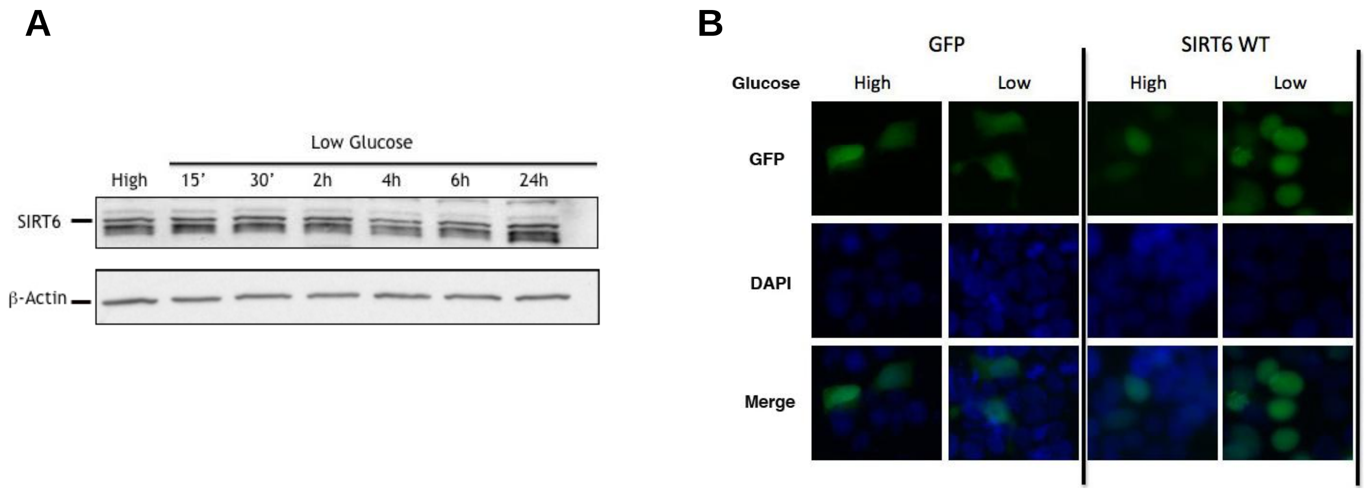


Figure S7 related to Figure 7.

Nutrient stress does not affect total protein levels or localization of SIRT6.

A. Total SIRT6 levels were assayed by Western blot, following glucose deprivation in ES cells. β-actin was used as loading control.

B. SIRT6-GFP was transfected into 293T cells, and localization of SIRT6 evaluated following glucose starvation. Note that SIRT6 localizes diffusely to the nucleus, as previously published (Mostoslavsky et al., 2006), and this pattern is not affected by nutrient stress. GFP-only vector was used as control (localizes both to nucleus and cytoplasm).

Supplemental Experimental Procedures

ChIPs and Q-RT-PCR

For Q-RT-PCR, total RNA was isolated using RNeasy Mini Kit (Qiagene) and cDNA was generated using QuantiTect Reverse Transcription Kit (Qiagene). Q-PCR was carried out using Brilliant SYBR Green QPCR Master Mix Kit (Stratagene). For ChIP assays, cells were fixed with 1% formaldehyde and harvested for whole-cell lysate preparation. Protein lysate was used for ChIP with the following antibodies: anti-SIRT6 antibody (Novus), anti-Hif1α antibody (Novus) and anti-H3K9Ac antibody (Abcam). ChIP-enriched DNA was analyzed by Q-PCR as described above. High resolution ChIP analysis was carried out as described in (Donner et al., 2007), using the following antibodies: RNA polymerase II **(**Santa Cruz Biotechnology), S5P-CTD **(**Covance) and S2P-CTD **(**Covance). The primers' sequences for all the RT-PCRs are included in Supplementary Experimental Procedures.

Polysome Profiling Analysis

Wildtype and KO ES cells were incubated with 0.1mg/mL cycloheximide for 10 minutes at 37°C in cell culture medium, after which cells were washed with ice-cold PBS containing 0.1mg/mL cycloheximide. Next, cytoplasmic extracts were prepared by douncing cells in 400uL of polysome lysis buffer (10mM NaCl, 15mM MgCl2, 10mM Tris-HCl (pH 7.5), 1.2% Triton X-100, 0.12% Deoxycholate, 0.1mg/mL heparin, and 0.1mg/mL cycloheximide). Cytoplasmic extracts were then layered over 15–45% sucrose gradients and centrifuged for 2.5 h at 37,000 rpm at 4°C in a Beckman SW41Ti rotor. Gradients were fractionated using an ISCO gradient fractionation system connected to an UV detector for continuous measurement of the absorbance at 254nm. Total RNA was isolated from each fraction using RNA STAT60 (Tel-test, Inc) and cDNA synthesis was performed on pooled RNA fractions using SuperScript II Reverse Transcriptase (Invitrogen). Subsequently, quantitative RT-PCR was performed to assess distribution of HIF1 alpha mRNA, which was normalized to 18S mRNA.

Supplemental Table 1.

PCR primers for the ChIP assays.

LDHB-ChIP-5'	AGAGAGAGCGCTTCGCATAG
LDHB-ChIP-3'	GGCTGGATGAGACAAAGAGC
ALDOC-ChIP-5′	AAGTGGGGCACTGTTAGGTG
ALDOC-ChIP-3′	GTTGGGGATTAAGCCTGGTT
PFKM-ChIP-5′	TTAAGACAAAGCCTGGCACA
PFKM-ChIP-3′	CAACCACAGCAATTGACCAC
LDHA-ChIP-5′	AGGGGGTGTGTGAAAACAAG
LDHA-ChIP-3′	ATGGCTTGCCAGCTTACATC
LDHA-ChIP-1Kb-5′	TGCAAGACAAGTGTCCCTGT
LDHA-ChIP-1Kb-3′	GAGGGAATGAAGCTCACAGC
Pkd4-ChIP-F	CTGTAGTCCCCCTTCCCTGT
Pdk4-ChIP-R	GAGCTTTTGGAGCAGACTGG
Hif1α-F	ACCTTCATCGGAAACTCCAAAG
Hif1α-R	CTGTTAGGCTGGGAAAAGTTAGG