Legends to Supplementary Figures

Supplementary Figure S1 (related to Figure 1). The mTORC1 pathway regulates glutamine metabolism via glutamate dehydrogenase

Glutamine uptake was determined in: **(A)** *Tsc2*-/- MEFs stably expressing empty vector (EV) or TSC2, and **(B)** HEK293T cells stably expressing Rheb-WT, the mutant S16H Rheb or empty vector (EV). Immunoblots showing increased S6K1 and S6 phosphorylation levels in HEK293T cells expressing Rheb (right panel). **(C)** Ammonia production in *Tsc2*-/- MEFs treated with rapamycin. **(D)** Schematic of the expected labeling patterns of TCA cycle intermediates from uniformly labeled glutamine (top panel). Direct glutamine contribution in *Tsc2*-/- MEFs after rapamycin treatment to α-ketoglutarate (m+5), succinate (m+4), malate (m+4) and citrate (m+4) using a [U-$^{13}C_5$]-glutamine tracer. (E) Direct glutamine contribution to Alanine (m+1) in DLD1 cells after rapamycin or AOA treatment using α-^{15}N-glutamine as a tracer. The mean is shown; error bars represent SEM (n > 3).

Supplementary Figure S2 (related to Figure 2). mTORC1 controls glutamate dehydrogenase activity by repressing SIRT4

SIRT4 protein levels in whole cell lysates of: **(A)** *Tsc2*-/- MEFs transfected with a non-targeting control siRNA (NTC), three individual siRNAs or a siRNA pool against SIRT4; **(B)** *Tsc2*-/- MEFs treated with rapamycin at indicated time points; **(C)** *Tsc2*-/- MEFs treated with the indicated compounds for 24h. The concentrations of the compounds were: rapamycin 20ng/mL; U0126 10μM; KU0063794 1μM; and **(D)** *Tsc2*-/- MEFs treated with the indicated compounds for 24h. The concentrations of the compounds were: rapamycin 20ng/mL; LY294002 20μM; PI-103 1μM; KU0063794 1μM. **(E)** Xenograft tumors after inoculating ELT3 cells expressing vector (V3) or *Tsc2* (T3) into nude mice. **(F)** Immunoblot analysis of SIRT4 in ELT3 xenograft tumors treated with or without rapamycin. ELT3 cells were inoculated into nude mice and when tumors reached 100 mm^2, mice were randomly assigned to i.p., rapamycin at a dose of 1 mg/kg, or sterile PBS 3 times per week for 2 weeks.

Supplementary Figure S3 (related to Figure 3). SIRT4 is regulated at the mRNA level in an mTORC1-dependent fashion.

(A) Immunoblots analysis from whole cell lysates of *Tsc2*-/- MEFs transfected with a non-targeting control siRNA (NTC), or with four individual siRNAs against CREB2. **(B)** *SIRT4* mRNA levels in *Tsc2*-/- MEFs transfected with non-targeting siRNA (NTC) or with siRNA pools against either CREB-1 or CREB2. The mean is shown; error bars represent SEM (n > 3).

Supplementary Figure S4 (related to Figure 4). mTORC1 regulates the stability of CREB2

(A) *CREB2* mRNA levels in *Tsc2*-/- MEFs treated with rapamycin (Rapa) 20ng/mL for 24h. The mean is shown; error bars represent SEM (n > 3). **(B)** Immunoblot analysis of CREB2 in *Tsc2*-/- MEFs treated with rapamycin (Rapa) 20ng/mL or U0126 10μM. Cells were harvested at the indicated time points. **(C)** Western blotting of *Tsc2*-/- MEFs transfected with non-targeting siRNA (NTC) or with siRNA pools against either CREB-1 or CREB2, and then treated with rapamycin (Rapa) 20ng/mL for 24h. **(D)** CREB2 protein in whole cell lysates from *Tsc2*-/- cells treated with the indicated compounds for 24h. The concentrations of the compounds were: rapamycin (Rapa) 20ng/mL; LY294002 20μM; PI-103 1μM; KU0063794 1μM. **(E)** Immunoblot analysis of *Tsc2*+/+ MEFs transfected with a non-targeting control siRNA (NTC) or two siRNAs against mTOR. Forty-eight hours after transfection cells were serum starved for 18h followed by insulin (100nM) stimulation for eight hours. **(F)** *Tsc2*+/+ MEFs were serum starved for 18h and pre-treated with the indicated drugs for 30 min, followed by addition of insulin (100nM) for 2 or 6h. The concentrations of the compounds were: rapamycin (Rapa) 20ng/mL; MG132 20μM. Protein lysates were resolved by immunoblot using the antibodies indicated. **(G)** HEK293E cells expressing CREB2 mutants as indicated.

Supplementary Figure S5 (related to Figure 5). SIRT4 represses bioenergetics and proliferation of Tsc2-/- MEFs

(A) Cell viability of control of SIRT4 expressing *Tsc2*+/+ MEFs deprived of glucose and supplemented with DM-αKG (7mM) for 48h. **(B)** Growth curves of control or SIRT4 expressing *Tsc*2-/- MEFs cultured in standard media lacking pyruvate. Cell number was measured every 24h for four consecutive days. **(C)** Cell number of control or SIRT4 expressing *Tsc2* wildtpe (WT) or *Tsc2*-/- MEFs cultured in standard media lacking pyruvate for 72h. Low panel: Immunoblot analysis of HA-SIRT4 and α-Tubulin. **(D)** Immunofluorescence of *Tsc2*-/- expressing control vector or SIRT4-HA. Cells were fixed as explained in the experimental procedures and then were reacted with anti-HA. MitoTracker was used as a specific mitochondrial marker. The mean is shown; error bars represent SEM (n > 3).

Supplementary Figure S6 (related to Figure 6). SIRT4 is a potential tumor suppressor

Immunoblot analysis of xenograft tumors of *Tsc2*-/- cells expressing control vector or SIRT4.

Supplementary Figure S7 (related to Figure 7). The combination of glutamine metabolism inhibitors with glycolytic inhibition is an effective therapy to kill Tsc2-/- and PTEN-/- cells

Cell viability of: *PTEN*-/- MEFs **(A)** or *PTEN*+/+ *MEFs* ***(B)*** *after 48h treatment with* the indicated doses of Mechlorethamine hydrochloride with or without EGCG (50μM). **(C)** *Tsc*2-/- MEFs transduced with an empty vector were given the indicated doses of Mechlorethamine hydrochloride with or without BPTES (10μM). Cell viability was measured 48 hours post treatment via PI-exclusion. **(D)** *Tsc2*-/- MEFs re-expressing *Tsc2* were treated as in (C). **(E)** Cell viability of *Tsc2*-/- MEFs treated with mechlorethamine (Mechlo) 20μM with or without BPTES (10μM) cultured in standard media lacking pyruvate and supplemented with OAA (2mM) or pyruvate (10mM) for 48h. **(F)** *Tsc*2-/- MEFs transduced with an empty vector were treated with 2-DG (10mM) in the presence or absence of EGCG (50μM) or BPTES (10μM). Cell viability was measured every twenty-four hours for three days via PI-exclusion. **(G)** *Tsc2*-/-

MEFs re-expressing *Tsc2* were treated as in (F). The mean is shown; error bars represent SEM (n > 3).

Supplementary Table S1 Putative transcription factor-recognition sites on the human *SIRT4* promoter

Gene Symbol [a, b]	Gene Name
AML-1a	Acute myeloid leukemia 1a
AP-1	Activator protein-1
Cdx1	Caudal type homeobox 1
CREB2	cAMP response element binding-2
c-Rel	c-Reticuloendotheliosis
deltaE	Delta E Transcription Factor
E2F	Adenovirus E2 promoter-binding factor
GATA-1	GATA sequence binding protein-1
GATA-2	GATA sequence binding protein-2
HFH-1	Hepatocyte nuclear factor-3 homologue 1
HSF2	Heat shock protein factor 2
LSF	Late SV40 Factor
M2F1	Myeloid zinc finger protein 1
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells
Nkx-2	Homeobox protein Nkx-2
Oct-1	Octamer binding protein-1
p300	E1A binding protein p300
Sox-5	SRY-related HMG box-5
Sp1	Specificity protein 1
SRY	Sex-determining region
v-Myb	Myeloblastosis viral oncogene homolog (avian)

[a)] Putative transcription factors were identified using TFSEARCH software. The minimum cut-off threshold score was set to 85 points (out of 100). All listed genes were derived from vertebrate species.

[b)] Nomenclature for gene symbols is based on designations from the NCBI sequence database.

Extended Experimental Procedures

Cell Viability Measurements

All cell viability experiments were conducted with propidium iodide (PI) exclusion assay. In brief, PI was added to culture media (1 μg/mL) for 5 minutes prior to collection. The media from each sample was collected and then the plates were washed briefly with saline. The saline was added to the media collection tube, and then the remaining cells on the plate were detached with trypsin. The cells were then collected and also put into the original media collection tube. The entire tube, which now has the original media with saline wash and trypsinized cells, was centrifuged and the pellet was analyzed for PI-exclusion via FACS (Beckon Dickinson). Additionally, the cells were washed with media lacking glucose and all amino acids (1/2 volume of incubating media) prior to adding the experimental media, which contained dialyzed FBS. For mechlorethamine experiments, cells were plated overnight (~15-18 hours), and media was changed for new media without pyruvate and with or without glutamine/ glucose. 8 hours after the change, cells were given different doses of mechlorethamine.

Antibodies, Chemicals, Plasmids

The antibodies used for this study are the following: Ki-67 antibody from BioGenex (San Ramon, CA). p53, HIF-1α, Raptor, p70, 389(P) p70, S6(P), Akt-473, Akt, α-Tubulin, 4EBP1, PTEN, TSC2, 202(P)204(P) ERK1/2, ERK1/2, 51(P) eIF2a, CREB, and cleaved PARP were all purchased from Cell Signaling Technologies. Antibody to CREB2 was purchased from Santa Cruz, Inc. Antibody to GDH was purchased from Abcam. SIRT4 and FLAG-M2 were purchased from Sigma. Rictor antibody was from Bethyl Laboratories. GAPDH antibody was purchased from Ambion. PAR antibody was from Calbiochem. The following chemicals were used in this study: Rapamycin (Calbiochem), EGCG (Calbiochem), LY294002 (Calbiochem), Cycloheximide (Calbiochem), methylpyruvate (Sigma), aminooxyacetate (Sigma), DM-α-ketoglutaric acid (Sigma), sodium pyruvate (Sigma), Mechlorethamine Hydrochloride (Sigma), succinic acid (Sigma), glutamate (Sigma), U0126 (Selleckchem), 3-Methyladenine

(Sigma), Oligomycin (Calbiochem), Insulin (Sigma), PI-103 (Selleckchem), MG132 (Sigma), DPQ (Santa Barbara Technology), biotin-NAD^+ (Trevigen), Lipofectamine and RNAi MAX (Invitrogen), Bromobimane (Sigma). *SIRT4* was cloned into pBabe, *TSC2* was cloned into pLPCX, and the *TSC2* containing viruses were used to reconstitute *Tsc2-/- p53-/-* MEFs. Rheb wild- type and S16H, were cloned into pLPCX2.

siRNA transfections

Twenty five (25) nM siRNAs were transfected in cells right after being seeded at a density of 30-50% confluency depending on experiments using Lipofectamine RNAiMAX (Invitrogen) according to the manufacturer's protocols. Cells were harvested 36-60 hours post-transfection as described in the Figure legends. All siRNAs used were obtained from Dharmacon as follows: siCREB2 #1, D-042737-01; siCREB2 #2, D-042737-02; siCREB2 #4, D-042737-04; siCREB2 #17, D-042737-17; siRaptor SMART-Pool, M-058754; siRictor SMART-Pool, M-064598; siCREB1 #1, D-048375-01; siSIRT4 #2, D-065606-02; siSIRT4 #3, D-065606-03; siSIRT4 #17, D-065606-17; siSIRT4 #18, D-065606-18; siSIRT4 #4 D-048375-04; siFBXW1B #1, D-058886-01; siFBXW1B #2, D-058886-02; siFBXW1B #3, D-058886-03; siFBXW1B #4, D-058886-04; siFRAP SMART-Pool, M-065427; siCREB1 #1, D-040959-01; siCREB1 #2, D-040959-02; siCREB1 #3, D-040959-03, siCREB1 #4, D-040959-04.

Retroviral infections

With Lipofectamine 2000 (Invitrogen), retroviruses were generated by transfecting 15 μg of pLPCX or pBabe-based plasmids into VSVG 293T cells 24 hours post-seeding onto 10 cm dishes at a density of 90% confluency. Viruses were collected on days 4, 5, and 6 post-transfection, and pooled for infecting cells. Depending on virus titers, retroviruses were infected at least three times every 24 hours in actively growing cells. Non-infected cells were eliminated later in the presence of 2-5 μg/ml of puromycin.

Bioinformatic meta-analysis of SIRT4 expression

Microarray expression data from six independent data sets (Figure 6f) corresponding to tumor samples of different origin including bladder (Dyrskjot et al., 2004), breast (TCGA), colon (TCGA), gastric (Cho et al., 2011), ovarian (Bonome et al., 2008) and thyroid (He et al., 2005) were downloaded from TCGA portal https://tcga-data.nci.nih.gov/tcga/ and the Oncomine repository at http://www.oncomine.org/ to examine the relative mRNA expression levels of SIRT4 between normal and cancer samples. The distributions of log2 median-centered signal intensities were plotted using boxplots and differential gene expression was computed using the Welch two sample t-test, which is appropriate for subsets of unequal variances. Only the tumor sets which showed the same differential mode of expression in at least three independent datasets were included in this analysis.

A univariate cox proportional hazard regression model (Andersen et al., 1982) was applied to correlate gene expression of SIRT4 with time to metastasis in a breast carcinoma dataset of n=195 samples (Symmans et al., 2010) and the Likelihood ratio test, Wald test, and Score (logrank) test were all used to compute the P value (*P < 0.04 for all three tests). To visualize the result obtained from this analysis, the samples were ranked according to SIRT4 gene expression (Supplementary Fig. 6a) and Kaplan-Meier curves (Supplementary Fig. 6b) were plotted for breast carcinomas with the lowest (<25th percentile) versus highest (>25th percentile) SIRT4 expression giving a P value of 0.02 (logrank test) (Supplementary Fig. 6b).

Immunofluorescence assays

Cells were seeded onto poly-L-lysine- and fibronectin-coated glass coverslips in 12-well tissue culture plates at a density of 30% confluency. The coverslips were fixed with 4% methanol-free formaldehyde (Polysciences) in PBS for 30 min, rinsed, and permeabilized three times with 0.05% Saponin (Sigma) in PBS. The coverslips were then blocked for 1 hour with the blocking buffer (50:50 mixture of 0.05% Saponin buffer and Li-COR blocking buffer) and incubated overnight at 4 ^{o}C with anti-HA.11 antibody (1:100 dilution, Covance, #MMS-101P) along with MitoTracker (1:1000 dilution, Invitrogen) in the blocking buffer. After rinsing three times with the Saponin

buffer, the coverslips were incubated for 1 hour at room temperature in the dark with the secondary antibodies conjugated with with Alexa568 (1:1000 dilution in the blocking buffer), washed four times with PBS, and mounted onto the glass slides with a mounting medium (Dako). Images were taken with a 60x objective using a spinning disk confocal microscopy (Nikon), processed, and analyzed using the MetaMorph® program.

Glutathione Measurement

Bromobimane staining was used to measure reduced glutathione levels using flow cytometry.

NAD Measurement

NAD levels were measured using the NAD/NADH kit from Abcam (ab65348) following the manufacturer's instructions.

Cell Lysis and Immunoprecipitations

Cells washed once with cold PBS were solubilized on ice either in a regular lysis buffer (40 mM HEPES [pH 7.4], 1mM EDTA, 120 mM NaCl, 10 mM β-glycerophosphate, 1 mM NaF, 1 mM Na_3VO_4, and 0.3% CHAPS) or in a low-salt lysis buffer (40 mM HEPES [pH 7.4], 1mM EDTA, 10 mM β-glycerophosphate, 1 mM NaF, 1 mM Na_3VO_4, and 0.3% CHAPS) supplemented with protease inhibitors (250 μM PMSF, 5 μg/ml Pepstatin A, 10 μg/ml Leupeptin, and 5 μg/ml Aprotinin). Cleared cell lysates were obtained by centrifugation at 10,000rpm for 10 min at 4^{o}C and 0.7-1.2 mg of the lysates were used for immunoprecipitations. For this, cell lysates were incubated with primary antibodies for 2 hours at 4°C followed by 1-2 hour further incubation with 50% slurry of protein A/G-sepharose presaturated with the lysis buffer. After rinsing three times with the regular or low-salt lysis buffer, immunoprecipitated proteins were recovered from the beads by boiling for 10 min in sample buffer, and analyzed by immunoblotting.

ADP-ribosylation assay

Mono-ADP ribosylation levels were determined as previously described (Mao et al., 2011) with slight modifications. Cells were transfected with 6-Biotin-NAD+ (Lonza transfection protocol). 24h after transfection cells were treated with rapamycin for 24h and harvested with IP buffer. Mitochondria were purified using the isolation kit from Pierce. Poly-ADP ribosylated proteins from mitochondrial samples were cleared using PAR antibody (1:200) with protein A beads for 1h. Avidin coated beads (1:10) (Sigma) were added and incubated for 1h at 4°C. Mono-ADP ribosylated protein was developed using Streptaviding-HRP (Abcam).

ATP and ADP measurements

To measure ATP and ADP amounts, the cells were lysed in somatic releasing buffer (Sigma). Whenever possible, all experiments were conducted in the cold room and all reagents were at ~4° C prior to use. The total ATP amounts were measured via luciferase activity (Sigma) with linear ranges determined for both the samples and standard controls. The ADP levels were measured by adding excess amounts of phosphoenolpyruvate (PEP) and pyruvate kinase (Both from Sigma). This reaction, which converts all the ADP in a sample to ADP, was allowed to progress for 40 minutes in room temperature. The total ATP in this sample was measured via luciferase and the total ADP was determined by subtracting the total nmoles of ATP in the sample (1st reaction) from the second reaction, which contains both ATP and ADP. The ATP/ADP ratio was then determined.

When plating the cells for both ATP and viability assays, the media was changed when the cell confluency was about 70-80%. This equated to about 650,000 *Tsc2-/-* MEFs per 10cm plate. Deprivation was done typically 18-20 hours post plating. It has been our experience that more confluent conditions will take more time to deplete the ATP and induce cell death, while more sparse plating will take less time. To be consistent, we conducted all of our experiments when the cells were at about 70-80% confluency.

Quantitative RT-PCR analysis

Total cellular RNA was purified from cultured cells using the RNeasy mini kit (Qiagen)

following the manufacturer‟ s protocol. For quantitative real-time PCR (qRTPCR), RNA was reverse-transcribed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturer‟ s instructions. The resulting cDNA was analyzed by qRT-PCR using the QuantiTect SYBR Green qPCR System (Qiagen). A QuantiTect Primer Assay was used to amplify the target gene, while the β-actin primers (β-actin forward, ACCCAGATCATGTTTGAGACCT; and β -actin reverse, GCAGTAATCTCCTTCTGCATCC) were used as a normalization control. All reactions were run on an ABI 7900HT Fast Real-Time PCR instrument with a 15 min hot start at 95°C followed by 40 cycles of a 3-step thermocycling program: denaturation: 15 s at 94°C, annealing: 30 s at 55°C and extension: 30 s at 70°C. Melting curve analysis was performed at the end of every run to ensure that a single PCR product of the expected melting temperature was produced in a given well. A total of 3 biological replicates x 4 technical replicates were performed for each treatment group. Data was analyzed using the comparative C_t method ($\Delta\Delta C_t$ method).

Metabolite analysis of spent medium

Glucose and glutamine concentrations were measured in fresh and spent medium (after 24 hours of culture) using a Yellow Springs Instruments (YSI) 7100. Glucose and glutamine data is presented as net decrease in concentration, and levels were normalized to cell number. The media used for these experiments did not contain pyruvate and were supplemented with 10% dyalized FBS.

Ammonia Measurements

Ammonia levels in culture media were measured using the BioProfile FLEX analyzer (Nova Biomedical). Briefly, fresh media were added to a 6-well plate of cells and metabolite levels in the media were measured 6-9 hr later and normalized to the number of cells in each well.

Immunohistochemistry

Sections were deparaffinized, incubated overnight with primary antibodies at 4°C in a humidified chamber and then rinsed and incubated with biotinylated secondary

antibodies for 30 minutes at room temperature. Slides were developed using the Histostain-Plus kit (Invitrogen, Grand Island, NY), and were counterstained with Gill's hematoxylin.

In vivo manipulations

2 million *Tsc2-/-p53-/-* or *Tsc2+/+p53-/-* MEFs stably expressing SIRT4-HA or vector control pBabe were inoculated bilaterally into the posterior back region of 6-8 week-old immunodeficient CD-1 nude mice (Taconic, Hudson, NY). Tumor length, width, and depth were measured with a Vernier caliper by an investigator blinded to the experimental conditions. 2.5 millions V3 or T3 ELT3 cells were inoculated bilaterally into the posterior back region of 6-week-old immunodeficient CB17 SCID mice (Taconic). Tumors were harvested when they reached 150 mm^2. *Tsc2*+/- mice in A/J background 7-mo old were treated with either Vehicle or Rapamycin (Biomol) at a dose of 6 mg/kg every other day for 3 days (2 injections) (Supplementary Fig. 2f).
2.5 millions ELT3 cells were inoculated bilaterally into the posterior back region of 6-wk-old immunodeficient CB17 SCID mice (Taconic). When tumors reached 100 mm^2, mice were randomly assigned to i.p. rapamycin (Biomol) at a dose of 1 mg/kg, or sterile PBS 3 times per week for 2 weeks (Fig. 2d). The animal studies were approved by the Animal Care and Use Committee of Children's Hospital, Boston.

Anchorage-Independent Growth

5000 cells were grown 6-well plates containing 0.6% agarose. Plates were incubated at 37°C and 5% CO2. Cells were refed once a week and after 21-30 days, colonies from 10 different fields in each of two wells were counted for each treatment, and the average number of colonies per well was calculated. Colonies were stained by adding 0.005% Crystal violet for more than 1h at room temperature, and photographed under a light microscope at magnification x100.

Labeling, flux and metabolite level measurements

Labeled tissue cultures were washed with saline and metabolism was quenched with -20ºC cold 65% methanol. After cell scraping in 65% methanol, -20ºC cold chloroform

was added and the samples were vortexed at 4ºC to extract metabolites. Phase separation was achieved by centrifugation at 4ºC. Methanol phase was separated and dried applying constant airflow. Dried metabolite samples were stored at -80ºC. Metabolites were derivatized with methoxyamine (TS-45950 Thermo Scientific) for 90min at 40ºC and subsequently with N-(tert-butyldimethylsilyl)-N-methyl-trifluoroacetamide, with 1% tert-Butyldimethylchlorosilane (375934-Sigma) for 60min at 60ºC. Isotopomer distributions and metabolite levels were measured with a 6890N GC system (Agilent Technologies) combined with a 5975B Inert XL MS system (Agilent Technologies). Isotopomer distributions were analyzed using the Matlab based software Metran (Antoniewicz et al., 2007). Total ion counts were normalized with the internal standard norvaline and cell number to yielded metabolite levels. Glutamate-to-αKG flux was calculated based on glutamine and glutamate uptake or secretion rates.

Chromatin immunoprecipitation

A total of $2x10^6$ cells were treated with or without rapamycin for 24 h, and were then cross-linked with 3.7% formaldehyde (Sigma) at room temperature for 10 min. Cells were incubated with 0.125M glycine to terminate cross-linking, washed twice with PBS, and lysed in SDS nuclear lysis buffer (1% SDS, 10mM EDTA, and 50mM Tris-HCl, pH 8.1) for 10 min on ice. Sonicated lysates were diluted in ChIP dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.2mM EDTA, 167mM NaCl, and 16.7mM Tris-HCl, pH 8.1), and incubated with 10 μg of rabbit IgG (Pierce) and protein A agarose beads overnight at 4C with gentle rocking. The cleared supernatants were mixed with either 2 μg of an anti- CREB2 rabbit polyclonal antibody (Santa Cruz Biotechnology) or with pre-immune rabbit IgG overnight at 4C. Antibody/protein/DNA complexes were co-precipitated with protein-A beads. Protein/DNA conjugates were eluted from the beads complexes using Elution buffer (100mM NaHCO3 and 1% SDS) for 30 min. Cross-links were reversed in 5M NaCl. RNA and protein were removed by incubation first with 10 μg DNase-free RNase-A at 37C for 1 h, and then with 20 μg proteinase K at 50C for 4 h. DNA was recovered by phenol/chloroform extraction and ethanol precipitation. A DNA fragment encompassing the indicated region of the human

SIRT4 promoter was amplified using 35 cycles of PCR at 94C for 30 s, 55C for 30 s, and 72C for 30 s. All amplified products were resolved on a 2% agarose gel.

Protein half-life

Cells were treated with 100 µg/ml CHX for the indicated times in Figures 4d and Supplementary 4g. Protein was analyzed by western blotting.

GDH activity

Glutamate dehydrogenase activity was measure by an in vitro reaction using the BioVision kit, according to the manufacturer's protocol. We optimized the readout by immunoprecipitating GDH with the anti-GDH antibody from Abcam after harvesting. As a control, we performed siRNA experiment to knockdown GDH (data not shown), this experiment showed specificity to measure the activity of the enzyme.

In vivo ubiquitination assay

HEK293E cells were co-transfected with a plasmid expressing CREB2 along with pHis-Ub by Lipofectamine (Invitrogen) according to the manufacturer's instructions. At 48 hr post transfection, cells were treated with 20 µM MG132 for 6 hr. Cells were lysed in urea buffer [8 M Urea, 0.1 M NaH_2PO_4, 0.1 M Tris-HCl (pH 8.0), 0.05% Tween-20 and 10 mM imidazole (pH 8.0)]. Two mg of protein was incubated with Ni-NTA agarose-beads (Qiagen) overnight. Beads were extensively washed with denaturing wash buffer twice [8 M Urea, 0.1 M NaH_2PO_4, 0.1 M Tris-HCl (pH 8.0), 0.05% Tween-20, 20 mM imidazole (pH 8.0)] and then with native wash buffer [0.1M NaH_2PO_4, 0.1 M Tris-HCl (pH 8.0), 0.05% Tween-20, 20 mM imidazole (pH 8.0)]. Protein was dissolved in Laemmli buffer and resolved by SDS-PAGE. Monoclonal antibody FLAG M2 (SIGMA) was used to detect ubiquitinated CREB2.

References

Andersen, P. and Gill, R. (1982). Cox's regression model for counting processes, a large sample study. *Annals of Statistics* 10, 1100-1120.

Antoniewicz MR, Kelleher JK, Stephanopoulos G (2010). Accurate assessment of amino acid mass isotopomer distributions for metabolic flux analysis. *Anal Chem*. 79(19):7554-9.

Bonome T, Levine DA, Shih J, Randonovich M, Pise-Masison CA, Bogomolniy F, Ozbun L, Brady J, Barrett JC, Boyd J, Birrer MJ (2008). A gene signature predicting for survival in suboptimally debulked patients with ovarian cancer. *Cancer Res.* 68(13):5478-86.

Cho JY, Lim JY, Cheong JH, Park YY, Yoon SL, Kim SM, Kim SB, Kim H, Hong SW, Park YN, Noh SH, Park ES, Chu IS, Hong WK, Ajani JA, Lee JS (2011). Gene expression signature-based prognostic risk score in gastric cancer. *Clin Cancer Res.* 17(7):1850-7.

He H, Jazdzewski K, Li W, Liyanarachchi S, Nagy R, Volinia S, Calin GA, Liu CG, Franssila K, Suster S, Kloos RT, Croce CM, de la Chapelle A (2005). The role of microRNA genes in papillary thyroid carcinoma. *Proc Natl Acad Sci U S A.* 102(52):19075-80.

Dyrskjot L, Kruhoffer M, Thykjaer T, Marcussen N, Jensen JL, Møller K, Orntoft TF (2004). Gene expression in the urinary bladder: a common carcinoma in situ gene expression signature exists disregarding histopathological classification. *Cancer Res*. 64(11):4040-8.

Mao Z, Hine C, Tian X, Van Meter M, Au M, Vaidya A, Seluanov A, Gorbunova V (2011). SIRT6 promotes DNA repair under stress by activating PARP1. *Science*. 332(6036):1443-6.

Symmans WF, Hatzis C, Sotiriou C, Andre F, Peintinger F, Regitnig P, Daxenbichler G, Desmedt C, Domont J, Marth C, Delaloge S, Bauernhofer T, Valero V, Booser DJ, Hortobagyi GN, Pusztai L (2010). Genomic index of sensitivity to endocrine therapy for breast cancer. *J Clin Oncol.* 28(27):4111-9.