Supplementary Experimental Procedures

Antibodies

Antibodies against G6PD (Bethyl Laboratories; Abcam), SIRT2 (Sigma), GFP (Abmart), HA (Santa Cruz), Flag (ShanghaiGenomics), PARP (Cell Signalling), Caspase-3 (Cell Signalling), and p38 MAPK phosphorylation (Cell Signalling) were purchased commercially. To generate a pan-Acetyl-Lysine antibody, Chicken Ovalbumin was acetylated was described previously (Guan et al, 2010). Purified acetylated OVA was used to immunize rabbits. Anti-serum was collected after four doses of immunization. To generate a site-specific antibody to detect the acetylated K403 of G6PD [α-acG6PD(K403)], synthesized peptide EAVYTK^{ac}MMTKK (GL Biochem) was coupled to KLH as antigen to immunize rabbit. Anti-serum was collected after four doses of immunization.

Plasmids

The cDNA encoding full-length human G6PD, SIRT1, SIRT2, and SIRT3 were cloned into Flag, HA, GFP or His-tagged vectors (pcDNA-Flag; pcDNA3-HA; pEGFP-C3; pSJ3). The CobB plasmid was a generous gift from Dr. Wang Qijun (Fudan University, Shanghai, China). Point mutations of G6PD and SIRT2 were generated by site-directed mutagenesis using a QuickChange Site-Directed Mutagenesis kit (Stratagene). DNA fragments of full-length human G6PD were sub-cloned into pSJ3 encoding an N-terminal 6×His tag. All expression constructs were verified by DNA sequencing.

Cell culture and treatment

HEK293T cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) (Invitrogen, Shanghai, China) supplemented with 10% new born bovine serum (Biochrom, Germany) in the presence of penicillin, streptomycin, and 8 mM L-glutamine (Invitrogen). MEFs were kept in DMEM supplemented with 10% fetal bovine serum (Biochrom, Germany) in the presence of penicillin, streptomycin. Plasmid transfection was carried out either by the calcium phosphate method or lipofectamine 2000 (Invitrogen). Tricostatin A (TSA, 0.5 μM) and Nicotinamide (NAM, 5-15 mM) were added to the culture medium 18 and 4 hrs before cell harvest, respectively. Glucose free medium was prepared with DMEM base (GIBCO, #11966) and supplemented with glucose (Sigma) of different concentrations as indicated. Oxidative agents, including H_2O_2 (300 μM or as indicated, Sinopharm) and menadione (50 μM or as indicated, Sigma) were added to the culture medium to induce oxidative stress.

Animals

C57BL6 mice (6-8 weeks of age, weighing 20-25 g) were purchased from Fudan Animal Center. Sirt2 knock-out (KO) mice on a B6 background were purchased from the Jackson Laboratory, and maintained at Fudan Animal Center. Animals were given unrestricted access to a standard diet and tap water. Animal experiments were performed at Fudan Animal Center in

accordance with the animal ware fare guidelines.

Immunoprecipitation and western blotting

Cells were lysed in ice-cold NP-40 buffer [50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 0.1% NP-40) containing protease inhibitor cocktail (Roche). Immunoprecipitation was carried out either by incubating Flag beads (Sigma) at 4°C with lysate for 3 hrs or by incubating appropriate antibody with cell lysate for 1 hr, followed by incubating with Protein-A beads (Upstate) for another 2 hrs at 4°C before beads were washed for three times with ice-cold NP-40 buffer. Flag, HA and GFP western blotting was blocked with 5% fat-free milk (BD Biosciences), and standard western blot protocols were adapted.

For acetylation western blotting, 50 mM Tris (pH 7.5) with 10% (V/V) Tween-20 and 1% peptone (Sigma) was used for blocking, and 50 mM Tris (pH 7.5) with 0.1% peptone was used to prepare primary and secondary antibodies.

RNA isolation and quantitative real-time PCR

Total RNA was isolated from cultured cells using Trizol reagent (Invitrogen) following the manufacturer's instructions. RNA was reverse transcribed with random primers and preceded to real-time PCR with gene-specific primers in the presence of SYBR Premix Ex Taq (TaKaRa). β-actin was used as a housekeeping control. Primer sequences were present in the Supplementary Table S1.

Enzyme concentration determination

Protein concentrations were determined by the Bradford method (Bradford, 1976) using a Bio-Rad Protein Assay Kit (Bio-Rad). Relative enzyme concentrations for immuno-precipitated enzymes were quantified by measuring the band intensity of western blots by using Amersham Bisciences ImageQuant TL 2005v software.

Protein expression and purification

CobB, wild-type G6PD and its mutants were expressed in BL21 (DE3). Cells were grown at 37ºC till OD600 reached 0.5-0.6, and then the culture was induced at 16ºC overnight with 0.5 mM of IPTG. The cells were harvested, washed with PBS and stored at −80°C for further analysis.

Recombinant proteins were purified by using affinity chromatography. Cells were resuspended in lysis buffer [25 mM HEPES (pH 8.0), 150 mM NaCl, protease inhibitors (Roche)]. Cells were then lysed following three passes through an ice-cold French pressure cell press and the lysate was cleared by centrifugation at 40,000g for 1 hr. The hexahistidine fusion proteins were purified on a nickel-charged column (HisTrap HP, GE healthcare). After washing with a 20 mM imidazole-containing buffer, the fusion proteins were eluted [25 mM HEPES (pH8.0), 150 mM NaCl and 250 mM imidazole]. Protein was concentrated and further purified by gel filtration (Superdex 200, GE

healthcare). Purity of recombinant protein was checked by 10% SDS-PAGE. Purified proteins were then stored at −80°C in 10% glycerol for further analysis.

***In vitro* deacetylation assay**

Equimolar of purified recombinant CobB and immuno-percipitated Flag-G6PD were incubated at 37°C in HEPES buffer [40 mM HEPES (pH8.0), 6 mM $MgCl_2$, 1 mM DTT, 10% Glycerol) with or without 1 mM NAD^+ for 2 hrs or as the indicated time. Flag-tagged G6PD were washed 3 times with NP-40 buffer before eluted with Flag peptide and subjected to the enzymatic activity assay and western blot analysis.

Partial proteolysis assays

Recombinant G6PD (0.2 mg/mL) was incubated with chymotrypsin (Worthington; 1000:1 in mass unit) or clostripain (Worthington; 400:1 in mass unit) on ice for indicated time periods. Undigested samples served as zero time point. The reaction was stopped by addition of SDS loading buffer. Samples were immediately incubated at 95°C for 10 min, followed by 10% SDS-PAGE separation. The SDS-PAGE gel was stained by Coomassie Blue and digitized by using Typhoon FLA 9500 (GE healthcare).

Protein melting curve analysis

The thermodynamic stability of recombinant protein was determined as

previously described (Hudson et al, 2012). Briefly, 45 μL of 1 μM purified protein (in 25 mM HEPES, 150 mM NaCl at pH 8.0) was mixed with 15 μL SYPRO-Orange (20X, Sigma). 45 μl buffer (25 mM HEPES, 150 mM NaCl, pH 8.0) mixed with 15 μl of 20X SYPRO-Orange was used as control. The mixture was aliquoted in triplicate (20 μL per well) into a 96-well plate. Data of melting curves were collected by using ABI 7500 (Applied Biosystems). Melting curve fluorescent signal was acquired between 20°C and 70°C using a ramping rate of 0.03 °C s−1. Melting temperatures (Tm) were determined by fitting the data with Boltzmann model. Data were presented as normalized relative fluorescence ± S.D.

Measurement of intracellular ROS levels

ROS production was determined by using a fluorescent dye 2', 7'-dichlorofluorescein diacetate (H_2DCF-DA, Sigma). Briefly, cells were grown in six-well plates at a density of 4×10^4 cells per well. Afterward, cells were washed with PBS and incubated with 10 μM H_2DCF-DA at 37°C for 30 min to load the fluorescent dye. Cells were washed twice with PBS and trypsinized for specified treatments. Fluorescence (Ex.488nm, Em.525nm) was monitored by a SpectraMax M5 Microplate Reader (Molecular Devices).

Cell survival assay

Cells were seeded in six-well plates at a density of 4×10^4 cells per well. After

incubation with increasing doses of menadione (Sigma) for 3 hrs, the remaining adherent cells were trypsinized and countered by using a hemocytometer (ShanghaiQiujing).

Measurement of NADPH and $NADP^+$ levels

The intracellular levels of NADPH and $NADP^+$ were measured by enzymatic cycling methods as previously described (Wagner & Scott, 1994; Zerez et al, 1987). In brief, 1.5×10^6 cells were seeded in 10 cm dishes. On the next day, cells were lysed in 400 µL of extraction buffer (20 mM NAM, 20mM $NaHCO_3$, 100 mM Na_2CO_3) and centrifuged at 1,200g for 15 min. For NADPH extraction, 150 µL of the supernatant was incubated in a heating block for 30 min at 60°C. 160 µL of NADP-cycling buffer (100 mM Tris-HCl, pH8.0; 0.5 mM thiazolylblue; 2 mM phenazine ethosulfate; 5 mM EDTA) containing 1.3U of G6PD was added to a 96-well plate containing 20 µL of the cell extract. After incubation for 1 min at 30°C in darkness, 20 µL of 10 mM G6P was added to the mixture, and the change in absorbance at 570 nm was measured every 30 sec for 10 min at 30°C in a SpectraMax M5 Microplate Reader (Molecular Devices). All the samples were run in triplicate. The concentration of $NADP^+$ was calculated by subtracting NADPH (heated sample) from the total of $NADP^+$ and NADPH (unheated sample).

SIRT2 activity assay

Activity of SIRT2 was measured by using SIRT2 direct fluorescent screening assay kit (Cayman). Briefly, HEK293T cells were transfected with a plasmid encoding Flag–SIRT2 or a vector control. 36hrs post transfection, cells were collected and immunoprecipitated using the Flag-beads (Sigma). The purified SIRT2 protein was eluted using Flag peptide and was subsequently used as a substitution of recombinant human SIRT2 in the protocol provided by the manufacturer.

Mouse erythrocyte isolation

Erythrocytes were isolated as previously described (Hanson et al, 2008). Briefly, blood was taken by cardiac puncture from mice. Whole blood was centrifuged at 500 g at 4°C for 10 min, and the plasma, buffy coat, and uppermost erythrocytes were removed by aspiration. The remaining erythrocytes were washed three times in buffer containing 21.0 mM Tris (pH 7.4), 4.7 mM KCl, 2.0 mM $CaCl_2$, 140.5 mM NaCl, 1.2 mM $MgSO_4$, 5.5 mM glucose and 0.5% bovine albumin fraction V. Isolated mouse erythrocytes were maintained in RPMI1640 supplemented with 10% fetal bovine serum (Biochrom, Germany) and 25 mM glucose, pH7.4.

Supplementary References

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Analytical Biochemistry* **72:** 248-254

Choudhary C, Kumar C, Gnad F, Nielsen ML, Rehman M, Walther TC, Olsen JV, Mann M (2009) Lysine acetylation targets protein complexes and co-regulates major cellular functions. *Science* **325:** 834-840

Guan KL, Yu W, Lin Y, Xiong Y, Zhao S (2010) Generation of acetyllysine antibodies and affinity enrichment of acetylated peptides. *Nat Protoc* **5:** 1583-1595

Hanson MS, Stephenson AH, Bowles EA, Sridharan M, Adderley S, Sprague RS (2008) Phosphodiesterase 3 is present in rabbit and human erythrocytes and its inhibition potentiates iloprost-induced increases in cAMP. *Am J Physiol Heart Circ Physiol* **295:** H786-793

Hudson JJ, Chiang SC, Wells OS, Rookyard C, El-Khamisy SF (2012) SUMO modification of the neuroprotective protein TDP1 facilitates chromosomal single-strand break repair. *Nat Commun* **3:** 733

Kotaka M, Gover S, Vandeputte-Rutten L, Au SW, Lam VM, Adams MJ (2005) Structural studies of glucose-6-phosphate and NADP+ binding to human glucose-6-phosphate dehydrogenase. *Acta Crystallogr D Biol Crystallogr* **61:** 495-504

Wagner TC, Scott MD (1994) Single extraction method for the spectrophotometric quantification of oxidized and reduced pyridine nucleotides in erythrocytes. *Anal Biochem* **222:** 417-426

Zerez CR, Lee SJ, Tanaka KR (1987) Spectrophotometric determination of oxidized and reduced pyridine nucleotides in erythrocytes using a single extraction procedure. *Anal Biochem* **164:** 367-373