Supplementary Materials

Materials and Methods

Plasmids

Full-length and truncated cDNA of TDG cloned in plasmids pET28 and pGEX-4T-2 vectors have been described[1]. Full-length TDG (residues 1–410) and truncated hTDG constructs containing TDG-ΔN(111–410) and TDG(67–308) cloned in pGEX-4T-2 vector (GE Health Healthcare) were transformed into BL21 Star cells (Stratagene) to express glutathione-S-transferase (GST) tagged proteins. pET28-TDG-FL was transformed into Rosetta *E. coli* cells to produce His-tagged full-length TDG protein. To express green fluorescent protein (GFP) tagged hTDG in human cells, *hTDG* cDNA was amplified by polymerase chain reaction (PCR) method using template pET28-TDG-FL and primers listed in Table S1 in the supplementary data. The PCR product was digested with SacI and BamH1 and ligated with digested pEGFP-N1 (Clonetech) to yield pEGFP-TDG. To express His-tagged TDG in human cells, *hTDG* cDNA was cut out from pET28-TDG-FL by digestion with BamH1 and XbaI and ligated with digested pcDNA3.1 (Invitrogen) to yield pcDNA3-TDG.

Full length human SIRT1 (SIRT1-FL) in pECE vector and N-terminus truncated SIRT1 (SIRT1-NT with residues 7-83 deleted) cloned in pcDNA3.1 vector were obtained from Addgene. *SIRT1* cDNA was amplified by PCR using template SIRT1-NT and primers listed in Table S1 in the supplementary data. The PCR product was digested with BamH1 and XhoI and ligated into the digested pGEX-4T-2 (GE Healthcare) to yield pGEX-SIRT1-NT construct. Plasmid pCIN4-p300 was obtained from Dr. Jianyuan Luo (University of Maryland).

Cell transfection and cell extract preparation

For immunoprecipitation, HEK293T cells (5×10^6 cell/10 cm dishes) were cotransfected with GFP-hTDG and FLAG-SIRT1 plasmids (5 μg each) using Fugene HD (Invitrogen) according to the manufacturer's instructions and allowed to grow for 72 hrs. Cells were harvested, washed with phosphate-buffered saline (PBS), and lysed by using 1 ml of lysis buffer [50 mM Tris HCl, pH 7.4, 50 mM NaCl, 1 mM EDTA, 1% Triton X-100, and 10 μl protease inhibitor cocktail (P8340, Sigma-Aldrich)] for 10 cm dish containing 10^6–10^7 cells. The whole cell extracts were quickly frozen using liquid nitrogen and stored at -80^{o}C. The protein concentration was determined by Bradford method (Bio-Rad).

To isolate acetylated TDG from human cells, His-hTDG and p300 plasmids were transiently transfected into HEK293T cells. Cell extracts were prepared as above and acetylated TDG was purified by Ni-column (Qiagen). The majority of Ac-TDG was found in the flow-thru fraction which was then aliquoted and stored at -80^{o}C.

To investigate *in vivo* TDG deacetylation, GFP-hTDG plasmid (5 μg) was transiently transfected into HEK293T cells in the absence or presence of SIRT1-FL plasmid (5 μg) as above. SIRT1 specific inhibitor EX-527 (1 μM) (Tocris Bioscience)[2] was added 6 hrs before cell harvest. Equal amounts of cell extracts were subjected to immunoprecipitation by anti-GFP (Abcam) and Wetern blot was probed by α-acetyl lysine antibody (Calbiochem).

GST pull-down assay was performed as described previously[1].

Detection of TDG mRNA Level by RT-PCR

Total RNA from MEF cells was isolated by the RNeasy kit method (Qiagen). 100 ng RNA was used as a template for reverse transcription-quantitative PCR (RT-qPCR) reactions using primers for hTDG and actin (Table S1). Real time PCR was performed using the Light Cycler 480 II Detection System (Roche) with iScript™ SYBR® Green One-Step Kit (BioRad). The reactions were carried out in initial step for RT at 50°C for 10 min followed by PCR cycles (95°C for 30 sec, 60°C for 30 sec, and 70°C for 45 sec). The mRNA level of *Tdg* was calculated relative to that of *actin* as ΔCt which is the difference between the number of cycles required to go above background in *Tdg* and *actin* samples. The fold difference of *Tdg* mRNA levels of WT cells over Sirt1 KO cells was calculated according to the formula $2^{\Delta Ct(KO)- \Delta Ct(WT)}$. The reactions were carried out in duplicate and data are averaged from three independent experiments.

SIRT1 decetylase activity assay

SIRT1 decetylase activity was determined with SIRT1 Direct Fluorescent Screening Assay Kit (Cayman Chemical) according to the manufacturer's instructions. The reaction was set up with 30 nM SIRT1 in deactylase buffer containing 50 mM Tris-HCl, pH 8.0, 137 mM sodium chloride, 2.7 mM KCl, 1 mM $MgCl_2$, and 3 mM NAD^+. The deacetylation reaction was initiated by adding 125 μM substrate which is a p53 peptide Arg-His-Lys-Lys(e-acetyl)-AMC (7- amino-4-methoxy coumarin). After 45-min incubation at room temperature, 50 μl of developing solution was added for additional 30 min at room temperature. The fluorophore was analyzed using excitation wavelength of 350-360 nm and emission wavelength of 450-465 nm in fluorescence spectrophotometer Cary Eclipse. Increasing amounts of TDG (15, 30, and 60 nM) were added to investigate the influence of TDG on SIRT1 deacetylase activity. Reactions without enzyme or with 30 nM TDG were used as negative controls.

REFERENCES

(1) Guan, X., Madabushi, A., Chang, D.-Y., Fitzgerald, M. E., Shi, G., Drohat, A. C., and Lu, A.-L. (2007) The human checkpoint sensor Rad9-Rad1-Hus1 interacts with and stimulates DNA repair enzyme TDG glycosylase. *Nucleic Acids Res. 35*, 6207-6218.

(2) Solomon, J. M., Pasupuleti, R., Xu, L., McDonagh, T., Curtis, R., DiStefano, P. S., and Huber, L. J. (2006) Inhibition of SIRT1 catalytic activity increases p53 acetylation but does not alter cell survival following DNA damage. *Mol. Cell Biol. 26*, 28-38.

Table S1. Oligonucleotides used

Name	Sequence	Purpose
GST-SIRT1-For	5'CGCTGGATCCATGGCGGACGAGGCG	hSIRT1 5' primer
GST-SIRT1-Rev	5'CGCTATATGCGGCCGCCTATGATTTGTTTGATG	hSIRT1 3' primer
GFP-TDG-For	5'CGCTGAGCTCATCGCCACCATGGAAGCGGAGAAC	hTDG 5' primer
GFP-TDG-Rev	5'CGCTGGATCCCGAGCATGGCTTTCTTCTTC	hTDG 3' primer
T40	5'AATTGGGCTCCTCGAGGAATTTGCCTTCTGCAGGCATGCC[a]	TDG substrate
G40	5'CCCGAGGAGCTCCTTAAGCGGAAGACGTCCGTACGGGGCC	TDG substrate
FU-28	5'-GTGTCACCACTGCTCA(FU)GTACAGAGCTG-3'	TDG substrate
G-28	5'-CAGCTCTGTACGTGAGCAGTGGTGACAC-3'	TDG substrate
fC-28	5'-GTGTCACCACTGCTCA(fC)GTACAGAGCTG-3'	TDG substrate
caC-28	5'-GTGTCACCACTGCTCA(caC)GTACAGAGCTG-3'	TDG substrate
TDG-For	5'CAGAAATATCAGCCACGAATA	qPCR Primer
TDG-Rev	5'TGAAGCCCAAATTCCAAGTT	qPCR Primer
Actin-For	5'ACCAACTGGGACGACATGGA	qPCR Primer
Actin-Rev	5'TACATGGCTGGGGTGTTGAA	qPCR Primer

[a]The base which mismatches with the complementary strand is underlined.

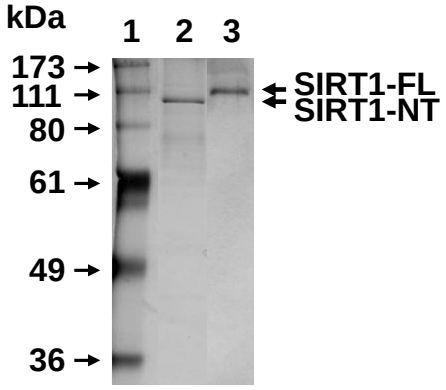

Figure S1 Purification of FLAG-tagged hSIRT1

FLAG-tagged hSIRT1 was expressed in HEK293T cells and purified by M2 affinity column (Sigma/Aldrich) followed by SP chromatography. Lane 1 contains the molecular weight markers. Lane 2 contains SIRT1-NT and lane 3 contains SIRT1-FL. The proteins were fractionated on a 10% polyacrylamide gel in the presence of SDS and stained with silver. The apparent sizes based on the mobility of SIRT1 proteins on SDS-PAGE appear larger than their actual sizes. Arrow marks the SIRT1 proteins.

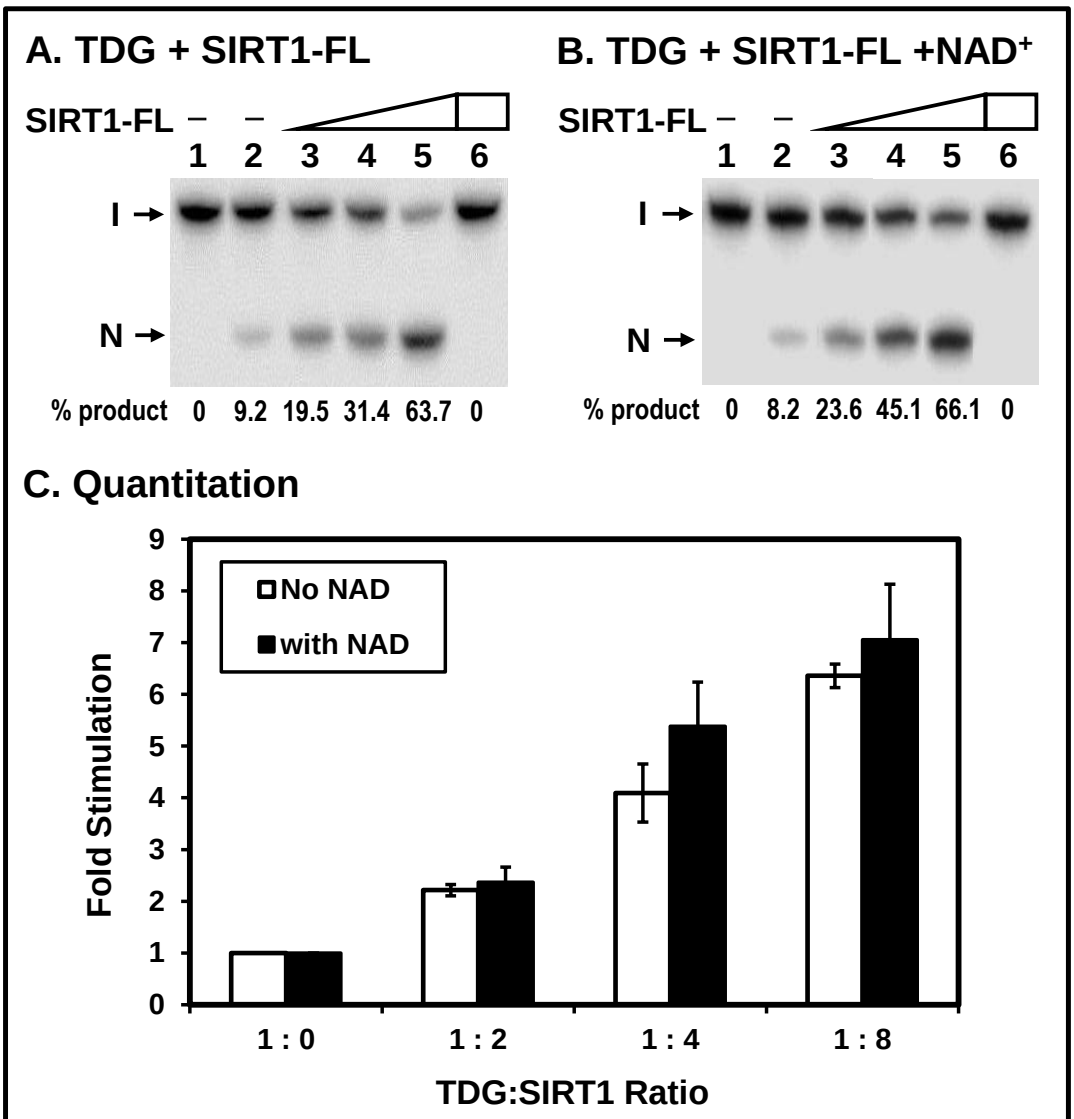

Figure S2 Stimulation of TDG glycosylase activity by hSIRT1 is independent of NAD+

Human SIRT1-FL enhances TDG activity on T/G-DNA in the absence (A) or presence of NAD+ (B). Lane 1, T/G-containing DNA substrate. Lane 2, 0.18 nM T/G-containing DNA substrate was incubated with 0.5 nM untreated hTDG. Lanes 3-5 are similar to lane 2 but with added 1, 2, and 4 nM hSIRT1-FL. Lane 6, T/G-containing DNA substrate was incubated with 4 nM without TDG. Arrows mark the intact DNA substrate (I) and the cleavage product (N). Percentage (%) of product generated is shown below each lane. (**C**) Quantitative analyses of the fold of stimulation on the TDG glycosylase activity by hSIRT1-FL in the absence of NAD+ (white bars) or in the presence NAD+ (black bars). The error bars reported are the standard deviations of the averages.

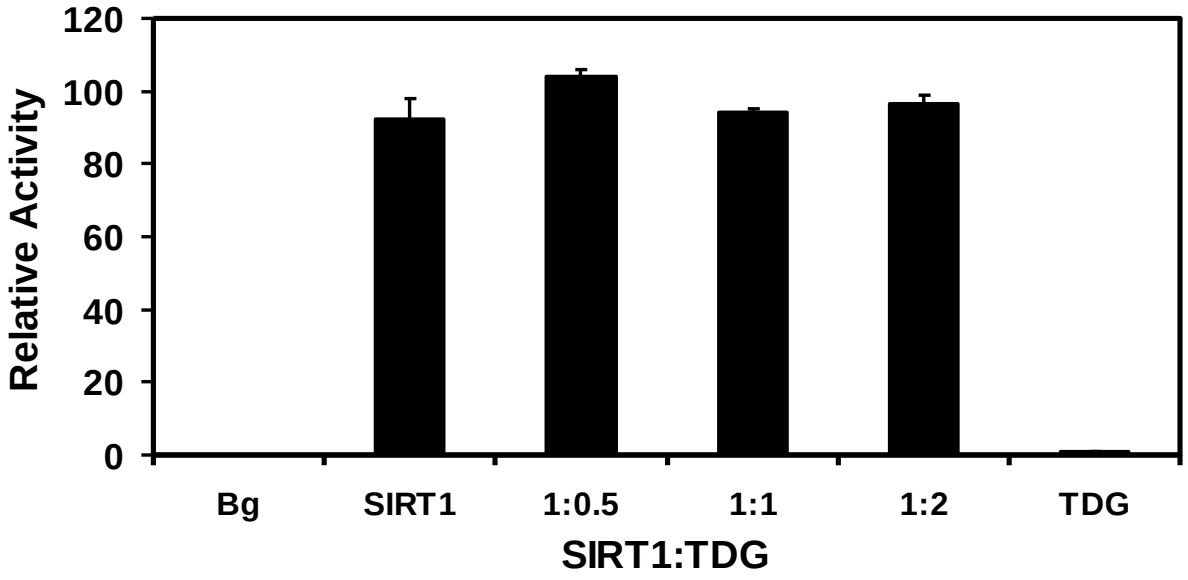

Figure S3 TDG does not affect the SIRT1 deacetylase activity

SIRT1 decetylase activity was determined with SIRT1 Direct Fluorescent Screening Assay Kit (Cayman Chemical) according to the manufacturer's instructions. Column 1 is the background (Bg). Columns 2 and 6 are reactions with 30 nM SIRT1 and 30 nM TDG, respectively. Columns 3-5 are reactions with 30 nM SIRT1 in the presence of increasing amounts of TDG (15, 30, and 60 nM).

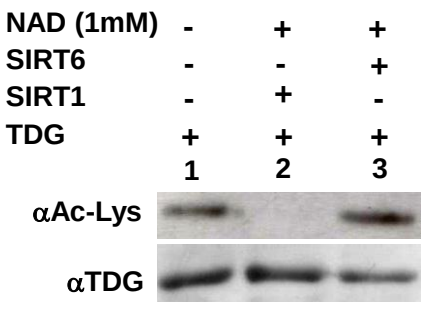

Figure S4 SIRT6 cannot deacetylate TDG

His-hTDG and p300 plasmids were transiently transfected into HEK293T cells. Acetylated TDG was purified and incubated with SIRT1-FL or SIRT6 in the presence of NAD^+. Western blotting was performed with antibody against acetyl-lysine (α-AcLys) or TDG (α-TDG) to evaluate TDG acetylation.