Supporting Information



Supplementary Fig 1. Analysis of SIRT1 promoter area. A schematic representation of the constructs used in transcription activity assay.



Supplementary Fig 2. Normalized luciferase activity under the control of a series of the human

SIRT1 promoter fragments in Hela cells.





В



Supplementary Fig 3. Functional cooperation between PPAR γ and SIRT1. (A) Cells were transfected with epitope-tagged construct encoding Myc-SIRT1 and immunoprecipitated (*IP*) first with an Myc-specific antibody followed by Western blotting (*WB*) with a PPAR γ antibody; similar analysis comparing the interaction of wild type SIRT1 (*WT*) and SIRT1_{H363Y} (*HY*) with Flag-PPAR γ demonstrating significantly reduced interaction with the deacetylase domain mutation. (B) reciprocal interaction analysis by first immunoprecipitation (*IP*) of lysates with a Flag-epitope antibody followed by Western blotting (*WB*) with a Myc-specific antibody again demonstrating reduced affinity of the SIRT1 (*HY*) for PPAR γ .



Supplementary Fig 4. **SIRT1 binds to p300**. Cell lysates from Hela cells were subjected to immunoprecipitation using anti-SIRT1 antibodies. The immunoprecipitated complexes were separated by SDS-PAGE and subjected to immunoblot analysis using the indicated antibodies.



Supplementary Fig 5. **SIRT1 deacetylates PPAR** γ in vitro in an NAD-dependent fashion. Hela cells were transfected with expression plasmids encoding Flag-PPAR γ and p300, and PPAR γ was immunoprecipitated (IP) from protein lysates using the Flag-epitope tag. SIRT1 was not added to the immunoprecipitated PPAR γ for 1 hour in the presence or absence of indicated concentrations of either NAD or nicotinamide. Levels of acetylated PPAR γ were determined by Western blot (*WB*) analysis using an antibody that recognizes acetyl-lysine (AcLys) residues.



B





С



Supplementary Fig 6. The silencing of p300 in 2BS cells prevented the appearance of senescence-associated features. 2BS cells infected with the pSuper neo (Vector) or pSuper-shRNA were analyzed for the β -gal sataining. (A)Western blot analysis of p300 silencing in p300shRNA-transfected cells compared with vector-transfected cells. (B) Infected cells were

stained for SA- β -gal staining. (C) Real-time PCR analysis of SIRT1 expression in p300shRNA-insfected 2BS cells. Each experiment was performed at least three times. GAPDH transcript was used as a control. Each bar depicts data from three independent PCR reactions (mean \pm S.D.).



Supplementary Fig 7. Levels of PPAR γ acetylation are increased by the SIRT1 inhibitor NAM. Transfected cells or endogenous cells were grown to 60% confluence and then treated with 10mM nicotinamide (Acros Organics) dissolved in the growth medium. Cells were incubated with the nicotinamide treatment for 24 h, after which they were used for subsequent experiments.





Supplementary Fig 8. Alterations of SIRT1 protein expression levels were determined in senescent animal tissues. (A) Western blot analysis of SIRT1 expression in extracts from brain and kidney tissues in young (Y) and senescent (S) Balb/c mice, with actin as a loading control (n=3 per group). (B) Real-time PCR analysis of SIRT1 mRNA derived from brain and kidney tissues in young and senescent Balb/c mice, GAPDH transcript was used as a loading control (n=3 per group).