Supporting Information

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SI Materials and Methods

Cell Culture and Treatments. Human embryonic kidney (HEK293T) cells, human lung cancer (H1299) cells, and the human colon cancer cell lines HCT116 ($p53^{+/+}$) and HCT116 ($p53^{-/-}$) cells were maintained in DMEM supplemented with 10% FBS and 100 μg/mL streptomycin-penicillin (HyClone). Cells were treated with Adr, etoposide, cisplatin (Sigma), or UV-C for the indicated times at various concentrations.

Plasmids Construction. GST-SIRT1 was constructed by inserting the SIRT1 fragment into vector pGEX-4T3 with *Bam*H1 and *Not*1. The N terminus of SIRT1 was inserted into vector pGEX-4T3 by using *Bam*H1 and *Mfe*1 (New England Biolabs). The middle fragment and C terminus of SIRT1 were cloned by using primers as follows:

Middle fragment sense: 5′ CGCggatccGATGCTGTGAAATTACTGCA 3′;
Middle fragment antisense: 5′ ATAAGAATgcggccgcGGCATATTCACCACCT 3′; C terminus sense: 5′ CGCggatccAAACTTTGCTGTAACCCTGT 3′;
C terminus antisense: 5′ ATAAGAATgcggccgcTGATTTGTTTGATGGATAGT 3′.

Flag-tagged WT-Set7/9 and H297A were from the W.G. laboratory, and Set7/9 was separately subcloned into pGEX-4T3, EGFP, and pET28 vectors. The substitution mutants of SIRT1 were constructed by using site-directed mutagenesis kit (Stratagene) following manufacturer's instructions.

Luciferase Assay. The activity of p53 was determined by a Promega Luciferase Assay System (Promega) as described in ref. 1.

RNA Interference (RNAi). Sequences of RNAi oligonucleotides are as follows:

Nonspecific small interfering RNA (siRNA), UUCUCCGAACGUGUCACGU
Set7/9 siRNA sense strand: 5′-GGGCACCTGGACGATGACGGA-3′
SIRT1 siRNA sense strand: 5′-GTTGGATGATATGACACTG-3′.

All RNAi oligonucleotides were purchased from Shanghai GenePharma Company. These RNAi oligonucleotides were transfected into cells by using the Lipofectamine 2000 transfection kit (Invitrogen) according to the manufacturer's instructions.

Western Blotting. Protein expression was detected by Western blotting as described with minor modifications. Equal amounts of proteins (20–150 μg) were size fractionated by 6–15% SDS-polyacrylamide gel electrophoresis. The antibodies used were anti-SIRT1, anti-GAPDH, anti-p53, anti-GFP, anti-Myc, anti-GST (Santa Cruz), anti-Flag (Sigma), anti-Set7/9 (Diagenode), anti–acetyl-lysine (Upstate Biotechnology), anti–pan-methyl-lysine (Abcam), and anti-acetyl-p53 (K382) (Cell Signaling).

Co-immunoprecipitation. Cells were harvested and then lysed in lysis buffer (1% Nonidet P-40, 150 mM NaCl, 50 mM Tris at pH 7.5, 5 mM EDTA, 0.05% SDS, 1 mM PMSF, and a 1% mixture of protease inhibitors) on ice for 30 min. After centrifugation at 4 °C at 15,294 × *g* for 15 min, antibodies were added to the supernatant and rolled at 4 °C overnight. Protein G or A Agarose (GE Healthcare) was then added to the samples, and the samples were rolled at 4 °C for 2 h. After the beads were washed three times with lysis buffer, the pellets were dissolved into 2× SDS loading buffer after centrifugation and boiled at 100 °C for 5 min. Proteins were analyzed by Western blotting with different antibodies.

RT-PCR and Real-Time PCR. Total RNA was extracted by TRIzol reagent (Invitrogen) and quantified by spectrophotometer. cDNA was synthesized from 2 μg of RNA with oligo $(dT)_{18}$ primers by using Quant Reverse Transcriptase (TIANGEN) and measured by PCR or quantitative real-time PCR. Primers used in this study were purchased from Augct Company.

1. Zhao Y, et al. (2006) Acetylation of p53 at lysine 373/382 by the histone deacetylase inhibitor depsipeptide induces expression of $p21^{Waf1/Cip1}$. *Mol Cell Biol* 26:2782–2790.

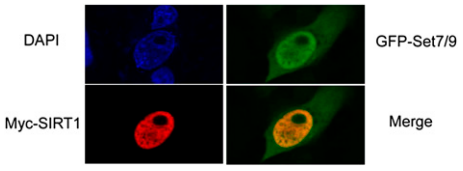

Fig. S1. GFP-tagged Set7/9 and Myc-tagged SIRT1 were cotransfected into HEK293T cells, and immunofluorescence was then performed 24 h after the transfection. DAPI, blue color; GFP, green color; Myc, red color; Merge, green and red.

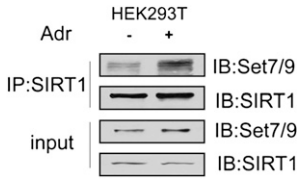

Fig. S2. HEK293T cells were treated with 1 μM Adr for 6 h. Cell lysates were prepared for coimmunoprecipitation with anti-SIRT1 and probed with anti-Set7/9.

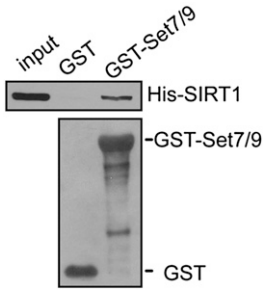

Fig. S3. GST-tagged Set7/9 was used to pull down His-tagged SIRT1 in vitro.

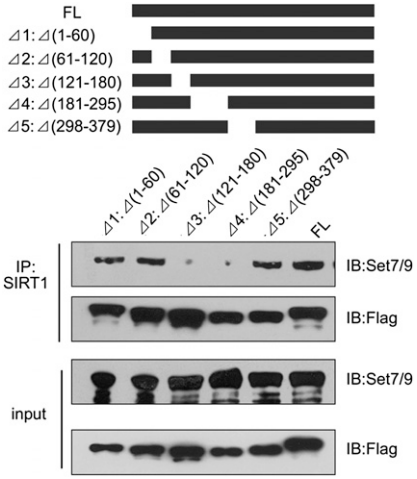

Fig. S4. Full-length or different deletion mutants of Flag-SIRT1 were transfected into HEK293T cells and co-IP was performed with anti-SIRT1. Western blotting was performed by using anti-Set7/9.

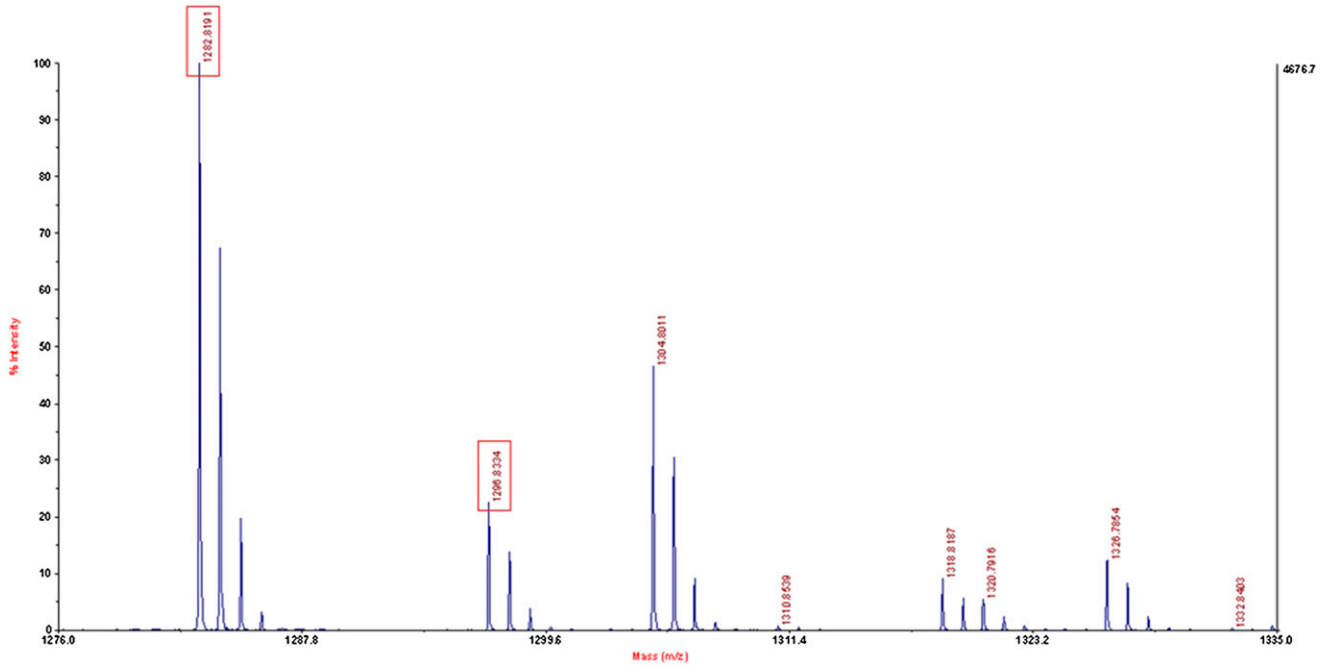

Fig. S5. Peptides containing different lysines from the N terminus of SIRT1 were incubated with Set7/9 and subjected to mass spectrometry. Only the peptide containing K233/235/236/238 showed signs of methylation (red frame).