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Supplemental Information

A SIRT2-Selective Inhibitor Promotes c-Myc Oncoprotein Degradation and Exhibits Broad Anticancer Activity

Hui Jing, Jing Hu, Bin He, Yashira L. Negrón Abril, Jack Stupinski, Keren Weiser, Marisa Carbonaro, Ying-Ling Chiang, Teresa Southard, Paraskevi Giannakakou, Robert S. Weiss, and Hening Lin

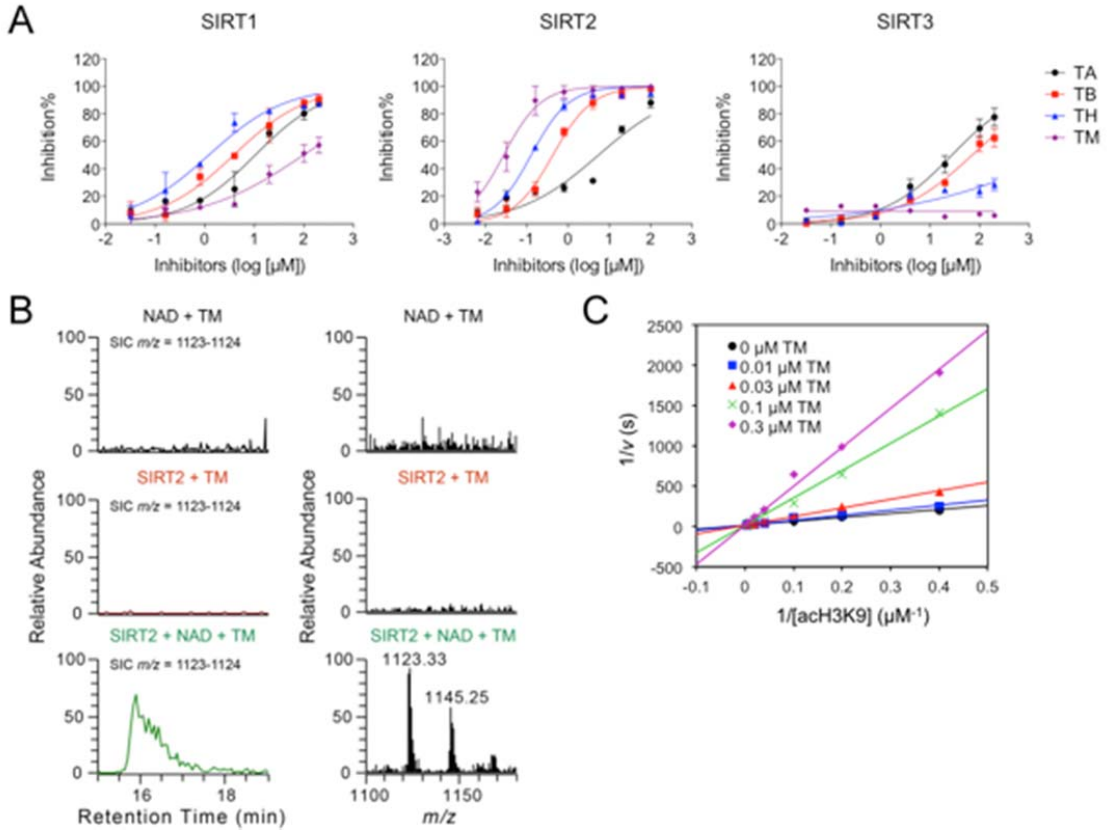


Figure S1. Related to Figure 1. (**A**) Dose-responsive curve for TA, TB, TH, TM and M against SIRT1-3. (**B**) LC-MS detection of the covalent intermediate formed by TM and NAD. The selected ion chromatogram (SIC) (*m/z* = 1123-1124) was shown on the left, the mass spectrum was shown on the right. The data from the reaction mixture containing 100 μM NAD, 100 μM TM, 1 mM DTT, and 20 mM pyridinium formate (pH 7.0) or the mixture containing 50 μM SIRT2, 100 μM TM, 1 mM DTT, and 20 mM Pyridinium formate (pH 7.0) were shown as negative controls. (**C**) Double reciprocal plot with varied TM and acH3K9 concentrations. Data was fit to competitive inhibition using Graphpad Prism. Error bars represent mean ± sd.

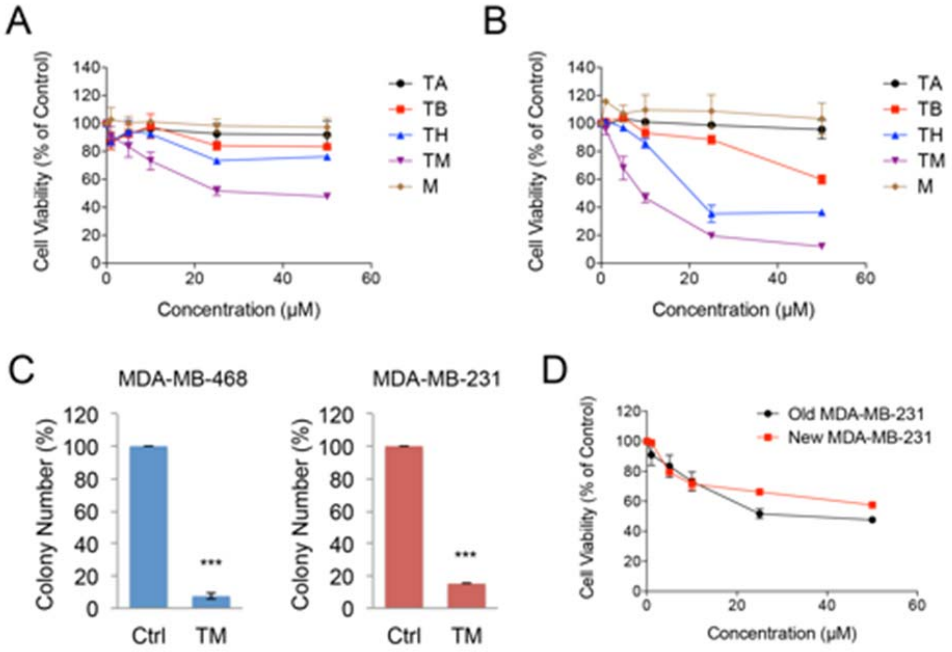


Figure S2. Related to Figure 2. (**A and B**) Cell viability of MDA-MB-231 (**A**) and HeLa (**B**) cells treated with ethanol or indicated inhibitors (1, 5, 10, 25, 50 μM) for 72 hr. (**C**) Soft agar colony formation of MDA-MB-468 and MDA-MB-231 cells treated with ethanol or TM (25 μM). The y axis represents percent colony number relative to ethanol-treated cells. Statistics, two-tailed Student's *t*-test. (**D**) Comparison of the sensitivity of two batches of MDA-MB-231 cells to TM treatment. The old MDA-MB-231 cell line has been maintained in our laboratory for over 2 years; while the new MDA-MB-231 cell line has been recently purchased from ATCC. Cells were seeded in 96-well plate one day before TM treatment at a density of 3,000/well. On the day of treatment, cells were incubated with media containing 0, 1, 5, 10, 25, and 50 μM for TM for 3 days. CellTiter-Blue® assay was performed to assess the cell viability. Error bars represent mean ± sd. ***$p < 0.001$.

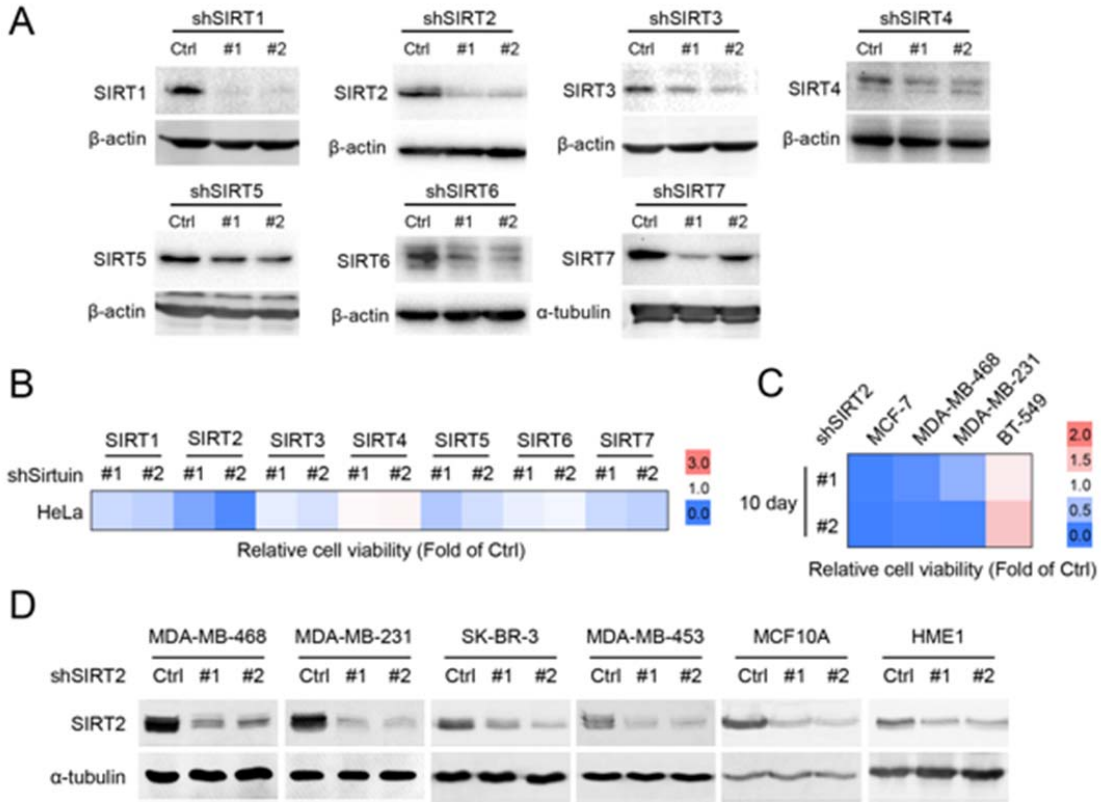


Figure S3. Related to Figure 3. (**A**) Representative results showing the knockdown efficiency of SIRT1-7 in HeLa cells. Cells were infected with lentivirus carrying Luciferase shRNA and shRNAs against SIRT1-7 for 72 hr before analyzed by Western blot for sirtuin levels. (**B**) Cytotoxicity effects of knocking down SIRT1-7 in HeLa cells after 72 hr of lentiviral infection. (**C**) Cytotoxicity effects of SIRT2 knockdown in MCF-7, MDA-MB-468, MDA-MB-231 and BT-549 cells at day 10 after the infection. (**D**) SIRT2 knockdown efficiency in Figure 3B and Figure S3C was confirmed by Western blot (the first row). The α-tubulin level was used as internal standard of total protein amount.

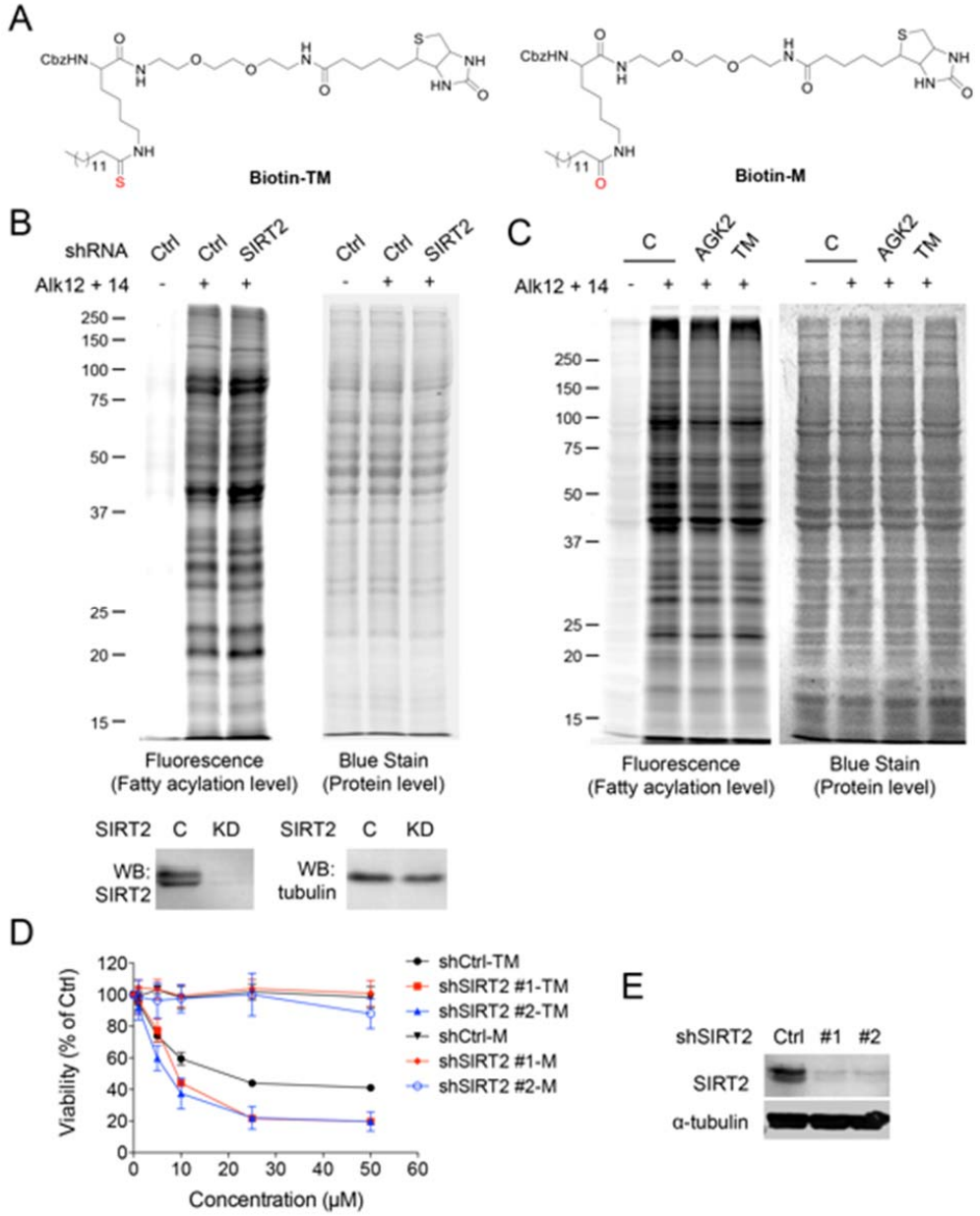


Figure S4.

Related to Figure 4. (**A**) Structures of Biotin-TM and Biotin-M. (**B**) Global protein fatty-acylation in HEK293T cells with Ctrl and SIRT2 knockdown. Protein fatty acylation was detected by a metabolic labeling method using alkyne-tagged fatty acid analogs Alk12 (50 μM) and Alk14 (50 μM) as previously reported (Jiang et al., 2013). (**C**) Global protein fatty acylation in HEK293T cells treated with the ethanol, AGK2 (25 μM) or TM (25 μM) for 6 hr in the presence of Alk12 (50 μM) and Alk14 (50 μM). (**D**) Effect of SIRT2 knockdown on the sensitivity of MDA-MB-231 cells to TM. MDA-MB-231 cells were infected with lentiviral Luciferase shRNA and SIRT2 shRNAs, respectively, for 24 hr before being treated with different concentrations of TM for another 72 hr. Cell viability was measured by CellTiter-Blue® assay. (**E**) SIRT2 knockdown in (**D**) was confirmed after 72 hr of infection by Western blot. Error bars represent mean ± sd.

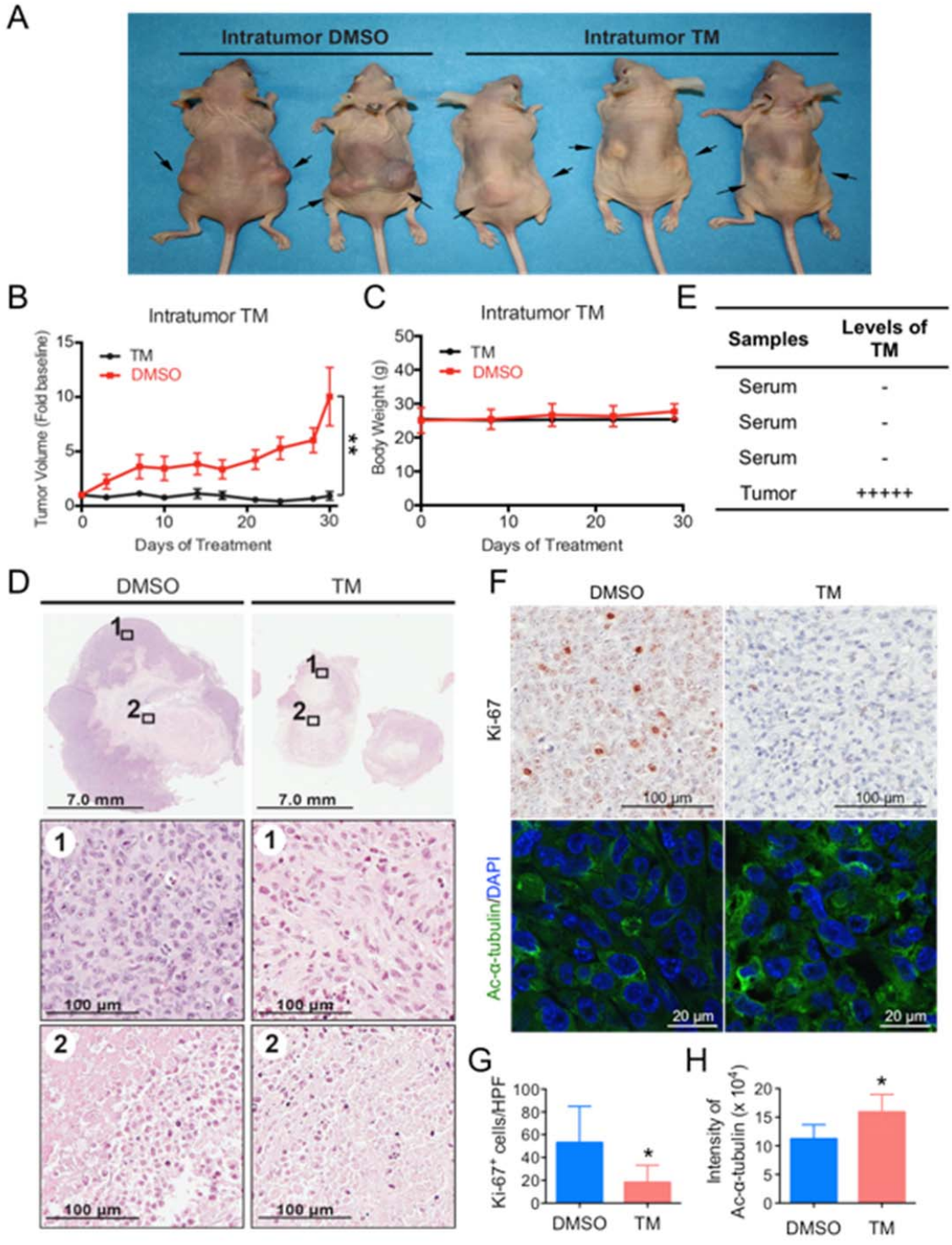


Figure S5. Related to Figure 5. Analysis of tumor growth and histopathological findings of xenografted mice treated by intratumor TM injection. Mice bearing MDA-MB-231 human breast cancer xenograft were divided into two groups and treated by direct intratumor injection with either

the control vehicle solvent (DMSO) or TM (0.75 mg TM in 50 uL DMSO; n = 5) three times per week. Tumors were collected after 30-day treatment. (**A**) Gross findings at necropsy after 30 days of intratumor treatment with either DMSO or TM. (**B**) Tumor growth chart. Statistics, paired Student's *t*-test. (**C**) Mouse body weight chart. (**D**) Hematoxylin and eosin staining of tumor tissues after 30 days of treatment with either DMSO or TM. (**E**) Detection of TM in mouse serum and tumor tissues by mass spectrometry. (**F**) Representative images of Ki-67 immunohistochemistry staining and acetyl-α-tubulin (K40) immunofluorescence staining of tumor tissues after 30 days of treatment with either DMSO or TM. (**G**) Quantification of Ki-67^+ cells in (**F**). The y axis represents Ki-67^+ cells per high power field (10 HPFs/tumor for all the tumors analyzed, n = 4 for DMSO, n = 6 for TM). Statistics, unpaired Student's *t*-test. (**H**) Quantification of acetyl-α-tubulin fluorescence intensity in (**F**). The y axis represents integrated intensity per cell. (10 HPFs/tumor for all the tumors analyzed, n = 4 for DMSO, n = 6 for TM). Statistics, unpaired Student's *t*-test. Error bars represent mean ± sd. *$p < 0.05$, **$p < 0.01$.

Table S1. Related to Figure 7. Top four correlated genes from molecular target Compare analysis of the NCI-60 assay data of TM. The data set used is the MT series.

Rank	**Mol. Target ID**	**Gene**	**Correlation value**	**Target pattern description**
1	MT18283	*MYC*	0.503	c-Myc phosphorylation level at T58 and S62
2	MT18332	*MYC*	0.493	c-Myc protein level
3	MT11065	*FGFR2*	0.477	Fraction of DNA methylation at *FGFR2* 5' UTR
4	MT1125	*CDC25A*	0.465	Relative mRNA levels of *CDC25A*

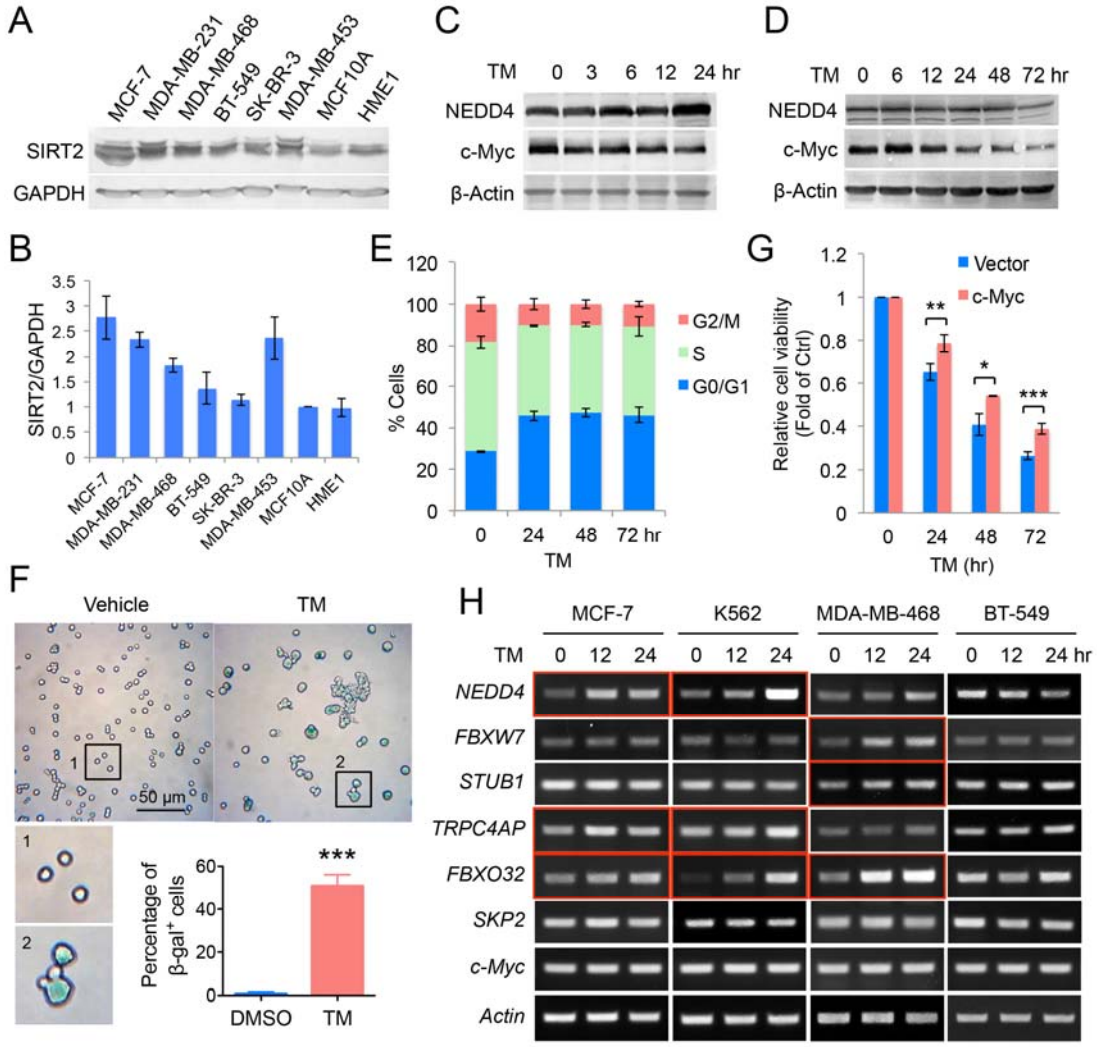
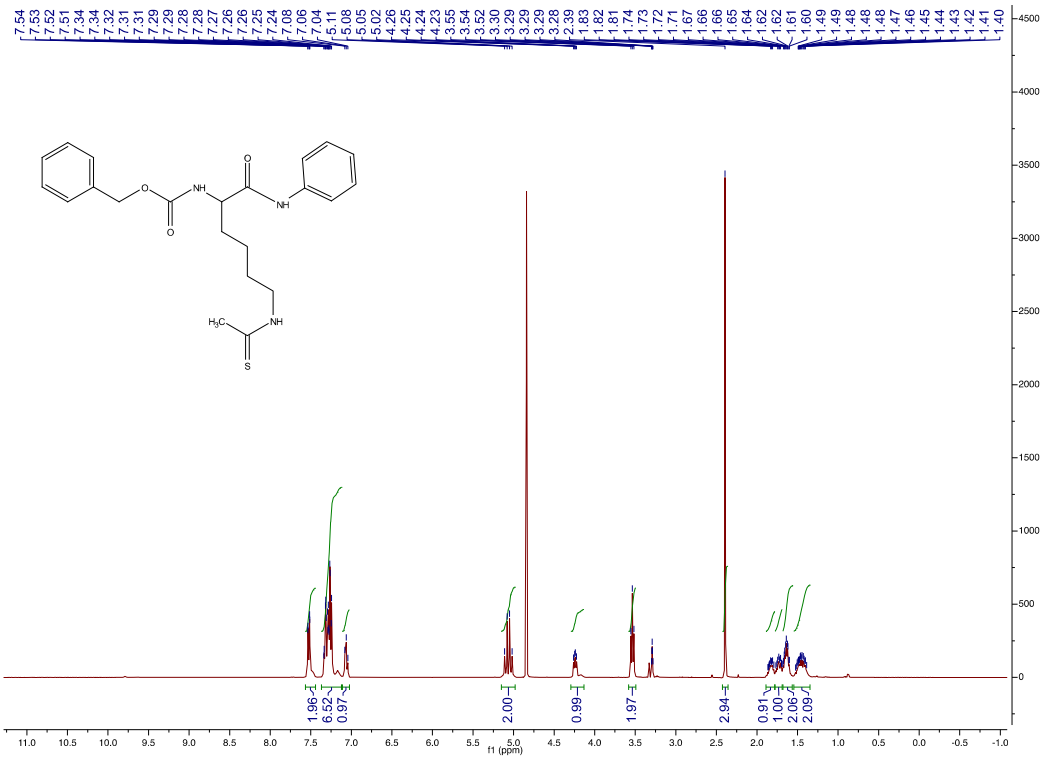


Figure S6. Relate to Figure 8. (**A, B**) SIRT2 levels in different human normal and breast cancer cell lines. Western blot analysis of SIRT2 level (**A**) and semi-quantification of SIRT2 level relative to GAPDH level (**B**). (**C, D**) Effects of TM on c-Myc and NEDD4 protein levels in K562 (**C**) and MDA-MB-468 (**D**) cells. Cells were treated as indicated. (**E**) Cell cycle distribution of K562 cells treated with TM (25 μM) for 0, 24, 48 or 72 hr. The graph shows the percentage of cells for each cell cycle phase as assessed by propidium iodide (PI) staining-coupled flow cytometry. (**F**) Acidic β-gal (β-gal) staining in K562 cells treated with TM (25 μM) for 5 days. Quantification (right panel) was shown as percentage of β-gal^{+} cells. Statistics, two-tailed Student's *t*-test. (**G**) Effect of c-Myc overexpression on the cytotoxicity effect of TM. MCF-7 cells transfected with pCDH vector or pCHD-c-*Myc* for 12 hr were treated with TM (25 μM) for another 0, 24, 48 or 72 hr, followed by CellTiter-Blue® assay. (**H**) Effect of TM on the transcript levels of various E3 ligases of c-Myc. MCF-7, K562, MDA-MB-468 or BT-549 cells were treated with TM (25 μM) for the indicated time. PCR was performed for the assessment of transcript levels of E3 ligases (*NEDD4, FBXW7, STUB1, TRPC4AP, FBXO32, SKP2*), *c-Myc* and *Actin*. Statistics, two-tailed Student's *t*-test. Error bars represent mean ± sd. *p < 0.05, **p < 0.01, ***p < 0.001.

A



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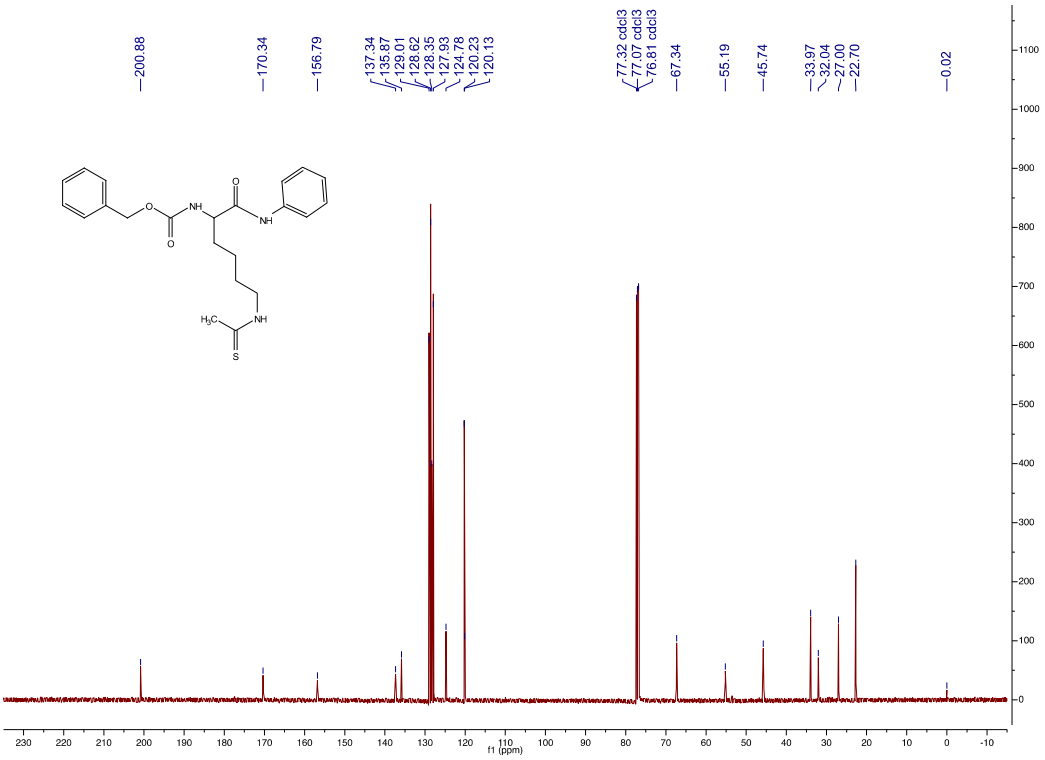

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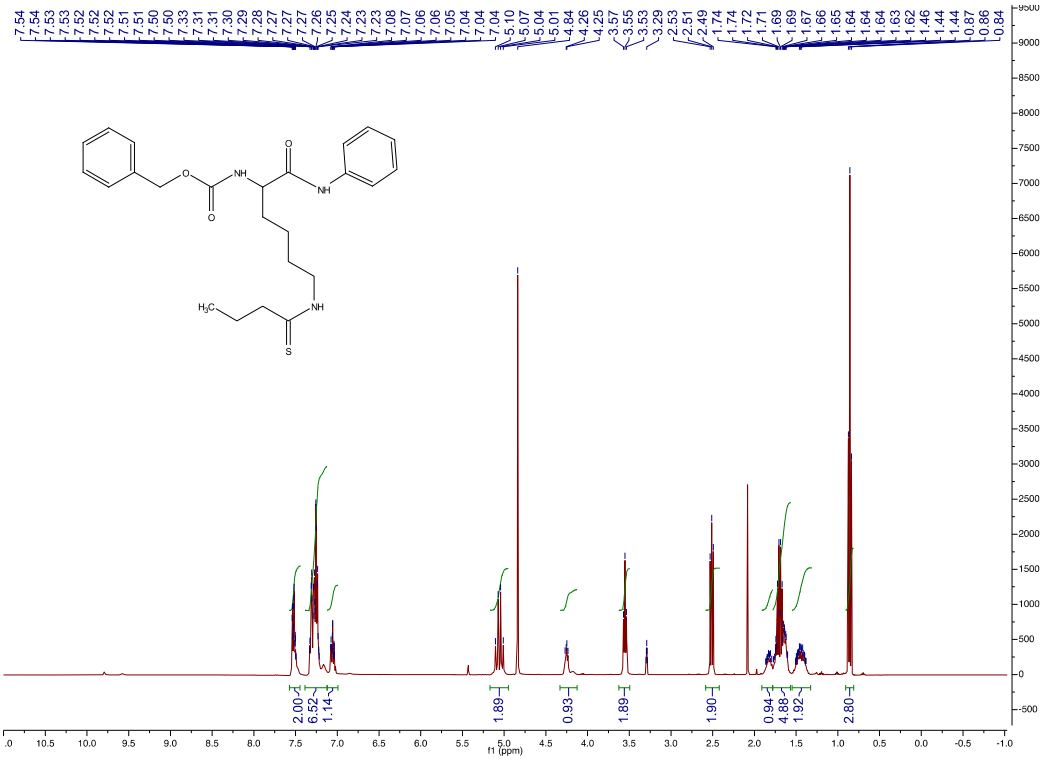

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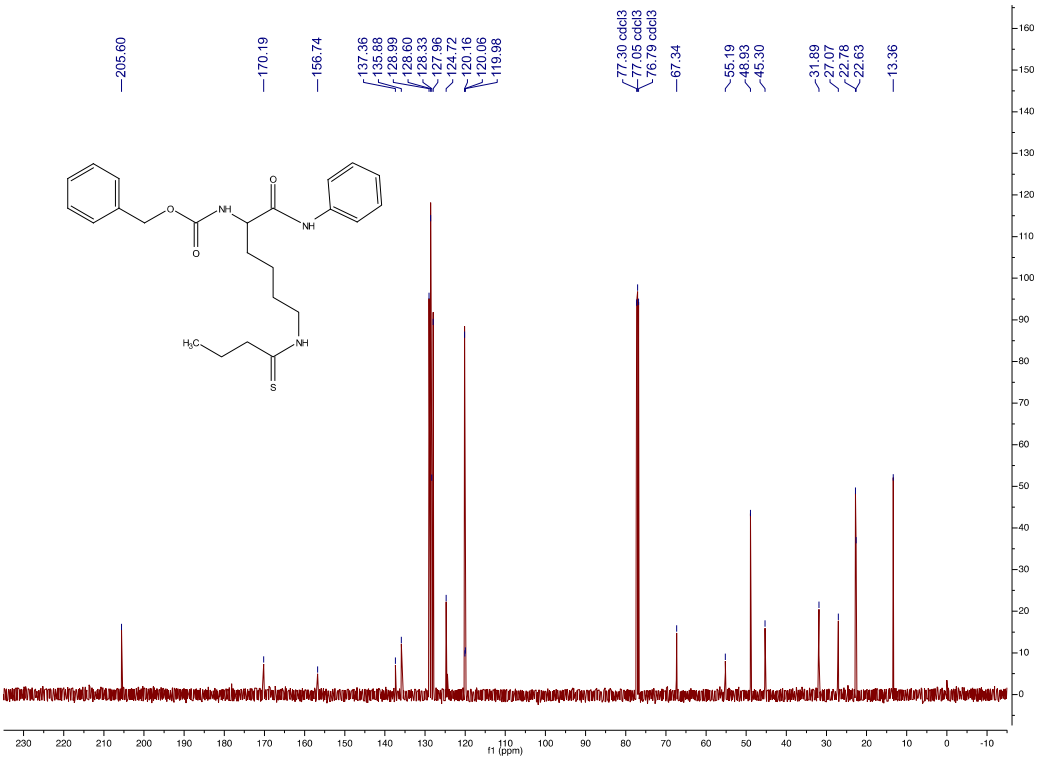

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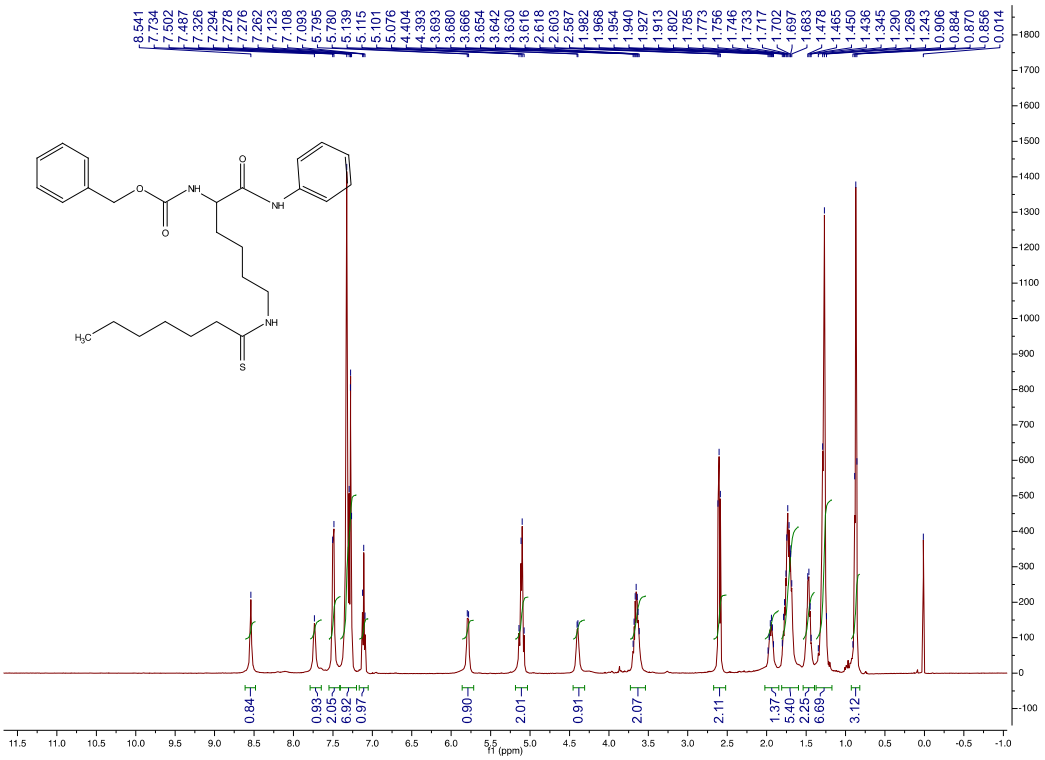
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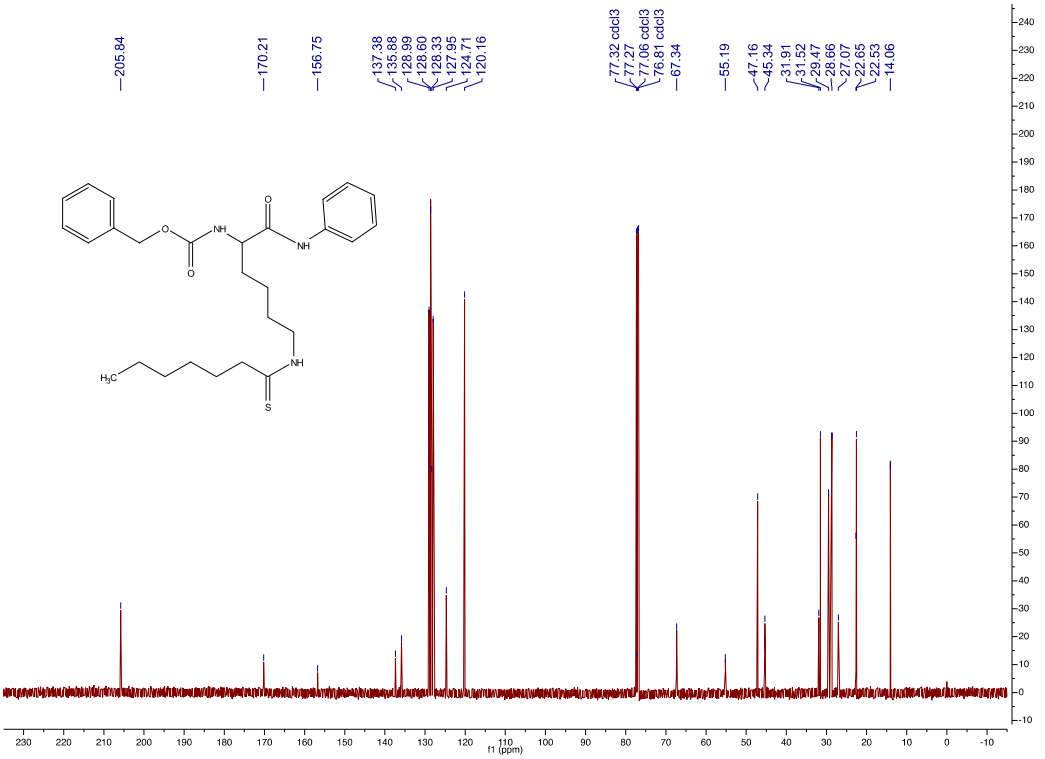
H (ppm)

F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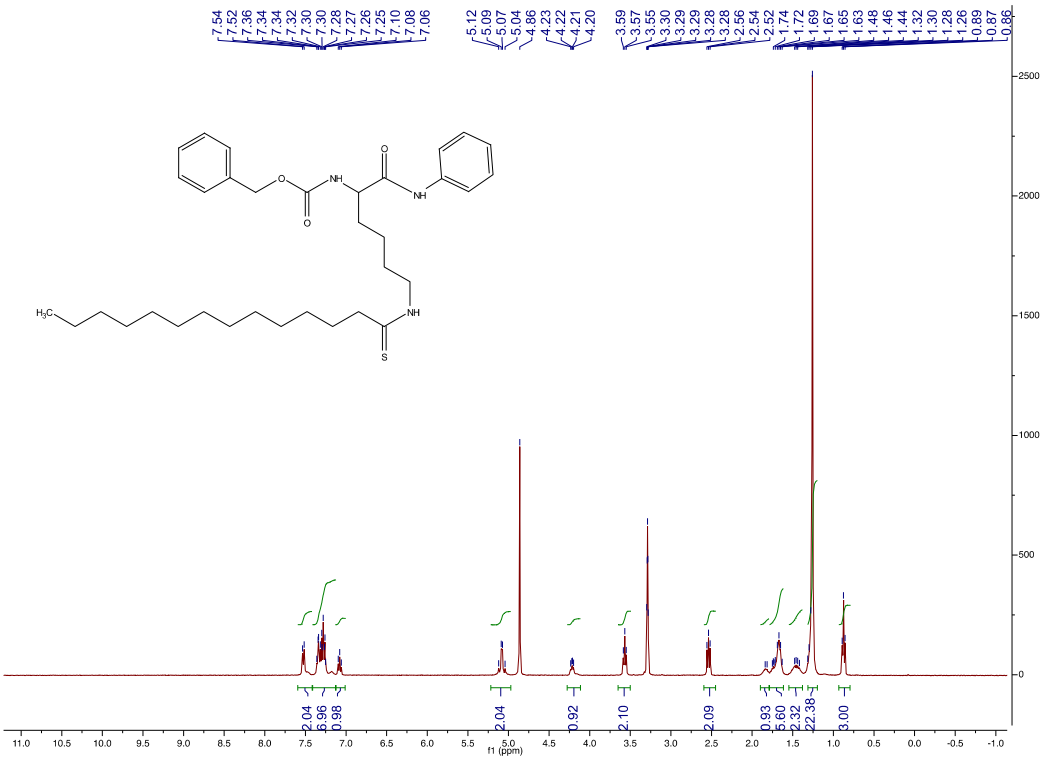
G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3.59 3.57 3.55 3.30 3.29 3.29 3.28 3.28 2.56 2.54 2.52 1.74 1.72 1.69 1.67 1.65 1.63 1.48 1.46 1.44 1.32 1.30 1.28 1.26 0.89 0.87 0.86
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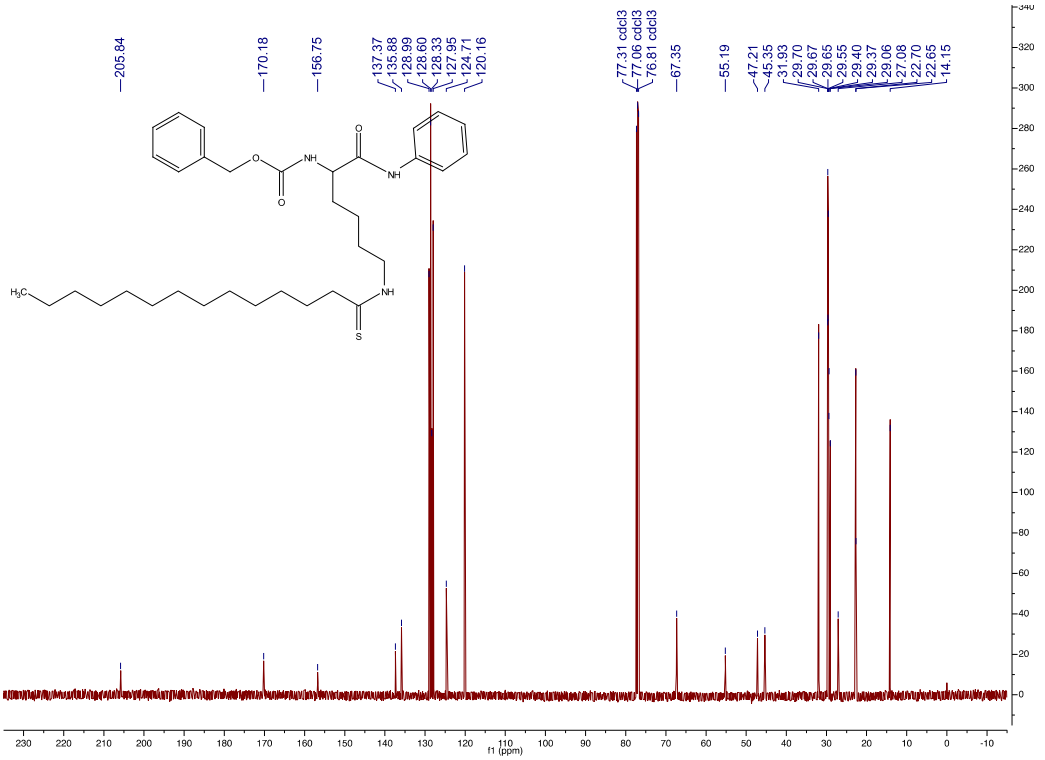
f1 (ppm)

H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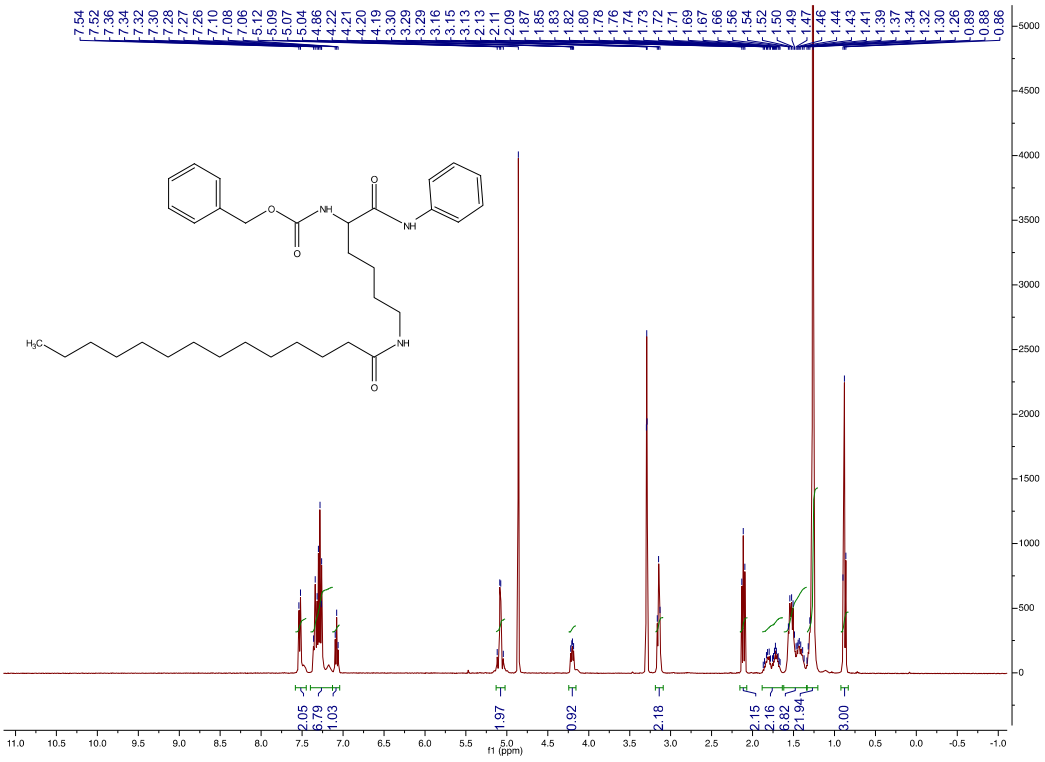
f1 (ppm)

I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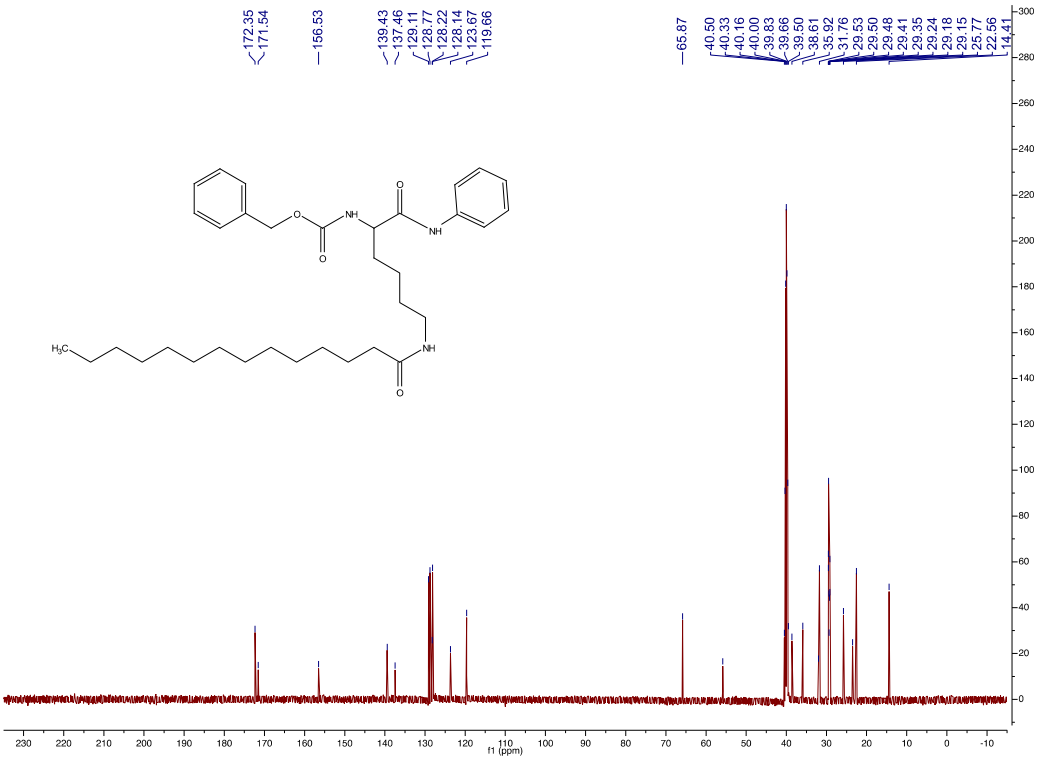
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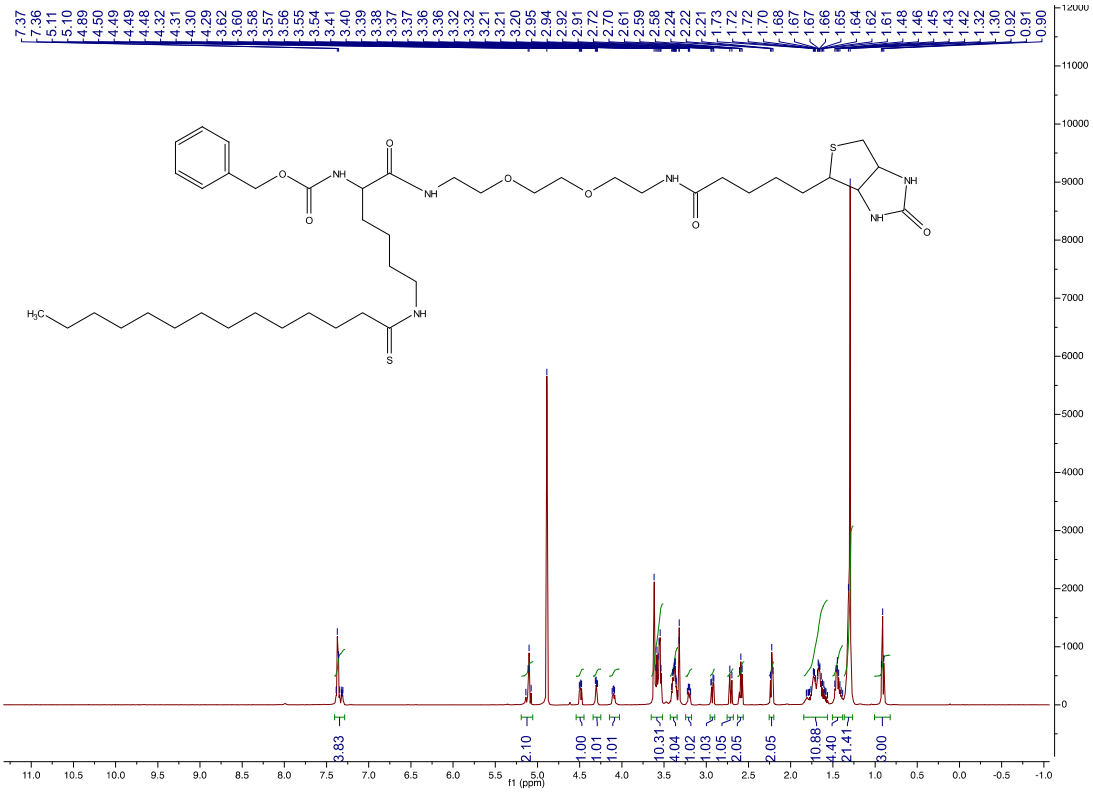
K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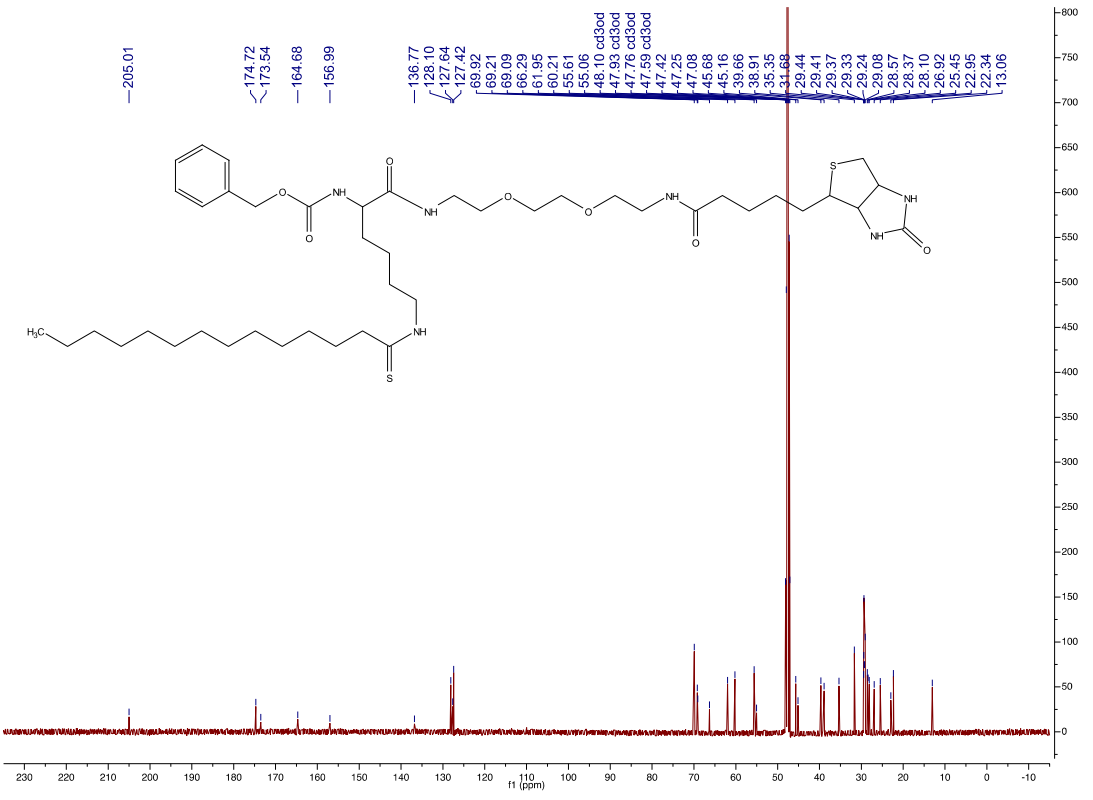
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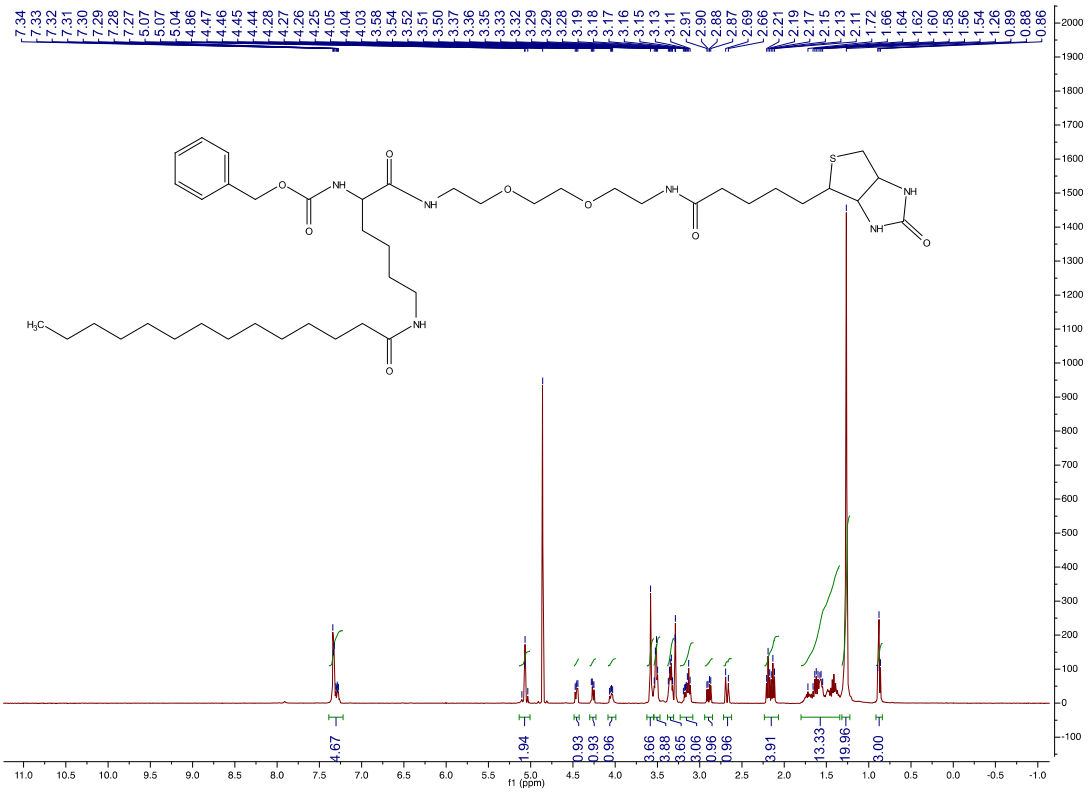


N

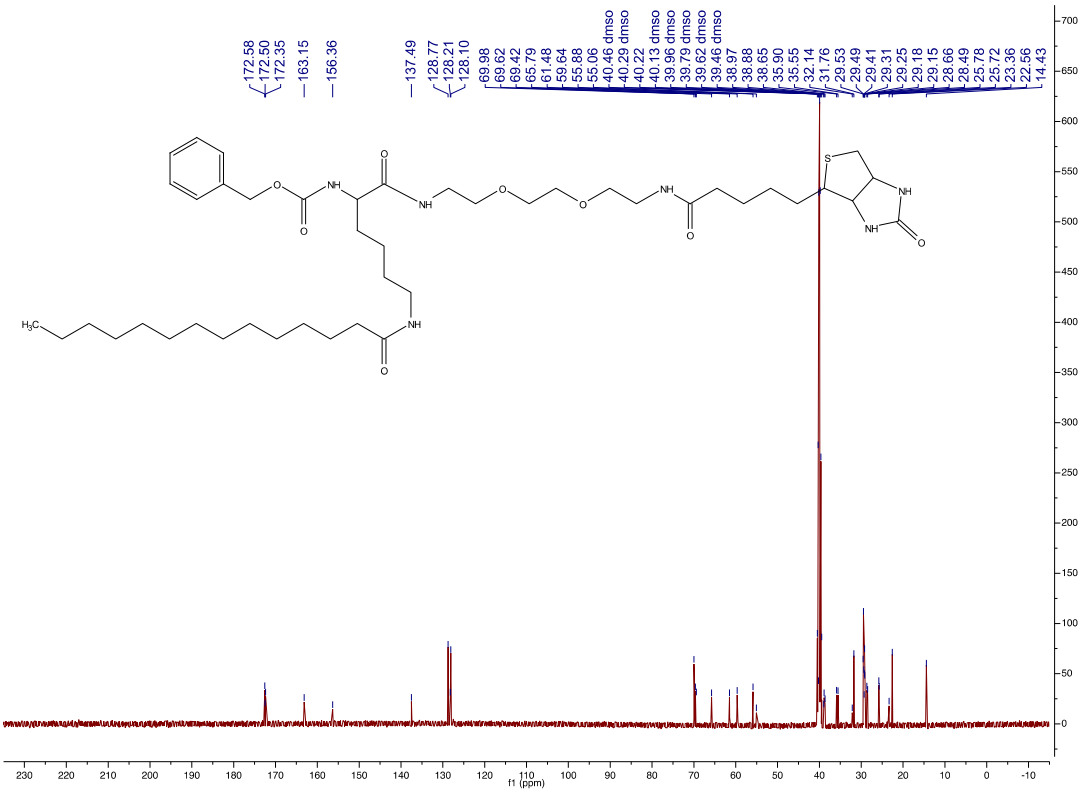


Figure S7. Relate to **Synthesis of Compounds used in the study** in the section of **Experimental Procedures.** NMR spectra of the synthesized compounds. (**A**) ^{1}H NMR spectrum of Compound **TA.** (**B**) ^{13}C NMR spectrum of Compound **TA.** (**C**) ^{1}H NMR spectrum of Compound **TB.** (**D**) ^{13}C NMR spectrum of Compound **TB.** (**E**) ^{1}H NMR spectrum of Compound **TH.** (**F**) ^{13}C NMR spectrum of Compound **TH.** (**G**) ^{1}H NMR spectrum of Compound **TM.** (**H**) ^{13}C NMR spectrum of Compound **TM.** (**I**) ^{1}H NMR spectrum of Compound **M.** (**J**) ^{13}C NMR spectrum of Compound **M.** (**K**) ^{1}H NMR spectrum of Compound **Biotin-TM.** (**L**) ^{13}C NMR spectrum of Compound **Biotin-TM.** (**M**) ^{1}H NMR spectrum of Compound **Biotin-M.** (**N**) ^{13}C NMR spectrum of Compound **Biotin-M.**

Supplemental Experimental Procedures

Cloning, expression and purification of human sirtuins. Human SIRT1, SIRT3, SIRT5 and SIRT6 were expressed as previously described (Du et al., 2009; Jiang et al., 2013). Human SIRT2 (aa38-356) was cloned and inserted into pET28a vector for the expression of N-terminal His6-SUMO fusion protein. Then SIRT2 expression vector was introduced into an *E. coli* BL21. Successful transformation were selected by plating the cells on kanamycin (50 μg mL^{-1}) and chloramphenicol (20 μg mL^{-1}) luria broth (LB) plates. Single colonies were selected and grown in LB with kanamycin (50 μg mL^{-1}) and chloramphenicol (20 μg mL^{-1}) overnight at 37 °C. On the following day the cells were subcultured (1:1000 dilution) into 2 L of LB with kanamycin (50 μg mL^{-1}) and chloramphenicol (20 μg mL^{-1}). The cells were induced with 20 μM of isopropyl β-D-1-thiogalactopyranoside (IPTG) at an OD_{600} of 0.6 and grown overnight at 15 °C, 200 rpm. The cells were harvested by centrifugation at 8000 rpm for 5 min at 4 °C (Beckman Coulter refrigerated floor centrifuge) and passed through an EmulsiFlex-C3 cell disruptor (AVESTIN, Inc.) 3 times. Cellular debris was removed by centrifuging at 20,000 rpm for 30 min at 4 °C (Beckman Coulter). The supernatant was loaded onto a nickel column (Histrap, Ge Healthcare) pre-equilibrated with 20 mM Tris-HCl pH 8.0 with 500 mM NaCl. The protein was eluted with a linear gradient of imidazole (0-500 mM). The desired fractions were pooled, concentrated and buffer exchanged. The His6-SUMO tag was removed by overnight incubation at 4 °C with ULP1, followed by Ni-affinity column purification to remove any undigested SIRT2. The tag-free SIRT2 was further purified on a Superdex 75 column (Bio-Rad, Hercules, CA). The protein was eluted with 20 mM Tris-HCl, pH 8.0, 500 mM NaCl. After concentration, the target protein was frozen at -80 °C.

Reagents, antibodies and plasmids. All chemicals were obtained in the highest purity available. MG132 was from Cayman Chemical Co. (Ann Arbor, MI). Cycloheximide was purchased from Amresco (Euclid, OH). Trichostatin A (TSA) and AGK2 (2-Cyano-3-[5-(2,5-dichlorophenyl)-2-furanyl]-N-5-quinolinyl-2-propenamide) were obtained from Sigma-Aldrich (St. Louis, MO).

The anti-human SIRT1 antibody (3H10.2) was from EMD Chemicals Inc. (San Diego, CA). The anti-human SIRT2 (EPR1667), SIRT6 antibodies were from Abcam (Cambridge, MA). The anti-

human SIRT3 (C73E3), acetyl-p53 (Lys382) antibodies were obtained from Cell Signaling Technology (Danvers, MA). The anti-SIRT7 (C-3), c-Myc (9E10), NEDD4 (H-135), ubiquitin (P4D1), β-actin (C4) and the goat anti-mouse/rabbit IgG-horseradish peroxidase-conjugated antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The anti-SIRT4 (LS-C100490) antibody was purchased from LSBio, Inc. (Seattle, WA). The anti-SIRT5 antibody (Center) was from Abgent (San Diego, CA). The anti-acetyl-α-tubulin (6-11B-1), α-tubulin (B-5-1-2) antibodies, the anti-Flag M2 antibody conjugated with horseradish peroxidase and the anti-Flag M2 affinity gel were from Sigma-Aldrich.

The pLKO.1-puro lentiviral shRNAs constructs toward Luciferase and SIRT1-7 were purchased from Sigma-Aldrich. Luciferase shRNA (SHC007), SIRT1 shRNA1 (TRCN0000018980), SIRT1 shRNA2 (TRCN0000018981), SIRT2 shRNA1 (TRCN0000040221), SIRT2 shRNA2 (TRCN0000310335), SIRT3 shRNA1 (TRCN0000038890), SIRT3 shRNA2 (TRCN0000038893), SIRT4 shRNA1 (TRCN0000018948), SIRT4 shRNA2 (TRCN0000232894), SIRT5 shRNA1 (TRCN0000018544), SIRT5 shRNA2 (TRCN0000018545), SIRT6 shRNA 1 (TRCN0000378253) and shRNA 2 (TRCN0000232528), SIRT7 shRNA1 (TRCN0000359663), and SIRT7 shRNA2 (TRCN0000020254) were used. The scrambled siRNA and Stealth Select RNAi™ siRNA targeting SIRT2 (HSS117928 and HSS177042) were purchased from Invitrogen (Carlsbad, CA). To generate human SIRT2 with C-terminal Flag-tag expression vector, full-length human *SIRT2* cDNA was amplified by PCR and inserted into pCMV-tag-4a vector between BamHI and XhoI sites. A human c-Myc expression vector with N-terminal Flag-tag was obtained by PCR amplification of *Flag-c-Myc* and subcloning via BamHI and XhoI sites into pCMV-tag-4a vector.

Inhibition assay for SIRT1, SIRT2, SIRT3, SIRT5. Different concentrations (0.0064, 0.032, 0.16, 0.8, 4.0, 20, 100 and 200 μM) of TA~TM, and M were pre-incubated with 0.1 μM of SIRT1, 0.2 μM of SIRT2, 1 μM of SIRT3 or 1 μM of SIRT5, respectively, and 1 mM NAD in 20 mM Tris-HCl buffer (pH 8.0) with 1 mM dithiothreitol (DTT) at 37 °C for 15 min. Then 10 μM of acyl peptide (acetyl-H3K9 for SIRT1, SIRT2 and SIRT3; succinyl-H3K9 for SIRT5) was added to initiate the reactions. Then reactions were incubated at 37°C in a total volume of 60 μL (5 min for SIRT1, 5 min for SIRT2, 20 min for SIRT3, and 10 min for SIRT5). The reactions were stopped by adding 60 μL of an aqueous solution of 50% methanol containing 200 mM HCl and 320 mM acetic acid.

After quenching the sirtuin reactions, centrifugation was used to remove precipitated proteins and the supernatant was analyzed by HPLC with a reverse phase C18 column (Kinetex XB-C18 100A, 100 mm × 4.60 mm, 2.6 μm, Phenomenex) with a gradient of 0 % in 2 min, 0% to 20% in 2min, 20% to 40% B in 13 min and then 40% to 100% for 2 min at 0.5 mL/min. Product quantification was based on the area of absorbance monitored at 280 nm. The peak areas were integrated and the conversion rate was calculated from the peak areas as the fraction of the free H3K9 peptide from the total peptide.

All reactions were done in duplicate.

Determination of kinetic parameters for TM. For SIRT2 inhibition kinetics of TM, a mixture of acetyl-H3K9 (acH3K9) peptide substrate (2.5, 5, 10, 25, 50, 100, 187.5 μM), NAD (25, 50, 100, 250, 500, 1000, 1500 μM), TM (0, 0.01, 0.03, 0.1, and 0.3 μM), 20 mM Tris-HCl (pH 8.0) and 1 mM DTT was incubated at 37 °C. 1 mM NAD was used for determining the kinetic parameters for acH3K9 peptide, 100 μM of acH3K9 peptide was used for the determination of kinetic parameters for NAD. The reaction was started by adding 0.2 μM of SIRT2, and stopped after 5 min by adding 60 μL of an aqueous solution of 50% methanol containing 200 mM HCl and 320 mM acetic acid. The samples were analyzed by HPLC as described above and the initial velocity was calculated. The K_m and v_{max} were obtained from Michaelie-Menten plots using Graphpad Prism software.

Mass spectrometry detection of the stalled intermediate formed by TM and NAD. Reactions containing 50 μM SIRT2, 100 μM NAD, 100 μM TM, 1 mM DTT, and 20 mM pyridinium formate (pH 7.0) was reacted for 5 min at 37 °C. Controls were run in which NAD or SIRT2 was removed from the reaction mixture. Reactions were quenched with 1 volume of acetonitrile and the mixture was centrifuged to remove the precipitated protein. The supernatant was then analyzed by LC-MS using water and acetonitrile as solvents.

Inhibition assay for SIRT6. Different concentrations (0.0125, 0.05, 0.2, 0.8, 3.2, 12.8, 51.2, 204.8 μM) of TA~TM were pre-incubated with 1 μM of SIRT6 and 1 mM NAD in 20 mM Tris-HCl buffer (pH 8.0) with 1 mM DTT at 37°C for 20 min. Then 50 μM of myristoyl-H3K9 peptide (myrH3K9) was added to initiate the reactions. The reactions were incubated at 37 °C in a total volume of 60 μL for 1 hr. The reactions were stopped by adding 60 μL of an aqueous solution of 50% methanol containing 200 mM HCl and 320 mM acetic acid.

Inhibition assay for SIRT7. Different concentrations (0.0125, 0.05, 0.2, 0.8, 3.2, 12.8, 51.2, 204.8 μM) of TA~TM were pre-incubated with 1 μM of SIRT7 and 1 mM NAD in 150mM NaCl and 50 mM KH_2PO_4 buffer (pH 8.0) with 1 mM DTT at 37°C for 20 min. Then 10 μM myrH3K9 peptide and 0.083mg/mL tRNA were added to initiate the reactions. Then reactions were incubated at 37 °C in a total volume of 60 μL for 110 min. The reactions were stopped by adding 60 μL of an aqueous solution of 50% methanol containing 200 mM HCl and 320 mM acetic acid.

Cell culture and transfection. All cell culture media contained 10% (vol/vol) heat-inactivated fetal bovine serum (FBS; Invitrogen, Carlsbad, CA) and 1% penicillin-streptomycin (Invitrogen) unless otherwise specified. Human MCF-7, MDA-MB-231, MDA-MB-468, HeLa, HME1 cells were grown in DMEM media (Invitrogen). Human BT-549, SK-BR-3, MDA-MB-453 and K562 cells were grown in RPMI-1640 media (Invitrogen). The MCF-10A cells were cultured in mammary epithelial cell growth medium (MEGM; Lonza, Walkersville, MD) with supplements according to

manufacturer's instruction.

To overexpress SIRT2 or c-Myc in cells, the pCMV-tag-4a vector containing *SIRT2* or *c-Myc,* or pCDH vector containing *c-Myc* were transfected into cells using FuGene 6 (Promega, Madison, WI) according to manufacturer's protocol. Empty vector was transfected as negative control.

Soft agar colony formation assay. For colony formation in semisolid medium, 1.0×10^4 cells were plated in 0.3% low-melting point agarose (LMP, Invitrogen) onto 6-well plate coated with 1.2% LMP mixed with 2 × complete medium. For treatments, 2 × inhibitor was added to cells at the time of plating. The medium and inhibitor were replaced with fresh ones every 3 days. For colony formation of the SIRT2 KD cells, cells were transfected with the scrambled siRNA or SIRT2 siRNAs for 48 hr before plating in 6-well plate. Similarly, cell media was replaced every 3 days. After 14 days of incubation, colonies were photographed and counted with ImageJ.

Western blot analysis. Western blot analysis was performed as described previously (Jiang et al., 2013). The proteins of interest were detected using enzyme-linked chemiluminescence (ECL; Pierce Biotechnology Inc.) and visualized using the Storm Imager (GE Healthcare, Piscataway, NJ). Quantification of Western blots was done using the Quantity One software (Bio-Rad).

Biotin-TM/M pull-down assay. HEK293T cells were collected and lysed in lysis buffer containing 25 mM Tris, pH 7.4, 150 mM NaCl, 10% glycerol, 1% Nonidet P-40 and 1 × protease inhibitor cocktail (Sigma-Aldrich). The cell extract supernatant was collected after centrifugation at 14,000 g for 20 min at 4 °C. Cell lysates were incubated with 10 μM Biotin-TM or Biotin-M in the absence or presence of 1 mM NAD at 4 °C for 1 hr. The high capacity streptavidin resin (Pierce Biotechnology, Rockford, IL) was added to the mixture and incubated at 4 °C for another 1 hr. After centrifugation at 500 g for 2 min at 4 °C, the streptavidin resin was washed 3 times with 1 mL washing buffer (25 mM Tris, pH 7.4, 150 mM NaCl, 10% glycerol, 0.2% Nonidet P-40). The resin-bound proteins were then separated with SDS-PAGE and immunoblotted with anti-SIRT1 or anti-SIRT2 antibodies.

To assess the binding of TM to SIRT2 in cells, MCF-7 parental cells, Luciferase KD and SIRT2 KD cells were treated with 50 μM D-Biotin or Biotin-TM as indicated for 6 hr and then lysed in lysis buffer containing 1 mM NAD. Cell extract was collected, streptavidin pull-down and western blot analysis was performed as described above.

SIRT1 inhibition in cells. MCF-7 or MDA-MB-468 cells were treated with indicated test compounds in the presence of 200 nM TSA for 6 hr. The acetylation level of p53 protein was determined by western blot using anti-acetyl-p53 (K382) antibody. β-actin served as a loading control.

SIRT2 inhibition in cells. MCF-7 cells were treated with indicated inhibitors at for 6 hr after

being transfected with pCMV-tag-4a-*SIRT2* for 18 hr. Cells were collected and lysed in lysis buffer containing 25 mM Tris, pH 7.4, 150 mM NaCl, 10% glycerol, 4 mM $MgCl_2$, 0.2 mM DTT, 100 mM NAD, 1% Nonidet P-40 and 1 × protease inhibitor cocktail. And the cell lysates were subjected to western blot for the analysis of acetyl-α-tubulin (K40) and α-tubulin levels.

TM treatment of mice bearing human breast cancer xenotransplants. Two million MDA-MB-231 cells suspended in 100 μL 1 × PBS and 100 μL Matrigel were injected subcutaneously on the flanks of female Ncr Nu/Nu mice. Following the injections, mice were permitted to recover and monitored biweekly, including tumor measurement using calipers. Once the majority of tumors reached a threshold size of 200 mm^3, mice with intraperitoneal (IP) or intra-tumor (IT) injections of vehicle alone (DMSO) or inhibitor (TM in DMSO) over one month. IP injections of 1.5 mg TM in 50 μL DMSO were given daily. IT injections of 0.75 mg TM in 50 μL DMSO per tumor were given 3 days per week. After one month of treatment or if mice met humane endpoint criteria, mice were euthanized by CO_2 asphyxiation. Tissues were collected, fixed with 10% neutral-buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). H&E-stained sections were scanned using an Aperio ScanScope and analyzed by a veterinarian certified in anatomic pathology by the American College of Veterinary Pathologists blinded to treatment group. Serum, tumor tissues and organs were snap frozen in liquid N_2 and stored at -80 °C for subsequent analyses.

TM treatment of MMTV-*PyMT* mice. MMTV-*PyMT* transgenic female mice on a pure FVB/N background were obtained from the Jackson Laboratory and treated beginning at 6 weeks of age with daily IP injections of vehicle (DMSO) or 1.5 mg TM in 50 μL DMSO over one month. Mice were monitored daily for tumor development and health status, and tumor size was measured twice per week. After one month of treatment or if mice met humane endpoint criteria, mice were euthanized by CO_2 asphyxiation and necropsied. Tissues were collected and analyzed as described above.

Ubiquitination assay. MCF-7 cells were transfected with pCMV-tag-4a or pCMV-tag-4a-*c-Myc*, respectively. 18 hours after transfection, cells were treated with 25 μM TM for 6 hr in the presence of proteasome inhibitor MG132 (10 μM). Immunoprecipitation was performed with the cell lysates by anti-Flag M2 affinity gel as described previously (He et al., 2014). The gel-bound proteins were resolved on SDS-PAGE and detected with anti-ubiquitin antibody. The c-Myc level in total cell lysates was used as input control.

Reverse transcription (RT)-PCR analysis of mRNA levels. Total RNA was extracted from vehicle-, TM- or M-treated cells using RNeasy Mini Kit (Qiagen, CA, USA) according to the manufacturer's instructions. The concentration and purity of total RNA were determined by using the NanoDrop (Thermo Fisher Scientific Inc, Wilmington, DE). cDNA was synthesized using SuperScript

III reverse transcriptase (Invitrogen). Amplification of genes of interest was performed using Herculase II Fusion DNA Polymerase (Agilent Technologies, Santa Clara, CA) with the gene-specific primers shown below. 10 µl of each PCR product were analyzed by gel electrophoresis on a 2% agarose gel.

Primer target	Direction	Sequence
c-Myc	Forward	GGCTCCTGGCAAAAGGTCAGAGT
c-Myc	Reverse	CTGCGTAGTTGTGCTGATGTGT
NEDD4	Forward	TCAGGACAACCTAACAGATGCT
NEDD4	Reverse	TTCTGCAAGATGAGTTGGAACAT
Actin	Forward	CATGTACGTTGCTATCCAGGC
Actin	Reverse	CTCCTTAATGTCACGCACGAT
STUB1	Forward	AGCAGGGCAATCGTCTGTTC
STUB1	Reverse	CAAGGCCCGGTTGGTGTAATA
SKP2	Forward	ATGCCCCAATCTTGTCCATCT
SKP2	Reverse	CACCGACTGAGTGATAGGTGT
TRPC4AP	Forward	ACAAGCACACGCTTCTTGC
TRPC4AP	Reverse	CTGACACCTTTCGAGTCGCC
FBXW7	Forward	CGACGCCGAATTACATCTGTC
FBXW7	Reverse	CGTTGAAACTGGGGTTCTATCA
FBXO32	Forward	GCCTTTGTGCCTACAACTG
FBXO32	Reverse	CTGCCCTTTGTCTGACAGAAT

Immunofluorescence of cultured cells. MDA-MB-231 cells were treated with ethanol, M (25 µM) or TM (25 µM) for 6 hr. Immunostaining was performed and images were acquired by confocal microscopy as previously described (Mabjeesh et al., 2003).

Flow cytometry. For cell cycle analyses, MCF-7 or K562 cells were treated with 25 µM for 0, 24, 48 and 72 hr. Cells were spun down, washed with PBS, fixed with 70% ethanol overnight, and then washed with PBS. RNA was degraded with RNAse A and DNA was stained with propidium iodide (Invitrogen). Samples were analyzed on a BD LSR-II. Cell cycle analysis was performed with FlowJo flow cytometry analysis software (Tree Star, Inc., Ashland, OR).

Cellular senescence staining. MCF-7 or K562 cells were treated with ethanol or 25 µM TM. After 5 days of treatment, cells were stained for senescence as previously described (Debacq-Chainiaux et al., 2009).

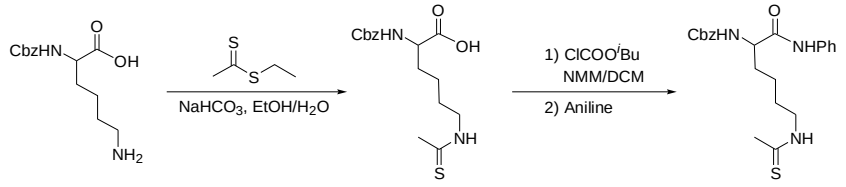
Immunofluorescence and Immunohistochemistry of tumor sections. Formalin-fixed, paraffin-embedded (FFPE) tumors were sectioned, dewaxed and submitted to heat mediated antigen retrieval in 0.01 M citrate buffer for 50 min. For immunofluorescence, sections were incubated with anti-acetyl-α-tubulin, followed by Alexa Fluro-488 conjugated secondary antibodies from Invitrogen and cell nuclei counterstaining with DAPI Fluoromount-G® from SouthernBiotech. Fluorescent

images were taken using Zeiss LSM880 inverted confocal microscopy (Carl Zeiss Inc., Thornwood, NY). For immunohistochemistry, sections were incubated with anti-Ki67 Clone MM1 (Vector Laboratories) antibody followed by biotinylated polyclonal rabbit anti-mouse (DAKO). Color was developed using 3,3'-Diaminobenzidine tetrahydrochloride substrate from Invitrogen and counterstained with hematoxilin. Images were scanned using an Aperio ScanScope.

Synthesis of compounds used in the study

General methods. Reagents were obtained from Aldrich or Acros in the highest purity available and used as supplied. ^{1}HNMR was performed on INOVA 400/500 spectrometer. LCMS was carried out on a SHIMADZU LC and Thermo LCQ FLEET MS with a Sprite TARGA C18 column (40 × 2.1 mm, 5 μm, Higgins Analytical, Inc.) monitoring at 215 and 260 nm. Solvents used in LCMS were water with 0.1% acetic acid and acetonitrile with 0.1% acetic acid.

1. Synthetic Route for TA

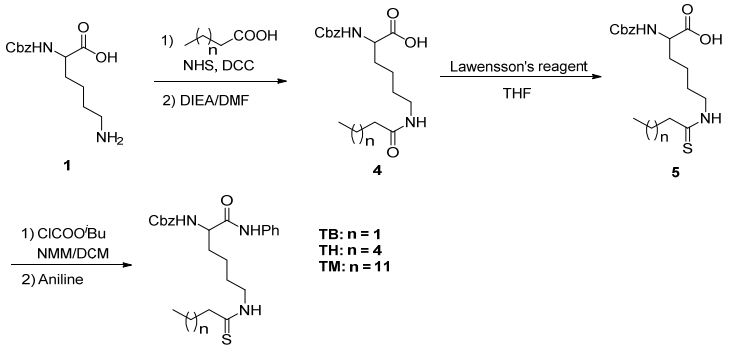


1 2 TA

Synthesis of compound 2. To a solution of Z-Lys-OH (2.8 g, 10 mmol) in ethanol (100 mL) was added 20 mL of 10% (w/v) Na_2CO_3 aqueous solution at 0°C. The reaction mixture was allowed to warm to room temperature (rt) while stirred extensively. Ethyl dithioacetate (1.32 g, 11 mmol) was added and the reaction mixture was stirred overnight at rt. Solvent was evaporated and then the crude product was acidified to pH **=** 2 with 3 M HCl on ice and extracted with DCM (3 x 100 mL). The organic phase was washed with brine (2 x 30 mL), dried with Na_2SO_4, and evaporated to obtain compound **2,** which was directly used in the next step without further purification.

Synthesis of compound TA. To a solution of compound **2** (3.38 g, 10 mmol) and N-methylmorpholine (1.1 ml, 10 mmol) in dry dichloromethane (100 mL) at 0°C was added dropwisely iso-butylchloroformate (1.3 ml, 10 mmol). The reaction mixture was stirred for 30 min at 0°C. Aniline (1.09 ml, 12 mmol) was added at 0°C and the reaction mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure and the resulting residue was purified by flash chromatography on silica gel (Hexane/ethyl acetate = 2/1) to afford the expected compound **3** (3.95 g, 95.5% yield). ^{1}H NMR (400 MHz, CD_3OD): δ 7.53-7.51 (m, 2H), 7.34-7.22 (m, 7H), 7.08-7.04(t, *J* =7.2Hz, 1H), 5.06 (q, *J*=8.0Hz, 2H), 4.26-4.22 (m, 1H), 3.54 (t, *J*=7.1Hz, 2H), 2.39 (s, 3H), 1.89-1.76(m, 1H), 1.78-1.70 (m, 1H), 1.66-1.58 (m, 2H), 1.55-1.35 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 200.88, 170.34, 156.79, 137.34, 135.87, 129.01, 128.62, 128.35, 127.93, 124.78, 120.23, 120.13, 67.34, 55.19, 45.74, 33.97, 32.04, 27.00, 22.70.LCMS (ESI) calcd. for C22H28N3O3S $[M+H]^+$ 414.2, obsd. 414.3.

2. Synthetic Route for TB, TH, and TM



Synthesis of compound 4. To the solution of acid (30 mmol) in anhydrous *N, N'*-dimethylformamide (DMF, 20 mL) was added *N*-hydroxysuccinimide (NHS, 3.45 g, 30 mmol) with stirring at rt. Then *N, N'*-dicyclohexylcarbodiimide (DCC, 6.19 g, 30 mmol) in anhydrous DMF (20 mL) was added to the reaction. After stirring for 2 hr, the reaction mixture was filtered. The filtrate was added to a solution of Z-Lys-OH (8.4 g, 30 mmol) with *N, N*-diisopropylethylamine (DIEA, 5.2 mL, 30 mmol) in anhydrous DMF (50.0 mL) at room temperature. The resulting reaction mixture was stirred overnight. Then 44 mL water and 26 mL 1 M HCl was added to the reaction mixture to adjust pH to 2~3. The mixture was extracted ethyl acetate (3 x 200 mL) and washed brine (2 x 100 mL). The organic layer was dried over anhydrous sodium sulfate. After removal of the solvents in vacuum, the residue was purified by flash chromatography on silica gel (DCM/MeOH = 20:1) to afford the expected compound **4** (85% yield).

Synthesis of compound 5. To a solution of compound **4** (20 mmol) in THF (100 mL) was added Lawesson's reagent (8.0 g, 20 mmol) at room temperature. The reaction mixture was stirred overnight under nitrogen (monitored by LCMS). After removal of THF using a rotary evaporator, the residue was purified by silica gel column (DCM/MeOH = 20:1) to give the product as a white solid (76% yield).

Synthesis of compound TB, TH and TM. To a solution of compound **5** (10 mmol) and N-methylmorpholine (1.1 ml, 10 mmol) in dry dichloromethane (100 mL) at 0°C was added dropwisely iso-butylchloroformate (1.3ml, 10 mmol). The reaction mixture was stirred for 30 min at 0°C. Aniline (1.09 ml, 12 mmol) was then added at 0°C and the reaction mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure and the resulting residue was purified by flash chromatography on silica gel (Hexane/ethyl acetate= 2/1) to afford the expected compound **TB, TH, and TM**.

TB (91% yield) ^{1}H NMR (400 MHz, CD_3OD): δ 7.57-7.45 (m, 2H), 7.39-7.12 (m, 7H), 7.12-6.99 (m, 1H), 5.05 (q, *J* = 12.5 Hz, 2H), 4.27-4.23 (m, 1H), 3.55 (q, *J* = 12.5 Hz, 2H), 2.51 (t, *J* = 7.2Hz, 2H), 1.91-1.78 (m, 1H), 1.78-1.57 (m, 5H), 1.55-1.33 (m, 2H), 0.86 (t, *J* = 7.2 Hz, 3H).. ^{13}C NMR (126 MHz, $CDCl_3$): δ 205.60, 170.19, 156.74, 137.36, 135.88, 128.99, 128.60, 128.33, 127.96, 124.72, 120.16, 120.06, 119.98, 67.34, 55.19, 48.93, 45.30, 31.89, 27.07, 22.78, 22.63, 13.36. LCMS (ESI) calcd. for C24H32N3O3S $[M+H]^+$ 442.2, obsd. 442.3;

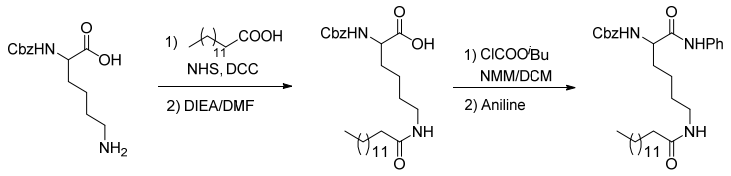
TH (89% yield) ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.54 (s, 1H), 7.73 (s, 1H), 7.49 (d, *J* = 7.5 Hz, 2H), 7.41-7.20 (m, 7H), 7.11 (t, *J* = 7.4 Hz, 1H), 5.79 (d, *J* = 7.5 Hz, 1H), 5.19 -5.03 (m, 2H), 4.45-4.31 (m, 1H), 3.70-3.62 (m, 2H), 2.60(t, *J* = 7.5 Hz, 2H), 1.98-1.91 (m, 1H), 1.81-1.60 (m, 5H), 1.54-1.41 (m, 2H), 1.36-1.18 (m, 6H), 0.87 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$):

δ 205.84, 170.21, 156.75, 137.38, 135.88, 128.99, 128.60, 128.33, 127.95, 124.71, 120.16, 67.34, 55.19, 47.16, 45.34, 31.91, 31.52, 29.47, 28.66, 27.07, 22.65, 22.53, 14.06. LCMS (ESI) calcd. for $C_{27}H_{38}N_3O_3S$ $[M+H]^+$ 484.3, obsd. 484.3;

TM (91% yield) ^{1}H NMR (400 MHz, CD_3OD): δ 7.53 (d, *J* = 8.0 Hz, 2H), 7.41-7.12 (m, 7H), 7.08 (t, *J* = 7.4 Hz, 1H), 5.22-4.97 (m, 2H), 4.22 (dd, *J* = 8.8, 5.4 Hz, 1H), 3.57 (t, *J* = 7.1 Hz, 2H), 2.54 (t, *J* = 7.6 Hz, 2H), 1.90-1.79(m, 1H), 1.79-1.61 (m, 5H), 1.55-1.37 (m, 2H), 1.26 (s, 20H), 0.87 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 205.84, 170.18, 156.75, 137.37, 135.88, 128.99, 128.60, 128.33, 127.95, 124.71, 120.16, 67.35, 55.19, 47.21, 45.35, 31.93, 29.70, 29.67, 29.65, 29.55, 29.40, 29.37, 29.06, 27.08, 22.70, 22.65, 14.15.

LCMS (ESI) calcd. for $C_{34}H_{52}N_3O_3S$ $[M+H]^+$ 582.4, obsd. 582.4;

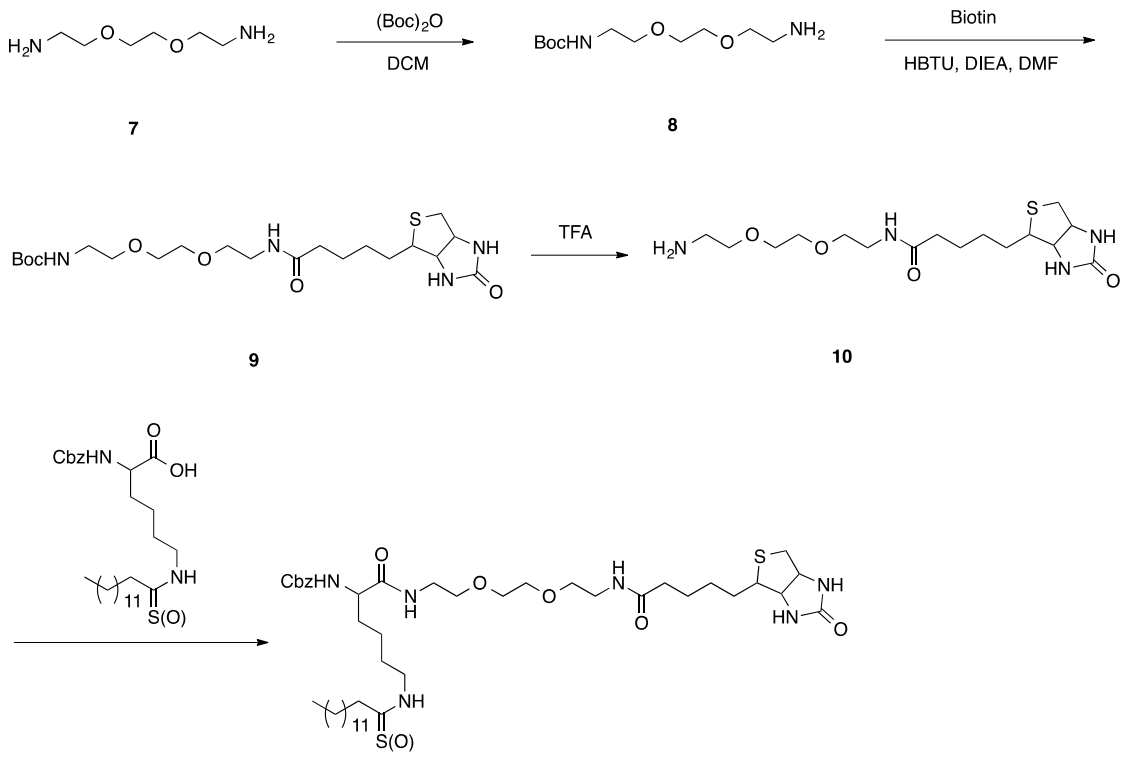
3. Synthesis of compound M



1 **6** **M**

The synthesis of compound **6** followed the method using in the synthesis of compound **4.** To a solution of compound **6** (4.9 g, 10 mmol) and *N*-methylmorpholine (1.1ml, 10 mmol) in dry dichloromethane (100 mL) at 0°C was added dropwisely iso-butylchloroformate (1.3 ml, 10 mmol). The reaction mixture was stirred 30 min at 0°C. Aniline (1.09 ml, 12 mmol) was added at 0°C and the reaction mixture was stirred overnight at rt. The solvent was removed under reduced pressure and the resulting residue was purified by silica gel chromatography (DCM/MeOH = 50:1) to afford the expected compound **M** (5.14 g, 91% yield). ^{1}H NMR (400 MHz, CD_3OD): δ 7.53 (d, *J* = 7.9 Hz, 2H), 7.40-7.13 (m, 7H), 7.08 (t, *J* = 7.4 Hz, 1H), 5.13-5.02 (m, 2H), 4.20 (dd, *J* = 8.5, 5.5 Hz, 1H), 3.15 (t, *J* = 6.7 Hz, 2H), 2.11 (t, *J* = 7.6 Hz, 2H), 1.88-1.64 (m, 2H), 1.62-1.34 (m, 6H), 1.32-1.21 (s, 20H), 0.87 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d6): δ 172.35, 171.54, 156.53, 139.43, 137.46, 129.11, 128.77, 128.22, 128.14, 123.67, 119.66, 65.87, 55.85, 38.61, 35.92, 31.98, 31.76, 29.53, 29.50, 29.48, 29.41, 29.35, 29.24, 29.18, 29.15, 25.77, 23.48, 22.56, 14.41.LCMS (ESI) calcd. for $C_{34}H_{52}N_3O_4$ $[M+H]^+$ 566.4, obsd. 566.5;

4. Synthetic Route for Biotin-TM and Biotin-M



Synthesis of Compound 8. To a solution of compound **7** (14.8 g, 100 mmol) in DCM (200 mL) was added 100 mL of di-tert-butyl dicarbonate (2.18 g, 10mmol) in DCM at 0°C. The reaction mixture was allowed to warm to rt and stirred extensively overnight. The organic phase was washed with water, until all the unreacted compound **7** was extracted. After drying over Na_2SO_4 and concentration under vacuum the Boc-protected compound **8** was quantitatively obtained.

Synthesis of Compound 9. To a solution of Biotin (2.2 g, 9 mmol) and HBTU (3.41 g, 9 mmol) in DMF (30 mL) was added DIEA (3.6 mL, 20 mmol) at room temperature with stirring for 30 min. Then compound **8** was added to the resulting mixture. The reaction mixture was stirred extensively overnight. After removal of the solvents under reduced pressure, the residue was purified by flash chromatography on silica gel (DCM/MeOH = 20:1 then 10:1) to afford the expected compound **9** (3.5 g, 81% yield).

Synthesis of Compound 10. To 20 mL of TFA was added the compound **9** (2 g, 4.2 mmol) and the resulting mixture was stirred for 30 min at room temperature. After removing the solvent under vacuum the deprotected compound **10** was quantitatively obtained and used in the next step without further purification.

Synthesis of Compound Biotin-TM and Biotin-M. The synthesis followed the method using in the synthesis of **TM**. The solvent used to dissolve the compound **10** is DMF instead of DCM.

Biotin-TM (81% yield) ^{1}H NMR (500 MHz, CD_3OD): δ 7.41-7.29 (m, 5H), 5.18-5.05 (m, 2H), 4.49 (dd, *J* = 7.8, 4.8 Hz, 1H), 4.30 (dd, *J* = 7.9, 4.5 Hz, 1H), 4.10 (dd, *J* = 8.7, 5.4 Hz, 1H), 3.66-3.52 (m, 10H), 3.44 -3.34 (m, 4H), 3.21 (dt, *J* = 9.9, 5.6 Hz, 1H), 2.93 (dd, *J* = 12.7, 5.0 Hz,

1H), 2.71 (d, *J* = 12.7 Hz, 1H), 2.59 (t, *J* = 7.4 Hz, 2H), 2.22 (t, *J* = 7.4 Hz, 2H), 1.86-1.55 (m, 10H), 1.48-1.38 (m, 4H), 1.35-1.25(m, 20H), 0.91 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, CD_3OD): δ 205.01, 174.72, 173.54, 164.68, 156.99, 136.77, 128.10, 127.64, 127.42, 69.92, 69.21, 69.09, 66.29, 61.95, 60.21, 55.61, 55.06, 45.68, 45.16, 39.66, 38.91, 35.35, 31.68, 29.44, 29.41, 29.37, 29.33, 29.24, 29.08, 28.57, 28.37, 28.10, 26.92, 25.45, 22.95, 22.34, 13.06. LCMS (ESI) calcd. for $C_{44}H_{75}N_6O_7S_2$ $[M+H]^+$ 863.5, obsd. 863.6.

Biotin-M (83% yield)^{1}H NMR (400 MHz, CD_3OD): δ 7.39-7.22 (m, 5H), 5.10-5.04 (m, 2H), 4.46 (dd, *J* = 7.9, 4.9 Hz, 1H), 4.27 (dd, *J* = 7.8, 4.5 Hz, 1H), 4.05 (dd, *J* = 8.8, 5.3 Hz, 1H), 3.58 (s, 4H), 3.52 (q, *J* = 5.2 Hz, 4H), 3.37-3.32 (m, 4H), 3.19-3.11 (m, 3H), 2.89 (dd, *J* = 12.7, 5.0 Hz, 1H), 2.68 (d, *J* = 12.7 Hz, 1H), 2.19 (t, *J* = 7.5 Hz, 2H), 2.13(t, *J* = 7.5 Hz, 2H), 1.80-1.34 (m, 14H), 1.26 (s, 20H), 0.88 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, DMSO): δ 172.58, 172.50, 172.35, 163.15, 156.36, 137.49, 128.77, 128.21, 128.10, 69.98, 69.62, 69.42, 65.79, 61.48, 59.64, 55.88, 55.06, 38.97, 38.88, 38.65, 35.90, 35.55, 32.14, 31.76, 29.53, 29.49, 29.41, 29.31, 29.25, 29.18, 29.15, 28.66, 28.49, 25.78, 25.72, 23.36, 22.56, 14.43. LCMS (ESI) calcd. for $C_{44}H_{75}N_6O_8S$ $[M+H]^+$ 847.5, obsd. 847.8.

Supplemental References

Debacq-Chainiaux, F., Erusalimsky, J. D., Campisi, J., and Toussaint, O. (2009). Protocols to detect senescence-associated beta-galactosidase (SA-betagal) activity, a biomarker of senescent cells in culture and in vivo. Nat. Protoc. *4*, 1798-1806.

Du, J., Jiang, H., and Lin, H. (2009). Investigating the ADP-ribosyltransferase activity of sirtuins with NAD analogues and 32P-NAD. Biochemistry *48*, 2878-2890.

He, B., Hu, J., Zhang, X., and Lin, H. (2014). Thiomyristoyl peptides as cell-permeable Sirt6 inhibitors. Org. Biomol. Chem. *12*, 7498-7502.

Jiang, H., Khan, S., Wang, Y., Charron, G., He, B., Sebastian, C., Du, J., Kim, R., Ge, E., Mostoslavsky, R.*, et al.* (2013). SIRT6 regulates TNF-alpha secretion through hydrolysis of long-chain fatty acyl lysine. Nature *496*, 110-113.

Mabjeesh, N. J., Escuin, D., LaVallee, T. M., Pribluda, V. S., Swartz, G. M., Johnson, M. S., Willard, M. T., Zhong, H., Simons, J. W., and Giannakakou, P. (2003). 2ME2 inhibits tumor growth and angiogenesis by disrupting microtubules and dysregulating HIF. Cancer Cell *3*, 363-375.