Supporting Information for

A Chemical Antibody Approach to Reveal Sirt6-targeted Histone H3 Sites in Nucleosomes

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Methods

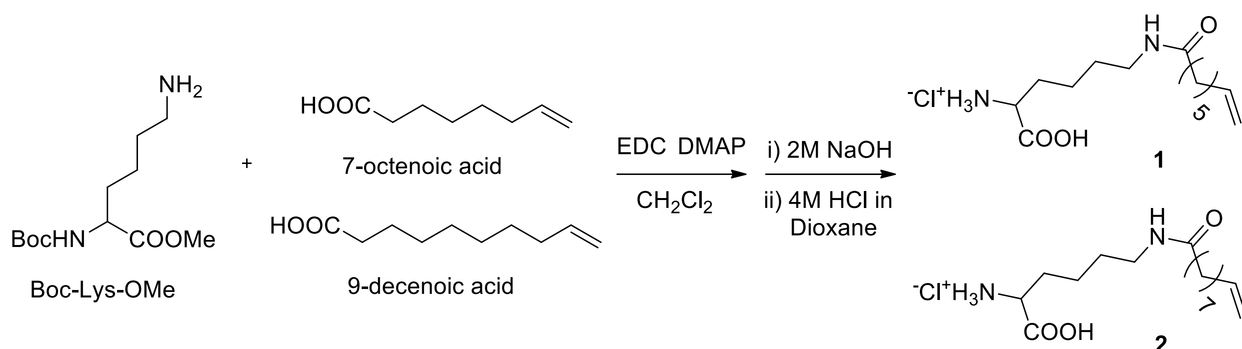
Expression and purification of Histone H3 mutants with acetyl-lysine

Human H3 gene fragment was cloned into the pETDuet-1 vector, after an N-terminal His tag and a selective TEV digestion site. And Cysteine 110 was mutated to Alanine. Amber stop codon was introduced at various lysine sites by site-directed mutagenesis. pETDuet-1 mutant H3 vectors were used to co-transform *E. coli* BL21(DE3) strain with pEVOL-mmAckRS. Single colonies were picked and inoculated in 2YT medium (100 ug/ml Ampicillin 34 ug/ml chloramphenicol) at 37 °C. When OD reached to 0.6, 0.5 mM IPTG, 5 mM Nicotinamide, 0.2 % (w/v) L-arabinose and 5 mM AcK were added into cell culture. Cells were harvested 10 hours later (expression at 37 °C) after induction and sonicated in lysis buffer (50 mM Tris, 500 mM NaCl, 0.1 % Triton X-100, pH 8.0). Inclusion bodies were collected and washed 3 times with lysis buffer and 3 times with pellet wash buffer (50 mM Tris, 500 mM NaCl, pH 8.0). 6 M guanidinium chloride buffer (6 M guanidinium chloride, 20 mM Tris, 250 mM NaCl, pH 8.0) was then added to dissolve inclusion bodies. Suspensions were then centrifuged (11000 g, 40 min, 4 °C, Beckman Coulter Allegra X-15R Centrifuge) and supernatants were collected and run through Ni-NTA purification under denatured condition. Acetyl-H3 proteins were concentrated to 1 ml in elution buffer (50 mM Tris-HCl, 100 mM NaCl, 250 mM imidazole, 6 M Urea, pH 7.8), and precipitated out by adding 9 ml of acetone (90 % v/v). Acetyl-H3 proteins were re-dissolve in arginine buffer (2 M arginine, 80 mM Tris-HCl, 1 M NaCl, pH 7.8) for further reactions.

Deacetylations of acetyl-H3 proteins by Sirt1, Sirt2, Sirt6, and sir2tm

Sirt1 and Sirt2 were expressed as described in one of our recent publications.[1] Sir2Tm was produced according to literature.[2] H3 mutant samples (6.4 uM) were mixed with sfGFP (1 mg/ml) as an inner standard to make the reaction stock solution (adjusted to 30 ul using arginine buffer). Then the mixtures were distributed evenly to six aliquots (5 ul), labeled as control, sir2tm, Sirt1, Sirt2, and Sirt6, respectively. All the reaction tubes were supplied with DTT (1 mM), NAD^+ (1 mM) and the corresponding sirtuins (400 nM) except the control. Water was added to the reaction tubes to make the final volume to be 20 μL. The deacylation reactions were performed at 37 °C overnight. The reaction mixtures were directly subjected to SDS-PAGE gel and transferred to nitrocellulose membrane with standard semi dry western blot protocol (Bio-Rad Trans-Blot Turbo transfer system). The membrane was coated with 5 % fat-free milk (10 mL) for 2 h at room temperature and then treated with pan-acetylation antibody from PTM bio-lab (#PTM-101) overnight at 4 °C (1:2000, 5 mL). The membrane then was washed by PBST buffer (PBS with 0.1 % tween-20, 10 mL) on the shaker six times with 10 min intervals. Then the membrane was treated with secondary antibody (1: 10000, 5 mL) from Jackson ImmunoResearch (West Grove, PA) at room temperature for 1 h. The membrane then was washed by PBST buffer (PBS with 0.1% tween-20, 10 mL) on the shaker three times with 10 min intervals. And then the result was visualized with Pierce ECL Western Blotting Substrate (#32106). Images were taken by ChemiDoc XRS+ system from Bio-Rad.

Synthesis of N$^{\varepsilon}$-7-octenoyl-L-Lysine 1 (OcK) and N$^{\varepsilon}$-9-decenoyl-L-Lysine (DeK) 2



Boc-Lys-OMe was synthesized based on our previously reported method. [3] Boc-Lys-OMe (3.43 g, 13.2 mmol) was dissolved in 40 ml anhydrous CH_2Cl_2, and the solution was injected into a round bottom flask containing 7-octenoic acid (0.94 g, 6.6 mmol) or 9-decenoic acid(1.13 g, 6.6 mmol). EDC (3.26 g, 17 mmol) and DMAP (64.8 mg, 0.53 mmol) were dissolved in 10 ml anhydrous CH_2Cl_2, and injected into reaction flask. The mixture was stirred at room temperature overnight under nitrogen gas flow. And the resulting 7-octenoyl and 9-decenoyl Boc-Lys-OMe were purified by silica gel column chromatography.

7-octenoyl and 9-decenoyl Boc-Lys-OMe were then dissolved in 8 ml THF. 2M NaOH aqueous solution (20 ml) was added dropwise over 30 minutes into the reaction flasks cooled by ice-water bath. To complete the reaction, the mixtures were stirred in ice-water bath for 1 hour and for another two hours at room temperature. Organic impurities were firstly washed away with ethyl ether (40 ml twice), then pH of the aqueous phase was adjusted to 3 by adding 3M HCl. Neutralized products were extracted with ethyl acetate (20 ml twice), and washed with water (40 ml twice) and dried by anhydrous $MgSO_4$. Dried solutions were concentrated to around 5 ml by evaporator in vacuo, into which 5 ml of 4 M HCl dioxane solution was added. The resulting suspensions were filter through bucker funnel, and the precipitated products were washed with dry acetone and collected as white solids, Koc (1.87 g 93 % yield for two steps) ^{1}H NMR (300 MHz, d6-DMSO) δ 8.43 (s, 3H), δ 7.84 (t, J=4.5 Hz, 1H), δ 5.68-5.83 (m, 1H), δ 4.87-5.01 (m, 2H), δ 3.8 (s, 1H), δ 2.98 (q, J=6 Hz, 2H), δ 1.92-2.05 (m, 4H), δ 1.71-1.81 (m, 2H), δ 1.14-1.51 (m, 10H); ^{13}C NMR (75 MHz, d-DMSO):172.36, 171.40, 139.20, 115.13, 52.23, 38.38, 35.79, 33.61, 29.99, 29.01, 28.62, 28.46, 26.68, 22.15). N$^{\varepsilon}$-9-decenoyl-L-Lysine **2** ^{1}H NMR (300 MHz, d6-DMSO) δ 8.46 (s, 3H), δ 7.86 (t, J=4.5 Hz, 1H), δ 5.67-5.83 (m, 1H), δ 4.84-5.00 (m, 2H), δ 3.78 (s, 1H), δ 2.97 (q, J=6 Hz, 2H), δ 1.90-2.05 (m, 4H), δ 1.70-1.81 (m, 2H), δ 1.12-1.49 (m, 14H); ^{13}C NMR (75 MHz, d-DMSO): 172,42, 171.35, 139.24, 115.07, 52.23, 38.38, 35.83, 33.60, 29.97, 29.09, 29.04, 28.99, 28.85, 28.67, 25.73, 22.15. (1.97 g, 90 % yield for two steps.)

Screening against mmPylRS mutants for OcK incorporation

Library with active-site mutations of the *Methanosarcina mazei* PylRS gene was constructed by site mutagenesis PCR which has randomization at active site residues (Y306NNK, L309NNK, C348NNK, Y/F/W384). NNK (N=A or C or G or T, K=G or T). The

following pairs of primers were used to generate the mmPylRS gene library, pBK-mmPylRS-348NNK-F, ACC ATG CTG AAC TTC NNK CAG ATG GGA TCG GGA TGC ACA CGG, pBK-mmPylRS-348NNK-R, AAA CTC TTC GAG GTG TTC TTT GCC GTC GGA CTC; pBK-mmPylRS-306-309-NNK-F, CTT GCT CCA AAC CTT NNK AAC TAC NNK CGC AAG CTT GAC AGG GCC CTG CCT, pBK-mmPylRS-306-309-NNK-R, CAT GGG TCT CAG GCA GAA GTT CTT GTC AAC CCT. Positive selection: library DNAs were used to transforme TOP10 electrocompetent cells containing plasmid pY+ to yield a cell library greater than 1×10^9 cfu, ensuring complete coverage of the pRSL library. Cells were plated on minimal agar plates containing 12 μg/mL tetracycline (Tet), 25 μg/mL kanamycin (Kan), 102 μg/mL chloramphenicol (Cm) and 1 mM OcK. After incubation at 37 °C for 72 h, colonies on the plates were collected and pRSL plasmids were extracted. Negative selection: the extracted plasmids from the positive selection were used to transform TOP10 electrocompetent cells containing plasmid pY- and plated on LB agar plates containing 50 μg/mL Kan, 200 μg/mL ampicillin (Amp), 0.2 % arabinose with or w/o 1 mM OcK. After incubation at 37 °C for 16 h. Survived cells from plates w/o OcK were pooled to extract plasmids for further selections. The alternative selections were repeated. Final positive selected colonies grew on LB plates with 102 μg/mL Cm, 25 μg/mL Kan, 12 μg/mL Tet, and with or w/o 1 mM OcK.

Incoporation of OcK into K48 of Ubiquitin

pETDuet vector containing C-terminally His-tagged Ubiqtuin (K48 codon was mutated to amber stop codon TAG) was used to co-transform *E. coli* BL21(DE3) with a pEVOL vector containing mmOcKRS. Single colony was picked and inoculated in 2YT medium (100 ug/ml Ampicillin 34 ug/ml chloramphenicol). When OD reached to 0.6, 0.5 mM IPTG, 5 mM Nicotinamide, 0.2 % (w/v) L-arabinose and 1 mM OcK were added into cell culture. Cells were harvested 5 hours later (expression at 37 °C) after induction and sonicated in lysis buffer (50 mM sodium phosphate, 250 mM NaCl, 0.1 % triton X-100, pH 7.4). Ub-K48oc was purified by Ni-NTA affinity resin. (15 mg/L, concentration determined by BCA assay) Sample for ESI-MS analysis was prepared by dialyzing into 20 mM NH_4HCO_3 (4 °C, pH 7.0) buffer and dried to pellets using the Genevac EZ-Bio Evaporator System. Ub-K48oc and w.t. Ubiquitin were both incubated with 0.2 mM pyrimidine-tetrazine-FITC (1XPBS buffer, pH 7.4) for 4 hours at room temperature. 100 % (w/v) TCA were added into reaction buffer (1:4 v/v), and the resulting suspensions were centrifuged at 13000 g and 4 °C for 20 mins (Eppendorf AG minispin). And the resulting protein pellets were washed with 100 % acetone for two times to get rid of residual dye. 15 ul 8 M urea buffer (8 M Urea, 20 mM Tris, 500 mM NaCl, pH 8.0) was added to dissolve protein pellets and samples were subjected to SDS-page gel analysis, FITC signals were blotted using ChemiDoc XRS+ system from Bio-Rad, which were compared with Coomassie blue staining results.

Incorporation of OcK into Histone H3

pETDuet mutant His-TEV-H3-C110A vectors were used to co-transform *E. coli* BL21(DE3) strain with pEVOL-mmOcKRS. Single colony was picked and inoculated in 2YT medium (100 ug/ml Ampicillin, 34 ug/ml chloramphenicol) at 37 °C. When OD reached to 0.6, 0.5

mM IPTG, 5 mM Nicotinamide, 0.2 % (w/v) L-arabinose and 1 mM OcK were added into cell culture. Cells were harvested 10 hours later (expression at 37 °C) after induction and sonicated in lysis buffer (50 mM Tris, 500 mM NaCl, 0.1 % Triton-X100, pH 8.0). Inclusion bodies were collected and washed 3 times with lysis buffer and 3 times with pellet wash buffer (50 mM Tris, 500 mM NaCl, pH 8.0). 6 M guanidinium chloride buffer (6 M guanidinium chloride, 20 mM Tris, 250 mM NaCl, pH 8.0) was then added to dissolve inclusion bodies. Suspensions were then centrifuged. (11000 g, 40 min, 4 °C, Beckman Coulter Allegra X-15R Centrifuge) and supernatants were collected and run through Ni-NTA purification under denatured condition. Purified Histone H3 mutants were dialyzed into MilliQ water at room temperature and dried to pellets by the Genevac EZ-Bio evaporation system, (yield ranging from 5 mg/L to 19 mg/L) concentrations were determined by Pierce BCA protein assay kit).

Expression and purification of GST-Sirt6

Human Sirt6 fragment was cloned from pQE-80l-Sirt6 (addgene #13739) into pGEX4t-3 vector with an N-terminal GST-tag. And the plasmid was used to transform *E. coli* BL21(DE3) strain. Single colony was picked and inoculated in LB medium (100 ug/ml ampicillin) at 37 °C. When OD reached to 0.6, cell culture were cooled down in ice-water bath before 0.5 mM IPTG was added. Cell culture was further incubated at 16 °C for 20 hours. Cell pellet was then re-suspended in lysis buffer (25 mM Tris, 250 mM NaCl, 1 mM PMSF, 1 mg/ml lysozyme, 1 mM EGTA, 10 mM DTT, pH 7.5). After sonication, cell lysate was centrifuged (11000 g, 40 min, 4 °C, Beckman Coulter Allegra X-15R Centrifuge) and supernatant was collected and run through GST affinity resin at 4 °C. Elution was collected and dialyzed into loading buffer (25 mM Tris, 20 mM NaCl, 2.5 % glycerol, 1 mM DTT, pH 7.5), and purified by Q Sepharose FF column FPLC (Buffer A: 20 mM Tris, 2.5 % glycerol, 1 mM DTT, pH 8.0, Buffer B: 20 mM Tris, 2.5 % glycerol, 1 mM DTT, 1 M NaCl, pH 7.5). Purified Sirt6 was concentrated using Amicon Ultra-4 Centrifugal Filter Units and dialyzed into storage buffer (25 mM Tris-HCl, pH 8.0, 100 mM NaCl, 0.05 % Tween-20, 1 mM DTT and 20 % glycerol pH 8.0) and stored in -80 °C. (final concentration 15.3 uM, determined by Pierce BCA protein assay kit)

Expression of recombinant human histone H2A, H2B, H3 and His-SUMO-TEV-H4.

pETDuet-1 vectors containing Human His-TEV-H2A, His-TEV-H2B, His-TEV-H3-C110A or His-SUMO-TEV-H4 were used to transform *E. coli* BL21(DE3) strain. Single colonies were picked and inoculated in 2YT medium (100 ug/ml Ampicillin 34 ug/ml chloramphenicol) at 37℃. When OD reached to 0.6, 0.5 mM IPTG was added to induce histone expression. Remaining protein purification steps were the same with the purification of recombinant mutant Histone H3.

Assembly of oc-H3-H4 tetramers

Pellets of His-TEV-H3 mutants and Histone His-SUMO-TEV-H4 were re-dissolved in 6 M guanidinium chloride buffer (6 M guanidinium chloride, 20 mM Tris, 250 mM NaCl, pH 8.0).

Concentrations were determined by UV absorption at 280 nm (Biotek synergy H1 plate reader) and coefficients found on Expasy. 37.5 ug His-SUMO-TEV-H4 and 25 ug His-TEV-H3 (molar ratio=1:1) were added together and diluted using 6 M guanidinium chloride buffer so that total protein concentrations were adjusted to 2 ug/ul. His-SUMO-TEV-H4 and His-TEV-H3 mixtures were dialyzed sequentially at 4 °C in 2 M NaCl buffer (2 M NaCl, 10 mM Tris, 1 mM EDTA, Ph 7.5), 1 M NaCl (1 M NaCl, 10 mM Tris, 1 mM EDTA, pH 7.5) and 0.25 M NaCl buffer (250 mM NaCl, 10 mM Tris, 1 mM EDTA, pH 7.5). Suspensions were centrifuged (13000 g 10 min, Eppendorf AG minispin) at 4 °C, supernatants were collected. Concentrations of His-TEV-H3-His-SUMO-TEV-H4 tetramers were determined by UV absorption at 280 nm (Tetramers were denatured by adding Guanidinium Chloride solids). TEV protease was added into His-TEV-H3-His-SUMO-H4 tetramer solution at ratio of 1: 30 (TEV protease: Histones w/w). After incubation at 4 °C for 24 hours, TEV digestion was confirmed to be finished by SDS-PAGE electrophoresis.

Deacylation assay on oc-H3-H4 tetramers.

Oc-H3-H4 tetramer concentrations were adjusted to 0.8 uM by adding 0.25 M NaCl buffer (250 mM NaCl, 10 mM Tris, 1 mM EDTA, pH 7.5). NAD+ (1 mM), DTT (1 mM), GST-Sirt6 (0.4 uM) were also added to tetramer reaction solutions. For each control assay, all ingredients were the same except that 0.25 M NaCl buffer was added instead of GST-Sirt6. All tetramer reactions were incubated at 37 °C for 3 hours. 10 mM nicotinamide and 50 mM Tris at pH 8.0 were added to quench the reaction. 20 mM iodoacetate was added to reaction solutions and the solution was incubated at room temperature for additional half an hour to remove DTT, which may interfere with tetrazine-FITC labeling. Then 0.2 mM Pyrimidine-Tetrazine-FITC (in 5 mM DMSO stock solution) was added to tetramer solutions. The labeling reaction was done at room temperature for 8 hours. Then TCA precipitation was applied to get rid of unreacted Tetrazine dye. Protein pellets were re-dissolved in 8 M Urea buffer (8 M Urea, 20 mM Tris, 500 mM NaCl, pH 8.0). Deacylation was monitored by directly detect fluorescent H3 bands from SDS-PAGE gel (using Bio-Rad Chemidoc XRS+).

Assembly of oc-nucleosomes

His-TEV-H2A and His-TEV-H2B pellets were re-dissolved by 6 M guanidinium buffer. Concentrations were determined by UV absorption at 280 nm (Biotek synergy H1 plate reader) and coefficients found on Expasy. Assembly procedure of His-TEV-H2A and His-TEV-H2B dimer was the same with His-TEV-H3-His-SUMO-TEV-H4 tetramers except that total protein concentration was 4 ug/ul. Mutant H3-H4 tetramers (6.6 uM), H2A-H2B dimer (13.2 uM) and 601-147 bp DNA (6.6 uM) (PCR product of a pUC19-601 DNA vector) were added together. NaCl concentrations were adjusted to 2 M using 5 M NaCl solution. Nucleosome was assembled by serial dilution using TE buffer (10 mM Tris, 1mM EDTA, pH 7.5) based on NEB EpiMark Nucleosome assembly protocol (http://www.neb.com/protocols/2012/06/04/epimark-nucleosome-assembly-kit-e5350). Assembly efficiencies were monitored by 5 % TBE Native PAGE gel shift assay.

Deacylation assay on oc-nucleosomes

Concentrations of oc-nucleosomes were adjusted to 0.66 uM by adding 0.25 uM NaCl buffer, 1 mM NAD^+ and 1 mM DTT. GST-Sirt6 (0.33 uM) was also added to deacylation reaction while 0.25 M NaCl buffer was added to control. All nucleosome reactions were incubated at 37 °C for 3 hours. 10 mM Nicotinamide and 50 mM Tris at pH 8.0 were added to quench the reaction. 20 mM iodoacetate was added to reaction solutions and the solution was incubated at room temperature for additional half an hour. Then 0.2 mM Pyrimidine-Tetrazine-FITC (in 5 mM DMSO stock solution) was added to nucleosome solutions. Labeling reactions lasted for 8 hours at room temperature. All reaction solutions were directly loaded onto 8 % TBE native PAGE gel. Fluorescence strength of separated nucleosome bands were directly blotted using Bio-Rad Chemidoc XRS+. EtBr staining was applied after FITC blot to reveal the total amount of acylated and non-acylated nucleosome.

Time-based deacylation assay on H3K4oc-, H3K9oc-, H3K18oc-, H3K23oc- and H3K27oc-nucleosomes.

Oc-nucleosome reaction solutions were prepared as the following recipe (0.66 uM Nucleosome, 0.33 uM GST-Sirt6, 1 mM NAD^+, 1 mM DTT, pH 7.5) and equally distributed to 4 tubes labeled as 0 hour, 0.5 hour, 1 hour, 2 hour. And the reactions were quenched at these different time-points by adding 10 mM nicotinamide, 50 mM Tris (pH 8.0) and 20 mM iodoacetate. After incubation at rt for 30 min, 0.2 mM Pyrimidine-Tetrazine-FITC (in 5 mM DMSO stock solution) was added to nucleosome solutions to label all 4 reactions stopped at different times. Fluorescence strength of separated nucleosome bands were directly blotted using Bio-Rad Chemidoc XRS+. EtBr staining was done after FITC blot to reveal the total amount of acylated and non-acylated nucleosome. Signal strengths were quantified using Bio-Rad Image Lab software. Ratios of strengths between FITC signal/EtBr signals were plotted against reaction time.

Sirt6 Deacetylation assay on H3K9ac-, H3K18ac-, and H3K27ac-nucleosomes.

Ac-nucleosomes were assembled as the same protocol for oc-nucleosomes. Deacetylation solutions were prepared as the following recipe (0.66 uM nucleosome, 0.33 uM GST-Sirt6, 1 mM NAD^+, 1 mM DTT, pH 7.5). Reactions were quenched after 3 hours of incubation at 37 °C by adding 10 mM nicotinamide, and were displayed by 5 % native PAGE electrophoresis. Nucleosome assemblies were confirmed by EtBr staining and nucleosome bands were then directly transferred to PVDF membrane using Bio-Rad semi-dry transmembrane protocol (Bio-Rad Trans-Blot Turbo transfer system). The membrane was coated with 5 % fat-free milk (10 mL) for 1 hour at room temperature and then treated with pan-acetylation antibody from PTM bio-lab (#PTM-101) overnight at 4 °C (1:2000, 5 mL). The membrane then was washed by PBST buffer (PBS with 0.1 % tween-20, 10 mL) on the shaker six times with 10 min intervals. Then the membrane was treated with second antibody (1: 10000, 5 mL) from Jackson ImmunoResearch at room temperature for 1 h. The membrane then was washed by PBST buffer (PBS with 0.1 % tween-20, 10 mL) on the shaker three times with 10 min intervals. And then the result was visualized with Pierce ECL ultra-sensitive

Western Blotting Substrate (#37070). Images were taken by ChemiDoc XRS+ system from Bio-Rad.

Sirt6 overexpression in 293T cell line and overall deacetylation level detection

Human sirt6 fragment was cloned from PQE-80l-Sirt6 (addgene #13739) into pEGFP vector. 8 ug pEGFP-sirt6 or pEGFP empty vector were added to 60mm 293T cell culture dishes and packed into cell by 20 ul lipofectamine 2000 (Thermo Fisher Scientific) reagent. After 12 h incubation, cell medium were changed, and nuclear extractions were obtained after 48 h according to previous protocol.[4] 10 ug of nuclear proteins were displayed by SDS-PAGE electrophoresis and blotted by site-specific anti-H3K9ac, anti-H3K18ac, anti-H3K27ac, anti-wtH3 (Acetyl-Histone H3 Antibody Sampler Kit #9927 Cell Signaling Technology, Inc.) and anti-Sirt6 antibodies (#A302-452A-T, Bethyl Labotaries).

References

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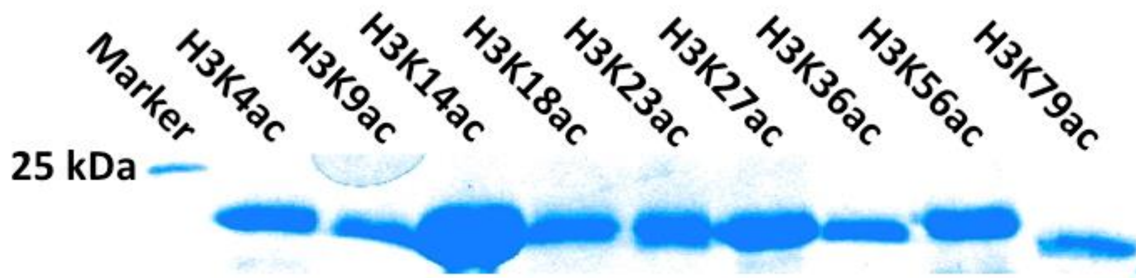
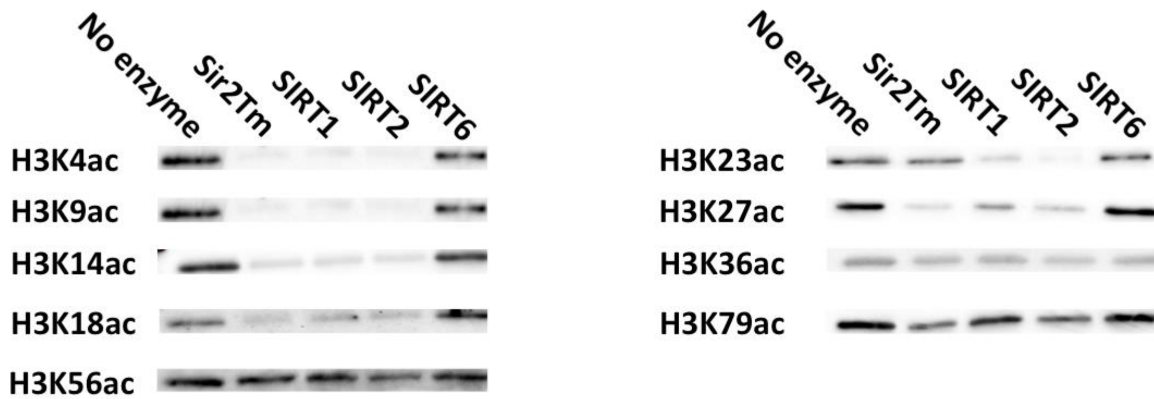
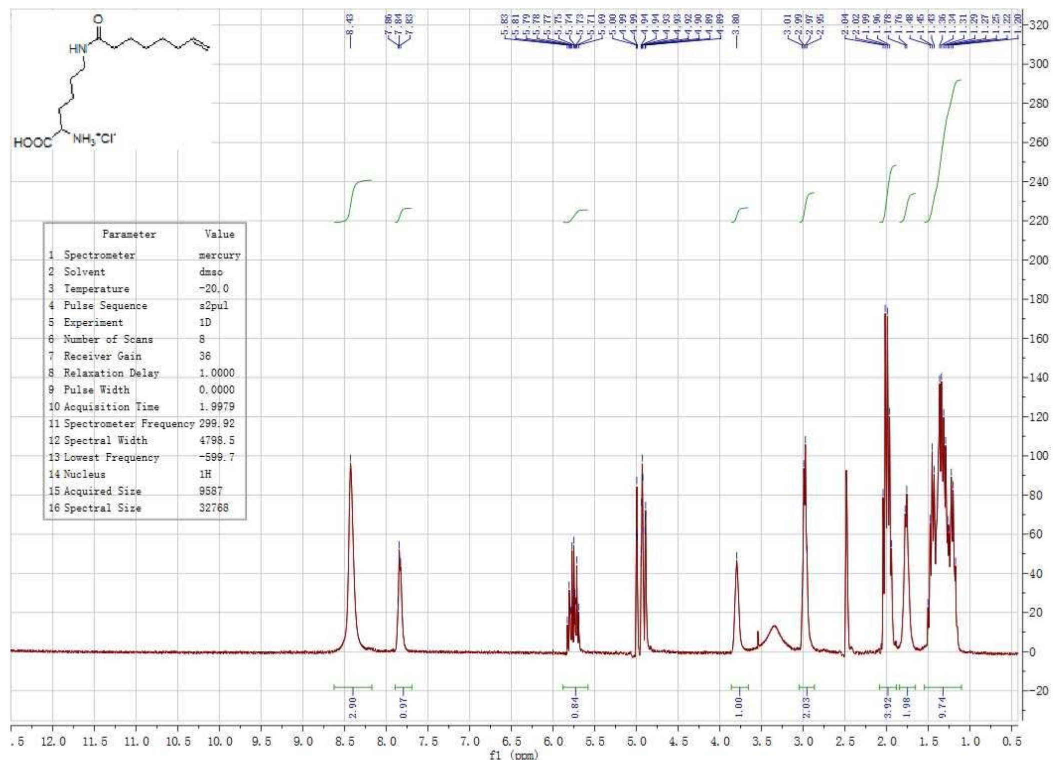
Supplementary Figure 1: SDS-PAGE analysis of purified acetyl-H3 proteins.

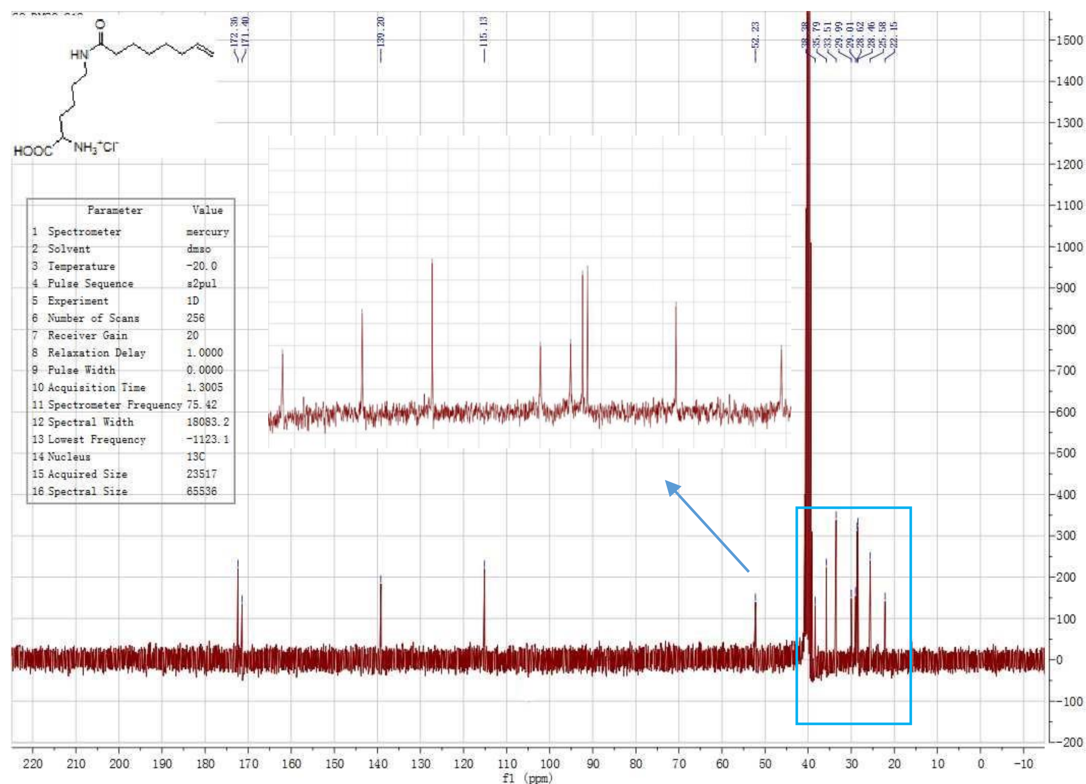


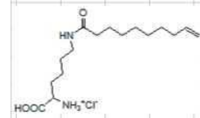
Supplementary Figure 2: Activities of sirtuins on recombinantly synthetized acetyl-H3 proteins. Please see the supplementary method section for conditions. The gels were probed by the pan anti-Kac antibody from PTM BioLabs that apparently doesn't recognize H3K36ac.



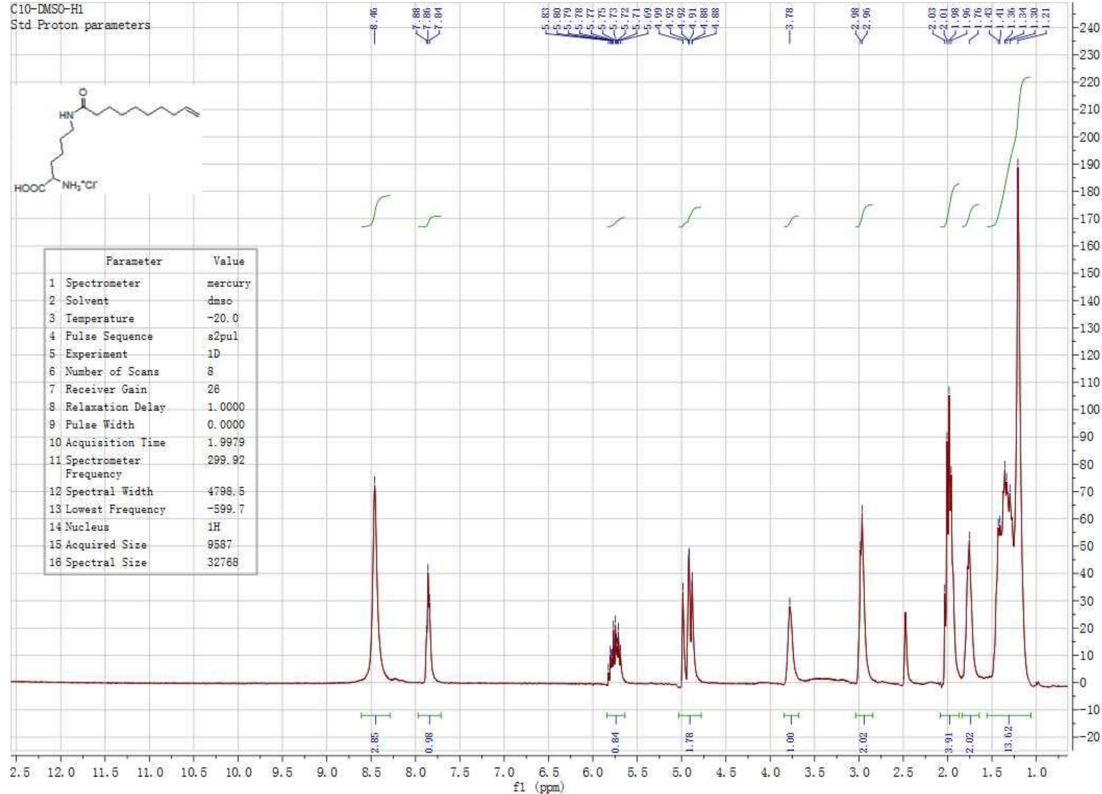
Supplementary Figure 3: 1H and 13C NMR of *N*ε-(7-octenoyl)-lysine (OcK) and *N*ε-(9-decenoyl)-lysine (DeK)







HN
O
HOOC
$NH_3^+Cl^-$



C10-DMSO-H1
Std Proton parameters
8.46
7.88
7.86
7.84
5.83
5.80
5.79
5.78
5.77
5.75
5.73
5.72
5.71
5.69
4.99
4.92
4.91
4.88
4.88
3.78
2.98
2.96
2.03
2.01
1.98
1.96
1.76
1.43
1.41
1.36
1.34
1.30
1.21
Parameter
Value
1 Spectrometer
mercury
2 Solvent
dmso
3 Temperature
-20.0
4 Pulse Sequence
s2pul
5 Experiment
1D
6 Number of Scans
8
7 Receiver Gain
26
8 Relaxation Delay
1.0000
9 Pulse Width
0.0000
10 Acquisition Time
1.9979
11 Spectrometer Frequency
299.92
12 Spectral Width
4798.5
13 Lowest Frequency
-599.7
14 Nucleus
1H
15 Acquired Size
9587
16 Spectral Size
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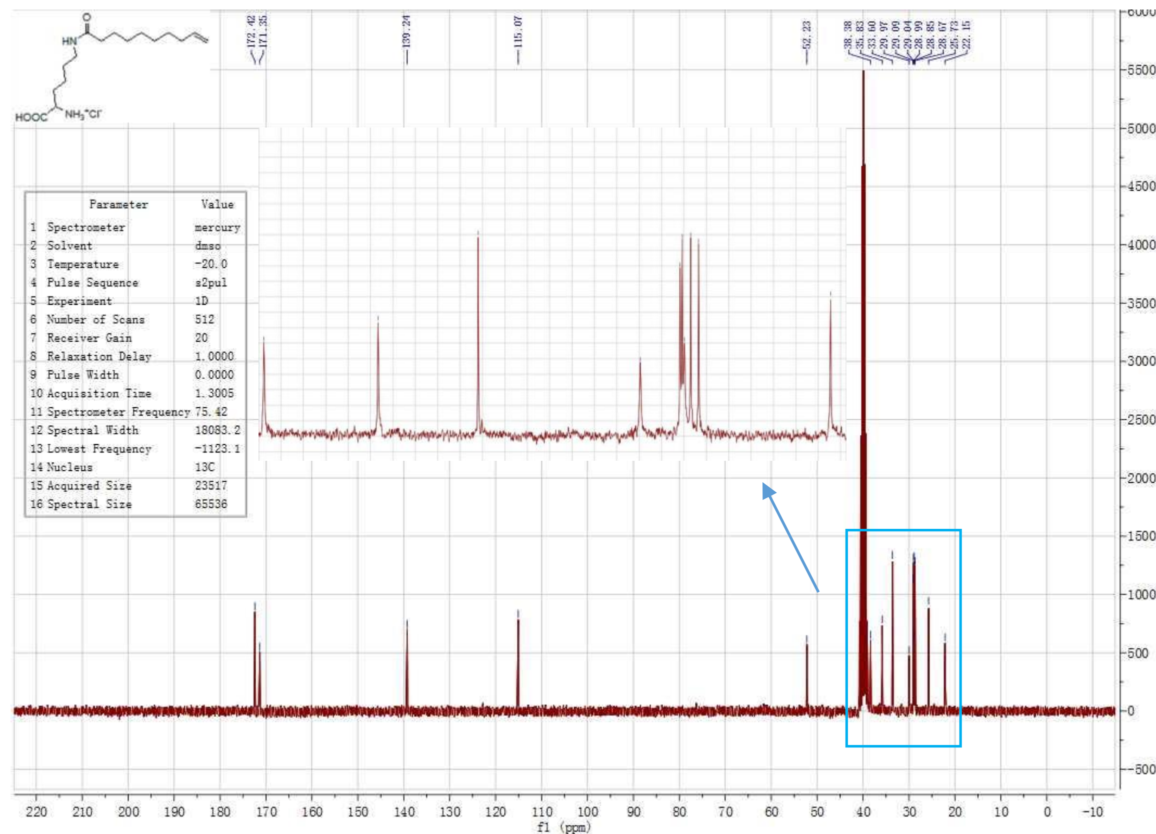
0.98
0.84
1.78
1.00
2.02
3.91
2.02
13.62
f1 (ppm)



HN
O
HOOC
$NH_3^+Cl^-$
172.42
171.35
139.24
115.07
52.23
38.38
35.83
33.60
29.97
29.09
29.04
28.99
28.85
28.67
25.73
22.15
Parameter
Value
1 Spectrometer
mercury
2 Solvent
dmso
3 Temperature
-20.0
4 Pulse Sequence
s2pul
5 Experiment
1D
6 Number of Scans
512
7 Receiver Gain
20
8 Relaxation Delay
1.0000
9 Pulse Width
0.0000
10 Acquisition Time
1.3005
11 Spectrometer Frequency
75.42
12 Spectral Width
18083.2
13 Lowest Frequency
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13C
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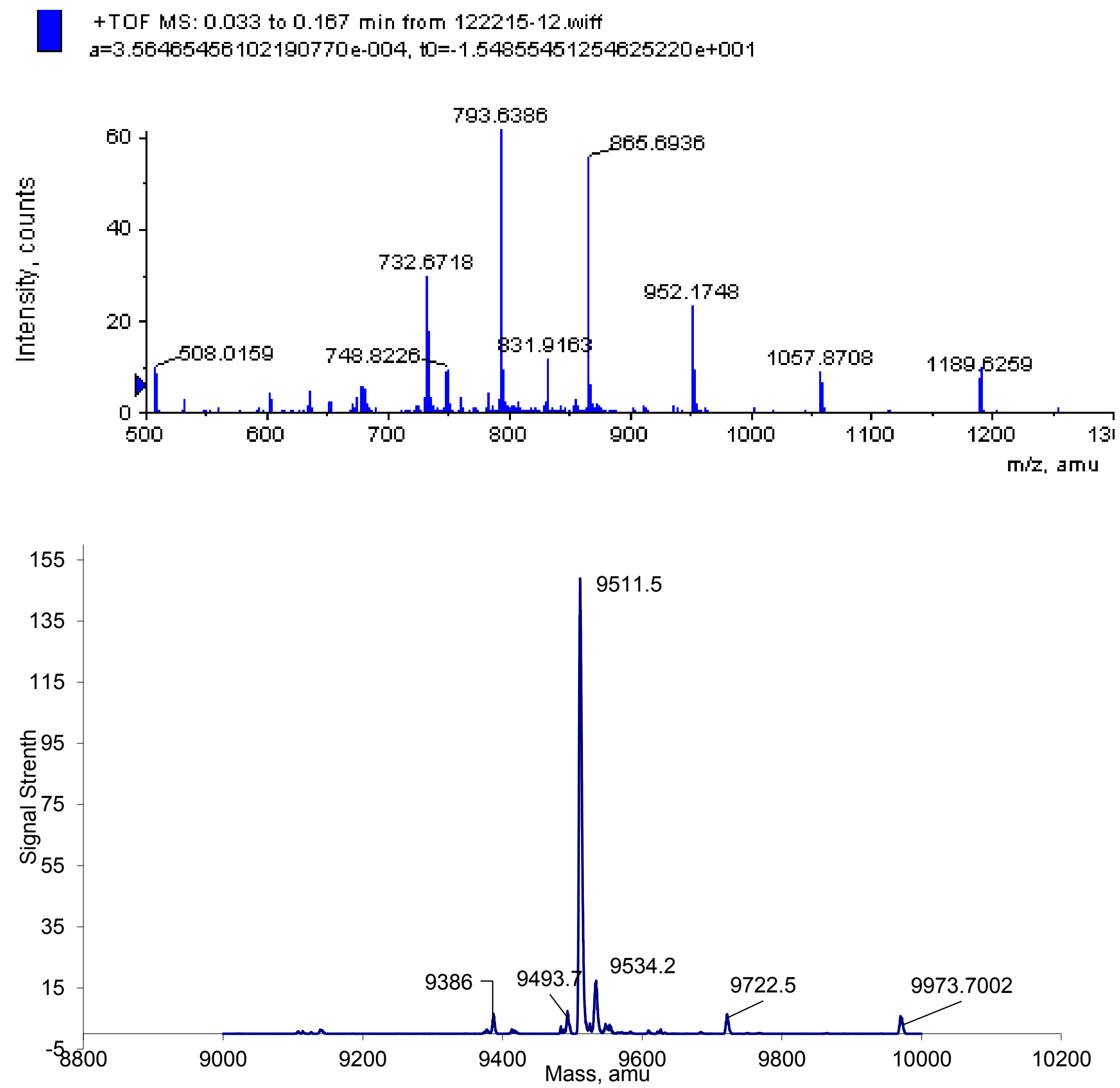
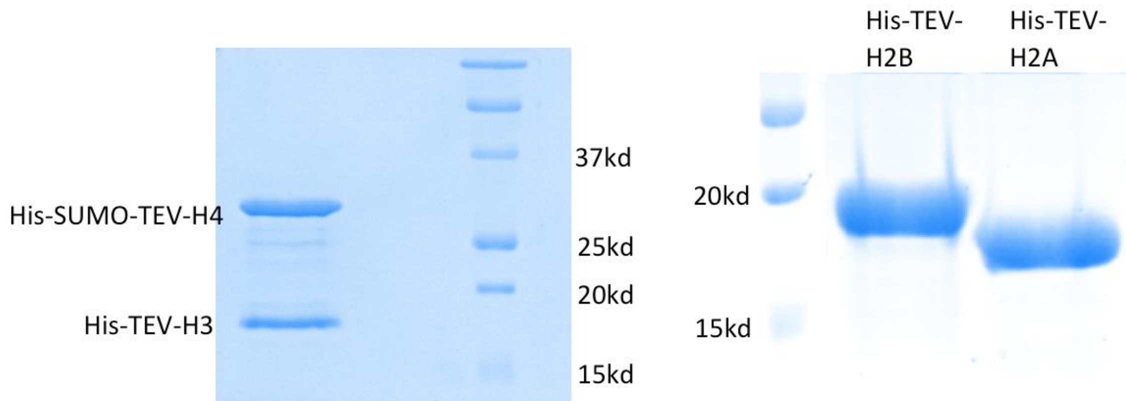
f1 (ppm)

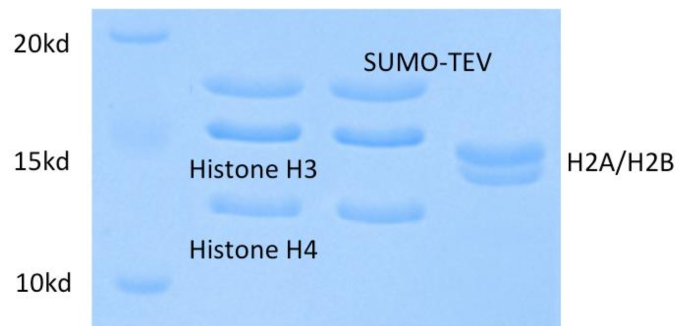
Supplementary Figure 4: ESI-MS of Ub-K48oc



Supplementary Figure 5: *In vitro* reconstitution of Histone H3/H4 tetramers and H2A/H2B dimers

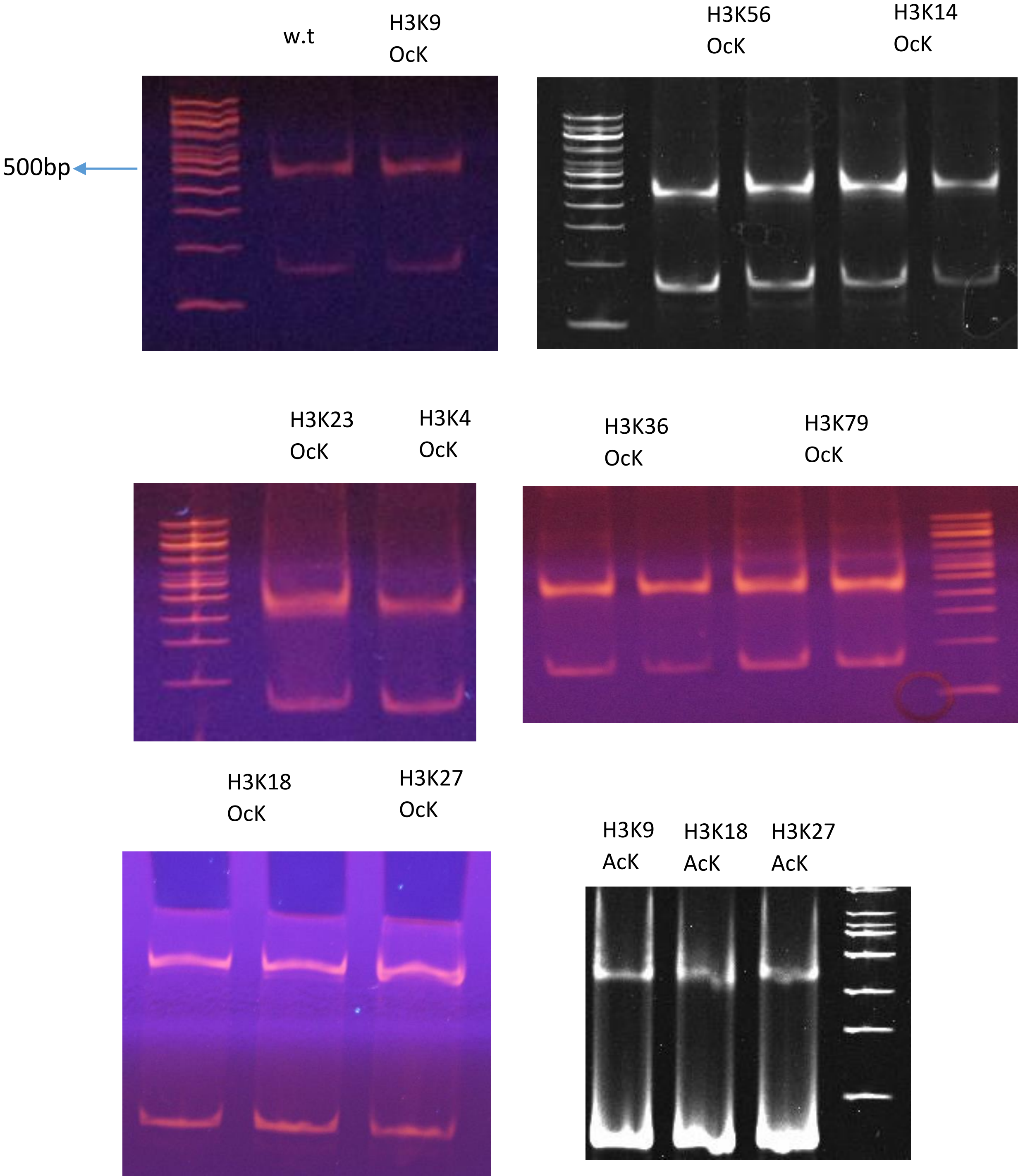


Before TEV protease digestion



After TEV protease digestion

Supplementary Figure 6: *In vitro* reconstitution of nucleosomes



Supplementary Figure 7: Comparative analysis of acetylation levels for four H3 lysine sites between Sirt6-transfected and control 293T cells.