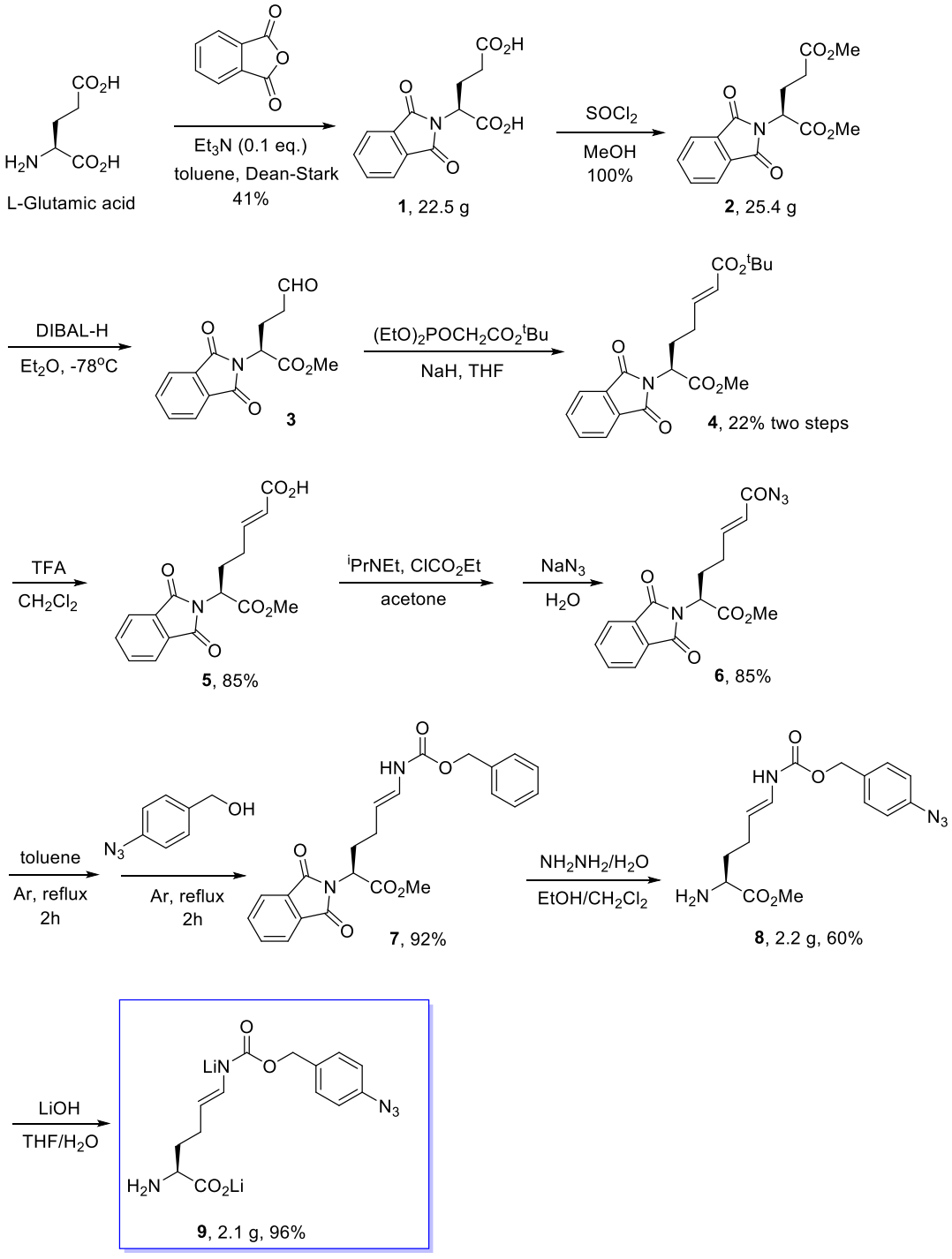
Supplementary Methods

All chemicals were either purchased from Sigma-Aldrich or provided by VMR. Pan anti-Kme2, anti-p53, anti-p53-K372me, anti-p53-K120ac, anti-H3K4Me2, and anti-H3 were purchased from Abcam. Pan anti-Kme2/Kme antibodies were purchased separately from Abcam and PTM Biolabs.

1. The synthesis of AcdK



To a 500 mL round bottom flask was added L-glutamic acid (30 g, 0.2 mol), phthalic anhydride (29.6 g, 0.2 mol), triethylamine (2.79 mL, 0.02 mol) and toluene (250 mL). The mixture was heated to reflux and the water formed in the reaction was collected using a Dean-Stark apparatus. After 24 h, the reaction was cooled to room temperature and solvent was removed under reduced pressure. EtOAc was added to dilute the reaction and pH was adjusted to 2 with HCl (3.0 M). Then the mixture was extracted with EtOAc (300 mL × 3) and the organic layers were dried and concentrated. The resulting solid was washed with EtOAc and then filtered to collect the solid. Drying the solid under high vacuum afforded the N-protected L-Glutamic acid **1** (22.5 g, 41% yield).

1 (22.5 g, 0.081 mol) was dissolved in MeOH (200 mL) and cooled to 0 ^{o}C. $SOCl_2$ (23.5 mL, 4 e.q.) was added dropwise. The mixture was warmed to room temperature naturally and stirred overnight. All the volatiles were removed under reduced pressure and then diluted with EtOAc. The organic layer was washed with aqueous $NaHCO_3$, dried, and concentrated to give methyl ester **2** (25.4 g, 100%).

2 (25.4 g, 0.083 mol) was dissolved in anhydrous diethyl ether (300 mL), cooled to -78^{o}C. DIBAL-H (99.8 mL, 1.0 M in hexane, 1.2 eq.) was added dropwise under argon protection. Stirring continued for another 1 h. Water (10 mL) was added. The reaction was warmed to room temperature and continually stirred for 1 h. The precipitate was removed with Celite and the filtrate was concentrated to produce the crude aldehyde **3**, which was used directly to the next step without further purification.

Anhydrous THF (80 mL) was added to a round bottom flask, and cooled to 0 ^{o}C. NaH (1.9 g, 60% dispersion in mineral oil, 1.2 eq.) was added carefully. Then tert-butyl 2-(diethoxyphosphoryl)acetate (11.99 g, 1.2 eq.) was added dropwise. After bubbles ceased to release, **3** (~ 0.0396 mol) in THF (20 mL) was added and the mixture was warmed to room temperature. After 2 h, NH_4Cl aqueous was added to quench the reaction. After diethyl ether extraction, the organic phase was dried and concentrated. The residue was purified via column chromatography with hexane/EtOAc (3:1 v/v) as eluent to give product **4** (6.9 g, 22% yield, two steps)

4 (6.9 g, 0.0184788 mol) was dissolved in CH_2Cl_2 (70 mL), cooled to 0 ^{o}C. Trifluoroacetic acid (14.15 mL, 10 eq.) was added dropwise. Then the solution was warmed to room temperature and stirred for 1.5 h. The reaction was diluted with CH_2Cl_2, washed with H_2O, then dried and concentrated. The residue was purified via column chromatography with hexane/EtOAc (1:1 v/v) as eluent to afford pure acid **5** (6.1 g, 85% yield).

5 (5.0 g, 0.015758 mol) was dissolved in acetone (150 mL), cooled to 0 ^{o}C and then added diisopropylethylamine (6.587 mL, 2.4 eq.). Ethyl chloroformate (3.3 mL, 2.2 eq.) was added dropwise and the solution was stirred at 0 ^{o}C for 1 h. Sodium azide (5.12 g, 5 eq.) in water (25 mL) was added and the reaction mixture was warmed to room temperature. After 1 h with stirring, brine was added and extracted with EtOAc. The organic layer was dried and concentrated. The residue was purified via column chromatography with hexane/EtOAc (3:1 v/v) as eluent to afford pure

acid **6** (4.6 g, 85% yield).

6 (4.6 g, 0.01344 mol) was dissolved in toluene, and heated to reflux for 2 h under argon protection. Then (2-azidophenyl)methanol (2 g, 1.0 eq.) was added and the mixture was continuously refluxed for another 2 h under argon protection. It was then cooled to room temperature. The solvent was removed under reduced pressure and the residue was purified via column chromatography with hexane/EtOAc (3:1 to 1:1 v/v) as eluent to afford product **7** (5.1 g, 92% yield).

7 (5.1 g, 0.011 mol) was dissolved in ethanol/dichloromethane (16 mL/25 mL) and hydrazine monohydrate (0.85 mL, 1.6 eq.) was added. The mixture was stirred at room temperature overnight. The white solid formed in the reaction was filtered away and the filtrate was concentrated. The residue was purified via column chromatography with methanol/ammonium hydroxide/dichloromethane (15:50:450 v/v/v) as eluent to afford amine **8** (2.2 g, 60% yield).

8 (2.2 g, 0.0066 mol) was dissolved in THF/H_2O (25 mL/12.5 mL) and LiOH (0.3168 g, 2 eq.) was added. After stirring for 1 h, the undissolved impurities were filtered away and the filtrate was concentrated under vacuum to afford amino acid **9** (2.1 g, 96% yield). ^{1}H NMR (CD_3OD, 300 MHz) 7.40 (d, 2H, *J* = 4.5 Hz), 7.07 (d, 2H, *J* = 4.5 Hz), 6.43 (dd, 1H, *J* = 0.9, 8.7 Hz), 5.12 (dd, 1H, *J* = 4.5, 8.7 Hz), 5.08 (s, 2H), 3.20 (dd, 1H, *J* = 0.9, 4.2 Hz), 2.12-2.07 (m, 2H), 1.76-1.73 (m, 1H), 1.60-1.56 (m, 1H). ^{13}C NMR (CD_3OD, 75 MHz) 181.4, 154.6, 139.8, 133.6, 129.3, 123.9, 118.6, 110.4, 65.5, 55.7, 36.0, 26.1.

The synthesis was further optimized to improve the overall yield. The finally optimized yields for each step was presented in **Figure 1C** in the main draft.

2. The PylRS mutant library construction

A *Methanosarcina mazei* PylRS (MmPylRS) gene library with codons for three active site residues Y306, L309, and C348 randomized as NNK nucleotides (N=A or C or G or T, K=G or T) and the codon for another active site residue Y384 randomized as codons for residues Y, F, and W were synthesized and cloned into a pBK vector that was originally developed by Schultz *et al*. Site-directed mutagenesis was first carried out to mutate Y384 MmPylRS to F and W in a PBK-MmPylRS plasmid that contains a wild type MmPylRS gene. The afforded two plasmids were equally mixed with the original plasmid to form the plasmid mix 1 that was used for the following mutagenesis to introduce random mutations at Y309, L309, and C348. The following pairs of primers were used to generate the mmPylRS gene library, including pBK-mmPylRS-348NNK-F (5'-ACC ATG CTG AAC TTC NNK CAG ATG GGA TCG GGA TGC ACA CGG-3'), pBK-mmPylRS-348NNK-R (5'-AAA CTC TTC GAG GTG TTC TTT GCC GTC GGA CTC-3'), pBK-mmPylRS-306-309-NNK-F (5'-CTT GCT CCA AAC CTT NNK AAC TAC NNK CGC AAG CTT GAC AGG GCC CTG CCT-3'), pBK-mmPylRS-306-309-NNK-R (5'-CAT GGG TCT CAG GCA GAA GTT CTT GTC AAC CCT-3'). Phusion DNA polymerase was used to PCR amplify the plasmid mix 1 with two primers pBK-mmPylRS-348NNK-F and pBK-mmPylRS-348NNK-R. The PCR product was phosphorylated by T4 polynucleotide kinase and then ligated by T4 ligase to form the plasmid mix 2. The plasmid mix 2 was used to transform Top10 cells and

amplied. It was then used to undergo Phusion DNA polymerase-catalyzed PCR amplification with primers pBK-mmPylRS-306-309-NNK-F and pBK-mmPylRS-306-309-NNK-R. The PCR product was then phosphorylated and ligated to afford the plasmid mix 3. This plasmid mix was then amplified in Top10 cells to give the final mutant pBK-MmPylRS library that was further used for the selection of AcdKRS. The parent vector of pBK-MmPylRS has a kanamycin (Kan) selection marker.

3. The selection of AcdKRS

A double sieved selection that has been generally used for the identification of mutant aminoacyl-tRNA synthetases specific for noncanonical amino acids in *E. coli* was employed. It is composed of three steps of positive selections staggered with two steps of negative selections between positive selections. For positive selections, the mutant pBK-MmPylRS library was used to transform TOP10 electrocompetent cells that contained the positive selection plasmid pY+ to yield a cell library greater than 1×10^9 cfu. pY+ was previously constructed that contains a gene coding $tRNA^{Pyl}$, a chloramphenicol (Cm) acetyltransferase gene with an amber mutation at its D112 position, a T7 RNA polymerase gene with two amber mutations at positions 1 and 107, and a green fluorescent protein GFP_{UV} gene under control of a T7 promoter. It has a tetracycline (Tet) selection marker. The transformed cells were plated on GMML (1×M9 Salts, 1% glycerol, 300 uM Leucine, 2 mM $MgSO_4$, 0.1 mM $CaCl_2$, and 0.2% NaCl) agar plates that also contained 12 μg/mL Tet, 25 μg/mL Kan, 102 μg/mL Cm, and 1 mM AcdK. After incubation at 37 °C for 72 h, colonies on the plates were collected and survived pBK-MmPylRS plasmids were extracted. Negative selection: the extracted pBK-MmPylRS plasmids from the positive selection were used to transform TOP10 electrocompetent cells containing plasmid pY- and plated on LB agar plates that contained 50 μg/mL Kan, 200 μg/mL ampicillin (Amp), and 0.2 % arabinose. pY- was previously constructed with an Amp selection marker. It contains genes coding $tRNA^{Pyl}$ and barnase with two mutations at Q2 and D44. After incubation at 37 °C for 16 h, survived cells from plates were pooled to extract plasmids for further selections.

4. The selected AcdK-specific clones and the construction of pEVOL-AcdKRS

Clones that can selectively charge $tRNA^{Pyl}$ were confirmed as shown in **Supplementary Figure 1**. They converged to be two clones L309T/D348G/Y384F and L309A/D348N/Y384F. The dominant clone is L309G/D348V/Y384F. The clone L309T/D348G/Y384F was PCR amplified and cloned into SpeI and SalI sites of the pEVOL vector to afford pEVOL-AcdKRS.

5. The expression of sfGFP-D134AcdK

The pBAD-sfGFP-D134TAG plasmid was a kind gift from Ryan Mehl at Oregon State University. This plasmid together with pEVOL-AcdKRS was used to transform BL21(DE3). A single colony was then picked and allowed to grow in 5 mL LB medium with 100 mg/mL Amp and 34 mg/mL Cm at 37 °C overnight. A 0.5 ml aliquot from the overnight culture was used to inoculate 50 mL 2YT medium with 100 mg/mL Amp and 34 mg/mL Cm. After growth to OD_{600} around 0.5, 0.2%

Arabinose was added to induce sfGFP expression with or without 0.5 mM AcdK. The induced cells were allowed to grow at 37 °C for 6 h and then the cells were collected by centrifugation (6,000 rpm for 15 min). The collected cells were resuspended in a 25 mL lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH 8.0) and sonicated in an ice water bath three times (4 min each, with a 5 min interval between each run). The lysate were clarified by centrifugation (10,000 rpm for 30 min) and the supernatant was decanted into a tube containing 2 mL Ni-NTA superflow resins. After incubation at 4 °C for 1 h, the mixture was loaded into an empty Qiagen Ni-NTA superflow cartridge. The resin was washed with 2 mL of the lysis buffer for 5 times and then 20 mL of a wash buffer (50 mM NaH_2PO_4, 300 mM NaCl, 20 mM imidazole, pH 8.0). The bound protein was finally eluted with an elution buffer (50 mM NaH_2PO_4, 300 mM NaCl, 250 mM imidazole, pH 8.0). The eluted protein was then concentrated and analyzed by SDS-PAGE. To analyze the purified protein by ESI-MS, the buffer of the purified protein was changed to 5 mM NH_4HCO_3 using an Amicon Ultra-15 Centrifugal Filter Devices (10,000 MWCO cut, Millopore). Protein concentrations were determined by the BCA method.

6. The synthesis of sfGFP-D134Km2 and sfGFP-D134Kme

The Purified sfGFP-D134AcdK were reduced with 5mM TCEP in PBS buffer (pH 7) at room temperature for 2 h, then undertook reductive amination with 100 mM methylamine or dimethylamine and 10 mM $NaCNBH_3$ for 8 h to generate sfGFP-D134Km2 and sfGFP-D134Kme. The final protein was purified with Ni-NTA resins to remove small molecule chemicals. The eluted protein was dialyzed against a 5 mM NH_4HCO_3 buffer and directly analyzed by ESI-MS.

7. Electrospray ionization mass spectrometry analysis of sfGFP-D134AcdK, sfGFP-D134AlK, sfGFP-D134Km2 and sfGFP-D134Kme

The purified sfGFP-D134AcdK, the product sfGFP-D134AlK from Staudinger reduction, and the products from reductive amination reaction as sfGFP-D134Kme and sfGFP-D134Kme2 were dialyzed against 10 mM ammonium bicarbonate (ABC) buffer, followed by the evaporation with lyopholizor to get protein powder. 40 % ACN/water with 0.1 % formic acid was used to dissolve the samples, and then injected to ESI-MS (Q Exactive Plus, Thermo Scientific, bought in Jan. 2016). The deconvolution data was analyzed by Protein Deconvolution Software (ThermoScientific, version 3.0).

8. The expression of H3K4AcdK, H3K9AcdK, and H3K36AcdK and corresponsing synthesis of H3K4me2, H3K9me2, and H3K36me2

The expression and purification of H3K4AcdK, H3K9AcdK, and H3K36AcdK followed same procedures. Plasmids pETduet-H3K4TAG, pETduet-H3K9TAG, and pETduet-H3K36TAG were constructed previously. They were used separately to transform *E. coli* BL21(DE3) cells that also contained the plasmid pEVOL-AcdKRS. LB agar plates with 100 μg/mL Amp and 34 μg/mL Cm were made to plate the transformed cells. A single colony was picked and grown in a 5 mL LB medium with 100 μg/mL Amp and 34 μg/mL Cm. This overnight culture was used to inoculate 2YT medium (250 mL). Cells were let grow at 37 °C for 2.5 h until OD_{600} reached 0.6. The induction of

protein expression was initiated by the addition of 1 mM IPTG, 0.2 % arabinose and 1 mM AcdK. 8 h after induction, cells were collected (4000 rpm for 20 min), washed with a lysis buffer (20 mM Tris, 500 mM NaCl, 0.1% Triton X-100, and pH 7.5; 20 mL) twice, and completely resuspended in the lysis buffer (35 mL). The resuspended cells were sonicated under ice bath for 3 times (2 min each time with an interval of 10 min) and centrifuged under 6000 rpm for 20 min. The supernatant was removed. The precipitate (inclusion body) was resuspended in a 25 mL wash buffer (20 mM Tris, 500 mM NaCl, and pH 7.5) for 10 times, with removing supernatant by centrifugation at 6000 rpm for 20 min after each resuspension. The inclusion body was further dissolved in histone solubilization buffer (6 M urea, 20 mM Tris, 500 mM NaCl, pH 7.5). The solution was centrifuged at 10000 rpm for 30 min. The collected supernatant was run through a 10 mL GE Healthcare Ni-NTA Fast Flow column which was further washed with 50 mL of histone wash buffer (6 M urea, 20 mM Tris, 500 mM NaCl, 20 mM imidazole, pH 7.5). A 10 mL histone elution buffer (6 M urea, 20 mM Tris, 500 mM NaCl, and pH 7.5) was used to elute the bound protein. The eluate was collected, concentrated, and analyzed by SDS-PAGE.

The synthesis of H3K4me2, H3K9me2, and H3K36me2 followed a protocol similarly to the synthesis of sfGFP-D134Kme2. 5 mM TCEP was added to each protein solution first to reduce the the incorporated AcdK for 2 h and then 100 mM dimethyllysine and 10 mM $NaCNBH_3$ were added to undergo reductive amination for 8 h. The final synthesis proteins were analyzed by SDS-PAGE and probed by both anti-H3 and pan anti-Kme2 antibodies in western blotting. To synthesize H3K4me and H3K9me, H3K4AcdK and H3K9AcdK were reduced with the addition of 5 mM TCEP first for 2 h and then 100 mM methylamine and 10 mM $NaCNBH_3$ were added for reductive amination for 8 h. The finally synthesized proteins were analyzed by SDS-PAGE and probed by anti-H3 and pan anti-Kme2/Kme antibodies in western blotting.

9. Tandem mass spectrometry analysis of GFP-N134Me2, H3K4Me2, H3K9Me2, and H3K36Me2

The site specific modifications to GFP (N134) and histone H3 (K4, K9, and K36) proteins were analyzed by LC-MS/MS. 10 μg of protein in 50mM Tris-HCl, 10mM $CaCl_2$ (pH 7.6) was denatured by incubating with 8 M urea at 37 °C for an hour, with 5 mM dithiothreitol (DTT) added for reduction of potential disulphide bonds. The sample was then diluted 8 folds in the same Tris buffer to a final urea concentration of 1 M. Except for the H3K4 modification sample which was digested by chymotrypsin (Promega, Madison, WI) at 25 °C, all other modified proteins were subjected to trypsin digestion by Mass Spectrometry Grade Trypsin Gold (Promega, Madison, WI) with 1:50 w/w at 37 °C for 18 hours. The digested peptides were loaded into a biphasic strong cation exchange/reversed phase capillary column with a pressure cell, followed by peptide separation in a C18 capillary column. The 2D back column was composed of 5cm of C18 reverse phase resin and 3 cm of strong cation exchange (SCX) resin. The back column was then connected to a 15-cm-long 100 um-ID C18 column and sprayed through a SilicaTip. Before SCX separation, a 1 h RP gradient from 100% Solvent A (95% H_2O, 5% acetonitrile, and 0.1% formic acid)

to 100% Solvent B (30% H_2O, 70% acetonitrile, and 0.1% formic acid) was configured to move peptides from C18 resin to SCX resin in the back column. The SCX LC separation was performed with five salt pulses containing increasing concentrations of ammonium acetate. Each salt pulse was followed by a 2 h reverse phase gradient from 100% Solvent A to 60% Solvent B. Peptides identities were analyzed in a LTQ ion trap mass spectrometer (Thermo Finnegan, San Jose, CA) over the range of 300-1700 m/z, and further subjected to collision induced dissociation (CID) fragmentation for MS/MS analysis.

The raw data of tandem mass spectra was first converted to MS2 file, followed by database searching in an in-house developed data processing pipeline based on ProLuCID (version 1.0) search algorism. Protein databases were generated including the target protein and 37 common contaminant proteins, as well as their reversed sequences to restrain false positive discovery to 0.01. Dimethylation modifications to N134 in GFP and K4/K9/K36 in H3 were included in the ProLuCID search by adding the mass increase, i.e, N134 (+42.1228) in GFP and K4/K9/K36 (+28.0532) in H3 proteins. DTASelect (v. 2.0) was applied to filter peptide/spectrum matches with the criteria of XCorr ≥ 1.8 for charge state +1, ≥ 2.5 for charge state +2, and ≥ 3.5 for charge state +3, and the DelCN ≥ 0.08.

10. The expression of LSD1.

The pET-15b-LSD1 plasmid was a gift from Professor Zigmund Luka at Vanderbilt University with the permission of using it from Professor Yang Shi at Harvard University. It contains a 6×His tagged LSD1 gene. For expression, *Escherichia coli* BL21(DE3) cells were transformed with pET-15b-LSD1. LSD1 expression and purification protocol was adopted from a previous publication with some modifications. The cells were grown overnight in 10 ml of LB Medium with 100 μg/mL Amp. Then this culture was inoculated in 1L 2YT medium with 100 μg/mL Amp for expression. When OD_{600} reached around 1.4-1.6, expression was induced by 0.5 mM IPTG. After the induction, expression was carried out at 25 °C for 7 h. After the expression, cells were spun down, washed once with cold Tris Buffer (100 mM, pH 7.7) and stored at -80 °C until further purification. To lyse cells, frozen cells were resuspended in 80 ml lysis buffer that contained 100 mM Tris (pH 7.5), 25 mM $MgCl_2$, 5 mM $CaCl_2$ 1 mM PMSF and 10 μg/mL DNaseI. After the sonication 14 mM β-mercaptoethanol was provided to lysis buffer. Then, the cell debris was pelleted at 10000 rpm for 40 min. Ammonium sulfate was then added to the supernatant to precipitate the expressed LSD1 (Ammonium sulfate precipitation was preferred over the Ni-NTA affinity purification because the low yield of the Ni-NTA method). The fraction between 26% and 36% saturation at 0 °C was collected. Then the pellet was re-dissolved in a buffer containing 20 mM Tris (pH 8.1), 50 mM NaCl, and 5 mM β-mercaptoethanol. During redissolving and protein concentration process precipitation of LSD1 was observed. After redissolving the pellet, solution was dialyzed against the redissolving buffer to remove remaining ammonium sulfate. FPLC purification was further carried out with Q Sepharose anion exchange column with a gradient from buffer A 20 mM Tris (pH 8.1), 50 mM NaCl, and 5 mM β-mercaptoethanol to buffer B with 20 mM Tris (pH 8.1), 1 M NaCl, and 5 mM β-

mercaptoethanol. Fractions around 20% of buffer B were collected to obtain purified LSD1.

11. LSD1 activities toward H3K4me2, H3K9me2, and H3K36me2

The H3K4me2 generated above was dialyzed against 4 L PBS buffer for 6 times, followed by the removal of precipitate. The supernatant was stored and directly used for the assay. To each 25 ul volume reaction, FAD was added to a final concentration of 10 μM, and LSD1 was provided to the final concentration of 5 μM. A control reaction was set up with the same amount of a H3 protein, FAD, but no LSD1 in a 25 μL volume. The assay was let to incubate at room temperature for 4 h, and was further analyzed by SDS-PAGE and then western blotting with anti-H3 and pan anti-Kme2 antibodies. Similar process was carried out for both H3K9me2 and H3K36me2.

12. The construction of pGEX-2T-GST-hp53-sfGFP-His, the expression of p53-K372AcdK, and the synthesis of p53-K372me2 and p53-K372me

The plasmid pGEX-2T-GST-hp53 was a kind gift from Dr. Wei Gu's group at Columbia Unviersity. Since it only gave μg expression, an sfGFP fusion with a 6×His tag for boosting up the overall p53 expression and easy separation was generated. Two primers (5'-ACG CGA GCT CGA GAA CCT GTA TTT CCA AGG TGC CAT G-3' and 5'-CCC AAG CTT TTA ATG GTG ATG ATG ATG GTG GCT GCC T-3' were used to amplify the sfGFP gene with a 6×His tag from pBAD-sfGFP that was a gift from Dr. Ryan Mehl's group at Oregon State University. Meanwhile, two primers (5'-GCC CAA GCT TCC GGG AAT TCA TCG TGA CTG AC-3' and 5'-ACT CGA GCT CGT CTG AGT CAG GCC CTT CTG TC-3') were used to PCR amplify the backbone of pGEX-2T-GST-hp53. Phusion DNA polymerase was used in both PCR reactions for maintaining low mutagenesis in the PCR products. Both PCR products were digested by HindIII and SacI and ligated together using T4 ligase. The constructed pGEX-2T-GST-hp53-sfGFP-His was sequenced confirmed and then used to undergo site-directed mutagenesis to introduce an amber mutation at K372 of p53. Two primers (5'-TAG AAG GGT CAG TCT ACC TCC CGC C-3' and 5'-GGA CTT CAG GTG GCT GGA GTG AG-3') were used to undergo PCR by Phusion DNA polymerase. The PCR product was phosphorylated and self ligated. The finally constructed pGEX-2T-GST-hp53(K372TAG)-sfGFP-His was sequenced confirmed. To express p53-K372AcdK, pGEX-2T-GST-hp53(K372TAG)-sfGFP-His and pEVOL-AcdKRS were used to cotransform *E. coli* BL21(DE3) cells. The transformed cells were grown in the 2YT medium. When OD_{600} reached 0.8, 0.2% arabinose, 1 mM IPTG, and 2 mM AcdK were provided to induce the expression of p53-K372AcdK. The expressed protein was purified by Ni-NTA and glutathione affinity resins sequentially. P53-K372AcdK was eluted from these two resins by 200 mM imidazole and 10 mM glutathione. The finally purified proteins were dialyzed against the PBS buffer (pH 7) to remove glutathione. After dialysis, it was then reacted with 5 mM TCEP for 2 h and then 100 mM dimethylamine in the presence of $NaCNBH_3$ for 8 h to generate p53-K372me2. For the synthesis of p53-K372me, dimethyllysine was simply replaced with methyllysine in the reductive amination step.

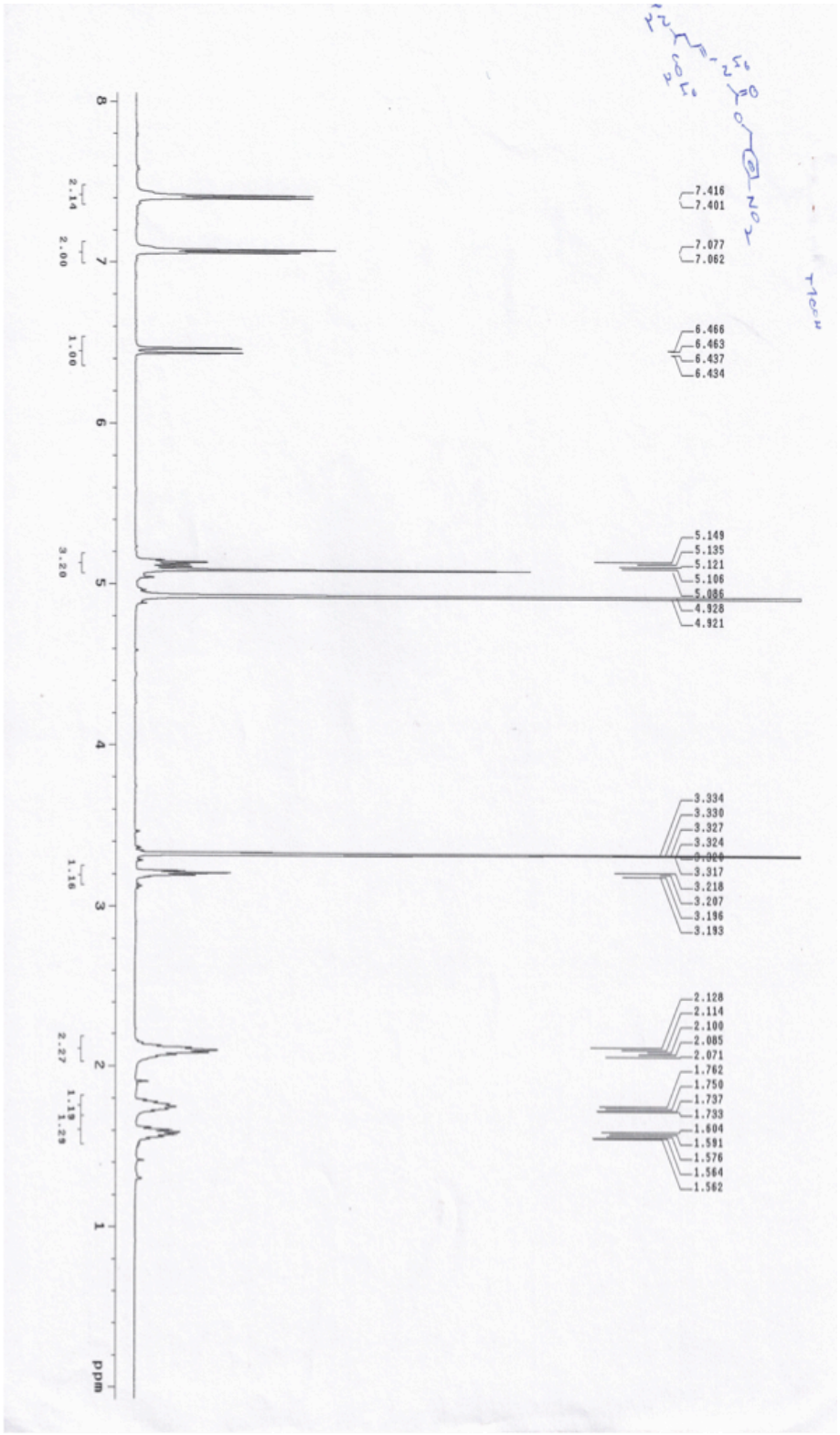
13. The expression of Tip60

The Tip60 expression plasmid pCIN4-FH-Tip60 in which Tip60 is fused with GST at its N-terminus was a kind gift from Dr. Wei Gu's group at Columbia University. It was used to transform *E. coli* BL21(DE3) cells. The transformed cells were grown at 37 degree in the LB medium to OD_{600} around 0.4-0.6. At this moment, the temperature was changed to 16 degree. 0.7 mM IPTG was then provided to induce the GST-Tip60 expression. After the induction, cells were continuously grown at 16 degree for 12 h. After expression, cells were collected and sonicated. The expressed GST-Tip60 was enriched in glutathione resins and eluted by 10 mM glutathione in 50 mM Tris buffer (pH 8).

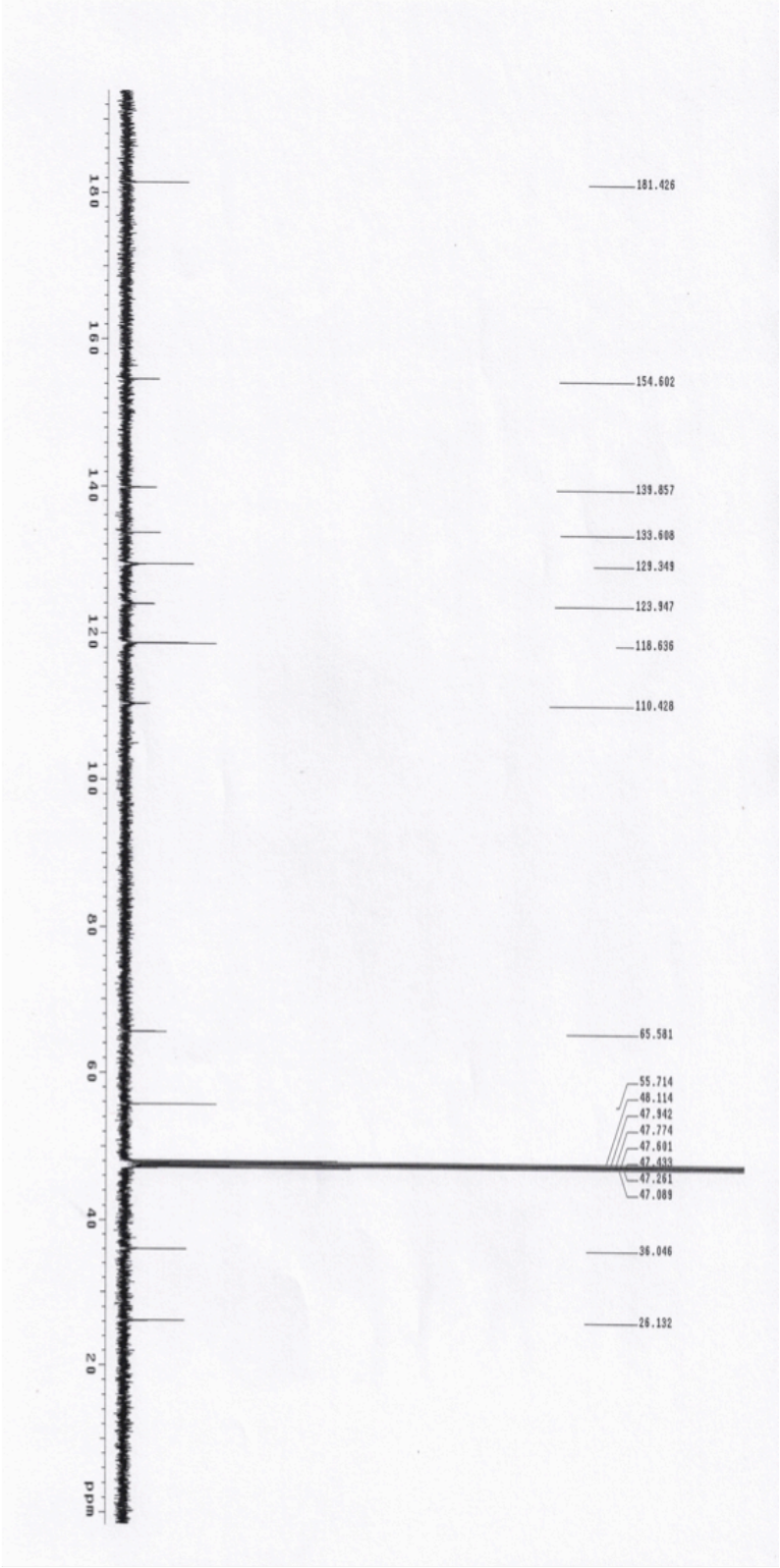
14. Tip60-catalyzed acetylation of p53 at K120

Purified p53 were reduced with 5mM TCEP in PBS buffer (pH 7) at room temperature for 2 h, then undertook reductive amination with 100 mM methylamine or dimethylamine and 10 mM $NaCNBH_3$ for 8 h. The final protein was purified with Ni-NTA resins to remove small molecule chemicals. Tip60 catalyzed acetylation was conducted in a 50 mM Tris (pH 8.0) buffered solution (20 μL) that contained 0.1 mM EDTA, 1 mM DTT, 330 nM TSA, 0.01% BSA, 0.01% Tween, and 0.1 mM acetyl-CoA for 2 h. The final concentration of Tip60 was 0.75 uM.

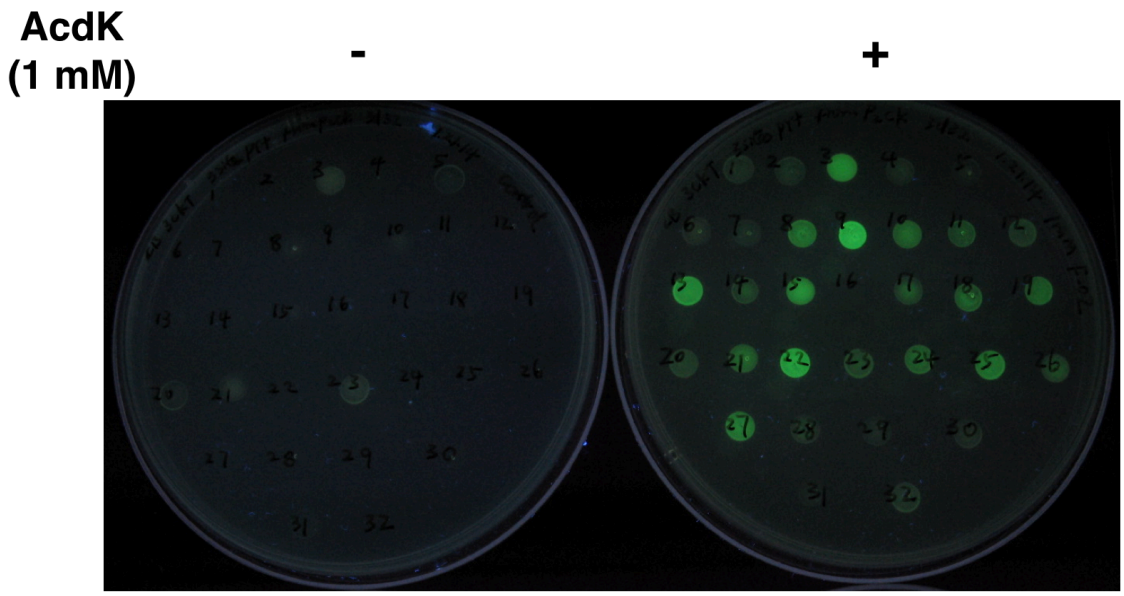
^{1}H NMR spectrum of compound 9



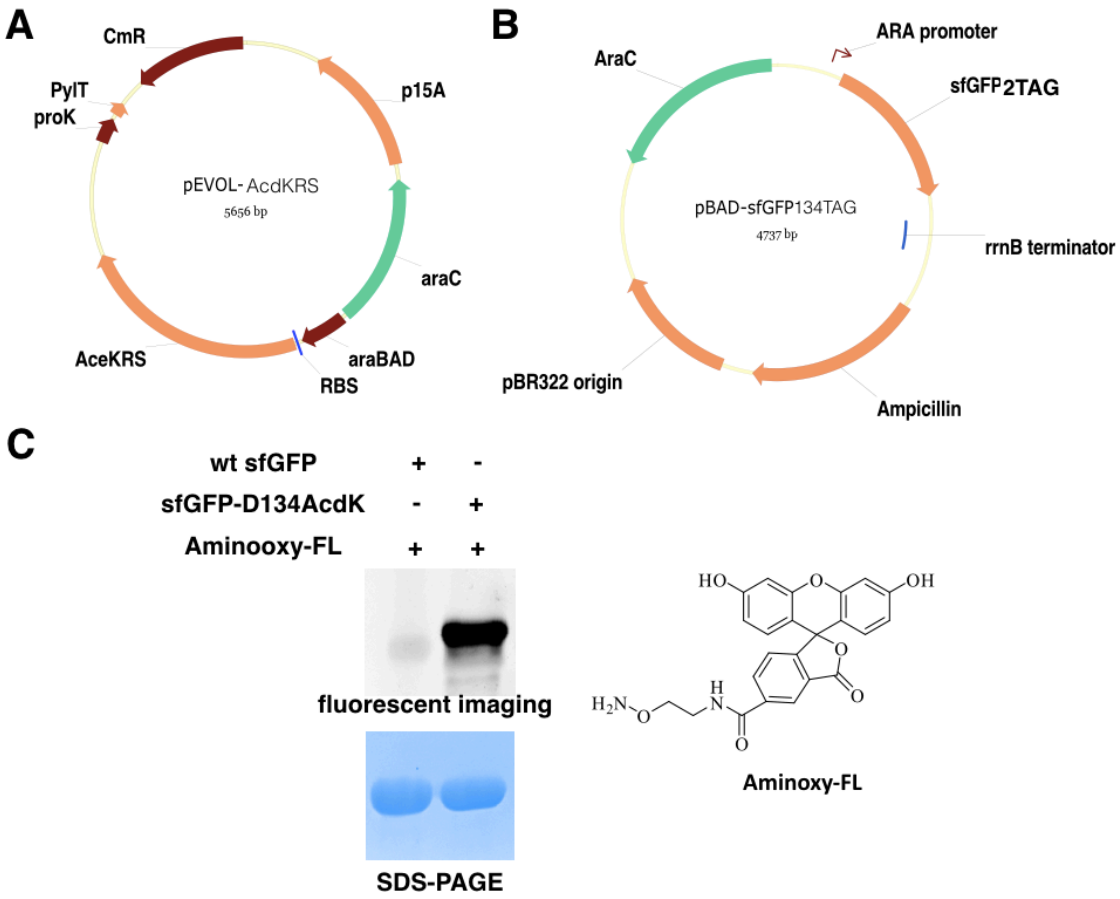
^{13}C NMR of compound 9



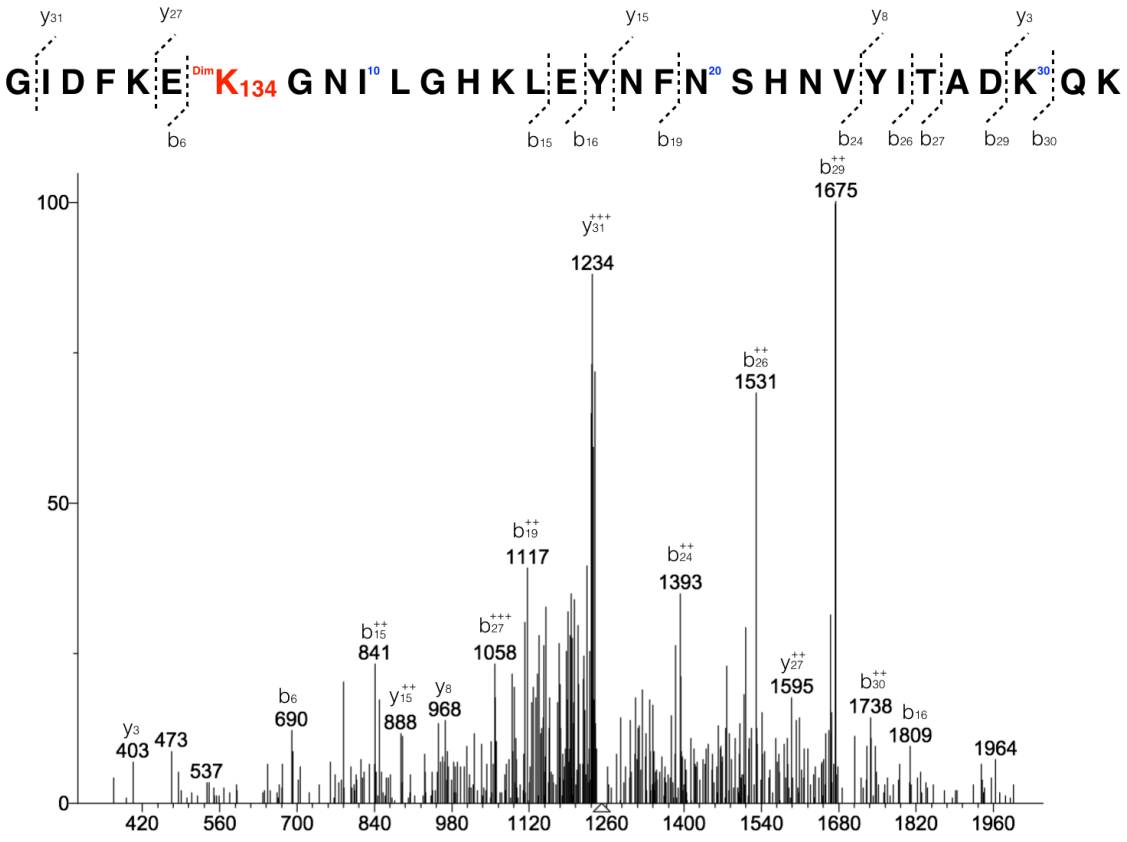
Supplementary Figures and their legends



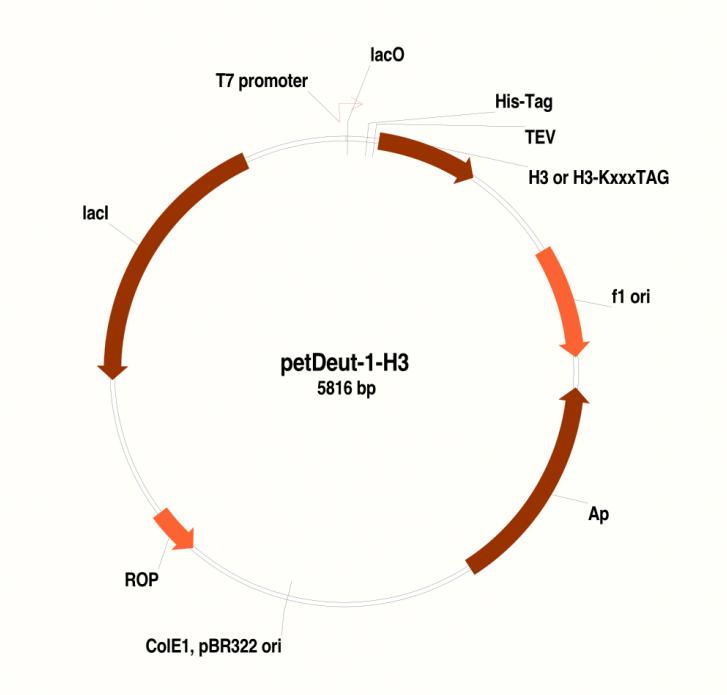
Supplementary Figure 1. Growth of selected clones from the third positive selection on LB plates supplemented without and with 1 mM AcdK. Both plates contained 102 μg/mL Cm, 25 μg/mL Kan, and 12 μg/mL Tet. All cells contained a pBK plasmid that contained a mutant PylRS gene and the pY+ plasmid that contained a $tRNA^{Pyl}$ gene, a chloramphenicol acetyltransferase gene with an amber mutation at D112, a GFP_{UV} gene under control of a T7 promoter, and a T7 RNA polymerase gene with two amber mutations at positions 1 and 107. The expression of GFP_{UV} was promoted by the suppression of two amber mutations in the T7 RNA polymerase. The fluorescent intensity of the expression of GFP_{UV} roughly represents the suppression efficiency at amber codons.



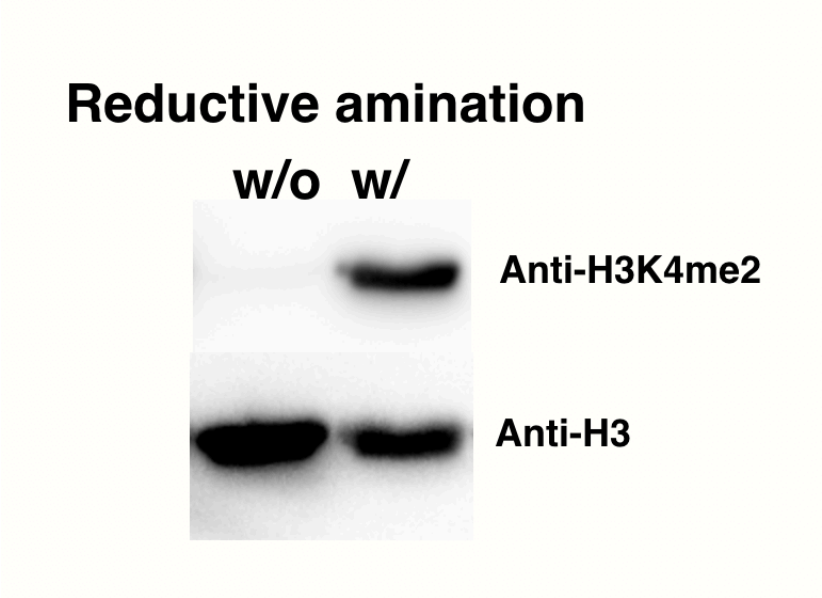
Supplementary Figure 2. Labeling purified sfGFP-D134AcdK with Aminoxy-FL. (**A**) The plasmid map of pEVOL-AcdKRS. (**B**) The plasmid map of pBAD-sfGFP134TAG. (**C**) The labeling of wild type sfGFP and purified sfGFP-D134AcdK with Aminoxy-FL.



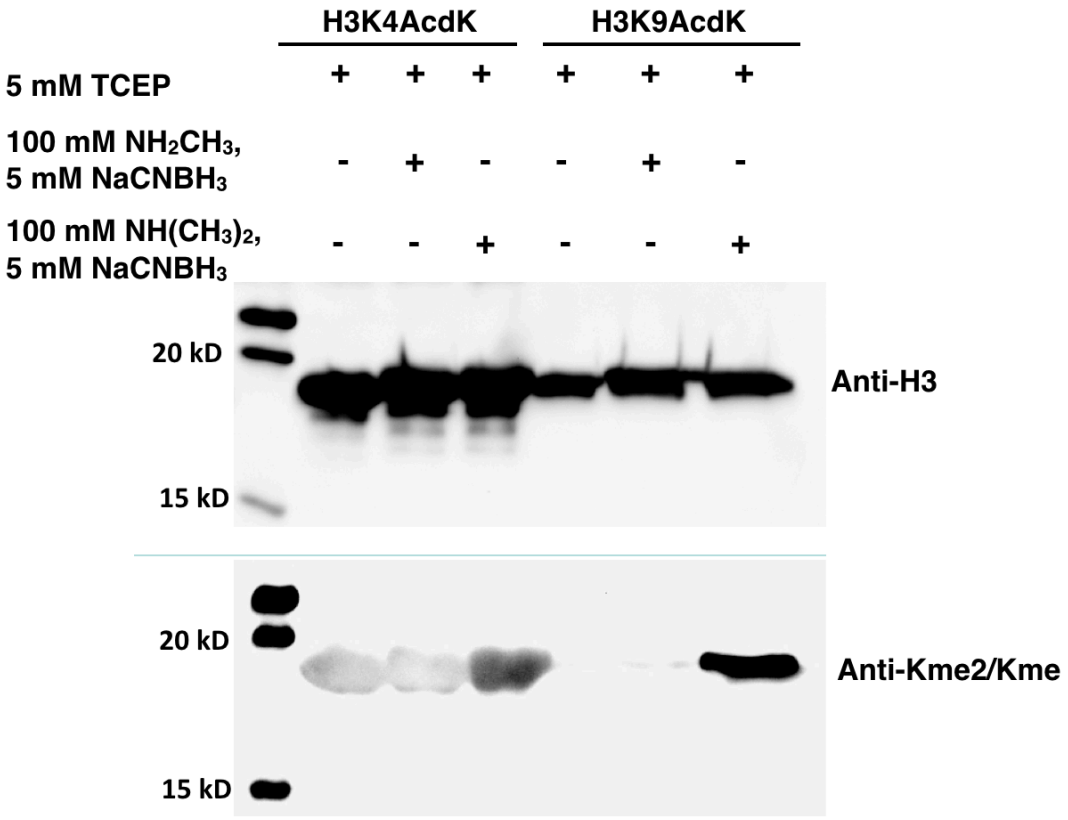
Supplementary Figure 3. Tandem mass spectrum of a Kme2-containing trypsinized fragment of sfGFP-D134Kme2.



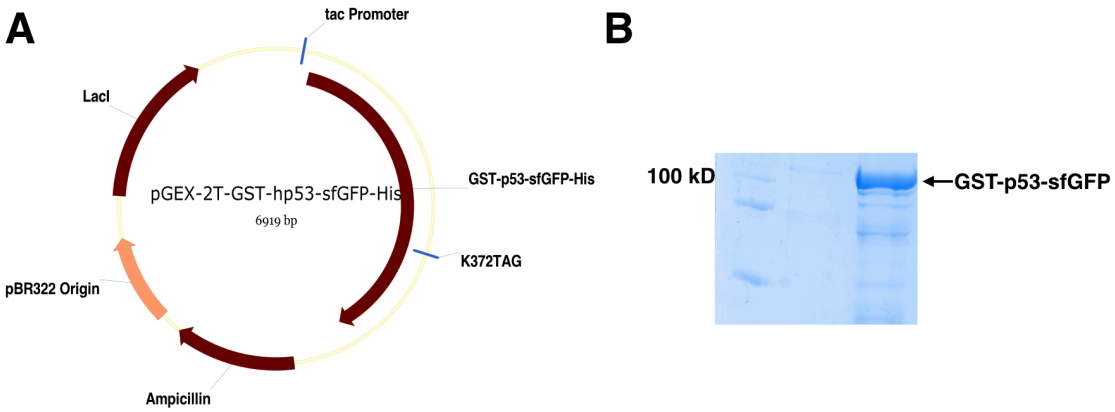
Supplementary Figure 4. The expression vector of H3 variants.



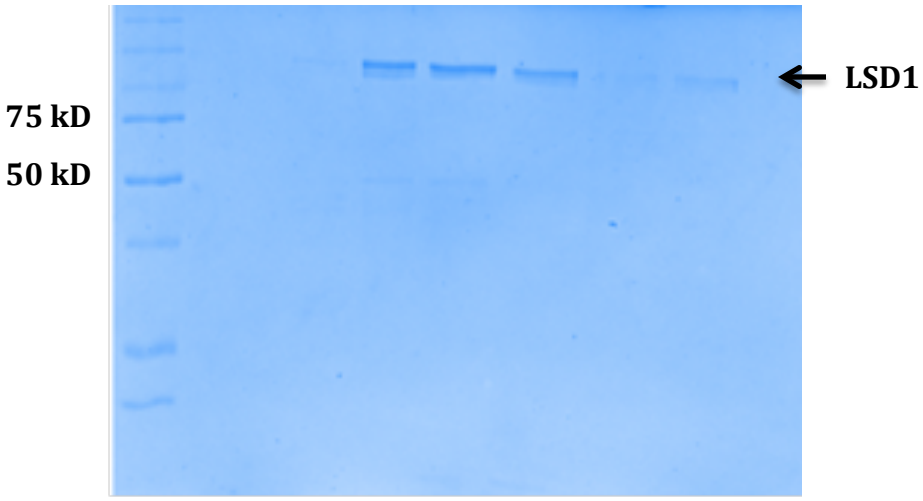
Supplementary Figure 5: The detection of Kme2 at H3K4 by anti-H3K4me2. H3K4AcdK was treated with or without Staudinger reduction and then reductive amination with dimethyllysine. The afforded proteins were then analyzed by SDS-PAGE and probes by anti-H3 and anti-H3K4me2 antibodies.



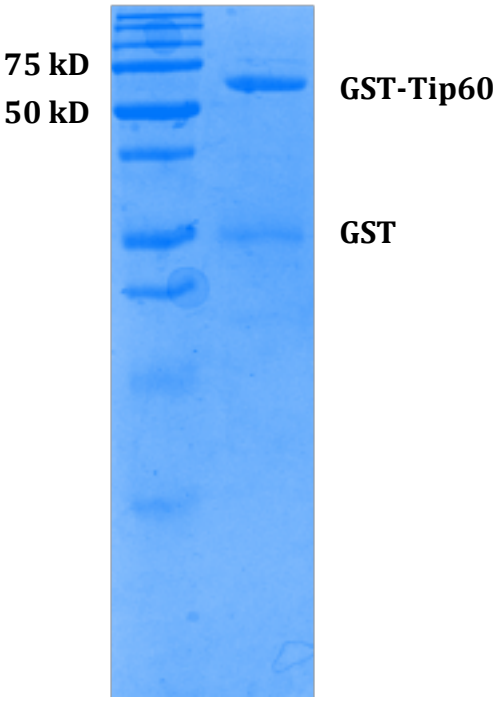
Supplementary Figure 6. Detection of mono- and dimethylation of histone H3 lysines. H3K4AcdK and H3K9AcdK were reacted with 5 mM TCEP for 2 h and then 100 mM methylamine or dimethylamine in the presence of 10 mM $NaCNBH_3$ for 8 h. They were then affinity-purified using Ni-NTA resins and analyzed by SDS-PAGE and western blotting. The total panel shows proteins probed by an anti-H3 antibody from Abcam and the bottom panel shows same proteins probed by a pan anti-Kme2/Kme antibody from abcam. The pan anti-Kme2/Kme antibody apparently detects Kme2 but not Kme. A pan anti-Kme2/Kme antibody from PTM biolabs led to a same result.



Supplementary Figure 7. The expression of p53-K372AcdK. (**A**) The expression vector of GST-p53-sfGFP with an amber mutation at K372. (**B**) GST-p53-sfGFP after its affinity-purified with both glutathione and Ni-NTA resins.



Supplementary Figure 8. Q-sepharose factions with LSD1.



Supplementary Figure 8. The expression of Tip60.