Supporting Information

Bioorthogonal Chemical Reporters for Monitoring Protein Acetylation

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List of Contents

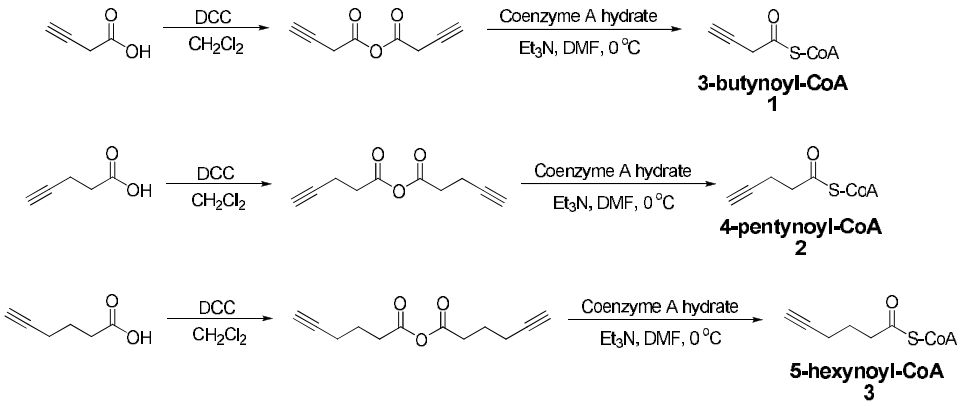
General Methods and Materials:

Unless otherwise noted, all the chemical reagents were purchased from either Sigma-Aldrich or Fisher Scientific. ^{1}H and ^{13}C NMR spectra were recorded on Bruker DPX-400 or Bruker AVANCE-600 instrument. Chemical shifts are reported in δ ppm, and *J* values are reported in Hz. MALDI-TOF mass spectra were acquired on Applied Biosystems Voyager-DE mass spectrometer. HPLC was conducted on Agilent 1100 series HPLC system with HPLC-grade acetonitrile (CH_3CN) and ultrapure water from Milli-Q Advantage A10 Purification System. In-gel fluorescence scanning was performed using Amersham Biosciences Typhoon 9400 variable mode imager (excitation 532 nm, 580 nm filter, 30 nm band-pass). All contrast/brightness adjustments on the images were applied to the whole gels and blots. All the image adjustments were performed in Photoshop.

HPLC-purified histone H3 peptide (aa 1-20 (L20Y): ARTKQTARKSTGGKAPRKQY, >90% purity based on HPLC analysis) and crude K^{14}-5-hexynoyl-histone H3 peptide (aa 1-20, >80% purity based on HPLC analysis) were purchased from the Proteomics Resource Center at the Rockefeller University. az-Rho, alk-12 and alk-16 were generously provided by Guillaume Charron and Paul Dossa in our lab (the synthetic methods for these compounds can be found in the supplementary reference 5). Recombinant histone H3 (human or *Xenopus laevis*) was purchased from Millipore. RPMI media 1640 and Dulbecco's modified Eagle media (DMEM) were purchased from Gibco. EDTA-free protease inhibitor was purchased from Roche Applied Science. Pre-stained protein ladder was purchased form Invitrogen. Pre-cast polyacrylamide gels (4-20% or 15% Criterion Tris-HCl gels) were purchased from Bio-Rad Laboratories. anti-histone H3 (C-16), anti-Hsp90 (F-8), anti-α Enolase (L-27), anti-Cofilin (FL-166), anti-moesin (E-10), anti-HMG-1 (W-18), anti-ADA (D-4) and anti-Cor1A (14.1) were purchased from Santa Cruz Biotechnology. anti-L-plastin was purchased from United States Biological. anti-Ku70 was purchased from Millipore. anti-acetylated lysine was purchased from Cell Signaling Technology. Secondary antibodies were purchased from Jackson ImmunoResearch Laboratories. Mass spectrometry grade trypsin was purchased from Promega. High-capacity streptavidin beads were purchased from Thermo Scientific.

Synthesis of alkynyl-acetyl-CoA analogs

***Scheme S1*. Chemical synthesis of alkynyl-acetyl-CoA analogs.**



2 mmol of 3-butynoic acid (synthesized from Jones oxidation of 3-butyn-1-ol[1]), 4-pentynoic acid (Fluka), or 5-hexynoic acid (Aldrich) was dissolved in 10 mL of anhydrous dichloromethane. To this solution was added 1 mmol of *N*,*N*′-dicyclohexylcarbodiimide (DCC) under argon, and the reaction was allowed to proceed at room temperature for 4 h. The anhydride products can be observed on TLC plate by using ethyl acetate/hexane (1/1) as the developing solvent system. The reaction mixture was then concentrated down to dryness, redissolved in anhydrous dimethylformamide (DMF) under argon and cooled to 4 ºC in ice-bath. To this reaction mixture was added 0.2 equivalent of coenzyme A·hydrate (Sigma, Cat. No: C4282) and 0.6 equivalent of triethylamine (Et_3N). The reaction was kept in ice bath and stirred for 30 min. Upon completion (as determined by LC/MS), the reaction was neutralized to pH 7 by dropwise addition of 0.1 N aqueous hydrochloride and then concentrated to dryness under high vacuum. The dried crude material was then redissolved in H_2O/CH_3CN (1/1) and filtered through the syringe filter devise (Millipore, Cat. No. SLCR013NL, 0.45 μm, hydrophilic PTFE, 13 mm). The filtrate was subjected to HPLC purification with the elution method set as 95% solution A/5% solution B to 50% solution A/50% solution B over 30 min (solution A: H_2O + 0.1% TFA; solution B: CH_3CN + 0.1% TFA). The alkynyl-acetyl-CoA analogs (**1**, **2** and **3**) were eluted at ~21 min under this elution condition.

Characterization of alkynyl-acetyl-CoA analogs

3-butynoyl-CoA (1): ^{1}H NMR (D_2O, 600 MHz) δ 8.58 (s, 1H), 8.32 (s, 1H), 6.11 (m, 1H), 5.35 (d, 1H, J = 6.2 Hz), 4.49 (s, 1H), 4.14 (m, 2H), 3.93 (m, 1H), 3.75 (m, 1H), 3.48 (m, 1H), 3.32-3.45 (m, 5H), 3.23 (m, 2H), 2.92 (m, 1H), 2.28 (m, 2H), 2.15 (m, 1H), 1.11 (m, 4H), 0.83 (s, 3H), 0.70 (s, 3H); ^{31}P NMR (D_2O, 161.9 MHz) δ 0.95, -9.65, -10.21; HMRS (ESI-TOF) calcd for $C_{25}H_{35}N_7NaO_{17}P_3S$ ($[M-3H+Na]^+$) 853.0926, found 853.1426.

4-pentynoyl-CoA (2): ^{1}H NMR (D_2O, 600 MHz) δ 8.72 (s, 1H), 8.66 (s, 1H), 8.48 (s, 1H), 6.35 (d, 1H, *J* = 5.3 Hz), 6.26 (d, 1H, *J* = 5.9 Hz), 5.21 (m, 2H), 4.72 (m, 1H), 4.64 (brs, 1H), 4.46 (brs, 1H), 4.92 (m, 2H), 4.29 (m, 2H), 4.06 (m, 1H), 3.89 (m, 1H), 3.63 (m, 1H), 3.49 (t, 2H, *J* = 6.5 Hz), 3.38 (m, 2H), 3.07 (m, 2H), 2.87 (m, 2H), 2.54 (m, 2H), 2.47 (t, 2H, *J* = 6.5 Hz), 2.38 (m, 1H), 0.97 (s, 3H), 0.84 (s, 3H); ^{31}P NMR (D_2O, 161.9 MHz) δ 0.93, -9.61, -10.15; HMRS (ESI-TOF) calcd for $C_{26}H_{41}N_7O_{17}P_3S$ ($[M+H]^+$) 848.1492, found 848.1428.

5-hexynoyl-CoA (3): ^{1}H NMR (D_2O, 600 MHz) δ 8.71 (s, 1H), 8.48 (s, 1H), 6.26 (d, 1H, *J* = 5.3 Hz), 4.91 (m, 2H), 4.63 (s, 1H), 4.30 (m, 2H), 4.06 (brs, 1H), 3.89 (m, 1H), 3.64 (m, 1H), 3.49 (t, 2H, *J* = 6.6 Hz), 3.37 (t, 2H, *J* = 6.3 Hz), 3.04 (t, 2H, *J* = 6.3 Hz), 2.77 (t, 2H, *J* = 7.3 Hz), 2.47 (t, 2H, *J* = 6.6 Hz), 2.38 (t, 1H, *J* = 2.4 Hz), 2.26 (td, 2H, *J* = 7.0, 2.8 Hz), 1.84 (m, 2H), 0.97 (s, 3H), 0.85 (s, 3H); ^{31}P NMR (D_2O, 161.9 MHz) δ 0.95, -9.76, -10.18; HMRS (ESI-TOF) calcd for $C_{27}H_{43}N_7O_{17}P_3S$ ($[M+H]^+$) 862.1649, found 862.1572.

Preparation of recombinant p300

Flag-tagged human p300 was prepared as previously described by baculoviral infection of SF9 cells[2]. Cells were lysed in lysis buffer (20 mM Tris, pH 7.5, 500 mM NaCl, 4 mM $MgCl_2$, 0.4 mM EDTA, 20% glycerol, 2 mM DTT, and supplemented with protease inhibitors). Clarified cell lysates were diluted to 300 mM NaCl, and NP-40 was added to 0.1% final concentration. Flag M2 agarose resin (Sigma) was added to the lysate and incubated for 4 hours at 4 ˚C. Resin was washed extensively with BC buffer (20 mM Tris, pH 7.9, 20% glycerol) containing 300 mM KCl, and p300 protein was eluted by 0.5 mg/ml Flag peptide in BC buffer containing 100 mM KCl. Protein concentration and purity were estimated by comparison to BSA standards on SDS gels stained with Gelcode Blue stain (Pierce).

MALDI-TOF analysis of p300-catalyzed acylation of histone H3 peptide *in vitro*

In vitro acylation reactions were carried out based on the reported procedure[3] with some modifications. 10 μL reaction solution containing 25 pmol H3 peptide, 20 μM acetyl-CoA or alkynyl-acetyl-CoA analogs, 50 ng of p300, 50 mM, pH 7.9 Tris buffer and 10% glycerol was incubated for 2 h at 30 °C. The peptide products were then extracted with ZipTip (Millipore) and eluted with 1.5 μL of 50% CH_3CN (with 0.1% TFA). Eluent (1 μL) mixed with the ionization matrix (1 μL), α-cyano-4-hydroxycinnamic acid, was spotted onto MALDI plates and subjected to MS analysis. The experimental results were shown in **Figure S1a**.

Stability of alkynyl-acetyl-CoA analogs

The stability of alkynyl-acetyl-CoA analogs were examined by incubating alkynyl-acetyl-CoA analogs (in 50 mM Tris buffer, pH 7.9) with or without 1 mM dithiothreitol at 30 °C as previously described[4]. The results were analyzed by analytical HPLC (100% solution A to 30% solution A/70% solution B over 30 min, solution A: H_2O + 0.1% TFA; solution B: CH_3CN + 0.1% TFA). As a comparison, we also examined the stability of acetyl-CoA under the same condition. CoA, acetyl-CoA and alkynyl-acetyl-CoA analogs can all be detected by UV at 210 nm. The half-life time for acetyl-CoA is >> 4 h (Tris buffer only) and 3 h (with DTT). 3-butynoyl-CoA was found to be quite unstable. The half-life time for 3-butynoyl-CoA is 30 min (Tris buffer only) and <10 min (with DTT). The half-life time for 4-pentynoyl-CoA is >> 4 h (Tris buffer only) and 1.5 h (with DTT). The half-life time for 5-hexynoyl-CoA is >> 4 h (Tris buffer only) and 3 h (with DTT). HPLC chromatographs are shown in **Figure S1b**.

***In vitro* acylation of histone H3 protein by p300 and mapping protein modification sites**

15 μL reaction solution containing histone H3 (0.3-1.7 μg), 160 μM acetyl-CoA or alkynyl acetyl-CoA analogs, 100 ng p300, 50 mM, pH 7.9 Tris buffer and 10% glycerol was incubated for 2 h at 30 °C. Following the *in vitro* reaction, proteins were separated on 15% SDS-PAGE and stained with coomassie brilliant blue R-250 staining solution (Bio-Rad). The gel slices containing histone H3 products were excised from each lane, washed with 50 mM ammonium bicarbonate (ABC) twice, destained with 50 mM ABC/CH_3CN (50/50) twice and dehydrated

with CH_3CN. After drying the gel slices in SpeedVac, gel slices were rehydrated with trypsin solution (2 μg of trypsin for each vial/gel slice) and incubated in 37 °C water bath for 18 h. Trypsin-digested peptides in solution were collected, dried in SpeedVac, resuspended in H_2O (with 0.1% TFA) and submitted samples to nano-HPLC/MS/MS analysis (Thermo LTQ-Orbitrap in the Proteomic Resource Center at Rockefeller University).

LC-MS analysis was performed with a Dionex 3000 nano-HPLC coupled to an LTQ-Orbitrap ion trap mass spectrometer (ThermoFisher). Peptides were pressure loaded onto a home-made 75 μm diameter, 15 cm C_{18} reverse phase column and separated with a gradient running from 95% buffer A (HPLC water with 0.1% formic acid) and 5% buffer B (HPLC grade acetonitrile with 0.1% formic acid) to 55% B over 30 min, next ramping to 95% B over 10 min and holding 95% B for 10 min. One full MS scan (300-2000 MW) was followed by 3 data dependent scans of the nth most intense ions with dynamic exclusion enabled. The spray voltage was set to 1.94 kV and the flow rate through the column was set to 0.25 μL/min.

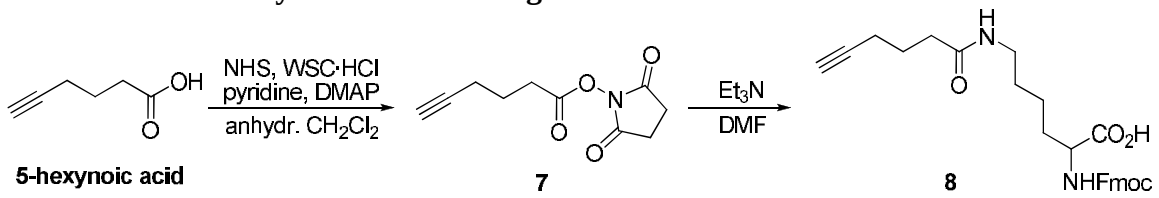
Raw tandem mass spectra were searched against the human IPI protein database version 3.56 using SEQUEST search engine (Thermo Scientific). Cysteine carbamido-methylation was searched as fixed modification, while methionine/tryptophan oxidation, asparagines/glutamine deamindation, lysine/serine/threonine/cysteine acetylation/3-butynoylation/4-pentynoylation/5-hexynoylation, N-terminal acetylation/3-butynoylation /4-pentynoylation/5-hexynoylation were searched as variable modifications. Peptide tolerance was set as 10.0 ppm, fragment ion tolerance was set as 1.0 AMU and trypsin was set as the digestion enzyme. The resulting searching files were then analyzed and visualized by Scaffold 2 proteome software. Each identified protein must contain at least 2 unique peptides that are exclusively assigned to this protein with a minimum protein identification probability of 99%. Each peptide spectrum must meet several selection thresholds including >95% for peptide identification probability, >1.0 for SEQUEST XCorr score, and $\pm$6 ppm for actual minus calculated peptide mass. The experimental results were shown in **Figure S1c-d**, and **Table S1**.

In-gel fluorescence detection of p300-catalyzed *in vitro* acylation

Following 2 h incubation at 30 °C, 15 μL *in vitro* acylation reaction was stopped by adding 10 μL of 4% SDS buffer (4% SDS, 150 mM NaCl, 50 mM triethanolamine, pH 7.4). CuAAC reaction was subsequently carried out by adding 2 μL of freshly-premixed "click chemistry cocktail"[5] (100 μM az-Rho[5], 1 mM TCEP, 100 μM (Tris[(1-benzyl-1H-1, 2, 3-triazole-4-yl)methyl] amine (TBTA)[6] and 1 mM $CuSO_4$) to the acylation reaction. CuAAC reactions were allowed to proceed at room temperature for 1 h, and proteins were subsequently separated on 15% SDS-PAGE. The gel was soaked in destaining solution (40% MeOH, 10% AcOH, 50% H_2O) for 1-2 h to remove the non-covalently bound az-Rho dye. After washing with dd H_2O, the gel was subjected to in-gel fluorescence scanning. The experimental results were shown in **Figure 1b** and **Figure S1e-f**.

Chemical synthesis of the building block (8) for K^{14}-(5-hexynoyl)-H3 peptide (1-20).

***Scheme S2*. Chemical synthesis of building block 8**



5-hexynoic acid **7** **8**

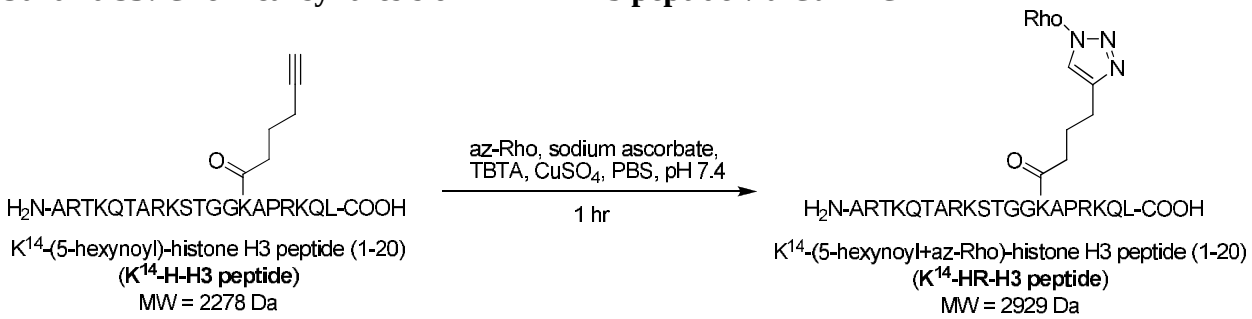
To the solution containing 5-hexynoic acid (9.1 mmol) in anhydrous CH_2Cl_2 (15 mL) was subsequently added N-hydroxysuccinimide (18.1 mmol), WSC·HCl (9.1 mmol), pyridine (10.9 mmol) and 4-dimethylamino-pyridine (1.1 mmol). The reaction was allowed to proceed at room temperature for overnight. The crude product was washed and extracted with CH_2Cl_2 and H_2O thrice. The combined organic layers were dried under vacuo and the crude product was separated by silica gel chromatography (ethyl acetate/hexane = 1/3). The yield of the pure compound **7** is 73%. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.92 (brs, 4H), 2.75 (t, 2H, *J* = 11.1 Hz), 2.33 (td, 2H, *J* = 10.3, 3.8 Hz), 1.99 (t, 1H, *J* = 3.8 Hz), 1.95 (t, 2H, *J* = 10.7 Hz); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 169.5, 168.6, 83.7, 70.2, 30.1, 26.0, 23.8, 18.0; HMRS (ESI-TOF) calcd for $C_{10}H_{12}NO_2$ ($[M+H]^+$) 210.0766, found 210.1063.

Compound **7** (1.8 mmol) was dissolved in 5 mL anhydrous DMF. To this solution was added commercial available Fmoc-lysine-OH·HCl (1.5 mmol) and triethylamine (1.8 mmol). The mixture was stirred at room temperature for 3 h. Upon the reaction was complete, remove all the solvents under vacuo. The dried residue was subjected to flash column chromatography ($MeOH/CH_2Cl_2$ = 1/9) and yield the pure compound **8** in 54% yield. ^{1}H NMR (d^4-MeOH, 600 MHz) δ 7.78 (d, 2H, *J* = 7.6 Hz), 7.66 (dd, 2H, *J* = 7.8, 7.8 Hz), 7.38 (dd, 2H, *J* = 7.5, 7.5 Hz), 7.30 (dd, 2H, *J* = 7.4, 7.4 Hz), 4.30-4.39 (m, 2H), 4.20 (t, 1H, *J* = 6.9 Hz), 4.12 (dd, 1H, *J* = 8.2, 4.7 Hz), 3.18 (t, 2H, *J* = 6.8 Hz) 2.18-2.39 (m, 6H), 1.76-1.81 (m, 3H), 1.49-1.55 (m, 2H), 1.38-1.45 (m, 2H); ^{13}C NMR (d^4-MeOH, 150 MHz) δ 179.1, 176.1, 159.4, 146.2, 146.0, 129.6, 129.0, 127.1, 121.7, 85.0, 71.1, 68.7, 57.0, 49.2, 40.9, 36.7, 33.6, 30.8, 26.8, 25.0, 19.5; HMRS (ESI-TOF) calcd for $C_{27}H_{31}N_2O_5$ ($[M+H]^+$) 463.2233, found 463.2160.

Pure compound **8** was utilized for solid-phase peptide synthesis of K^{14}-5-hexynoyl-histone H3 peptide (aa 1-20, >80% purity based on HPLC analysis). This peptide was synthesized by the Proteomics Resource Center at the Rockefeller University.

Preparation of K^{14}-(5-hexynoyl+az-Rho)-histone H3 peptide (1-20) and establishment of a standard curve for quantification of in-gel fluorescent intensity

***Scheme S3*. Chemical synthesis of K^{14}-HR-H3 peptide via CuAAC**



K^{14}-(5-hexynoyl)-histone H3 peptide (1-20)
(**K^{14}-H-H3 peptide**)
MW = 2278 Da

K^{14}-(5-hexynoyl+az-Rho)-histone H3 peptide (1-20)
(**K^{14}-HR-H3 peptide**)
MW = 2929 Da

Prepare the stock solution of peptide K^{14}-(5-hexynoyl)-histone H3 peptide (aa 1-20) [**K^{14}-H-H3 peptide**] (16 mg/mL) in dd H_2O. To start the synthesis of K^{14}-(5-hexynoyl+az-Rho)-histone H3 peptide [**K^{14}-HR-H3 peptide**] via CuAAC, an aliquot of K^{14}-H-H3 peptide stock solution (1 equiv.) was first added to 1×DIBCO's PBS buffer (133 μg peptide/100 μL PBS buffer) followed by subsequently adding 10 mM az-Rho (2 equiv.), 50 mM sodium ascorbate (3 equiv.), 10 mM TBTA (2 equiv.) and 50 mM $CuSO_4$ (2 equiv.) to this solution. Votex the reaction mixture and allow the reaction proceed at room temperature. Base on MALDI-TOF mass analysis, the reaction was complete in one hour. The product K^{14}-HR-H3 peptide was purified by preparative HPLC (running from 100% solution A to 15% solution A/85% solution B over 40 min, the product was eluted at 27.5 min. solution A: H_2O + 0.1% TFA; solution B: CH_3CN + 0.1% TFA). The peptide product was confirmed by MALDI-TOF. The molecular weight calculated for K^{14}-HR-H3 peptide is 2929, the observed value is 2930.4 ($[M+H]^+$).

To establish a standard curve for quantification of in-gel fluorescence intensity and for determining the kinetic parameters of 4-pentynoyl-CoA, 1.3 mg of HPLC-purified K^{14}-HR-H3 peptide was dissolved in 50 mM, pH 7.9 Tris buffer containing 10% glycerol to make a stock solution (4 nmol/μL). Perform a serial dilution from this stock to prepare several stock solutions of different concentrations ranging from 0.25 pmol/μL to 50 pmol/μL. Took 1 μL aliquot from serially diluted stock solutions individually and added into vials containing a mixture of 11 μL 50 mM, pH 7.9 Tris buffer, 10% glycerol and 8 μL 4% SDS buffer. Load K^{14}-HR-H3 peptide from these vials onto 15% Tris-HCl gel and separated by SDS-PAGE (200 Volt, 45 min). The gel was then soaked in destaining solution (40% MeOH, 10% AcOH, 50% H_2O) for 1 h followed by acquiring its fluorescent image. The resulting image was shown in **Figure S2a**. This experiment was performed in triplicate. The fluorescent intensity of each band was determined (or quantified) by ImageJ (free software). The determined fluorescent intensity versus the amount of K^{14}-HR-H3 peptide (or rhodamine dye) was plotted as shown in **Figure S2a**. The fluorescent intensity is linear respect to the amount of K^{14}-HR-H3 peptide when the amount of K^{14}-HR-H3 peptide is below 10 pmoles.

Determination of k_{cat} and K_M for 4-pentynoyl-CoA in p300-catalyzed *in vitro* acylation

Kinetic assays were carried out to obtain various initial rates at different 4-pentynoyl-CoA concentrations. The assay was performed by first pre-incubating a mixture of histone H3 protein (1 μg, Millipore) and 4-pentynoly-CoA (0.1, 0.5, 1, 2.5, 5, 10, 20 or 50 μM) in 11 μL of 50 mM, pH 7.9 Tris buffer (with 10% glycerol) at 30 °C for 10 min followed by adding 1 μL of full-length p300 (33 ng) to initiate reaction and incubated for the indicated time (15, 30 and 45 sec). The reaction was stopped by adding 8 μL of 4% SDS buffer with immediate vortexing. Once the whole assay (there are total 25 reactions including a negative control) was complete, adding freshly prepared "click chemistry cocktail" (100 μM az-Rho, 1 mM TCEP, 100 μM TBTA and 1 mM $CuSO_4$) to each reaction vial and allow CuAAC proceed at room temperature for 1 h. The reaction mixture was then loaded and separated on 15% Tris-HCl SDS-PAGE. The gel was then soaked in destaining solution (40% MeOH, 10% AcOH, 50% H_2O) for 1 h to remove non-covalently bound rhodamine dye followed by acquiring its fluorescent image. The resulting image was shown in **Figure S2b**. The assay was performed in triplicate. The fluorescent intensity of each band was quantified by ImageJ, and then based on the standard curve, each measured value was converted into the amount of rhodamine tag attached to histone H3 (pmoles). The concentration of rhodamine tags attached to histone H3 versus time was then plotted to obtain the initial rate at different concentrations of 4-pentynoyl-CoA. The initial rate (μM/sec)

versus various concentration of 4-pentynoyl-CoA (μM) was fitted to Michaelis-Menten equation and plotted using GraphPad Prism 5 to obtain the steady-state kinetic parameters, K_M and V_{max}, for 4-pentynoyl-CoA (shown in **Figure S2b**). The mean values of kinetic parameters derived from triplicate assays are K_M = 0.69 ± 0.12 (μM) and k_{cat} (= V_{max}/[p300]) = 2.10 ± 0.06 (sec^{-1}).

Preparation of sodium 3-butynoate (4), sodium 4-pentynoate (5) and sodium 5-hexynoate (6)
3-Butynoic acid, 4-pentynoic acid or 5-hexynoic acid (6 mmol) was dissolved in 20 mL ddH_2O. Aqueous NaOH solution (6 mmol, ~0.18N) was added dropwise to reaction mixture. The reaction mixture was then filtered through 0.45 μm membrane, frozen in liquid nitrogen and lyophilized to dryness. All the sodium forms of alkynyl-acetate analogs appeared as white powders. To prepare accurate stock solutions of sodium 3-butynoate, sodium 4-pentynoate and sodium 5-hexynoate, 10 mg of each were dissolved in 600 μL D_2O. 1.5 μL of anhydrous CH_3CN was added to each sample as an internal standard for peak integration. After gently vortexing and short spin, 400 μL of the well-mixed solution was transferred into NMR tube for ^{1}H-NMR analysis as shown in **Figure S3**. To normalize the concentration of each alkynyl-acetate analog added to the cells, we calculated the ratio of the peak integration value of the terminal alkyne proton relative to CH_3CN proton in each spectrum. Based on these ratios, we adjusted the concentration of acetate analogs to prepare 1M stock solutions.

Characterization of alkynyl-acetate analogs
To calibrate the chemical shifts of each alkynyl-acetate analog, MeOH (0.5 μL) was added to serve as a NMR calibration standard in D_2O. The characteristic peaks of MeOH in D_2O are δ2.61 ($C\underline{H_3}$) in ^{1}H-NMR and δ49.50 ($\underline{C}H_3$) in ^{13}C NMR.

Sodium 3-butynoate (4): ^{1}H NMR (D_2O, 600 MHz) δ 3.15 (d, 2H, J = 2.7 Hz), 2.46 (t, 1H, J = 2.6 Hz); ^{13}C NMR (D_2O, 150 MHz) δ 197.76, 101.88, 93.21; HMRS (ESI-TOF) calcd for $C_4H_4NaO_2$ ($[M+H]^+$) 107.0109, found 107.0416.

Sodium 4-pentynoate (5): ^{1}H NMR (D_2O, 600 MHz) δ 2.36-2.43 (m, 4H), 2.34 (t, 1H, J = 2.3 Hz); ^{13}C NMR (D_2O, 150 MHz) δ 194.33, 98.49, 82.72, 28.34; HMRS (ESI-TOF) calcd for $C_5H_6NaO_2$ ($[M+H]^+$) 121.0265, found 121.0184.
Sodium 5-hexynoate (6): ^{1}H NMR (D_2O, 600 MHz) δ 2.35 (t, 1H, J = 2.7 Hz), 2.27 (t, 2H, J = 7.4 Hz), 2.21 (td, 2H, J = 7.2, 2.6 Hz), 1.75 (pentet, 2H, J = 7.3 Hz); ^{13}C NMR (D_2O, 150 MHz) δ 195.88, 98.56, 82.53, 37.79, 30.50; HMRS (ESI-TOF) calcd for $C_6H_8NaO_2$ ($[M+H]^+$) 135.0422, found 135.0321.

Cell Culture
Jurkat T cells were cultured in tissue culture flasks in RPMI media supplemented with 10% fetal bovine serum (FBS), penicillin (100 units/mL) and streptomycin (0.1 mg/mL). RAW264.7 macrophages, NIH3T3 fibroblasts, HeLa cells, 293T cells, COS-7 cells and DC2.4 cells were cultured in petri dishes in DMEM media supplemented with 10% FBS, penicillin (100 units/mL) and streptomycin (0.1 mg/mL). Cells were incubated in a 5% CO_2 humidified incubator at 37 °C.

Metabolic labeling and preparation of total cell lysates
Jurkat T cells (20×10^6) were cultured in 4 mL of RPMI medium 1640 supplemented with 2% FBS, 1% penicillin and streptomycin and labeled with bioorthogonal chemical reporters (from 1

M stock solutions) at the indicated concentration. Trypan blue exclusion was used to determine the cell viability during metabolic labeling. Bioorthogonal chemical reporters did not appear to influence cell viability within the indicated labeling time. At the described time points, cells were harvested and washed twice with PBS. To prepare the total cell lysates, cell pellets were resuspended in 50 μL of pre-lysis buffer (7 mM PMSF, 10×EDTA-free protease inhibitors, 800 μM $MgCl_2$, 0.05% SDS, 10 mM triethanolamine, pH 7.4) followed by co-incubating with 0.7 μL of benzonase nuclease (Sigma) for 20 min. The cell suspensions were then lysed by adding 150 μL of 4% SDS buffer (4% SDS, 150 mM NaCl, 50 mM triethanolamine, pH 7.4) with subsequent vigorous vortexing. Insoluble cell debris was removed by centrifugation at 20000 g for 10 min. The supernatant was collected to yield total cell lysate. Protein concentration was determined by BCA assay.

CuAAC reactions and in-gel fluorescence scanning

For acquiring a typical profile of mammalian acetylome, 50 μg of total cell lysates was reacted with freshly pre-mixed "click chemistry cocktail" (100 μM az-Rho, 1 mM TCEP, 100 μM TBTA and 1 mM $CuSO_4$). The final protein concentration was typically 1 mg/mL in 4% SDS buffer. The reaction was allowed to stand at room temperature for 1 h. Proteins were then precipitated by $CHCl_3$-MeOH precipitation method and the pellet was washed thrice with chilled MeOH (-20 ºC). Dried protein pellets were resuspended in 4% SDS buffer and then separated by SDS-PAGE. The gel was subsequently subjected to in-gel fluorescence scanning to acquire the image.

Acid-extraction of core histones

The method for acid extraction of core histones was based on the reported protocol[7] with some modifications. To Jurkat T cell pellet (10×10^6 cells) was added 1 mL of ice-cold hypotonic lysis buffer (10 mM TEA, pH 7.4, 1 mM KCl, 1.5 mM $MgCl_2$, 1 mM PMSF, 10 mM SAHA) supplemented with 1×EDTA-free protease inhibitor cocktail. The resuspended cells were homogenized by an ice-cold tight-fitting dounce homogenizer and lysed by three cycles of freeze-thaw lysis. Intact nuclei were pelleted by spinning at 10000 g for 10 min at 4 °C. The supernatant was discarded and nuclear pellet was washed twice with ice-cold hypotonic buffer. The nuclear pellet was then resuspended in 0.4N H_2SO_4 and agitated overnight on rotator at 4 °C. The nuclear debris was pelleted by spinning at 16000 g for 10 min at 4 °C. The supernatant containing extracted core histones were collected and then precipitated with MeOH (5 volume) at -80 °C overnight. Precipitated histone proteins were spun down at 16000 g for 10 min at 4 °C and wash twice with 500 uL of ice-cold MeOH. Protein pellets were air-dried at room temperature and then resuspended in dd H_2O. The histone protein concentration was determined by BCA assay.

Histone H3 immunoprecipitation

Core histones (50 μg) extracted from Jurkat T cells described above were co-incubated with anti-histone H3 antibody (1 μg, Santa Cruz Biotechnology, C-16), 25 uL protein-G agarose bead slurry (Roche) at 4 °C on end-over-end rotator for 2 h. The beads were spun down (10,000 g, 30 sec) and washed thrice with ice-cold modified RIPA lysis buffer (1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 150 mM NaCl, 10 mM Tris, pH 7.4 in ddH_2O). The beads were then resuspended in 20 uL of 4% SDS lysis buffer and incubated with 2.5 uL of freshly-premixed "click chemistry cocktail" for 1 h at room temperature. 5 uL of 4×LDS loading buffer and 1 uL β-mercaptoethanol were added to each reaction. The mixtures were heated at 95 ºC for 5 min and

spun at 1000 g for 30 sec. 25 uL of the reaction supernatant was directly loaded onto 15% Tris-HCl gel and separated by SDS-PAGE. The gel was then destained for 2 h at room temperature and scanned by 9400 Typhoon imager.

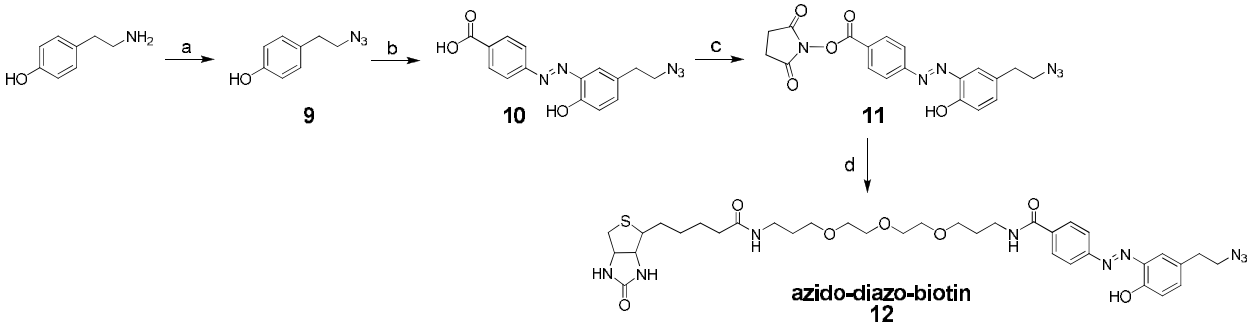
Immunoblotting

Proteins separated by SDS-PAGE were transferred onto PVDF membrane. After blocking with 5-10% milk in PBST (PBS with 1% Tween-20), the membrane was washed thrice with PBST and then coincubated with anti-histone H3 antibody (Millipore) or anti-Ac-Lys antibody (Millipore). The following procedures were based on the protocols provided by Millipore.

Chemical synthesis of azido-diazo-biotin

We employed the diazobenzene linker for CuAAC-based proteomics since this functional group can be efficiently cleaved by mild reduction with sodium dithionite ($Na_2S_2O_3$)[8,9].

***Scheme S4*. Synthesis of azido-diazo-biotin**



Scheme S4. Synthesis of azido-diazo-biotin (**12**): (a) TfN_3, $ZnCl_2$, TEA, CH_3CN, H_2O, 96% yield; (b) i. $NaNO_2$, 6M HCl, 4-aminobenzoic acid. ii. Et_3N, THF, 45~55% yield over two steps. (c) DCC, N-hydroxysuccinimide, THF, 79%; (d) biotin-PEG-NH_2, DMF, 80% yield.

4-(2-azidoethyl)phenol (9)[10]. To an ice-cooled suspension of NaN_3 (546 mg, 8.40 mmol) in CH_3CN (10 mL) was slowly added Tf_2O (1.17 mL, 7.00 mmol). The mixture was stirred at 0 ºC for 2 h. A solution of tyramine (800 mg, 5.83 mmol) and $ZnCl_2$ (79.3 mg, 0.58 mmol) in H_2O:CH_3CN (3:7) was added to the reaction mixture, followed by Et_3N (2.40 mL, 17.5 mmol) and freshly prepared N_3Tf. The reaction was kept stirring at rt for 8 h. The reaction mixture was then concentrated under vacuum, dissolved in EtOAc, and washed with H_2O twice. The organic layers were collected, dried over $MgSO_4$ and concentrated. The crude product was purified by flash column chromatography (25% EtOAc in hexane) to give colorless 4-(2-azidoethyl)phenol (915 mg, 5.61 mmol, 96%)[9a]. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.08 (d, 2H, J = 8.4 Hz), 6.78 (d, 2H, J = 8.4 Hz), 3.45 (t, 2H, J = 7.2 Hz), 2.82 (t, 2H, J = 7.2 Hz); ^{13}C-NMR ($CDCl_3$,100 MHz): δ 154.3, 130.1, 129.9, 115.4, 52.6, 34.4.

(E)-4-((5-(2-azidoethyl)-2-hydroxyphenyl)diazenyl)benzoic acid (10). Solid $NaNO_2$ (3.30 g, 6.55 mmol) was added to an ice-cooled suspension of 4-aminobenzoic acid in 6M HCl (40 mL). The resulting mixture was stirred at 0 ºC and turned into a yellow-colored solution. In the meantime, dissolving 4-(2-azidoethyl)phenol (**9**) (1.19 g, 7.30 mmol) in cooled THF (15 mL) at 0 ºC, and subsequently added K_2CO_3 to this solution to adjust pH to 8. After 40 min, the

diazonium salt solution was slowly added to compound 2 at 0 ºC, while the pH was kept around 8 by adding more K_2CO_3. Once the reaction was complete, removed all the solvents under vacuum. The concentrated residue was then re-dissolved in ethyl acetate, and washed with acidified H_2O (pH 2~3) for three times. The organic layers were collected, dried over $MgSO_4$ and concentrated. The crude product was purified by silica gel flash column chromatography (33% EtOAc in hexane and then 10% MeOH in CH_2Cl_2) to give yellow-colored compound **2** (1.27 g, 4.08 mmol, 56%). ^{1}H-NMR (CD_3OD, 600 MHz) δ: 8.24 (d, 2H, *J* = 8.5 Hz), 8.05 (d, 2H, *J* = 8.5 Hz), 7.89 (d, 1H, *J* = 1.9 Hz), 7.40 (dd, 1H, *J* = 8.3, 2.0 Hz), 7.04 (d, 1H, *J* = 8.4 Hz), 3.61 (t, 2H, *J* = 7.0 Hz), 2.97 (t, 2H, *J* = 7.0 Hz); ^{13}C-NMR (CD_3OD, 150 MHz) δ:176.6, 163.9, 163.8, 148.3, 144.9, 140.4, 139.6, 132.5, 131.3, 128.2, 61.4, 43.3. ESI-MS calcd. for $C_{15}H_{13}N_5O_3$ $[M-H]^-$: 310.0940, found 310.2.

(E)-2,5-dioxopyrrolidin-1-yl 4-((5-(2-azidoethyl)-2-hydroxyphenyl)diazenyl) benzoate (11): To compound **10** (164 mg, 0.53 mmol) dissolved in anhydrous THF (20 mL) was added DCC (119 mg, 0.58 mmol) and N-hydroxy-succinimide (66.7 mg, 0.58 mmol) under Ar. After the reaction was stirred at room temperature for 4 h and concentrated *in vacuo*. The crude solid residue was then dissolved in chilled ethyl acetate and the urea was filtered off. The filtrate was then concentrated and purified by silica gel flash column chromatography (33% EtOAc in hexane) to give yellow-colored compound **11** (170 mg, 79%). ^{1}H-NMR ($CDCl_3$, 600 MHz): δ 8.31 (d, 2H, *J* = 8.3 Hz), 8.00 (d, 2H, *J* = 8.3 Hz), 7.87 (d, 1H, *J* = 1.9 Hz), 7.31 (dd, 1H, *J* = 8.5, 2.0 Hz), 7.05 (d, 1H, *J* = 8.5 Hz), 3.60 (t, 2H, *J* = 7.0 Hz), 2.95-2.98 (m, 6H); ^{13}C-NMR ($CDCl_3$, 150 MHz): δ 169.1, 161.2, 154.3, 151.7, 137.4, 135.3, 133.6, 131.9, 130.0, 126.6, 122.4, 118.7, 52.4, 34.2, 25.7. ESI-MS calcd. for $C_{19}H_{16}N_6O_5$ $[M+H]^+$: 409.1260, found 409.3.

(E)-4-((5-(2-azidoethyl)-2-hydroxyphenyl)diazenyl)-N-(15-oxo-19-(2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-4,7,10-trioxa-14-azanonadecyl)benzamide (12): To compound **11** (13 mg, 0.03 mmol) dissolved in anhydrous DMF (3 mL) was added biotin-PEG-NH_2[11] (27.0 mg, 0.06 mmol). The reaction was complete after stirring at room temperature for 10 h and concentrated. The crude product was dissolved in CH_3CN:H_2O (1:1) and purified by reversed preparative HPLC column (CH_3CN: 5% to 40% in 10 min, then 40% to 100% in 40 min, compound **12** was eluted at 33 min) to give yellow-colored product (18.0 mg, 80%). ^{1}H-NMR (CD_3OD, 400 MHz): δ 8.44 (brs, 1H), 8.00 (brs, 4H), 7.83 (d, 1H, *J* = 2.0 Hz), 7.33 (dd, 1H, *J* = 8.5, 2.0 Hz), 6.99 (d, 1H, *J* = 8.4 Hz), 4.46 (dd, 1H, *J* = 7.7, 5.0 Hz), 4.27 (dd, 1H, *J* = 7.8, 4.4 Hz), 3.66-3.47 (m, 18H), 3.23 (t, 2H, J = 6.7 Hz), 3.16 (td, 1H, *J* = 4.6, 9.2 Hz), 2.92 (t, 2H, *J* = 7.0 Hz), 2.68 (d, 1H, *J* = 12.7 Hz), 2.16 (t, 2H, *J* = 7.3 Hz), 1.93 (t, 1H, *J* = 6.3 Hz), 1.89 (t, 1H, *J* = 6.3 Hz), 1.76-1.52 (m, 6H), 1.42 (t, 1H, *J* = 7.6 Hz), 1.38 (t, 1H, *J* = 7.7 Hz), 1.28 (brs, 2H), 0.89 (m, 1H); ^{13}C-NMR (CD_3OD, 100 MHz): δ 175.9, 169.1, 166.1, 154.3, 153.3, 139.0, 137.8, 135.8, 131.8, 131.3, 129.6, 123.4, 119.3, 71.5, 71.3, 71.2, 70.3, 63.3, 61.6, 57.0, 53.5, 41.0, 38.9, 37.8, 36.8, 35.1, 30.7, 30.4, 29.8, 29.4, 26.8. MALDI-TOF calcd. for $C_{35}H_{49}N_9O_7S$ $[M+Na]^+$: 762.8744, found: 762.26.

Proteomic analysis of alkynyl-acetate analogs-labeled proteins

3-Butynoate-, 4-pentynoate- or 5-hexynoate-labeled total cell lysates (15-25 mg) were diluted into 4% SDS buffer (the final protein concentration = 1 mg/mL) and reacted with freshly pre-mixed "click chemistry cocktail" (100 μM azido-diazo-biotin tag, 1 mM TCEP, 100 μM TBTA and 1 mM $CuSO_4$) for 2 h at room temperature (**Figure S7**). Proteins were then precipitated by

MeOH (5 volume) at -20 °C overnight. Precipitated proteins were centrifuged at 5,200 g for 30 min at 4 ºC and washed thrice with ice-cold MeOH. To capture the biotinylated proteins by streptavidin beads, the air-dried protein pellet was resuspended in 2-3 mL of 4% SDS buffer containing 10 mM EDTA and subsequently the protein suspension was diluted into NP-40 lysis buffer (1% NP-40, 150 mM NaCl, 50 mM Tris, pH 8.0) to reduce SDS concentration down to 1-1.5%. Pre-washed streptavidin beads were then incubated with this protein solution at room temperature for 1 h on end-over-end rotator. The captured proteins were sequentially washed thrice with the incubation buffer, modified RIPA lysis buffer (1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 150 mM NaCl, 10 mM Tris, pH 7.4), 1 × GIBCO's PBS (+ 0.2% SDS), PBS and ammonium bicarbonate (ABC). For cysteine reduction and alkylation, incubating the captured proteins with freshly-made 2 mM dithiothreitol in 8M urea for 10 min followed by alkylation for another 30 min with freshly prepared iodoacetamide (the final concentration is 6 mM). Wash the captured proteins with ABC buffer thrice. To release the proteins from the streptavidin beads, the beads were resuspended in ABC buffer and transferred to a dolphin tube. Pellet the beads, and elute the captured proteins by incubating the beads with elution solution (0.01% SDS, 25 mM sodium dithionite, 50 mM ABC buffer) for 1 h at room temperature. Spin down the beads and collect the eluent. Repeat the same elution procedure for 1-2 times. Concentrate the eluent by using the microcon centrifugal filter device (10 kDa NMWL). The concentrated eluent was then dried in SpeedVac. Resuspend the dried pellets in 1×LDS/5% β-mercaptoethanol. 60% volume of this resuspended solution was loaded onto SDS-PAGE for in-gel trypsin digestion, while the remaining sample was loaded onto another SDS-PAGE for validation of protein candidates by Western blot.

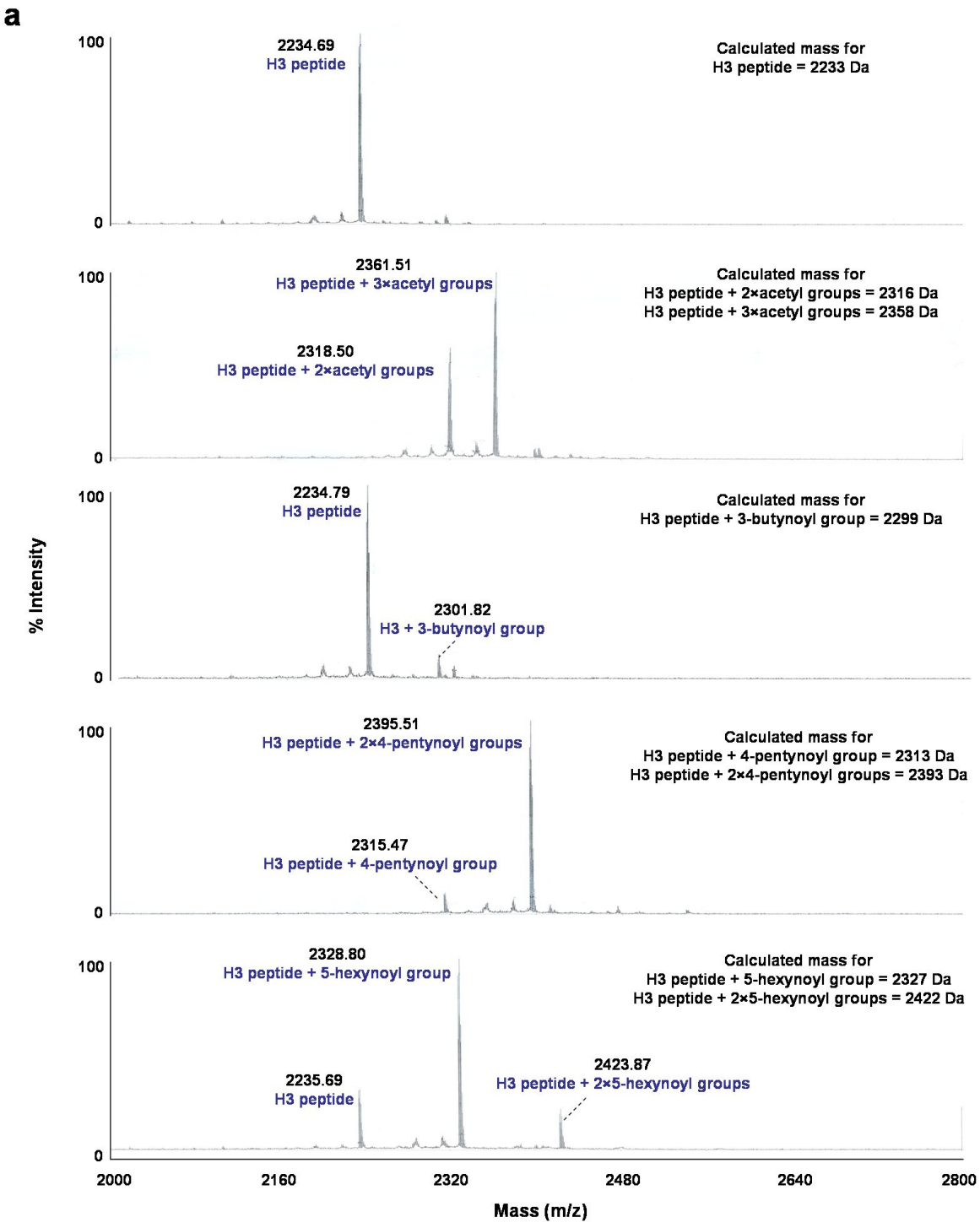
The resultant enriched sodium 3-butynoate-, sodium 4-pentynoate- or sodium 5-hexynoate-labeled proteome was visualized by coomassie blue staining. The gel images were shown in **Figure S7b** and **Figure S8a**. Each lane was sliced into 8 or 12 fractions. Each excised gel slice was placed in microcentrifuge tube. The gel slices were further cut into more pieces, washed with 50 mM ammonium bicarbonate (ABC) twice, destained with 50 mM ABC/acetonitrile (50/50) twice, and then dehydrated in 100% acetonitrile. After drying the gel pieces in a SpeedVac, gel pieces were rehydrated with trypsin solution (2 μg of trypsin for each vial/gel slice) and incubated in 37 °C water bath for 18 h. The eluted trypsin-digested peptides were then collected and dried in SpeedVac. Resuspended the dried eluents in H_2O (with 0.1% TFA) and submited the samples to nano-HPLC/MS/MS analysis (Thermo LTQ-Orbitrap in the Proteomic Resource Center at Rockefeller University).

Mapping the modification sites of 4-pentynoate-metabolically labeled proteins

For modification site-mapping experiments, 20 mg of 4-pentynoate-labeled-Jurkat T cell lysates was subjected to CuAAC reaction with azido-diazo-biotin, followed by MeOH precipitation for overnight at -20 °C. The precipitated proteins were pelleted, washed and air-dried. Resuspend the protein pellet in 8M urea. To reduce and alkylate cysteine residues, the proteins were first treated with 2 mM dithiothreitol for 30 min, and then treated with 6 mM iodoacetamide for 30 min. To remove the reducing and alkylating reagents, the proteins were precipitated in MeOH at -20 °C for overnight; afterwards, the protein pellet was washed thrice with ice-cold MeOH. Resuspend the air-dried protein pellet in freshly-prepared 8M urea. Upon the majority of protein was solublized, the concentration of urea was reduced to 1.5 M by diluting into 50 mM ABC buffer. Solubilized proteins were then subjected to trypsin digestion (1:50 w/w) in the presence of 20 mM methylamine at 37 °C for overnight. The extent of trypsin digestion was determined by

MALDI-TOF and SDS-PAGE. To enrich the biotinylated peptides, the trypsin-digested peptides were incubated with pre-washed streptavidin beads for 2 h at room temperature on end-over-end rotator. Wash the beads sequentially with 1.5 M Urea, 1×DIBCO's PBS (+ 0.2% SDS), 1×PBS and ammonium bicarbonate (ABC). To elute the bound peptides, the beads were transferred to dolphin tubes and then incubated with the elution buffer (0.01% SDS, 25 mM sodium dithionite, 50 mM ABC buffer) for 1 h at room temperature. Pellet the beads and collect the eluent. Repeat the cleavage step once. Dry the eluent in SpeedVac, and then resuspend the dried peptides in dd H_2O (+ 0.1% TFA). The peptides were cleaned up by C8 cartridge (Waters), eluted with 70% CH_3CN + 20% H_2O + 0.1% TFA and dried in SpeedVac. The details of analysis of raw tandem mass spectra and selection criterion for candidate proteins have been described, however, the mass of 258.1117, corresponding to the triazole tag (**Figure S7a**), was included here as a variable lysine modification in order to search for 4-pentynoate-modification sites. In addition, to investigate the labeling specificity of 4-pentynoate in cells, we searched for other amino acid residues that can be potentially modified by 4-pentynoate, including N-terminal amine, serine, threonine and cysteine. Moreover, to elucidate whether the chain length of 4-pentynoate could possibly be altered in cellular biosynthetic machineries; we searched for different chain-lengths of triazole tag as variable modifications on lysine residues (the set carbon number for acyl-moiety of triazole tag ranges from 1 to 18). The experimental results were shown in **Figure S9**.

Figure S1. **Characterization of p300-catalyzed *in vitro* acylation on histone H3 peptide and histone H3.** (a) Analysis of the crude *in vitro* acylation products of histone H3 peptide by MALDI-TOF mass spectrometry. 25 pmol of H3 peptide [aa 2-21 (L21Y): ARTKQTARKSTGGKAPRKQY] and 20 μM of acetyl-CoA or alkynyl-acetyl-CoA analogs were subjected to *in vitro* acetylation condition. The peptide products were extracted with ZipTip (Millipore) for MS analysis. (b) HPLC chromatographs for the stability experiments of acetyl-CoA and alkynyl-acetyl-CoA analogs. The time on the top left corner indicates the incubation time of peptide at 30 °C. These stability experiments were monitored by UV absorption (Abs) at 210 nm. (c) Crude *in vitro* acylation products of histone H3 (~1.7 μg) resolved on 15% SDS-PAGE. The gel slices containing histone H3 were later excised from the gels and subjected to in-gel trypsin digestion followed by MS analysis to determine the modification sites. (d) Selected MS/MS spectra of peptides derived from in-gel trypsin digestion of *in vitro* acylated histone H3 products. (e) In-gel fluorescent detection of *in vitro* histone H3 acylation with 4-pentynoyl-CoA under various p300 concentrations. Histone H3 (~1.7 μg) was co-incubated with 50 μM 4-pentynoyl-CoA and p300 of various concentrations at 30 °C for 10 min. The reaction was stopped by adding 2/3 reaction volume of 4% SDS buffer, followed by CuAAC with az-Rho. The reaction mixture was loaded onto 15% Tris-HCl and separated by SDS-PAGE. The gel was first soaked in destaining solution for 1.5 h and then subjected to in-gel fluorescence image acquisition. (f) In-gel fluorescent detection of *in vitro* time-dependent acylation of histone H3 and p300 with 4-pentynoyl-CoA. Histone H3 (~1.7 μg) were co-incubated with p300 [60 ng (top) or 120 ng (bottom)] and 4-pentynoyl-CoA [50 μM (top) or 160 μM (bottom)] at 30 °C for the indicated time. The reaction was stopped by adding 2/3 reaction volume of 4% SDS buffer (4% SDS, 150 mM NaCl, 50 mM triethanolamine, pH 7.4), followed by reacting with az-Rho via CuAAC, separated on 15% Tris-HCl SDS-PAGE, destaining for 1 h and in-gel fluorescence scanning. Comparable levels of protein loading are demonstrated by coomassie blue (CB) staining.


a
100
0
2234.69
H3 peptide
Calculated mass for
H3 peptide = 2233 Da
2361.51
H3 peptide + 3×acetyl groups
2318.50
H3 peptide + 2×acetyl groups
Calculated mass for
H3 peptide + 2×acetyl groups = 2316 Da
H3 peptide + 3×acetyl groups = 2358 Da
2234.79
H3 peptide
2301.82
H3 + 3-butynoyl group
Calculated mass for
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2395.51
H3 peptide + 2×4-pentynoyl groups
2315.47
H3 peptide + 4-pentynoyl group
Calculated mass for
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H3 peptide + 2×4-pentynoyl groups = 2393 Da
2328.80
H3 peptide + 5-hexynoyl group
2235.69
H3 peptide
2423.87
H3 peptide + 2×5-hexynoyl groups
Calculated mass for
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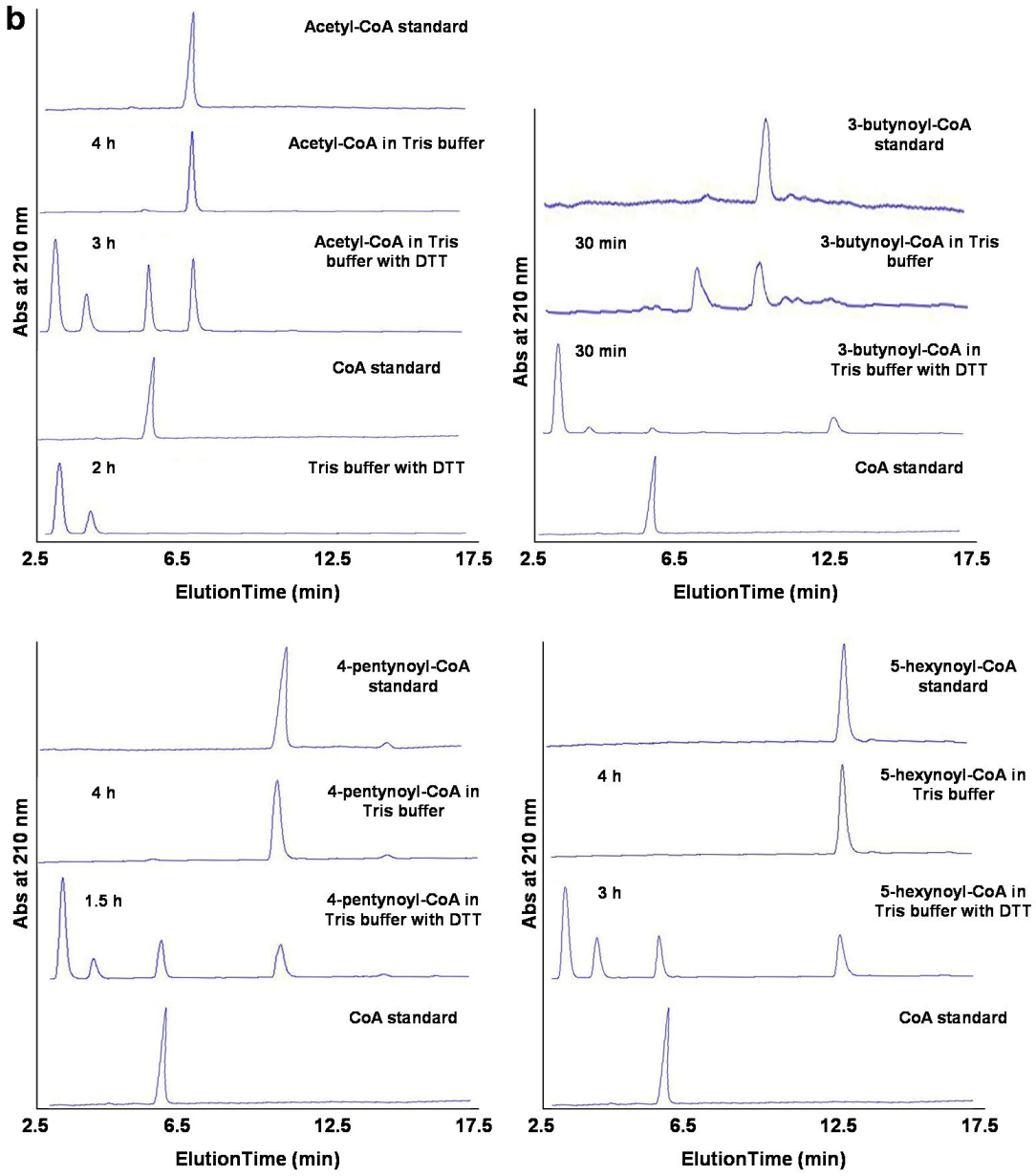
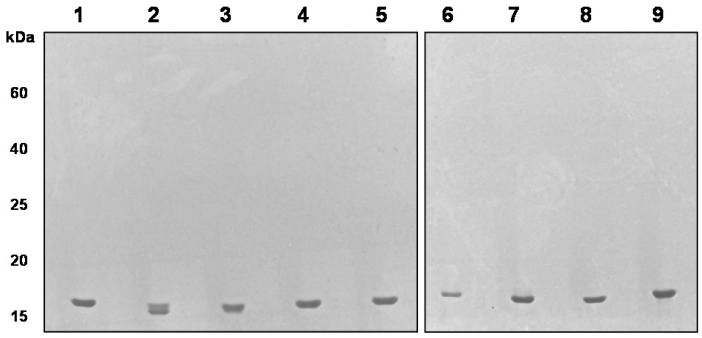
H3 peptide + 2×5-hexynoyl groups = 2422 Da
% Intensity
2000
2160
2320
2480
2640
2800
Mass (m/z)


b
Acetyl-CoA standard
4 h
Acetyl-CoA in Tris buffer
3 h
Acetyl-CoA in Tris buffer with DTT
CoA standard
2 h
Tris buffer with DTT
Abs at 210 nm
2.5
6.5
12.5
17.5
ElutionTime (min)
3-butynoyl-CoA standard
30 min
3-butynoyl-CoA in Tris buffer
30 min
3-butynoyl-CoA in Tris buffer with DTT
CoA standard
Abs at 210 nm
2.5
6.5
12.5
17.5
ElutionTime (min)
4-pentynoyl-CoA standard
4 h
4-pentynoyl-CoA in Tris buffer
1.5 h
4-pentynoyl-CoA in Tris buffer with DTT
CoA standard
Abs at 210 nm
2.5
6.5
12.5
17.5
ElutionTime (min)
5-hexynoyl-CoA standard
4 h
5-hexynoyl-CoA in Tris buffer
3 h
5-hexynoyl-CoA in Tris buffer with DTT
CoA standard
Abs at 210 nm
2.5
6.5
12.5
17.5
ElutionTime (min)

c

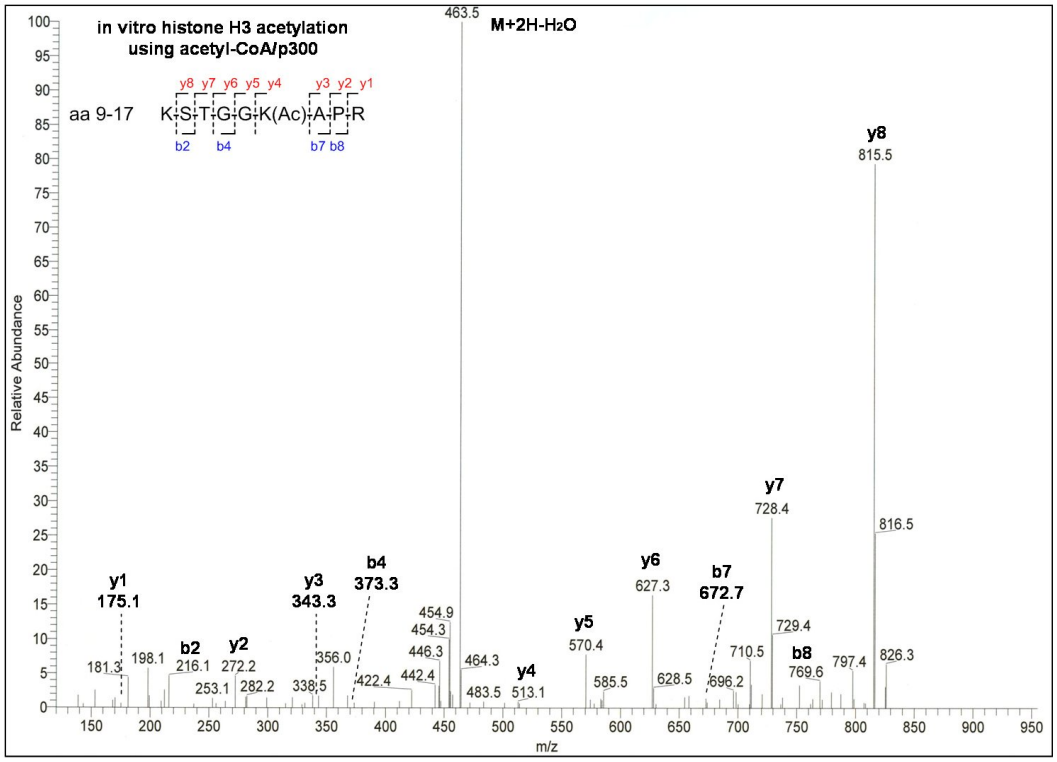


Coomassie blue gel (15% SDS-PAGE)

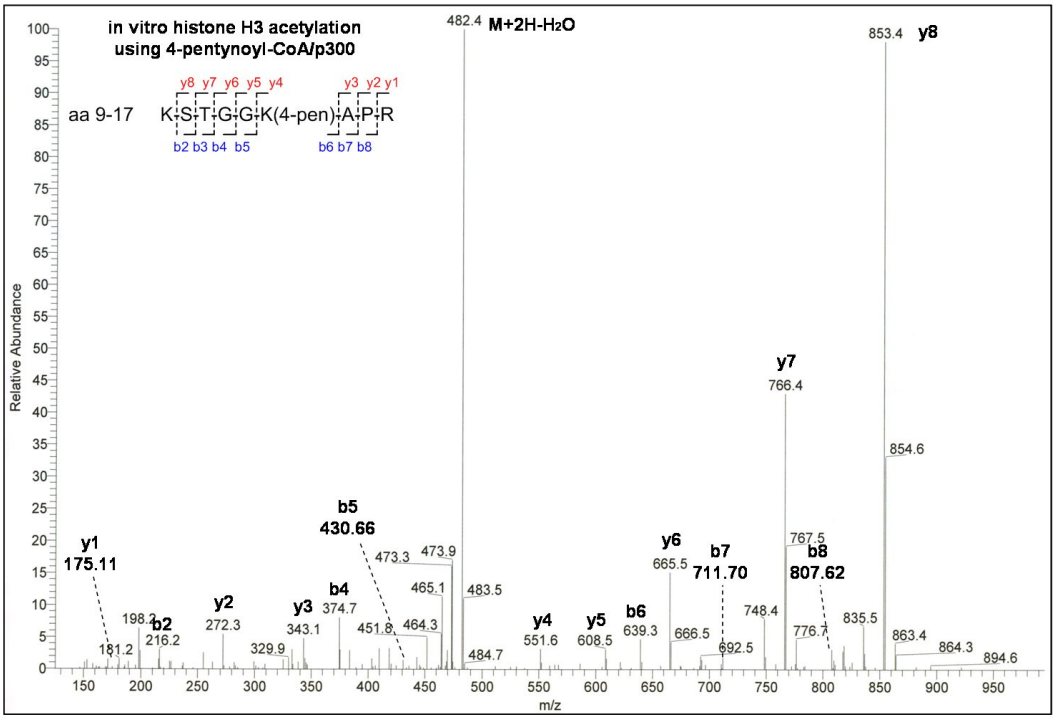
Lane 1: histone H3
Lane 2: histone H3 + acetyl-CoA + p300
Lane 3: histone H3 + 4-pentynoyl-CoA + p300
Lane 4: histone H3 + acetyl-CoA
Lane 5: histone H3 + 4-pentynoyl-CoA

Lane 6: histone H3 + 3-butynoyl-CoA
Lane 7: histone H3 + 3-butynoyl-CoA + p300
Lane 8: histone H3 + 5-hexynoyl-CoA
Lane 9: histone H3 + 5-hexynoyl-CoA + p300

d



d (continued)


in vitro histone H3 acetylation
using 4-pentynoyl-CoA/p300
aa 9-17 K S T G G K(4-pen) A P R
y8 y7 y6 y5 y4
y3 y2 y1
b2 b3 b4 b5
b6 b7 b8
M+2H-H2O
482.4
853.4 y8
y7 766.4
854.6
b5 430.66
y1 175.11
y6 665.5
b7 711.70
b8 807.62
767.5
y2 272.3
y3 343.1
b4 374.7
b2 216.2
y4 551.6
y5 608.5
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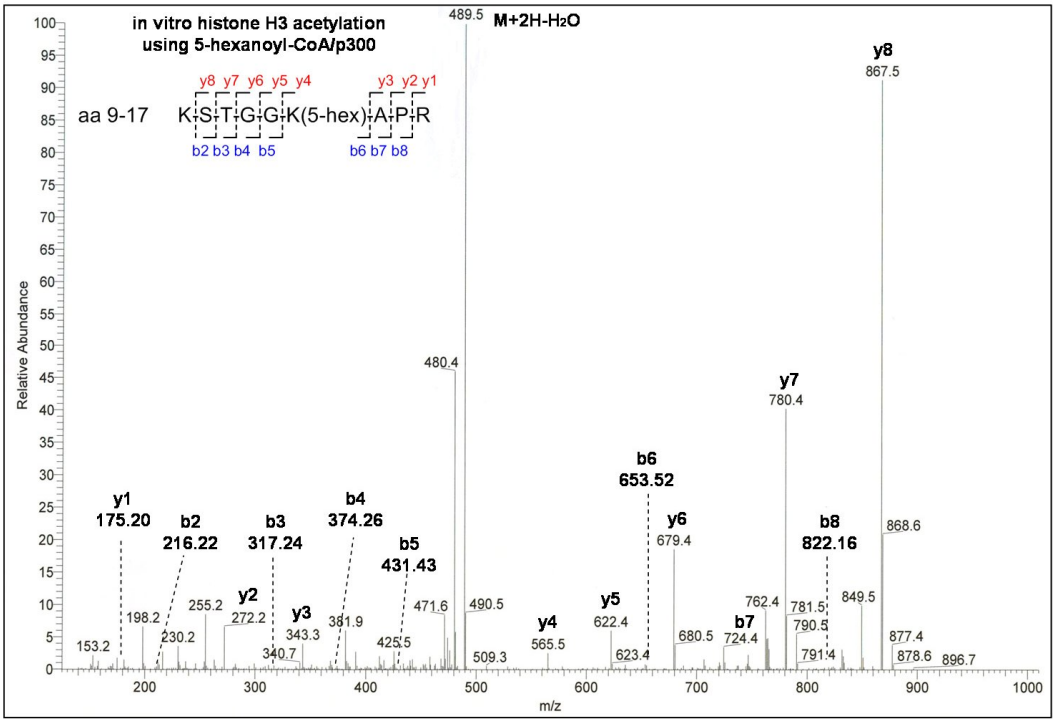
863.4
864.3
894.6
Relative Abundance
m/z


in vitro histone H3 acetylation
using 5-hexanoyl-CoA/p300
aa 9-17 K S T G G K(5-hex) A P R
y8 y7 y6 y5 y4
y3 y2 y1
b2 b3 b4 b5
b6 b7 b8
M+2H-H2O
489.5
y8 867.5
480.4
y7 780.4
b6 653.52
y1 175.20
b2 216.22
b3 317.24
b4 374.26
b5 431.43
y6 679.4
b8 822.16
868.6
y2 272.2
y3 343.3
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b7 724.4
762.4
849.5
Relative Abundance
m/z

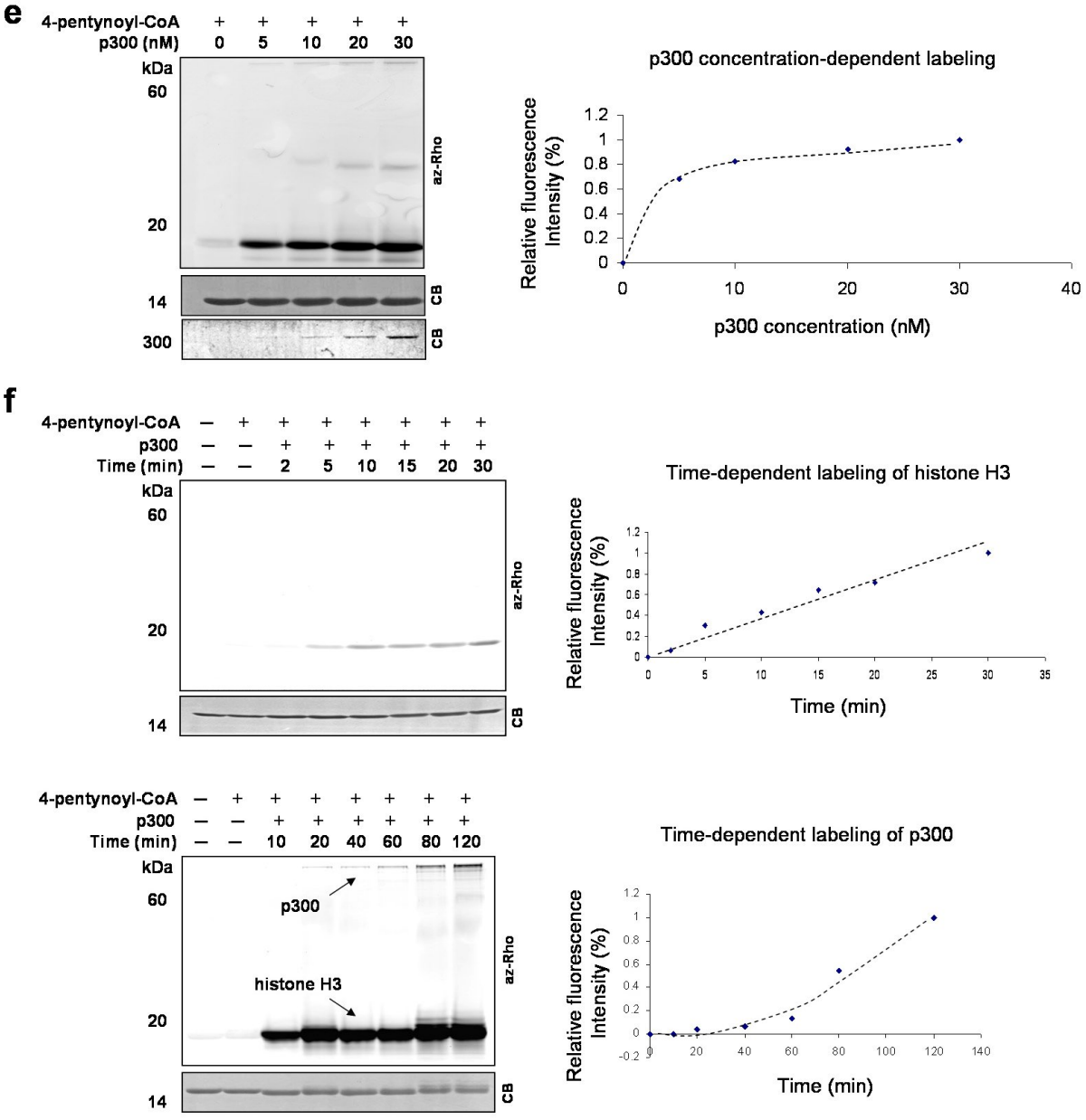

e
4-pentynoyl-CoA + + + + +
p300 (nM) 0 5 10 20 30
kDa
60
20
14
300
az-Rho
CB
CB
p300 concentration-dependent labeling
Relative fluorescence Intensity (%)
p300 concentration (nM)
f
4-pentynoyl-CoA — + + + + + + +
p300 — — + + + + + +
Time (min) — — 2 5 10 15 20 30
kDa
60
20
14
az-Rho
CB
Time-dependent labeling of histone H3
Relative fluorescence Intensity (%)
Time (min)
4-pentynoyl-CoA — + + + + + + +
p300 — — + + + + + +
Time (min) — — 10 20 40 60 80 120
kDa
60
20
14
p300
histone H3
az-Rho
CB
Time-dependent labeling of p300
Relative fluorescence Intensity (%)
Time (min)

Table S1*. List of peptide spectral counts acquired from in-gel trypsin digestion of *in vitro* acetylated and acylated histone H3.** The coomassie gel and selected MS/MS spectra were shown in **Figure S1c,d**. Raw tandem mass spectra were searched against the human IPI protein database version 3.56 using SEQUEST search engine (Thermo Scientific). Cysteine carbamidomethylation was searched as fixed modification, while methionine/tryptophan oxidation, asparagines/glutamine deamindation, lysine/serine/threonine/cysteine acetylation/4-pentynylation/5-hexynylation, N-terminal acetylation/4-pentynylation/5-hexynylation were searched as variable modifications. Each peptide spectrum must meet several selection thresholds including >95% for peptide identification probability, >1.0 for SEQUEST XCorr score, and ±6 ppm for actual minus calculated peptide mass. Those lysine-modified peptides listed in control experiments (H3+*AcCoA*, H3+2*** and H3+***3***) were derived from p300-independent acetylation/acylation (AcCoA: acetyl-CoA, ***2***: 4-pentynoyl-CoA, ***3***: 5-hexynoyl-CoA). For each chosen lysine-modified peptide, the spectral count ratio of p300-catalyzed modification/control must be greater than 2.

Peptide Sequence (K: modified residue)	Spectral Counts of Peptide						
	H3	H3+*AcCoA*	H3+*AcCoA*+p300	H3+*2*	H3+*2*+p300	H3+*3*	H3+*3*+p300
KQLATK (18-23)						4	10
KSAPATGGVK (27-36)	28	21	6	23	17	36	38
SAPATGGVK (28-36)	3	2		9	7	12	17
YRPGTVALR (41-49)	1	3	2	2	3	47	57
STELLIR (57-63)						7	16
KLPFQR (64-69)			1	1			8
EIAQDFK (73-79)	25	23	22	27	25	74	89
RVTIMPK (116-122)						1	
VTIMPK (117-122)	5	10	1	5	4	3	8
KSTGGKAPR (9-17, K14)		1	8		16	2	11
KSTGGKAPR (9-17, K9, K14)		18	60		12		2
STGGKAPR (10-17, K14)					9		
KQLATK (18-23, K18)			2		10	2	19
KQLATKAAR (18-26, K23)				1	3		
KQLATKAAR (18-26, K18, K23)		4	138		18		18
QLATKAAR (19-26, K23)			1				1
KSAPATGGVK (27-36, K27)		12	34	3	25	2	12
KSAPATGGVK (27-36, K27, K36)			4				
KSAPATGGVKKPHR (27-40, K27, K36)			34		6		
KSAPATGGVKKPHR (27-40, K27, K36, K37)			28				
RYQKSTELLIR (53-63, K56)		2	5				
RVTIMPKDIQLAR (116-128, K122)			2				
VTIMPKDIQLAR (117-128, K122)			2				

♦ Peptide sequences acquired from 3-butynoyl-CoA-participating in vitro histone H3 acylation are not included in this table. Based on MS/MS data, there were no histone H3 peptides modified by 3-butynoyl group.

***Figure S2*. Standard curve for quantification of in-gel fluorescence intensity and determination of the kinetic parameters for 4-pentynoyl-CoA in p300-catalyzed histone H3 acylation.** (a) (Left) The in-gel fluorescence image of a serially diluted K^{14}-HR-H3 peptide resolved on 15% Tris-HCl SDS-PAGE. (Right) A plot of in-gel fluorescence intensity versus various amount of K^{14}-HR-H3 peptide. The in-gel fluorescence intensity of each band on the left gel was determined by ImageJ. The measured values were then plotted versus the amount of K^{14}-HR-H3 peptide to give a standard curve (y = 10.86x when x<10 pmol). The curve appears non-linear when x is larger than 10 pmol. Notably, this fluorescence intensity was determined on the Tiff format of the original gel file. When the fluorescence intensity was determined based on the original gel format, the equation of the standard curve will be y = 2133.6x, x<10) (b) (Top) In-gel fluorescent image of the steady-state kinetic assay to determine the initial rates of p300-catalyzed histone H3 acylation at different 4-pentynoyl-CoA concentrations. The assay was carried out with 4-pentynoyl-CoA of various concentrations and allowed to proceed for various time lengths in order to obtain the initial rates at different 4-pentynoyl-CoA concentrations. The complete acylation reactions were then CuAAC with az-Rho for 1 h. Separated the reaction mixtures on 15% Tris-HCl SDS-PAGE, followed by destaining for 1 h and then acquiring the in-gel fluorescence image. (Bottom) A plot of initial rates versus 4-pentynoyl-CoA concentrations and the determined steady-state kinetic parameters for 4-pentynoyl-CoA.

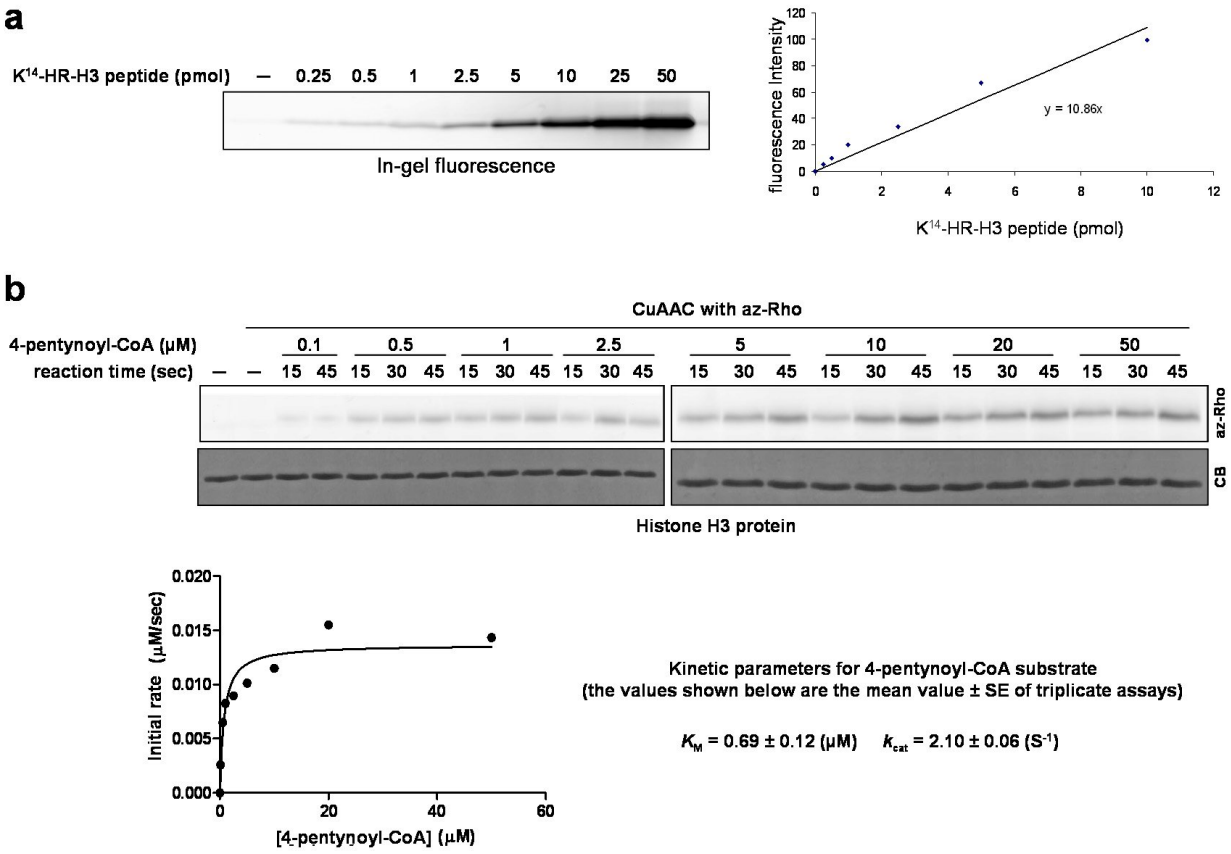


***Figure S3*. [1]H-NMR spectra of sodium 3-butynoate (4), sodium 4-pentynoate (5) and sodium 5-hexynoate (6).**

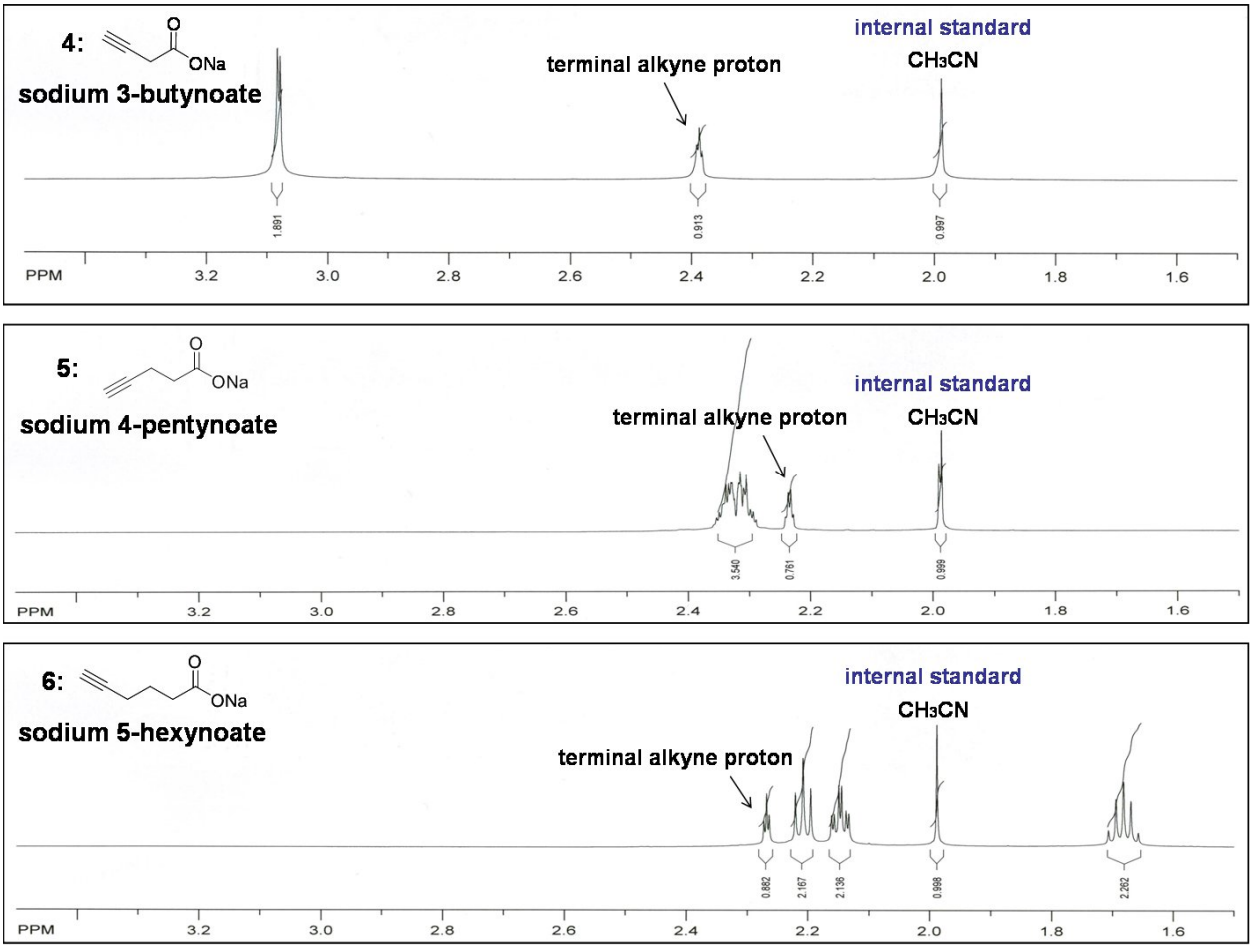
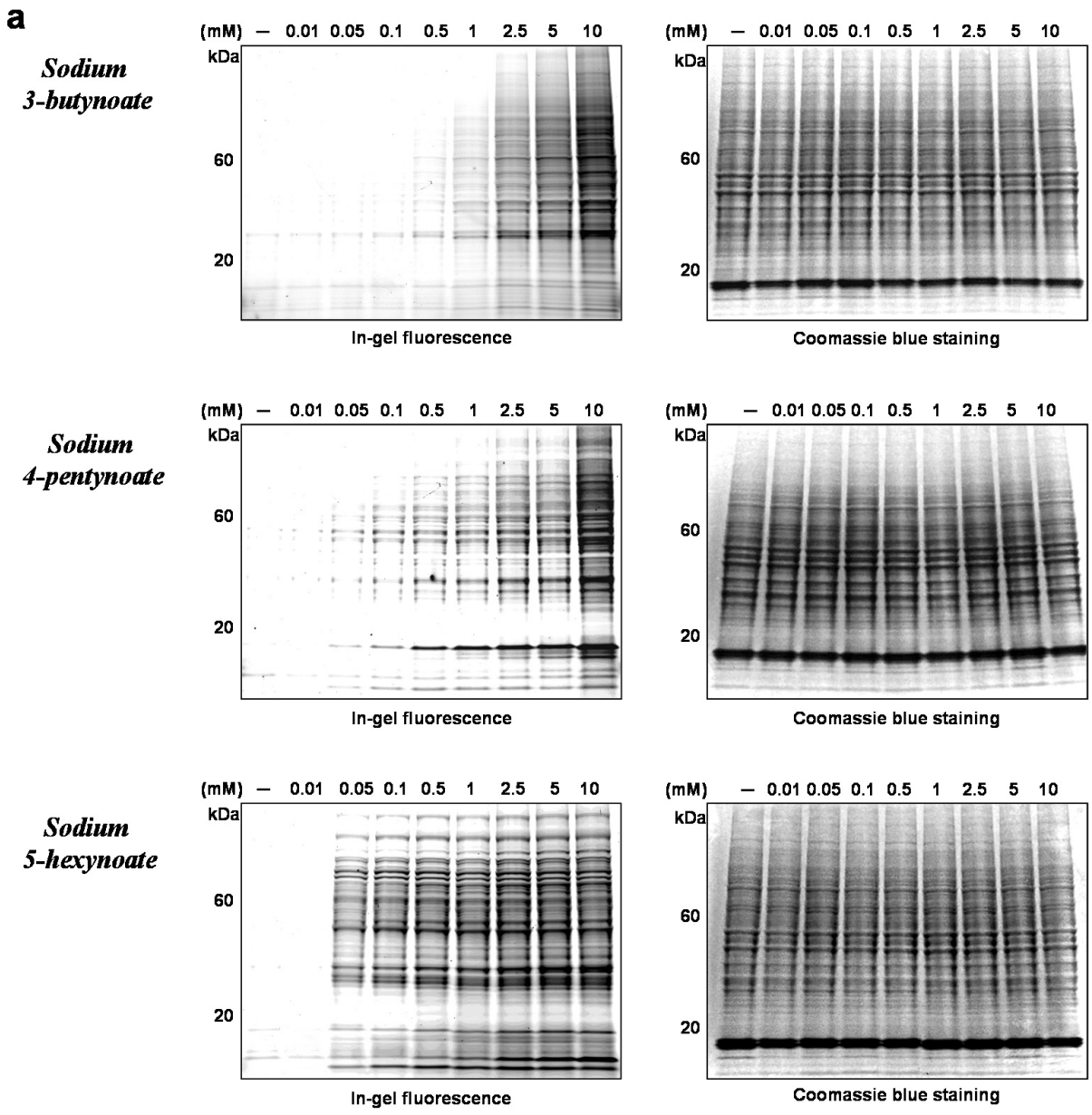
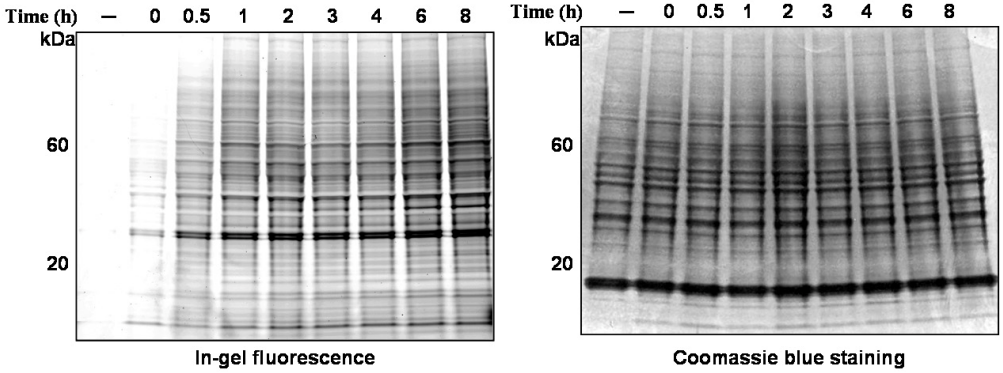


***Figure S4*. Time- and dose-dependent metabolic labeling of mammalian cells with sodium 3-butynoate (4), sodium 4-pentynoate (5) or sodium 5-hexynoate (6).** (a) Dose-dependent labeling of Jurkat T cells. Jurkat T cells were labeled with different concentrations of sodium 3-butynoate, sodium 4-pentynoate or sodium 5-hexynoate, and harvested after 6 h incubation. The lysates were reacted with az-Rho and analyzed by in-gel fluorescence scanning. (b) Time-dependent labeling of Jurkat T cells. 32 mL (4 mL × 8) of Jurkat T cells were labeled with 5 mM of sodium 3-butynoate, sodium 4-pentynoate or sodium 5-hexynoate for different time length. At each time point, 4 mL of labeling cells were collected, spun down and frozen in liquid nitrogen. Negative control (dd H_2O) was carried out in a separate flask. The lysates were reacted with az-Rho and analyzed by in-gel fluorescence scanning.

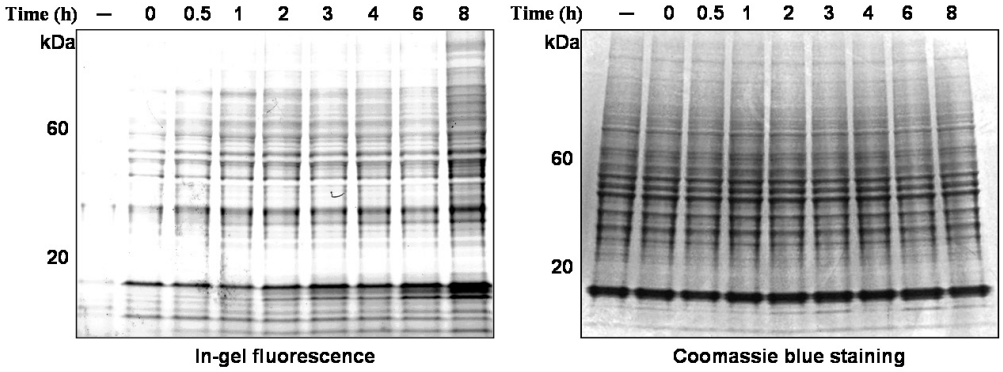


b

Sodium 3-butynoate



Sodium 4-pentynoate



Sodium 5-hexynoate

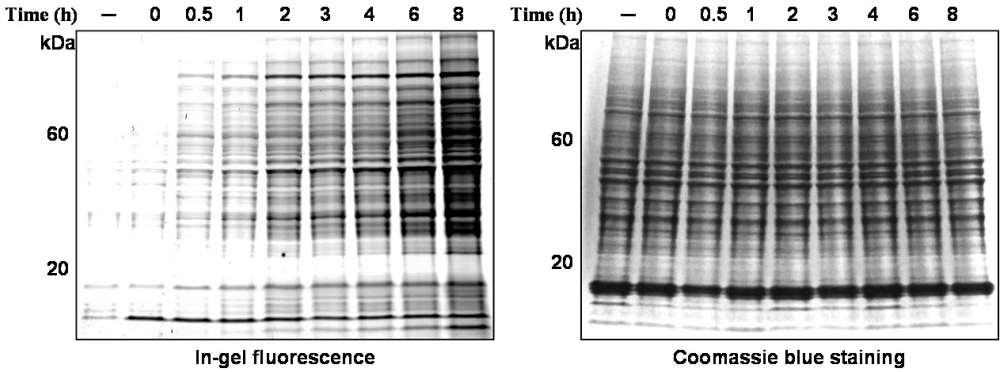
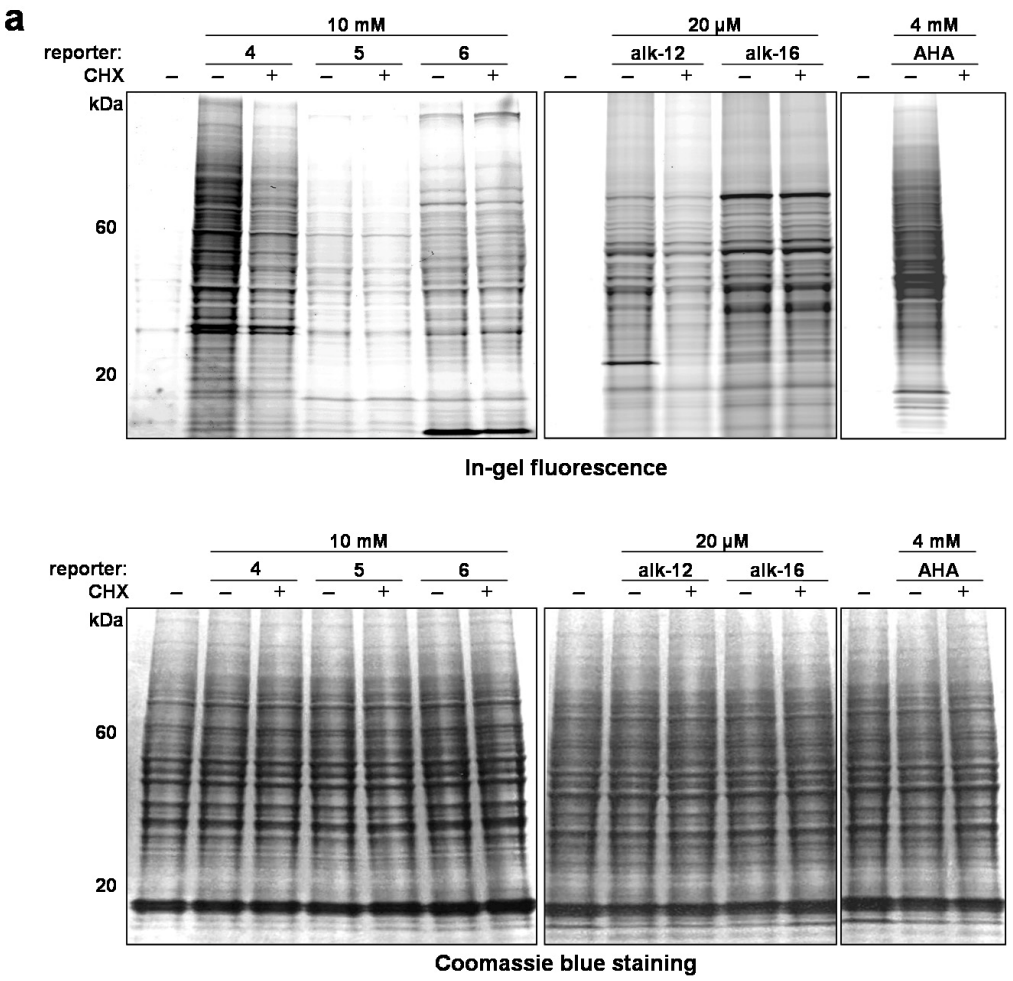


***Figure S5*. Specificity of sodium 3-butynoate (4), sodium 4-pentynoate (5) and sodium 5-hexynoate (6) metabolic labeling in mammalian cells**. (a) Inhibition of cellular protein biosynthesis by cycloheximide (CHX) in the presence of alkynyl-acetate analogs (10 mM), alkynyl-fatty-acid analogs (20 μM)[5] and AHA (azidohomoalanine, 4 mM)[12]. In this experiment, Jurkat T cells were either labeled with the chemical reporter alone for 1 h, or pre-treated with 100 μM CHX for 0.5 h and then labeled with the chemical reporter for additional 1 h. Cells labeled with AHA were cultured in HEPES-buffered saline (10 mM HEPES, 119 mM NaCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 5 mM KCl and 30 mM glucose)[12]. In the presence of 100 μM CHX, the incorporation of methionine surrogate, AHA, into proteins was completely blocked. As demonstrated in fatty-acylation by alk-12 (mainly target N-myristoylation) and alk-16 (mainly target cysteine palmitoylation), CHX diminished N-acylation, therefore, CHX can reveal the difference of labeling patterns between lysine acylation and N-terminal acylation labeled by alkynyl-acetate analogs in the leftmost figure. All the lysates were reacted with az-Rho and analyzed by in-gel fluorescence scanning. (b) Comparison of protein labeling profiles and labeling efficiencies among alkynyl-acetate analogs and alkynyl-fatty-acid analogs in Jurkat T cells. In this experiment, cells were labeled with 5 mM of sodium 3-butynoate, sodium 4-pentynoate and sodium 5-hexynoate and 20 μM of alk-12 and alk-16 for 6 h. The lysates were reacted with az-Rho and analyzed by in-gel fluorescence scanning. (c) Inhibition of HDACs by SAHA (suberoylanilide hydroxamic acid). SAHA induced changes in protein labeling profiles of total cell lysates and extracted core histones. Protein labeling levels were reduced due to the elevated acetylation levels. Jurkat T cells were either labeled with 10 mM alkynyl-acetate analog alone for 8 h, or co-incubated with 10 mM alkynyl-acetate analog as well as 10 μM SAHA for 8 h. The cell lysates and core histones were reacted with az-Rho, precipitated in MeOH at -80 °C overnight and analyzed by in-gel fluorescence scanning. SAHA was chemically synthesized by following the reported synthetic procedures[13]. The Western blot stained with anti-acetyl-Lys antibody showed the elevated acetylation level of histones in the presence of SAHA. (d) Inhibition of p300-dependent cellular labeling by 60 μM curcumin[14,15]. (Top) Protein labeling levels on core histones were reduced. (Bottom) Profiling total cell lysates showed many proteins have reduced labeling intensities. However, due to the cell toxicity of curcumin, non-specific protein labeling was also observed for sodium 3-butynoate (**4**). HeLa cells were either labeled with 10 mM alkynyl-acetate analogs alone for 4 h or pre-treated with 60 μM curcumin for 30 min then labeled with alkynyl-acetate analogs for additional 4 h. After metabolic labeling, HeLa cells were gently rinsed with ice-cold PBS before harvesting to remove those non-adherent cells. The cell lysates were reacted with az-Rho, precipitated in MeOH at -80 °C overnight and analyzed by in-gel fluorescence scanning. Although 100 μM curcumin can inhibit p300-mediated cellular labeling on histones more effectively than 60 μM curcumin[14,15], we observed significant toxicity to HeLa cells with this concentration.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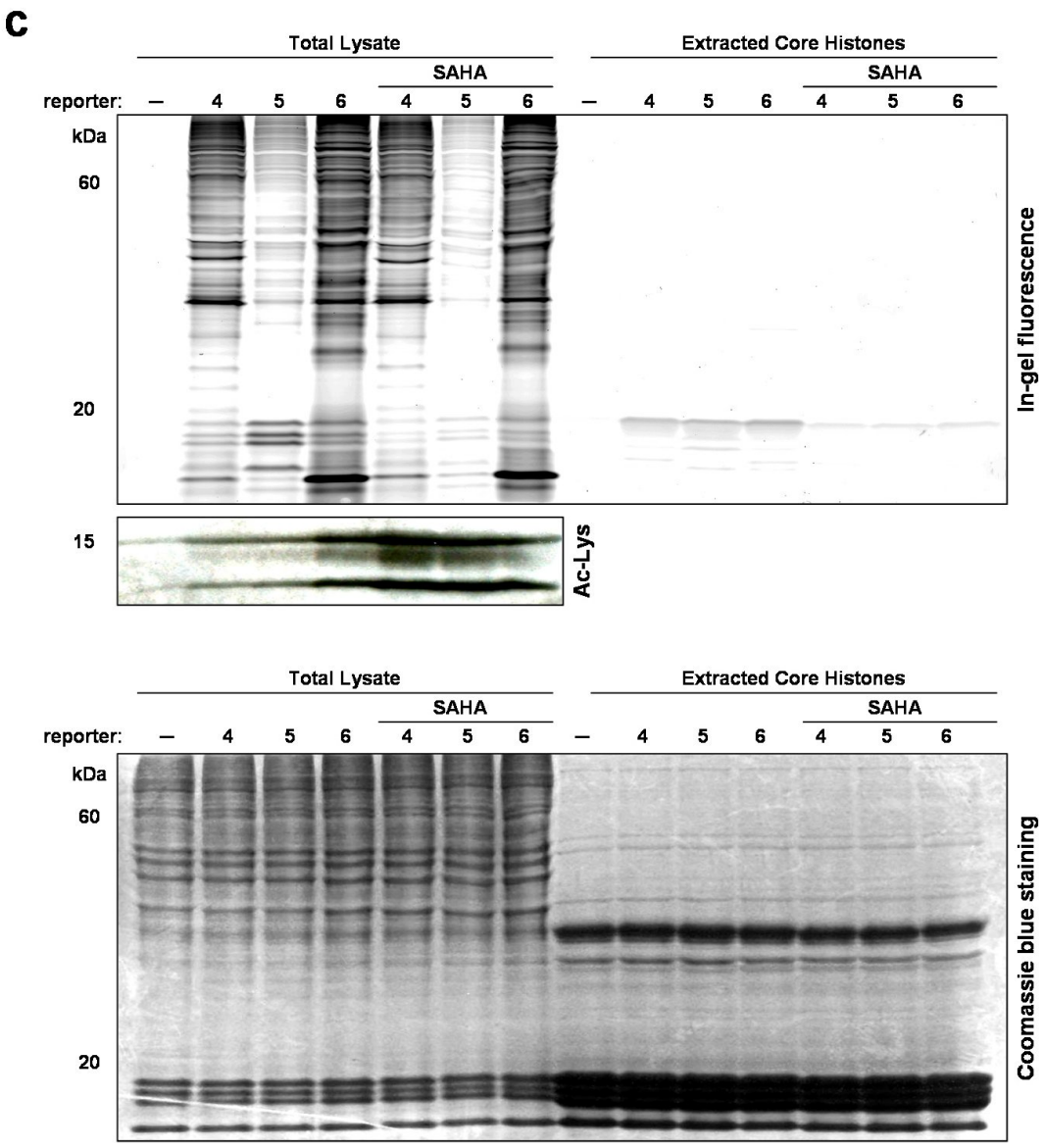
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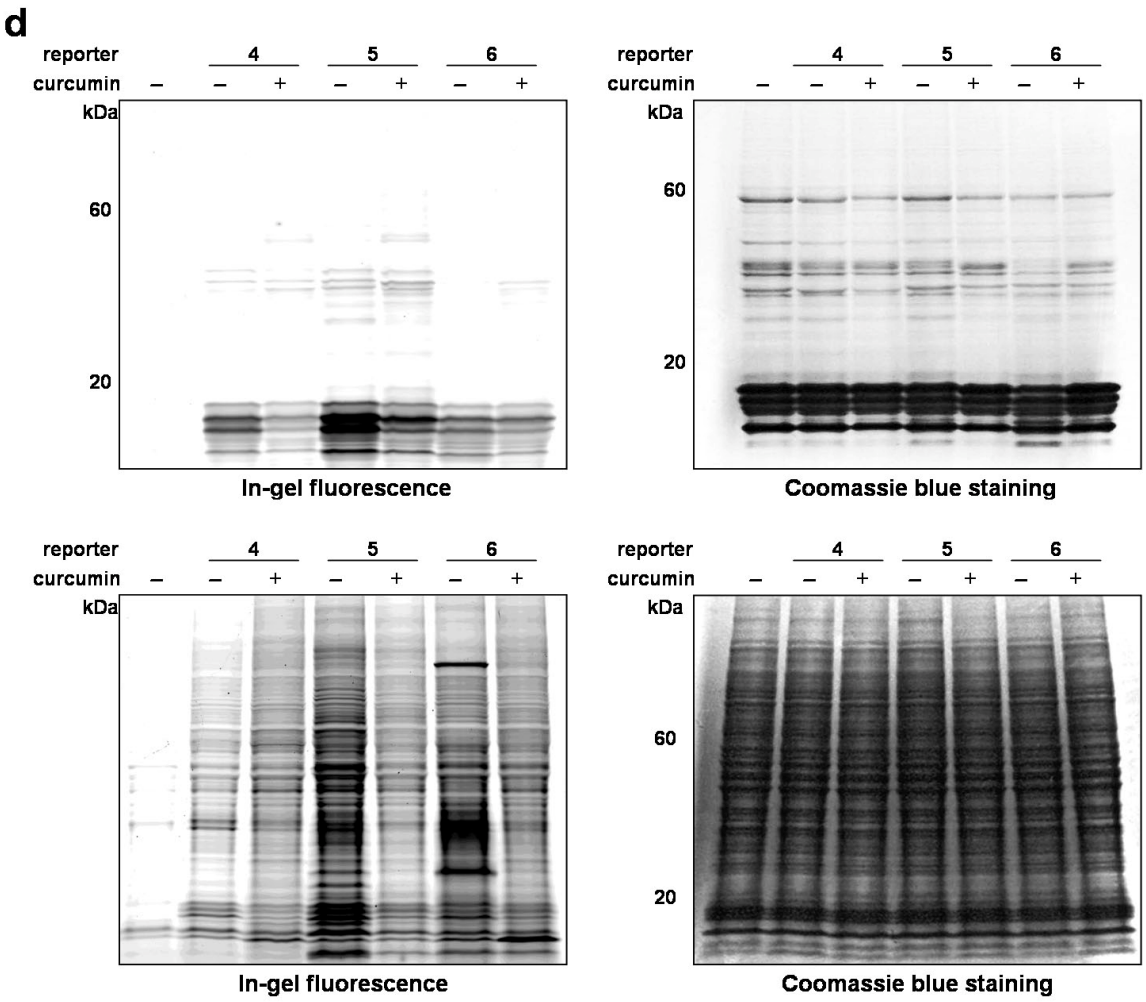
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***Figure S6*. In-gel fluorescence detection of acetylated proteins in different mammalian cell lines using alkynyl-acetate analogs.** ~50 µg of total cell lysates were reacted with az-Rho via CuAAC. All the different cell lines were labeled with 5 mM alkynyl-acetate analogs for 6 h.

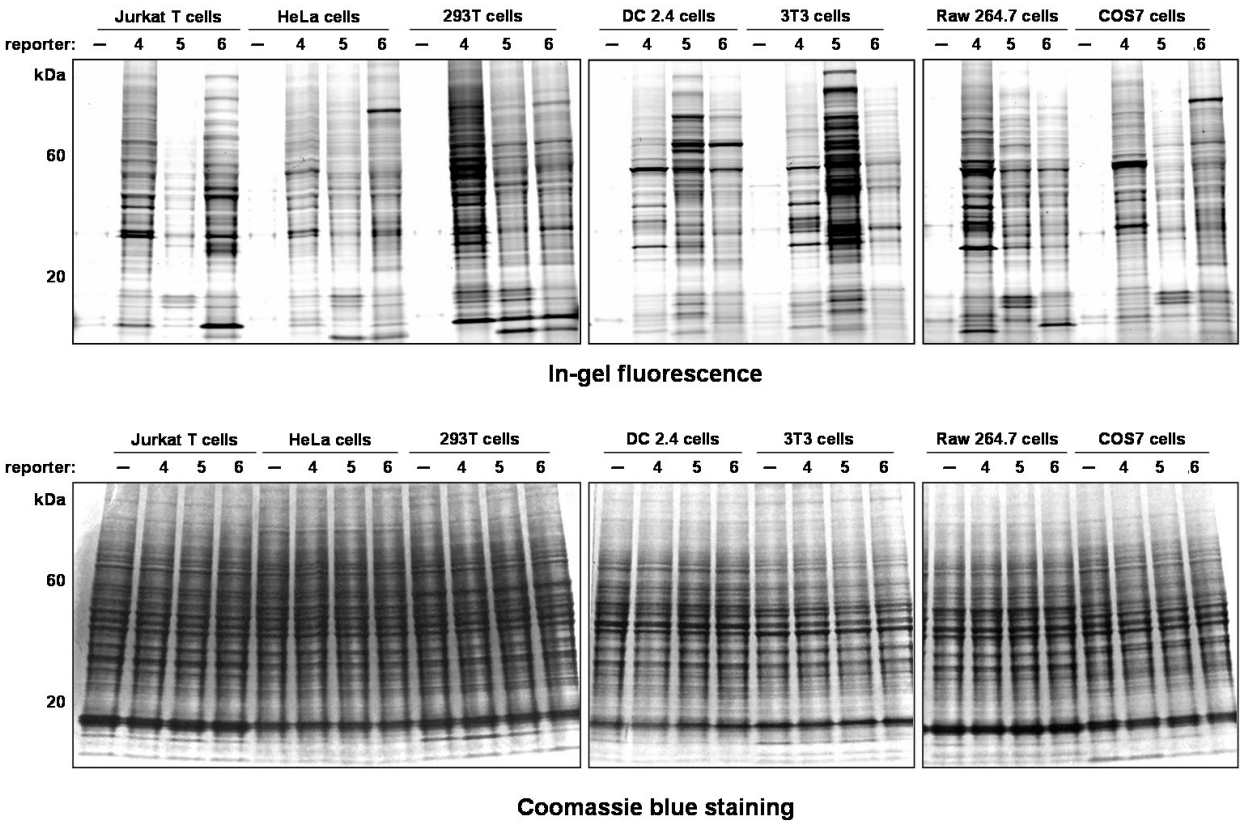
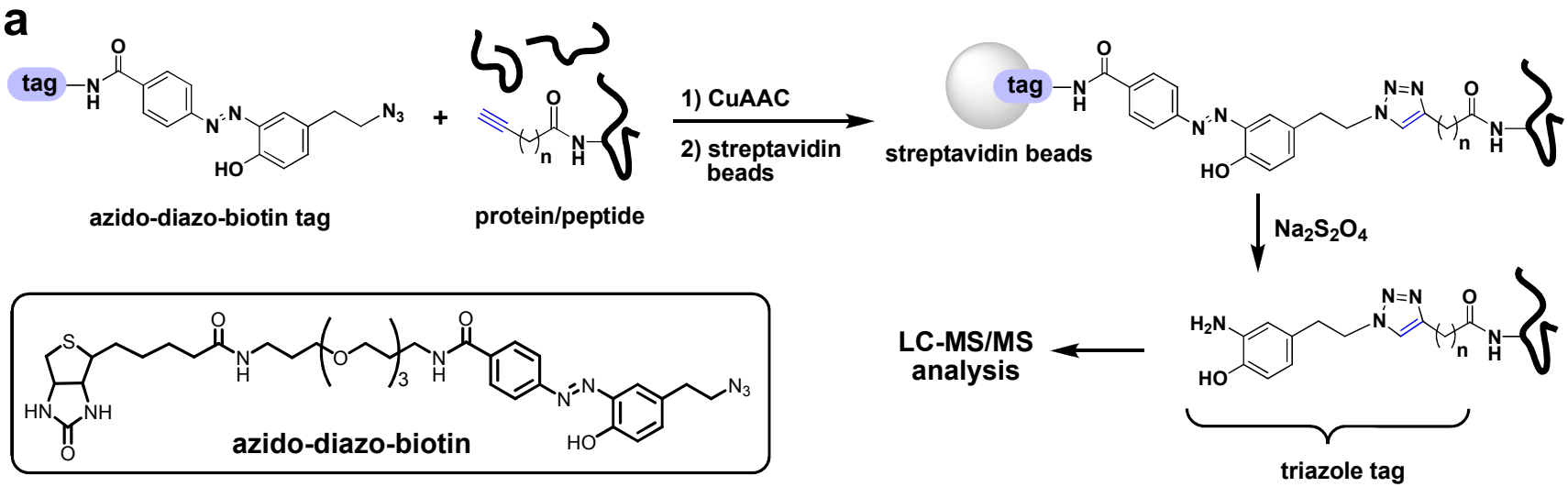
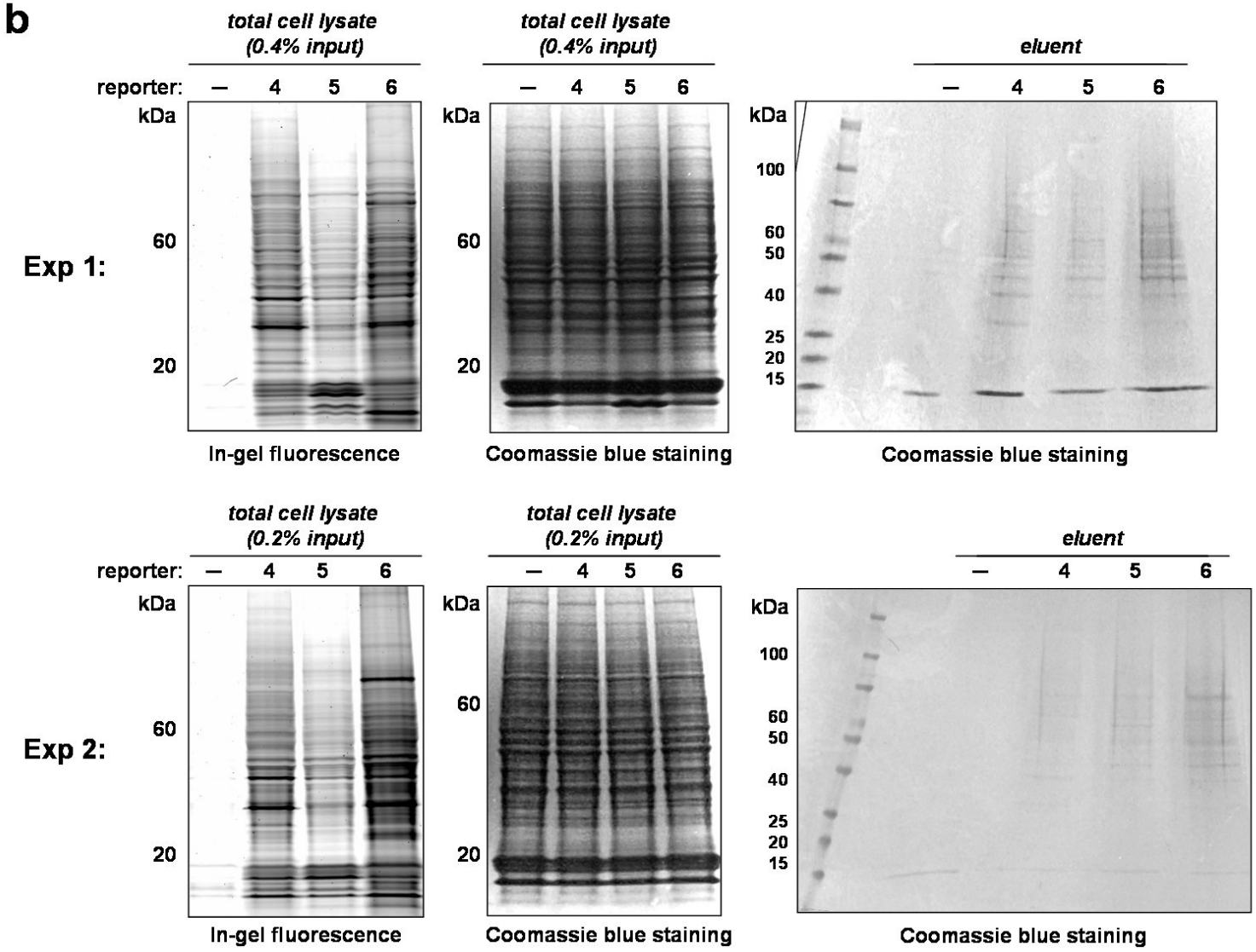


***Figure S7*. Proteomic analysis of sodium 3-butynoate (4)-, sodium 4-pentynoate (5)- and sodium 5-hexynoate (6)-metabolically labeled proteins in Jurkat T cells.** (a) Schematic of proteomic profiling of metabolically labeled proteins by employing the cleavable azido-diazo-biotin tag. (b) Profiles of enriched labeled proteins from two independent experiments. The in-gel fluorescence images (top and bottom panels) showed the metabolically-labeled protein profiles of Jurkat T cells, in which ~50 μg of total cell lysate were reacted with az-Rho via CuAAC. The coomassie blue-stained gels (top and bottom panels) showed the profiles of the eluted labeled proteins, in which the total cell lysates (12-20 mg) were reacted with azido-diazo-biotin via CuAAC followed by streptavidin-enrichment and $Na_2S_2O_4$-elution. (c) Confirming MS/MS-identified protein hits by Western blot analysis. Along with the input, an aliquot of the enriched protein eluent from control (dd H_2O) as well as from **4**, **5** and **6**-labeled cell lysates was loaded onto the gel, separated on SDS-PAGE and then transferred onto PVDF membrane. The proteins of interest were probed with their corresponding antibodies. (d) The Venn diagram shows the counts of unique and overlapped proteins labeled by different alkynyl-acetate analogs.

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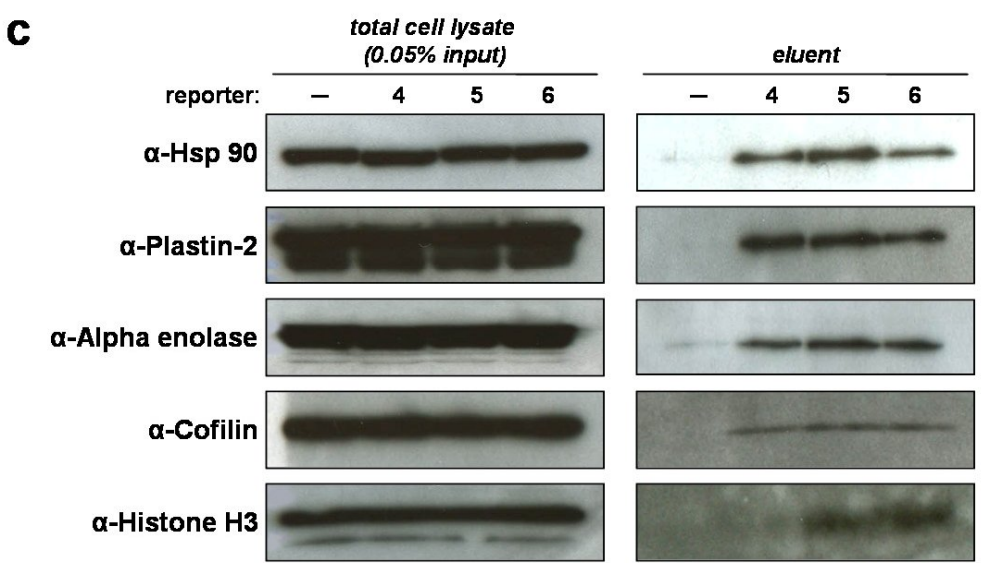
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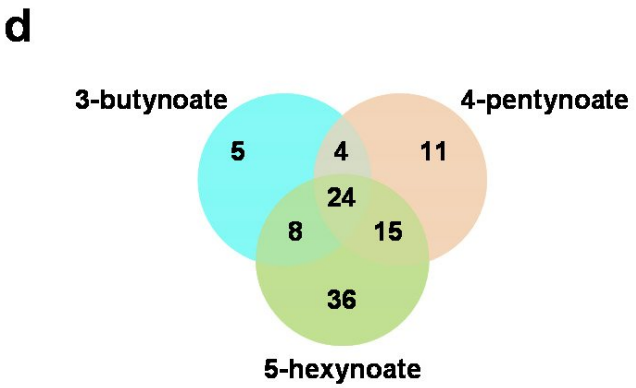
α-Histone H3


d
3-butynoate
4-pentynoate
5-hexynoate
5
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11
24
8
15
36

***Table S2*. Spectral count data of 3-butynoate (4)-, 4-pentynoate (5)- and 5-hexynoate (6)-metabolically labeled proteins derived from two independent proteomics experiments (Jurkat T cells).** Raw tandem mass spectra were searched against the human IPI protein database version 3.56 using SEQUEST search engine (Thermo Scientific). Cysteine carbamidomethylation was searched as fixed modification, while methionine/tryptophan oxidation, asparagines/glutamine deamindation, lysine acetylation and lysine 3-butynylation/4-pentynylation/5-hexynylation were searched as variable modifications. Each peptide spectrum must meet several selection thresholds including >95% for peptide identification probability, >1.0 for SEQUEST XCorr score, and $\pm$6 ppm for actual minus calculated peptide mass. Each protein listed has spectral count greater than or equal to 2, and the ratio of alkynyl-acetate analogs/control is greater than 3. The spectral counts from experiment #1 are colored in black, while the spectral counts from experiment #2 are colored in blue. The listing order of proteins is based on the molecular weight. This table also highlights those proteins that have been previously identified as lysine-acetylated proteins by Zhao and co-workers[16], Mann and co-workers[17] and Hake and co-workers[18]. Notably, the proteomic data reported by both Zhao *et al.* and Hake *et al*. were acquired from HeLa cells, while the proteomic data reported by Mann *et al.* were acquired from Jurkat T cells, A549 cells, MV4-11 cells. Note: dd H_2O was added to the cells as the negative control. The data of the negative control was not included in this table.

			Total spectral counts of peptides			Known acetylated proteins		
No	**Protein Name**	**Accession number**	**3-butynoate**	**4-pentynoate**	**5-hexynoate**	**Zhao**	**Mann**	**Hake**
1	Histone H4	IPI00453473	**31**	**45, 3**	**24, 5**	√	√	√
2	Histone H2A type 1-B/E	IPI00026272, IPI00031562, IPI00081836, IPI00216456, IPI00220855, IPI00255316, IPI00291764, IPI00552873, IPI00902514		**59**	**26**	√	√	√
3	Histone H2A type 2-C	IPI00339274		**2**	**26**	√	√	√
4	Histone H2B type 2-E	IPI00003935, IPI00152785, IPI00220403, IPI00515061	**3**	**6**	**5**	√		√
5	Histone H2B type 1-L	IPI00018534, IPI00020101, IPI00152906, IPI00303133, IPI00329665, IPI00419833, IPI00554798, IPI00646240, PI00794461, IPI00815755	**26**	**2**	**15**	√	√	√
6	Histone H3.1	IPI00465070	**2**	**4**	**3**	√	√	√

7	Histone H3.2	IPI00171611, IPI00219038, IPI00465070, IPI00909530	**10**	**5**	**8**	√		√
8	Histone H2A.x	IPI00219037		**11**		√	√	√
9	Profilin-1	IPI00216691		**3**	**3, 5**	√	√	√
10	Calcium-regulated heat stable protein 1	IPI00304409	**4**					
11	Peptidyl-prolyl cis-trans isomerase A	IPI00419585	**2**	**3**	**5, 4**		√	√
12	ubiquitin and ribosomal protein s27a precursor	IPI00179330, IPI00456429, IPI00719280	**3**	**2**			√	
13	Histone H2B type1-N	IPI00794461	**20**	**32**	**9**	√		√
14	Cofilin-1	IPI00012011	**2**		**2, 3**	√	√	√
15	Isoform 2 of DnaJ homolog subfamily C member 5	IPI00158847			**22**		√	
16	Protein DJ-1	IPI00298547	**24, 2**				√	
17	Peroxiredoxin-1	IPI00000874		**2**	**2**		√	
18	Glutathione S-transferase P	IPI00019755		**3**			√	
19	Isoform SNAP-23a of synaptosomal-associated protein 23	IPI00010438			**4**			
20	Rho GDP-dissociation inhibitor 2	IPI00003817	**4**	**6**	**5, 3**		√	
21	GTP-binding nuclear protein Ran	IPI00643041, IPI00795671		**2**			√	
22	GrpE protein homolog 1, mitochondrial	IPI00029557			**2**		√	
23	High mobility group protein 1-like 10	IPI00018755, IPI00419258	**2**	**15, 2**	**4**		√	
24	Highly mobility group protein B1	IPI00419258	**16**	**19**	**9**	√	√	√
25	Isoform 1 of deoxyuridine 5'-triphosphate nucleotidohydrolase, mitochondrial	IPI00013679, IPI00375015, IPI00749113			**3**		√	
26	14-3-3 proein zeta/delta	IPI00021263		**6, 2**	**12, 6**		√	
27	Glutathione S-transferase omega-1	IPI00019755	**139, 8**		**25, 8**		√	
28	Isoform Long of ES1 protein homolog, mitochondrial	IPI00024913, IPI00218482			**2**		√	
29	Thioredoxin-dependent peroxide reductase, mitochondrial	IPI00024919, IPI00374151			**2**		√	
30	28 kDa protein (Isoform B1 of heterogeneous nuclear ribonucleoproteins A2/B1)	IPI00396378, IPI00414696, IPI00874030	**10**	**8**	**14**		√	

31	14-3-3 proein epsilon	IPI00000816			**2**		√	
32	29 kDa protein	IPI00453476, IPI00549725		**3**			√	
33	Transcription factor A, mitochondrial	IPI00020928		**2**				
34	Isoform 2 of tropomyosin alpha-3 chain	IPI00218319, IPI00218320, IPI00382894, IPI00479185, IPI00477649, IPI00854592, IPI00909961		**2**	**3**		√	
35	Isoform 1 of triosephosphate isomerase	IPI00465028		**10**	**12**	√	√	
36	S-formylglutathione hydrolase	IPI00411706	**2**				√	
37	Isoform C1 of heterogeneous nucleaer ribonucleoproteins C1/C2	IPI00216592, IPI00477313			**3**		√	
38	Thioredoxin domain-containing protein 1	IPI00395887			**3**			
39	Isoform 1 of nucleophosmin	IPI00549248, IPI00658013		**5**		√	√	√
40	Glyceraldehyde-3-phosphate dehydrogenase	IPI00219018	**207, 12**	**71**	**238**		√	√
41	L-lactate dehydrogenase B chain	IPI00219217	**4**		**33, 6**		√	
42	Poly(rC)-binding protein 1	IPI00016610		**2**			√	
43	Bifunctional methylenetetrahydrofolate dehydrogenase/cyclohydrolase, mitochondrial	IPI00396378, IPI00414696, IPI00874030			**3, 5**		√	
44	Delta-aminolevulinic acid dehydratase	IPI00442121, IPI00790373			**2**			
45	Isoform A1-B of heterogeneous nuclear ribonucleoprotein A1	IPI00215965		**3**	**3**		√	√
46	Fructose-bisphosphate aldolase A	IPI00465439		**2**	**2**		√	
47	39 kDa protein	IPI00916260		**2**				
48	cDNA FLJ55792, highly similar to L-lactate dehydrogenase A chain	IPI00217966, IPI00607708, IPI00908791			**11, 3**		√	
49	Isoform 1 of isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial	IPI00030702, IPI00792971, IPI00909577, IPI00921820			**2**		√	
50	Adenosine deaminase	IPI00296441, IPI00872639	**2, 2**		**3**		√	
51	Actin, cytoplasmic 1	IPI00021439, IPI00021440, IPI00894498	**148**	**126**	**218**		√	√

52	Galphai2 protein	IPI00465121, IPI00748145			**8**			
53	Flap endonuclease 1	IPI00026215		**2**			√	
54	Guanine nucleotide binding protein (G protein), q polypeptide, isoform CRA_c	IPI00288947			**3**		√	
55	cDNA FLJ53975, highly similar to acetyl-CoA acetyltransferase, cytosolic	IPI00291419			**3**		√	
56	Phosphoglycerate kinase 1	IPI00169383	**9**	**12**	**39**		√	
57	Transcription Termination Factor, mitochondrial	IPI00016095			**101**			
58	Isoform alpha-enolase of Alpha-enolase	IPI00465248	**17**	**8**	**31**	√	√	
59	Heterogenous nuclear ribonucleoprotein H	IPI00013881	**3**		**5**		√	
60	Elongation factor 1-alpha 2	IPI00014424			**2**	√	√	√
61	Tubulin beta chain	IPI00011654	**34**	**42**	**94**		√	
62	Tubulin alpha-1C chain	IPI00218343	**64**	**46**	**103**		√	
63	Coronin-1A	IPI00010133	**13, 4**	**7**	**12**		√	
64	Isoform 1 of Heterogeneous nuclear ribonucleoprotein K	IPI00216049, IPI00216746, IPI00807545	**2**	**2**				
65	Trifunctional enzyme subunit beta, mitochondrial	IPI00022793			**50, 9**		√	
66	Isocitrate dehydrogenase [NADP], mitochondrial	IPI00011107		**7**	**36, 8**		√	
67	cDNA FLJ54554, highly similar to pyruvate kinase isozymes M1/M2	IPI00910979	**2**		**19**		√	
68	Protein RCC2	IPI00465044	**2**	**2**			√	
69	Serine hydroxymethyltransferase, mitochondrial	IPI00002520, IPI00748411, IPI00794549, IPI00794572, IPI00908824, IPI00909681			**2**		√	
70	59 kDa protein	IPI00302925, IPI00784090			**2**		√	
71	Isoform 3 of proto-oncogene tyrosine protein kinase LCK	IPI00515097			**19, 9**		√	
72	60 kDa heat shock protein, mitochondrial	IPI00784154	**3**	**3, 3**	**22, 8**		√	

73	Glucose-6-phosphate-isomerase	IPI00027497, IPI00908881, IPI00910781			**2**		√	
74	heterogeneous nuclear ribonucleoprotein L	IPI00027834			**4**		√	
75	Isoform 1 of Acyl-CoA synthetase family member 3, mitochondrial	IPI00166395, IPI00645533			**4, 4**		√	
76	NAD-dependent malic enzyme, mitochondrial	IPI00011201			**4**		√	
77	Lamin-B1	IPI00217975, IPI00790831			**2**		√	
78	Acyl-CoA synthetase family member 2, mitochondrial	IPI00304071		**47, 6**	**3**		√	
79	Acyl-CoA dehydrogenase family member 9, mitochondrial	IPI00152981, IPI00790834			**6**		√	
80	cDNA FLJ54957, highly similar to transketolase	IPI00643920, IPI00792641, IPI00793119			**2**	√	√	√
81	Plastin-2	IPI00010471	**22, 5**	**3**	**4, 6**		√	
82	ATP-dependent DNA helicase 2 subunit 1	IPI00644712	**2**	**2**	**2, 4**		√	√
83	cDNA FLJ51819, weakly similar ot long-chain fatty acid-CoA ligase, mitochondrial	IPI00304071, IPI00909579		**45**			√	
84	cDNA FLJ55574, highly similar to Calnexin	IPI00020984			**24, 11**		√	
85	Nucleolin	IPI00604620	**2**	**8**			√	
86	ATP-dependent DNA helicase 2 subunit 2	IPI00220834, IPI00871391	**2**	**2**	**4**		√	
87	Heat shock protein HSP 90-beta	IPI00414676	**6, 3**	**2, 3**	**16, 6**	√	√	√
88	Transferrin receptor protein 1	IPI00022462		**2**	**12**			
89	Isoform long of delta-1-pyrroline-5-carboxylate synthetase	IPI00008982, IPI00218547			**3**			
90	Endoplasmin	IPI00027230			**2**			
91	Heat shock 70kDa protein 4	IPI00002966	**2**		**2**		√	
92	Elongation factor 2	IPI00186290			**7**		√	
93	cDNA FLJ55599, highly similar to DNA replication licensing factor MCM3	IPI00013214	**2**				√	
94	Poly [ADP-ribose] polymerase 1	IPI00449049	**2**	**3**	**2**		√	

95	Ubiquitin-like modifier-activating enzyme 1	IPI00645078		**2**	**2**		√	
96	Leucine-rich PPR motif-containing protein, mitochondrial	IPI00783271			**4**		√	
97	DNA topoisomerase 2 (Fragment)	IPI00178667, IPI00218753, IPI00414101, IPI00879004	**2**				√	
98	Isoform 1 of clathrin heavy chain 1	IPI00024067, IPI00455383			**2**		√	
99	Isoform 2 of Nebulin-related anchoring protein	IPI00398162, IPI00478974, IPI00745227, IPI00786896		**15**				
100	Isoform 1 of myosin-9	IPI00019502	**10**		**9**		√	
101	Fatty acid synthetase	IPI00026781		**2**	**14**		√	
102	Cation-independent mannose-6-phosphate receptor	IPI00289819, IPI00903213			**2**		√	
103	Isoform 2 of filamin-A	IPI00302592, IPI00333541, IPI00644576, IPI00909642		**3**	**6**		√	
	Total protein numbers		**41**	**54**	**83**			

% of 3-butynoate-labeled known acetylated proteins/total 3-butynoate-labeled proteins (39/41) = 95.1%
% of 4-pentynoate-labeled known acetylated proteins/total 4-pentynoate-labeled proteins (48/54) = 88.9%
% of 5-hexynoate-labeled known acetylated proteins/total 5-hexynoate-labeled proteins (75/83) = 90.4%

***Figure S8*. Proteomic analysis of sodium 4-pentynoate (5)-metabolically labeled proteins (Jurkat T cells).** (a) Profiles of enriched labeled proteins. The in-gel fluorescence image (left) showed the protein-labeling profiles in Jurkat T cells, in which ~50 μg of total cell lysate were reacted with az-Rho via CuAAC. The coomassie blue-stained gels (right) showed the profiles of the eluted labeled proteins, in which the total cell lysates (25 mg) were reacted with azido-diazo-biotin via CuAAC followed by streptavidin-enrichment and $Na_2S_2O_4$-elution. (b) Confirming MS/MS-identified protein hits by Western blot analysis. Along with the input, an aliquot of the enriched protein eluent from control (dd H_2O) as well as from **5**-labeled cell lysates was loaded onto the gel, separated on SDS-PAGE and then transferred onto PVDF membrane. The proteins of interest were probed with their corresponding antibodies. (c) Pie charts show the distribution of biological functions and subcellular locations of 4-pentynoate-labeled proteins.

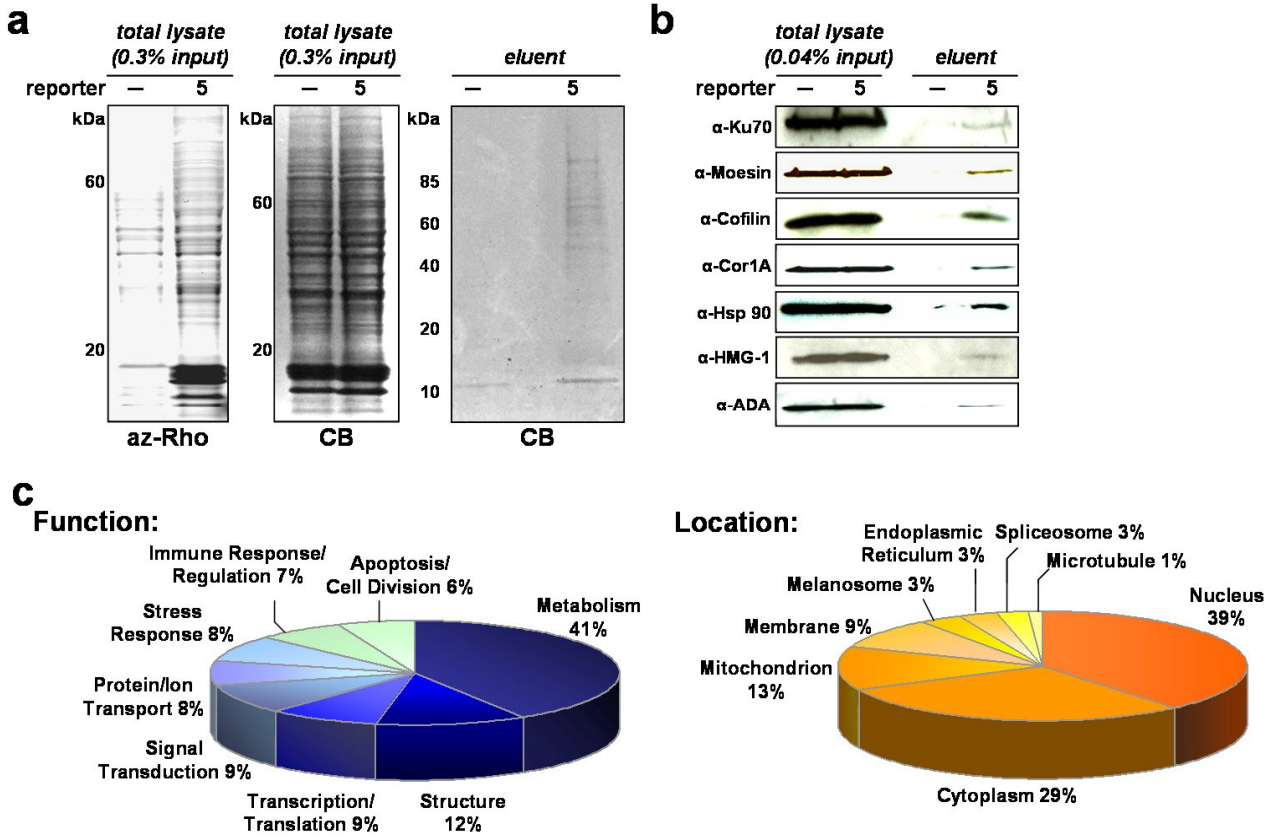


***Table S3*. Spectral count data of 4-pentynoate (5)-metabolically labeled proteins (Jurkat T cells).** As the stock solution of sodium 4-pentynoate was prepared in sterile dd H_2O, we added the same volume of dd H_2O into cells as a negative control. Raw tandem mass spectra were searched against the human IPI protein database version 3.56 using SEQUEST search engine (Thermo Scientific). Cysteine carbamidomethylation was searched as fixed modification, while methionine/tryptophan oxidation, asparagines/glutamine deamindation, lysine acetylation and lysine 4-pentynylation were searched as variable modifications. Each peptide spectrum must meet several selection thresholds including >95% for peptide identification probability, >1.0 for SEQUEST XCorr score, and $\pm$6 ppm for actual minus calculated peptide mass. Each protein listed here has spectral count greater than or equal to 2, and the ratio of 4-pentynoate/control is greater than 3. The listing order of proteins is based on the molecular weight. This table also highlights those proteins that have been previously identified as lysine-acetylated proteins by Zhao and co-workers[16], Mann and co-workers[17] and Hake and co-workers[18]. Notably, the proteomic data reported by both Zhao *et al.* and Hake *et al*. were acquired from HeLa cells, while the proteomic data reported by Mann *et al.* were acquired from Jurkat T cells, A549 cells, MV4-11 cells.

			Total spectral counts of peptides				
No	**Protein Name**	**Accession Number**	**Neg**	**4-pentynoate**	**Zhao**	**Mann**	**Hake**
1	Histone H4	IPI00453473	**18**	**297**	√	√	√
2	Histone H2A type 1-H	IPI00081836, IPI00216457, IPI00220855, IPI00255316, IPI00291764, IPI00339274, IPI00552873	**33**	**187**	√	√	√
3	Histone H2A.V	IPI00018278, IPI00218448	**0**	**14**	√	√	√
4	Hisotne H2B type 1-L	IPI00018534, IPI00020101, IPI00152906, IPI00303133, IPI00329665, IPI00419833, IPI00554798, IPI00646240, IPI00794461, IPI00815755	**0**	**184**	√	√	√
5	Hisotne H2B type 2-E	IPI00003935, IPI00152785, IPI00220403, IPI00515061	**0**	**14**	√	√	√
6	Isoform 2 of PEST proteolytic siganl-containing nuclear protein	IPI00060650, IPI00291608, IPI00796456	**0**	**5**	√	√	√
7	Activated RNA polymerase II transcriptional bcoactivator p15	IPI00221222	**0**	**5**		√	
8	Histone H3.1	IPI00465070	**0**	**22**		√	

9	Fatty acid-binding protein, epidermal	IPI00007797	**0**	**4**		√	
10	Acyl carrier protein, mitochondrial	IPI00022442	**0**	**5**		√	
11	Stathmin	IPI00479997, IPI00921996	**0**	**11**		√	
12	ubiquitin and ribosomal protein S27a precursor	IPI00179330, IPI00456429, IPI00719280	**0**	**26**		√	
13	Cofilin-1	IPI00012011	**0**	**9**	√	√	√
14	Ras-related C3 botulinum toxin substrate 2	IPI00010270, IPI00877663, IPI00878066	**0**	**4**		√	
15	Chromobox protein homolog 3	IPI00297579	**0**	**27**		√	
16	39S ribosomal protein L12, mitochondrial	IPI00005537	**0**	**4**			
17	Peroxiredoxin-1	IPI00000874, IPI00640741	**0**	**4**		√	
18	Histone H1.4	IPI00217467	**0**	**30**		√	
19	Histone H1x	IPI00021924	**0**	**8**		√	
20	Hisotne H1.5	IPI00217468	**0**	**31**		√	
21	Isoform 1 of Ras-related protein Rab-1A	IPI00005719, IPI00334174, IPI00873902, IPI00917079, IPI00922662	**0**	**3**			
22	Glutathione S-transferase P	IPI00219757	**0**	**27**		√	
23	Rho GDP-dissociated inhibitor	IPI00003817, IPI00792758	**0**	**12**		√	
24	GTP-binding nuclear protein Ran	IPI00643041, IPI00792352, IPI00795671, IPI00796462	**0**	**8**		√	
25	High mobility group protein B2	IPI00219097	**0**	**14**		√	
26	High mobility group protein B1	IPI00419258	**4**	**85**	√	√	√
27	Isoform 1 of Nuclear ubiquitous casein and cyclin-dependent kinases substrate	IPI00022145, IPI00872944	**0**	**12**			
28	Isoform 1 of 3-hydroxyacyl-CoA dehydrogenase type-2	IPI00017726	**0**	**6**			
29	Isoform 1 of deoxyuridine 5'-triphophate nucleotidohydrolase, mitochondrial	IPI00013679, IPI00375015, IPI00749113, IPI00793322	**0**	**90**		√	
30	14-3-3 protein zeta/delta	IPI00021263, IPI00879359	**0**	**3**		√	

31	Glutathione S-transferase omega-1	IPI00019755	**0**	**49**		√	
32	Thioredoxin-dependent peroxide reductase, mitochondrial	IPI00024919, IPI00374151	**0**	**3**		√	
33	Proteasome activator complex subunit 1	IPI00479722	**0**	**26**			
34	Isoform 1 of Acidic leucine-rich nuclear phosphoprotein 32 family member B	IPI00007423, IPI00759824	**0**	**7**		√	
35	29 kDa	IPI00453476, IPI00549725	**0**	**2**		√	
36	Putative uncharacterized protein PSME2	IPI00384051, IPI00746205	**0**	**3**			
37	14-3-3 protein epsilon	IPI00000816	**0**	**5**		√	
38	Isoform 1 of Triosephosphate isomerase	IPI00465028	**0**	**2**	√	√	
39	Complement component 1 Q subcomponent-binding protein, mitochondrial	IPI00014230	**0**	**4**		√	
40	Isoform 3 of Voltage-dependent anion-selective channel protein 2	IPI00216026, IPI00455531, IPI00737171, IPI00917420	**0**	**6**		√	
41	Isoform C1 of Heterogeneous nuclear ribonucleoproteins C1/C2	IPI00216592, IPI00909232	**0**	**10**		√	
42	Isoform 1 of Protein SET	IPI00072377, IPI00301311, IPI00844040, IPI00896523, IPI00917753	**0**	**29**		√	√
43	Isoform 1 of Nucleophosmin	IPI00549248	**2**	**33**	√	√	√
44	tropomyosin 3 isoform 1	IPI00183968, IPI00218319, IPI00218320, IPI00382894, IPI00477649, IPI00479185, IPI00642042	**0**	**8**	√	√	√
45	ADP/ATP translocase 2	IPI00007188	**0**	**5**		√	
46	MACRO domain-containing protein 1	IPI00155601	**0**	**2**		√	
47	DNA-(apurinic or apyrimidinic site) lyase	IPI00215911	**0**	**4**		√	
48	cDNA FLJ60094, highly similar to F-actin capping protein subunit beta	IPI00218782, IPI00641107, IPI00642256	**0**	**4**		√	

49	Isoform B1 of heterogeneous nuclear ribonucleoproteins A2/B1	IPI00396378, IPI00414696, IPI 00874030, IPI00916517	**4**	**18**		√	
50	Poly(rC)-binding protein 1	IPI00016610	**0**	**6**		√	
51	Isoform 1 of Prostaglandin reductase 2	IPI00167515	**0**	**33**			
52	Isoform 1 of Heterogeneous nuclear ribonucleoprotein D0	IPI00028888, IPI00220683, IPI00220684, IPI00220685, IPI00903278, IPI00915340	**0**	**9**		√	
53	H2A histone family, member Y isoform 2	IPI00059366, IPI00304171, IPI00744148	**0**	**13**			
54	Annexin A1	IPI00218918, IPI00549413	**0**	**4**		√	
55	Isoform A1-B of Heterogeneous nuclear ribonucleoprotein A1	IPI00215965, IPI00465365, IPI00797148	**3**	**19**		√	√
56	Isoform 1 of Heterogeneous nuclear ribonucleoprotein A3	IPI00419373, IPI00455134, IPI00909208	**0**	**2**		√	
57	Adenosine deaminase	IPI00296441, IPI00872639	**0**	**14**		√	
58	Synaptic vesicle membrane protein VAT-1 homolog	IPI00156689, IPI00184402, IPI00789469, IPI00797605	**0**	**4**			
59	Galphai2 protein	IPI00465121, IPI00748145	**0**	**4**			
60	Heterogeneous nuclear ribonucleoprotein G	IPI00304692, IPI00816796	**0**	**3**		√	
61	3-ketoacyl-CoA thiolase, mitochondrial	IPI00001539	**0**	**22**		√	
62	Flap endonuclease 1	IPI00026215	**0**	**7**		√	
63	Protein DEK	IPI00020021, IPI00871695	**0**	**7**		√	
64	cDNA FLJ59403, highly similar to Nucleosome assembly protein 1-like-4	IPI00017763, IPI00789437, IPI00798071	**0**	**3**			
65	Isoform 1 of Plasminogen activator inhibitor 1 RNA-binding protein	IPI00410693, IPI00412714, IPI00470497, IPI00470498	**0**	**3**		√	
66	Heterogeneous nuclear ribonucleoprotein F	IPI00003881	**0**	**5**		√	
67	Lupus La protein	IPI00009032, IPI00916802	**0**	**19**		√	
68	Calreticulin	IPI00020599	**4**	**14**		√	

69	Isoform 1 of Spliceosome RNA helicase BAT1	IPI00848161	**0**	**2**		√	
70	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial	IPI00420108	**0**	**9**		√	
71	Heterogeneous nuclear ribonucleoprotein H	IPI00013881, IPI00479191	**4**	**24**		√	
72	Elongation factor 1-alpha1	IPI00396485, IPI00472724	**9**	**41**		√	
73	Multifunctional protein ADE2	IPI00217223	**0**	**15**		√	
74	Histone acetyltransferase type B catalytic subunit	IPI00024719, IPI00651621	**0**	**3**			
75	cDNA FLJ53368, highly similar to Pyruvate kinase isozymes M1/M2	IPI00847989	**0**	**14**		√	
76	Tubulin beta-2C chain	IPI00007752	**0**	**8**		√	
77	isoform Beta of Lamina-associated polypeptide 2, isoforms beta/gamma	IPI00030131	**0**	**21**		√	
78	Trifunctional enzyme subunit beta, mitochondrial	IPI00022793, IPI00893108	**0**	**7**		√	
79	Isocitrate dehydrogenase [NADP], mitochondrial	IPI00011107	**4**	**34**		√	
80	Isoform 1 of Heterogeneous nuclear ribonucleoprotein K	IPI00216049, IPI00216746, IPI00807545, IPI00910458	**2**	**51**			
81	Coronin-1A	IPI00010133	**8**	**46**		√	
82	Calcium/calmodulin-dependent protein kinase type IV	IPI00430411	**0**	**5**		√	
83	6-phosphogluconate dehydrogenase, decarboxylating	IPI00219525, IPI00747533	**0**	**2**		√	
84	Isoform 2 of Protein disulfide-isomerase A6	IPI00299571, IPI00644989	**0**	**4**			
85	Dihydrolipoyl dehydrogenase, mitochondrial	IPI00015911, IPI00909143	**0**	**7**		√	
86	Non-POU domain-containing octamer-binding protein	IPI00304596	**0**	**20**		√	

87	Histone deacetylase 1	IPI00013774	**0**	**6**		√	
88	Serine hydroxymethyltransferase, mitochondrial	IPI00002520	**0**	**11**		√	
89	Protein RCC2	IPI00465044	**0**	**15**		√	
90	Inosine-5'-monophosphate dehydrogenase 2	IPI00291510	**0**	**14**		√	
91	cDNA FLJ56389, highly similar to Elongation factor 1-gamma	IPI00000875, IPI00738381, IPI00747497	**0**	**4**		√	
92	ATP synthase subunit beta, mitochondrial	IPI00303476	**0**	**7**		√	
93	Isoform 1 of Polypyrimidine tract-binding protein 1	IPI00179964, IPI00183626, IPI00334175	**0**	**10**		√	
94	D-3-phosphoglycerate dehydrogenase	IPI00011200, IPI00642548	**0**	**2**			
95	T-complex protein 1 subunit beta	IPI00297779	**0**	**13**		√	
96	Methylmalonate-semialdehyde dehydrogenase [acylating], mitochondria	IPI00024990	**0**	**11**			
97	Isoform M1 of Pyruvate kinase isozymes M1/M2	IPI00220644, IPI00479186	**18**	**115**		√	
98	Serine/threonine-protein kinase PAK 2	IPI00419979	**0**	**3**		√	
99	T-complex protein 1 subunit zeta	IPI00027626	**0**	**6**		√	
100	T-complex protein 1 subunit delta	IPI00302927, IPI00873222, IPI00893358, IPI00921414	**0**	**5**		√	
101	T-complex protein 1 subunit eta	IPI00018465	**0**	**5**		√	
102	Protein phosphatase 1G	IPI00006167	**0**	**2**		√	
103	Splicing factor 3A subunit 3	IPI00029764	**0**	**17**		√	
104	T-complex protein 1 subunit epsilon	IPI00010720, IPI00910316	**0**	**5**		√	
105	ATP synthase subunit alpha, mitochondrial	IPI00440493	**3**	**29**		√	
106	Isoform 1 of PC4 and SFRS1-interacting protein	IPI00028122	**0**	**9**			

107	cDNA FLJ56184, highly similar to Proto-oncogene tyrosine-protein kinase LCK	IPI00394952, IPI00515097	**0**	**9**		√	
108	T-complex protein subunit alpha	IPI00290566	**0**	**16**	√	√	
109	T-complex protein 1 subunit theta	IPI00784090	**0**	**23**		√	
110	60 kDa heat shock protein, mitochondiral	IPI00784154	**0**	**77**		√	
111	Isoform 1 of Heterochromatin protein 1-binding protein 3	IPI00642238	**0**	**2**		√	
112	Stress-induced-phosphoprotein 1	IPI00013894, IPI00871856	**0**	**6**		√	
113	Glucose-6-phosphate isomerase	IPI00027497, IPI00908881, IPI00910781	**0**	**16**		√	
114	Isoform 1 of Acyl-CoA syntetase family member 3, mitochondrial	IPI00166395, IPI00645533	**0**	**15**		√	
115	Heterogeneous nuclear ribonucleoprotein L	IPI00027834	**0**	**9**		√	
116	Isoform 2 of ATPase family AAA domain-containing protein 3A	IPI00295992, IPI00646144	**0**	**2**		√	
117	Lamin-B1	IPI00217975	**0**	**7**		√	
118	Isoform 1 of Poly(A) RNA polymerase, mitochondrial	IPI00153051, IPI00470416	**0**	**3**		√	
119	Moesin	IPI00219365, IPI00872814	**0**	**6**	√	√	
120	Acyl-CoA synthetase familiy member 2, mitochondiral	IPI00304071, IPI00909579	**0**	**221**		√	
121	Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial	IPI00021338	**0**	**16**		√	
122	Acyl-CoA dehydrogenase family member 9, mitochondrial	IPI00152981, IPI00790834	**0**	**3**		√	
123	Probable ATP-dependent RNA helicase DDX5	IPI00017617	**0**	**3**		√	
124	Plastin-2	IPI00010471	**22**	**181**		√	

125	ATP-dependent DNA helicase 2 subunit 1	IPI00644712	**3**	**15**		√	√
126	Isoform 1 of heat shock cognate 71 kDa protein	IPI00003865	**4**	**21**		√	
127	cDNA FLJ55574, highly similar to Calnexin	IPI00020984	**0**	**4**		√	
128	HSPA5 protein	IPI00003362	**0**	**10**		√	
129	Dolichyl-diphosphooligosaccharide-protein glycosyltransferase subunit 1 precursor	IPI00025874	**0**	**5**		√	
130	Stress-70 protein, mitochondrial	IPI00007765	**0**	**36**		√	
131	annexin VI isoform 2	IPI00002459, IPI00221226	**0**	**8**		√	
132	Isoform Beta of Nucleolar phosphoprotein p130	IPI00216654, IPI00292387, IPI00908873	**0**	**11**		√	
133	Isoform 5 of Interleukin enhancer-binding factor 3	IPI00219330, IPI00298788, IPI00298789, IPI00414335, IPI00418313, IPI00556173	**0**	**2**		√	
134	Nucleolin	IPI00604620	**0**	**307**		√	
135	Isoform 1 of Heterogeneous nuclear ribonucleoprotein M	IPI00171903, IPI00383296	**0**	**6**		√	
136	DEAD boz polypeptide 17 isoform 1	IPI00023785, IPI00651653	**0**	**23**		√	
137	FACT complex subunit SSRP1	IPI00005154	**0**	**5**		√	
138	cDNA FLJ61295, highly similar to Gamma-interferon-inducible protein Ifi-16	IPI00909417	**0**	**12**			
139	ATP-dependent DNA helicase 2 subunit 2	IPI00220834, IPI00871391	**0**	**7**		√	
140	Trifunctional enzyme subunit alpha, mitochondrial	IPI00031522	**0**	**4**		√	
141	Isoform Long of Delta-1-pyrroline-5-carboxylate synthetase	IPI00008982, IPI00218547	**0**	**2**			
142	Isoform 1 of Nucleolar RNA helicase 2	IPI00015953	**0**	**2**		√	
143	Isoform 1 of Transcription intermediary factor 1-beta	IPI00438229, IPI00438230	**0**	**4**		√	

144	Transitional endoplasmic reticulum ATPase	IPI00022774	**0**	**2**			
145	Isoform Short of Heterogeneous nuclear ribonucleoprotein U	IPI00479217, IPI00644079, IPI00644224, IPI00883857	**3**	**14**		√	
146	Heat shock protein 90 kDa alpha (cytosolic), class A member 1 isoform 1	IPI00382470, IPI00784295	**7**	**27**		√	√
147	DNA topoisomerase 1	IPI00413611, IPI00902597	**0**	**9**		√	
148	Putative pre-mRNA-splicing factor ATP-dependent RNA helicase DHX15	IPI00396435	**0**	**4**		√	
149	Endoplasmin	IPI00027230	**0**	**5**			
150	Heat shock 70 kDa protein 4	IPI00002966	**0**	**5**		√	
151	Elongation factor 2	IPI00186290	**0**	**22**		√	
152	Matrin-3	IPI00017297, IPI00789551	**0**	**11**		√	
153	cDNA FLJ55599, highly similar to DNA replication licensing factor MCM3	IPI00013214	**0**	**3**		√	
154	Importin subunit beta-1	IPI00001639	**0**	**7**		√	
155	splicing factor 3B subunit 2	IPI00221106, IPI00477803	**0**	**3**		√	
156	Retinoblastoma-associated protein	IPI00302829	**0**	**5**		√	√
157	Poly [ADP-ribose] polymerase 1	IPI00449049	**0**	**11**		√	
158	Isoform 1 of Presequence protease, mitochondrial	IPI00219613, IPI00787827, IPI00908889	**0**	**5**			
159	Ubiquitin-like modifier-activating enzyme 1	IPI00645078	**0**	**23**		√	
160	FACT complex subunit SPT16	IPI00026970	**0**	**9**		√	
161	importin 5	IPI00793443	**0**	**3**			
162	ATP-dependent RNA helicase A	IPI00844578	**0**	**8**		√	
163	Splicing factor 3B subunit 1	IPI00026089	**0**	**3**		√	
164	Leucine-rich PPR motif-containing protein, mitochondrial	IPI00783271	**0**	**2**		√	
165	Isoform 3 of DNA topoisomerase 2-alpha	IPI00218753, IPI00414101, IPI00478232, IPI00879004	**0**	**14**		√	
166	Isoform 1 of Myosin-9	IPI00019502	**0**	**18**		√	

167	Fatty acid synthase	IPI00026781	**0**	**61**		√	

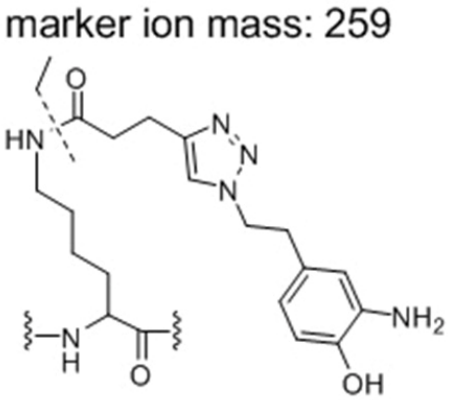
% of 4-pentynoate-labeled known acetylated proteins/total 4-pentynoate-labeled proteins (145/167) = 86.8%

***Figure S9*. Mapping the modification sites of 4-pentynoate (5)-labeled proteins in Jurkat T cells.** (a) MS/MS-identified 4-pentynoate modification sites on histones. (b)-(d) Selected MS/MS spectra of sodium 4-pentynoate-modified histone peptides.

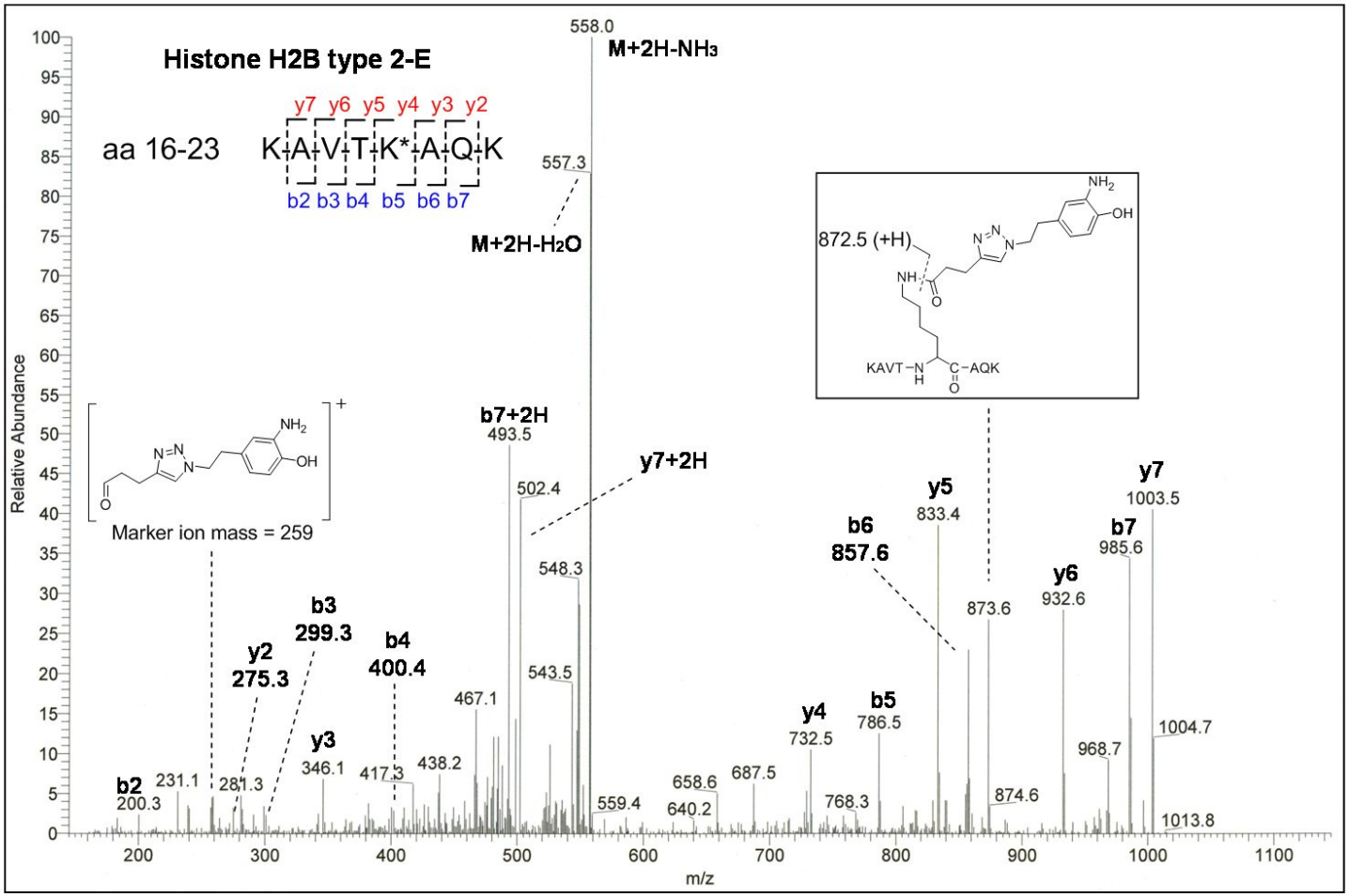
a

Histone	Peptide Sequence
Histone H2B	KGSK(Ac)**K***AVTK
	K(Ac)GSK(Ac)**K***AVTK
	KAVT**K***AQK
Histone H3	**K***STGGK(Ac)APR
	KQLAT**K***AAR
Histone H4	GG**K***GLGK(Ac)GGAK(Ac)R
	GGK(Ac)GLG**K***GGAK(Ac)R

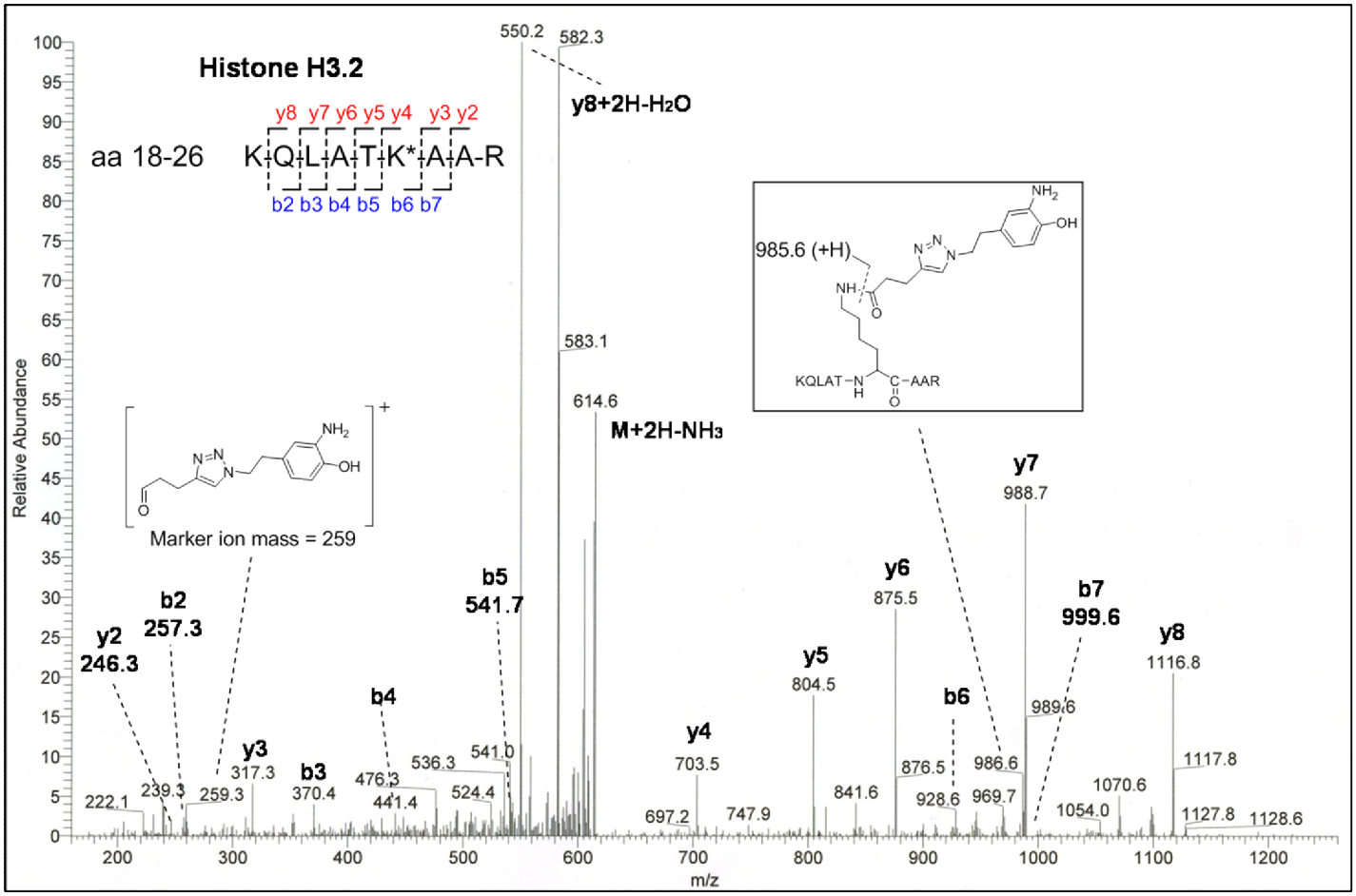
K* =



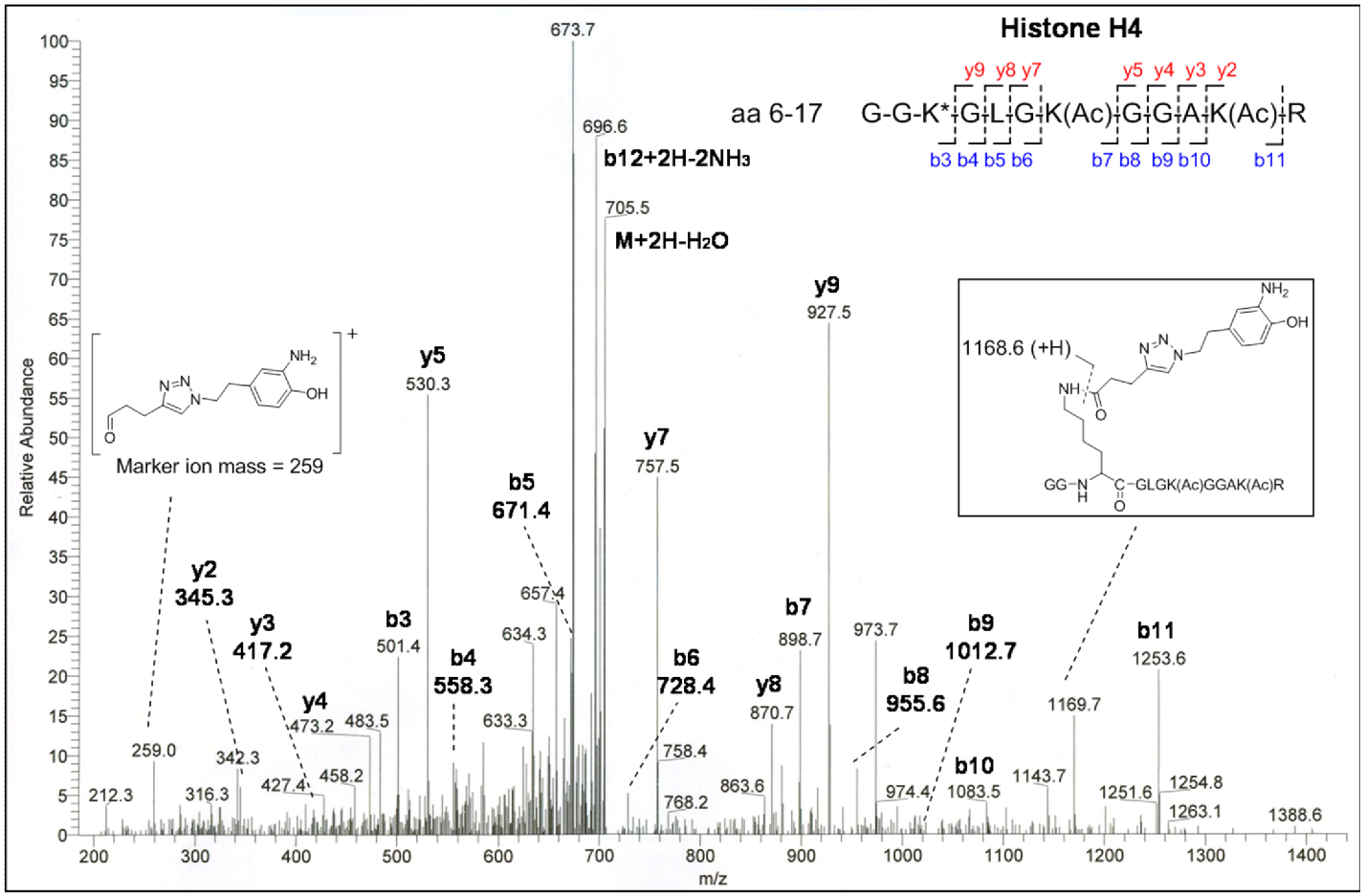
b


Histone H2B type 2-E
aa 16-23 K A V T K* A Q K
y7 y6 y5 y4 y3 y2
b2 b3 b4 b5 b6 b7
558.0
M+2H-NH3
557.3
M+2H-H2O
872.5 (+H)
KAVT–N H C–AQK
Marker ion mass = 259
b7+2H
493.5
y7+2H
502.4
548.3
543.5
467.1
b3
299.3
y2
275.3
b4
400.4
y3
346.1
417.3
438.2
b2
200.3
231.1
281.3
559.4
640.2
658.6
687.5
768.3
y4
732.5
b5
786.5
b6
857.6
y5
833.4
873.6
874.6
y6
932.6
968.7
b7
985.6
y7
1003.5
1004.7
1013.8
Relative Abundance
m/z

c


Histone H3.2
aa 18-26 K-Q-L-A-T-K*-A-A-R
y8 y7 y6 y5 y4 y3 y2
b2 b3 b4 b5 b6 b7
550.2
582.3
y8+2H-H2O
583.1
614.6
M+2H-NH3
985.6 (+H)
KQLAT-N-C-AAR
Marker ion mass = 259
Relative Abundance
m/z
y2 246.3
b2 257.3
y3 317.3
b3 370.4
b4 476.3
b5 541.7
y4 703.5
y5 804.5
y6 875.5
b7 999.6
y7 988.7
y8 1116.8
b6
222.1
239.3
259.3
441.4
524.4
536.3
541.0
697.2
747.9
841.6
876.5
928.6
969.7
986.6
989.6
1054.0
1070.6
1117.8
1127.8
1128.6

d


Histone H4
aa 6-17 G-G-K*-G-L-G-K(Ac)-G-G-A-K(Ac)-R
y9 y8 y7 y5 y4 y3 y2
b3 b4 b5 b6 b7 b8 b9 b10 b11
673.7
696.6
b12+2H-2NH3
705.5
M+2H-H2O
y9
927.5
y5
530.3
y7
757.5
b5
671.4
y2
345.3
y3
417.2
b3
501.4
b4
558.3
y4
473.2
483.5
657.4
634.3
633.3
b6
728.4
b7
898.7
y8
870.7
973.7
b9
1012.7
b8
955.6
b11
1253.6
1169.7
b10
1083.5
1143.7
1251.6
1254.8
1263.1
1388.6
758.4
768.2
863.6
974.4
259.0
342.3
427.4
458.2
212.3
316.3
Marker ion mass = 259
1168.6 (+H)
GG-N-C-GLGK(Ac)GGAK(Ac)R
Relative Abundance
m/z

Supplementary References

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