Supplementary Information

Importance of the 3'-phosphate group of acetyl-coenzyme A for efficient histone lysine acetyltranferase catalysis

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1. Synthesis of peptides

H3 peptides (residues 3-17) and H4 peptides (residues 13-27 or 1-20) were synthesised with C-terminal amide on Rink amide resin (0.78 mmol/g loading) employing automated Fmoc-SPPS chemistry on PurePep® Chorus Synthesizer. Coupling of amino acids were carried out by adding a mixture of amino acid (3.0 eq) activated with hexafluorophosphate azabenzotriazole tetramethyl uronium (HATU) (3.0 eq) and N,N-diisopropylethylamine (DIPEA) (5.0 eq) in dimethylformamide (DMF) at 75 °C for 12 min; Arg was coupled twice with this procedure. Fmoc deprotection was achieved by a solution of 20% (v/v) piperidine in DMF at 50 °C for 7 minutes. After completed elongation, the last Fmoc was removed and the resin was washed with dichloromethane and dried over diethyl ether. The peptide was subsequently cleaved from the resin using 95% (v/v) TFA, 2.5% (v/v) TIPS and 2.5% (v/v) MilliQ for 4 h. The crude peptides were precipitated with cold diethyl ether (−20 °C) and pelleted via centrifugation, purified by preparative HPLC, and lyophilised. Peptide stocks were prepared by measuring sequence specific absorbance at 205 nm with a NanoDrop 2000 spectrophotometer.

2. Synthesis of acetyl-3'-dephosphocoenzyme A

To a stirred solution of 3'-dephosphocoenzyme A (1.0 eq., 2.0 mgs) (purchased from Sigma-Aldrich, #D3385) in triethylammonium bicarbonate (TEAB) buffer, 50 mM, pH 8.5,) was added acetic anhydride (6.0 eq.) in a final reaction volume of 300 μL. The reaction mixture was left shaking at 37 °C for 2 h and monitored by MALDI-TOF MS. Upon completion, the reaction mixture was directly purified employing RP-HPLC with an isocratic gradient of 15% acetonitrile in MilliQ water (0.1% TFA (v/v)) for 30 min. Cosubstrate stocks were prepared by measuring molecule absorbance at 240 nm with a NanoDrop 2000 spectrophotometer.

3. Production of KAT enzymes

Recombinant KAT3B protein was purchased from Active Motif (cat. #81093). Recombinant KAT2A, KAT2B and KAT8 proteins were expressed as we described elsewhere.[1] KAT1 was expressed following a reported protocol.[2] Briefly, N-terminal His-tagged human KAT1 (amino acids 22-341) was transformed into *E. coli* Rosetta BL21 (DE3)pLysS cells and the expression was induced by addition of 1.0 mM

isopropyl-1-thio-D-galactopyranoside (IPTG) and cultured overnight at 15 °C. Harvested cells were resuspended in 50 mM sodium phosphate buffer pH 7.8, 250 mM NaCl, 5 mM imidazole, 5% glycerol, and 5 mM β-mercaptoethanol, and subsequently lysed by sonication. The cell lysate was then incubated with Ni-NTA beads, and the beads were washed with buffer (20 mM Tris-HCl pH 7.8, 250 mM NaCl, 20 mM imidazole and 5% glycerol). The protein was eluted with 20 mM Tris-HCl (pH 7.8, 250 mM NaCl, 250 mM imidazole and 5% glycerol). The eluted protein was concentrated using a spinfilter device (Cytiva, MWCO 10 kDa) and further purified by gel filtration chromatography using a Superdex 75 column and running buffer (20 mM Tris pH 7.5, 250 mM NaCl and 10 mM β-mercaptoethanol) at 0.5 ml min^{-1} flow speed. The purity of the eluted protein was assessed with SDS-PAGE. Pure fractions were pooled, rapidly flash-frozen and stored at -80 °C.

4. Enzyme assays

The time-course experiments KAT-catalysed reactions were performed by incubation of KAT2A (500 nM) and H3K14 peptide (50 μM) in the presence of AcCoA/dpAcCoA (150 μM); KAT2B (500 nM) and H3K14 peptide (50 μM) in the presence of AcCoA/dpAcCoA (150 μM); KAT1 (1 μM) and H4K12 peptide (50 μM) in the presence of AcCoA/dpAcCoA (150 μM); KAT8 (1 μM) and H4K16 peptide (100 μM) in the presence of AcCoA/dpAcCoA (300 μM); KAT3B (10 nM) and H4K5K12 peptide (10 μM) in the presence of AcCoA/dpAcCoA (30 μM); KAT3B (10 nM) and the H3K14 peptide (20 μM) in the presence of AcCoA/dpAcCoA (50 μM). All assays were performed in HEPES buffer (50 mM HEPES, 0.1 mM EDTA, 1 mM DTT, pH 8.0) in a final volume of 20 / 30 μL at 37 °C while shaking at 750 rpm and quenched by the addition of 10% (v/v) TFA at different time points. Quenched samples from three independent experiments were analysed by MALDI-TOF MS. The % conversion of the acetylation reaction was calculated from the ion chromatogram data by integrating peak areas using Flexanalysis software.

5. Enzyme kinetics studies

Determination of KATs kinetic parameters was performed in a total reaction volume of 20 μL using varying concentrations of the AcCoA/dpAcCoA (0-200 μM) and histone peptide (200 or 250 μM).

Reactions were initiated by the addition of KAT enzyme (4, 250 or 500 nM) and incubated at 37 °C for 30 min while shaking. Quenched samples were analysed by MALDI-TOF MS in triplicates. The % conversion of the methylation reaction was calculated from the ion chromatogram data by integrating peak areas using Flexanalysis software.

6. Western blot analysis

KAT assays were performed by incubating KAT2A (500 nM), KAT2B (500 nM) and KAT3B (10 nM) enzyme with 10 μM H3 full protein substrate in the presence of 30 μM AcCoA/dpAcCoA for 2 h; KAT1 (500 nM), KAT8 (500 nM) and KAT3B (20 nM) enzyme with 10 μM H4 full protein substrate in the presence of 50 μM AcCoA/dpAcCoA for 2 h at 37 °C in reaction buffer (50 mM HEPES, 0.1 mM EDTA, 1 mM DTT, pH 8.0). Reaction samples containing 1.8 μg histone were loaded and separated on 15% bis-tris acrylamide gradient gel with Tris/SDS/Glycine buffer, transferred to nitrocellulose membrane with transfer buffer (40% (v/v) MeOH, 20% (v/v) Tris/Glycine) and probed with primary antibody (Ab) for H4K12ac (ThermoFischer Scientific, #MA5-33388) or H3K14ac (ThermoFischer Scientific, #MA-24668) for 2 h. The membrane was blocked 1 h at room temperature for H4 and at 4 °C overnight for H3 with EveryBlot blocking buffer (BioRad) before primary Ab incubation. After primary Ab incubation, the membrane was probed with anti-rabbit HRP secondary antibody for 1 h. The membrane was stained with ECL substrate prior to detection and visualised with BioRad ChemiDoc imaging system. The same reaction samples and a negative control sample without a cosubstrate were used for Coomassie stain. Two independent Western blot experiments were carried out, showing that results are reproducible.

7. Molecular dynamics simulations

The X-ray structures of KAT1 (PDB ID: 2P0W), KAT2A (PDB ID: 1Z4R), KAT2B (PDB ID: 4NSQ), KAT3B (PDB ID: 5LKU), and KAT8 (PDB ID: 2GIV) and were imported into Maestro for refinement.[3] Chain B and Q were removed from the KAT1 structure and Cys101 was deacetylated. Prime was used to fill in loops between Arg201-Ser208 and Leu253-Pro261 for KAT8.[4] Missing side-chain atoms including hydrogens were added to both proteins using the Protein Preparation Wizard.[5] The prepared structures were subsequently exported. Using the build tools in Maestro, AcCoA was dephosphorylated to create dpAcCoA, which was exported as well.

The tleap tool in Amber was used to create topology and coordinate files using the Amber ff14SB[6] (protein), and GAFF2[7] (dpAcCoA relative to AcCoA) force fields. Charges for dpAcCoA and AcCoA were assigned based on electrostatic potential (ESP) fitting with Antechamber, which also assigned atom types. The ESP used in the fit was obtained with Gaussian09[8] based on HF/6-31G* optimised structures. This ESP was then used in a RESP[9] fitting procedure, performed by Antechamber. For the acetylated lysine, ffSB14 parameters determined by Papamokos et al. were used.[10] The proteins were solvated in cubic boxes with TIP3P[11] waters, with at least 12 Å between the proteins and the closest side. Sodium and chloride atoms were added to neutralise the system and obtain a concentration of 290 mM. Eight replica simulation series were performed of KAT8 and KAT1 bound to dpAcCoA and AcCoA using Amber22.[12] First, the systems were minimised for 1000 steps with restraints on the backbone heavy atoms. The systems were then gradually heated over 50 ps to 300K using the Langevin thermostat.[13] The pressure was then equilibrated for 50 ps before 110 ns of production simulation was performed. The final 100 ns was extracted for analysis in MDAnalysis.[14]

8. Supporting tables and figures

Table S1. Overview of the synthesised histone peptides used in this work.

Name	Sequence	$[M+H]^+$ found	m/z calculated
H3K14	TKQTARKacSTGG**K**APR	1629.0	1628.8
H4K12	SGRGKacGGKGLG**K**GGAKRHRK	2033.1	2033.3
H4K16	GGA**K**RHRKVLRDNIQ	1748.8	1748.0
H4K5K12	SGRG**K**GGKacGLG**K**GGAKacRHRKac	2119.3	2118.3

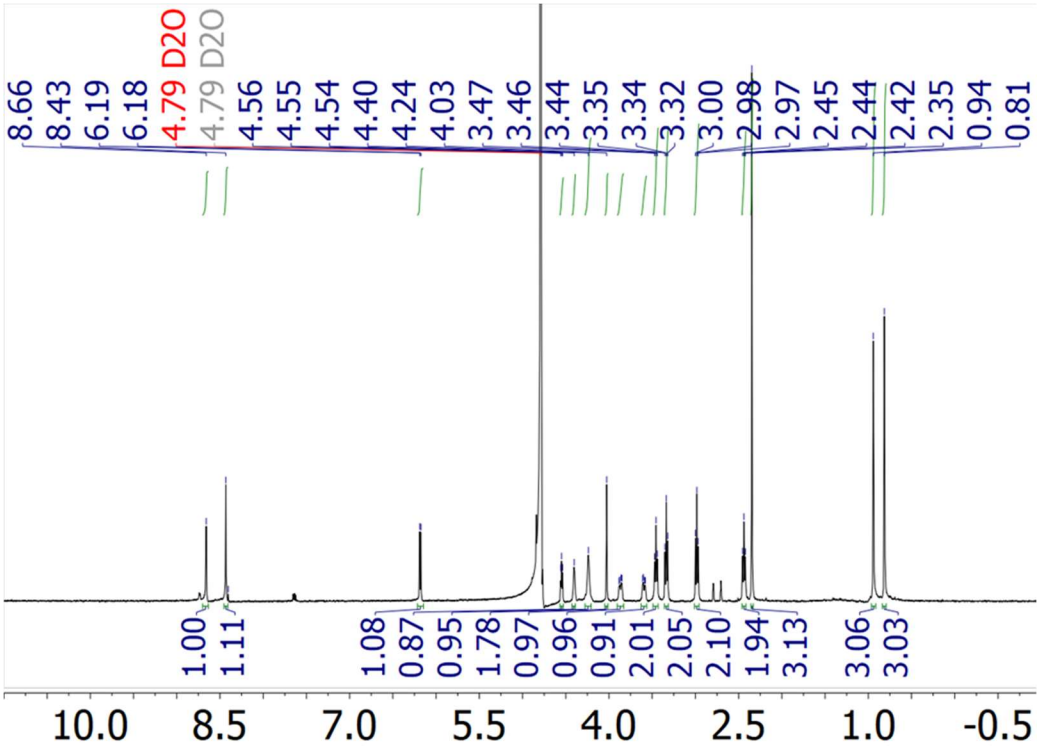

Figure S1. [1]H NMR spectrum of acetyl-3'-dephosphocoenzyme A.

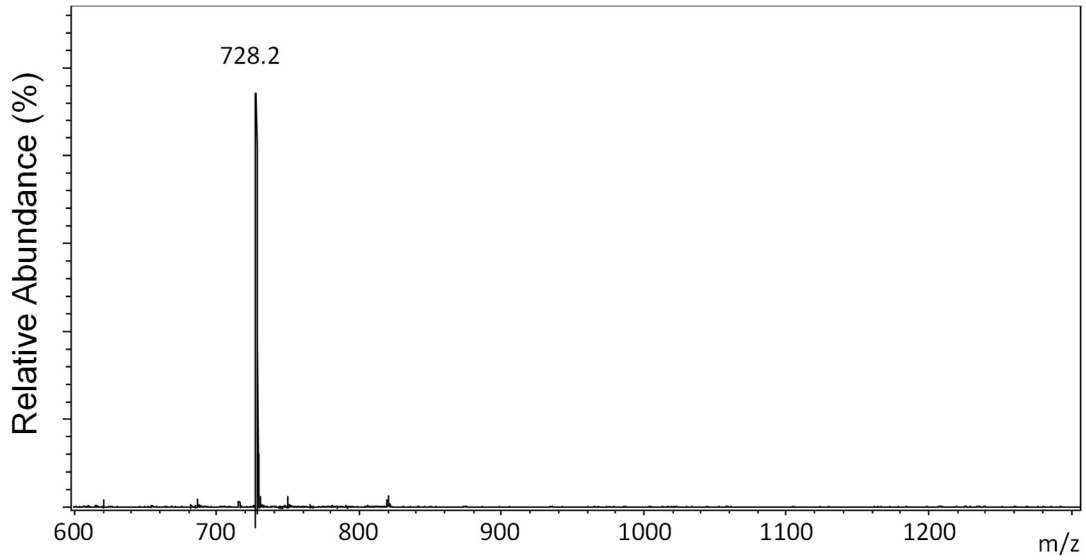

Figure S2. MALDI-TOF MS data for the synthetic acetyl-3'-dephosphocoenzyme A (*m/z observed* 728.2).

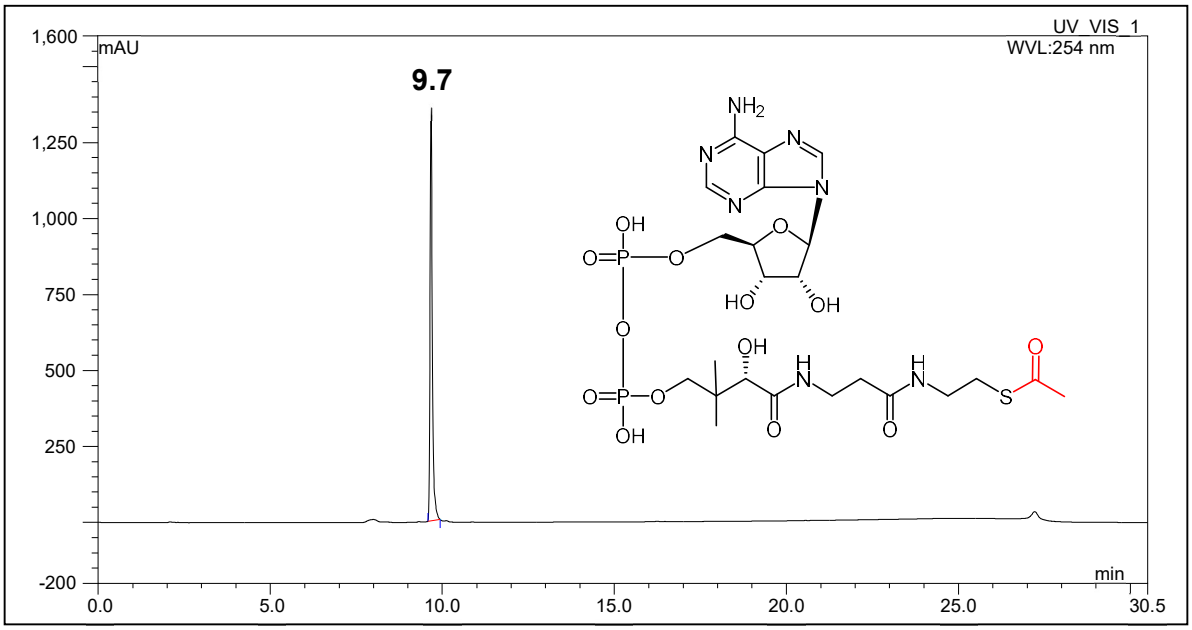

Figure S3. Analytical HPLC of synthetic acetyl-3'-dephosphocoenzyme A after RP-HPLC purification. The molecule elutes at 9.7 min.

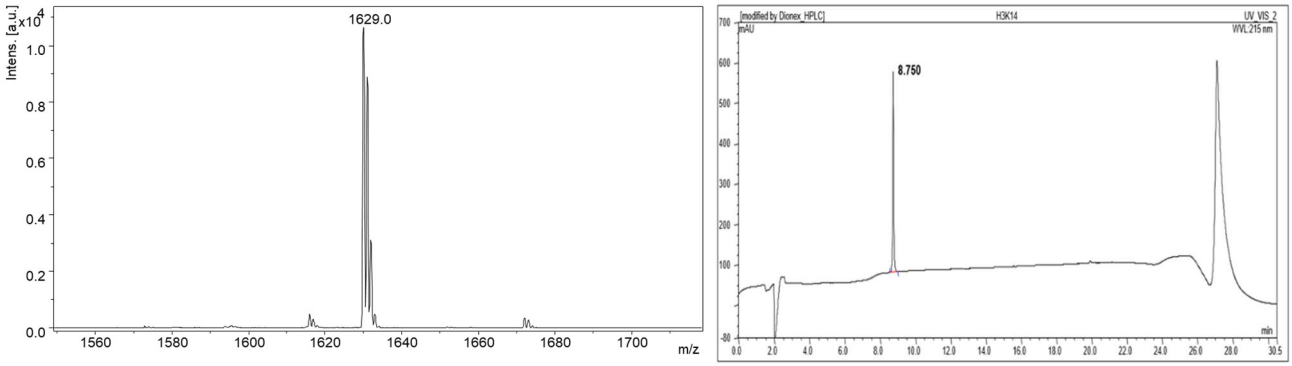

Figure S4. MALDI-TOF MS data for the H3K14 peptide (*m/z* 1628.9 observed) and analytical HPLC of the peptide after RP-HPLC purification.

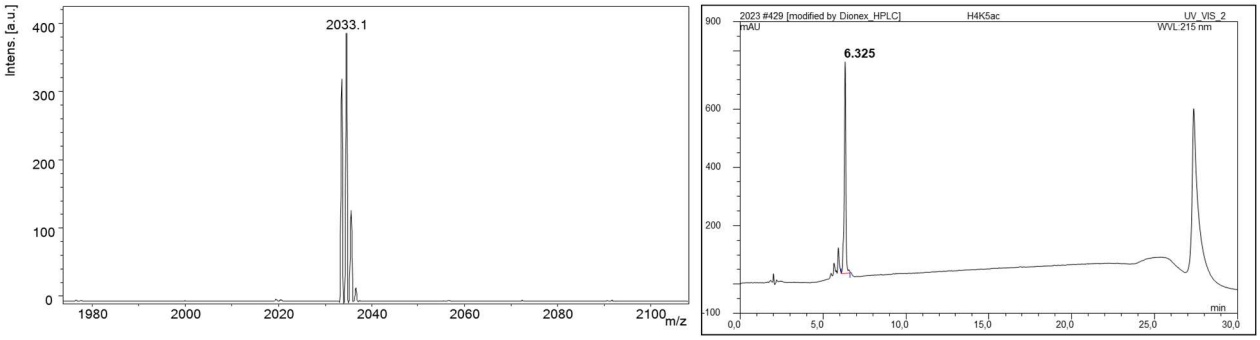

Figure S5. MALDI-TOF MS data for the H4K12 peptide (*m/z* 2033.1 observed) and analytical HPLC of the peptide after RP-HPLC purification.

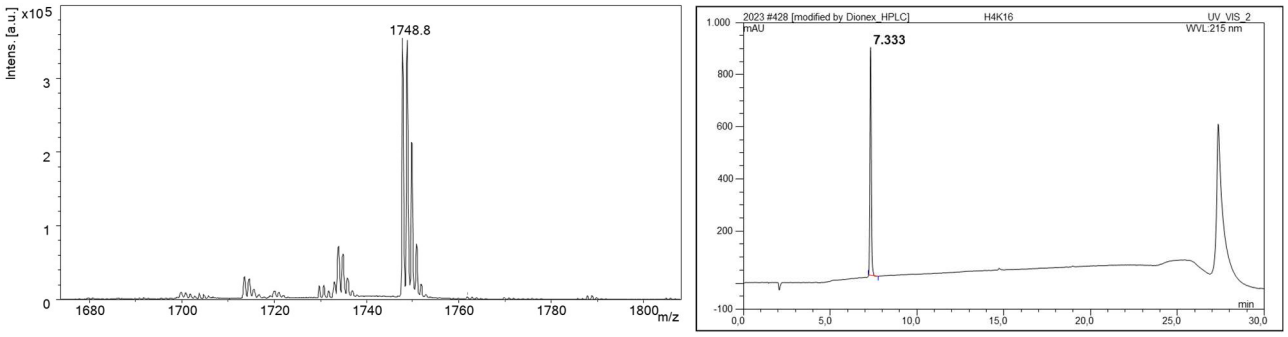

Figure S6. MALDI-TOF MS data for the H4K16 peptide (*m/z* 1748.8 observed) and analytical HPLC of the peptide after RP-HPLC purification.

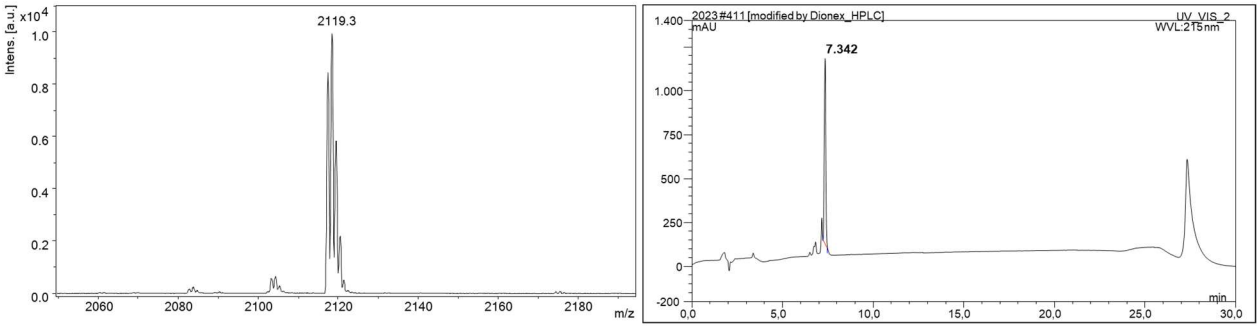

Figure S7. MALDI-TOF MS data for the H4K5K12 peptide (*m/z* 2119.3 observed) and analytical HPLC of the peptide after RP-HPLC purification.

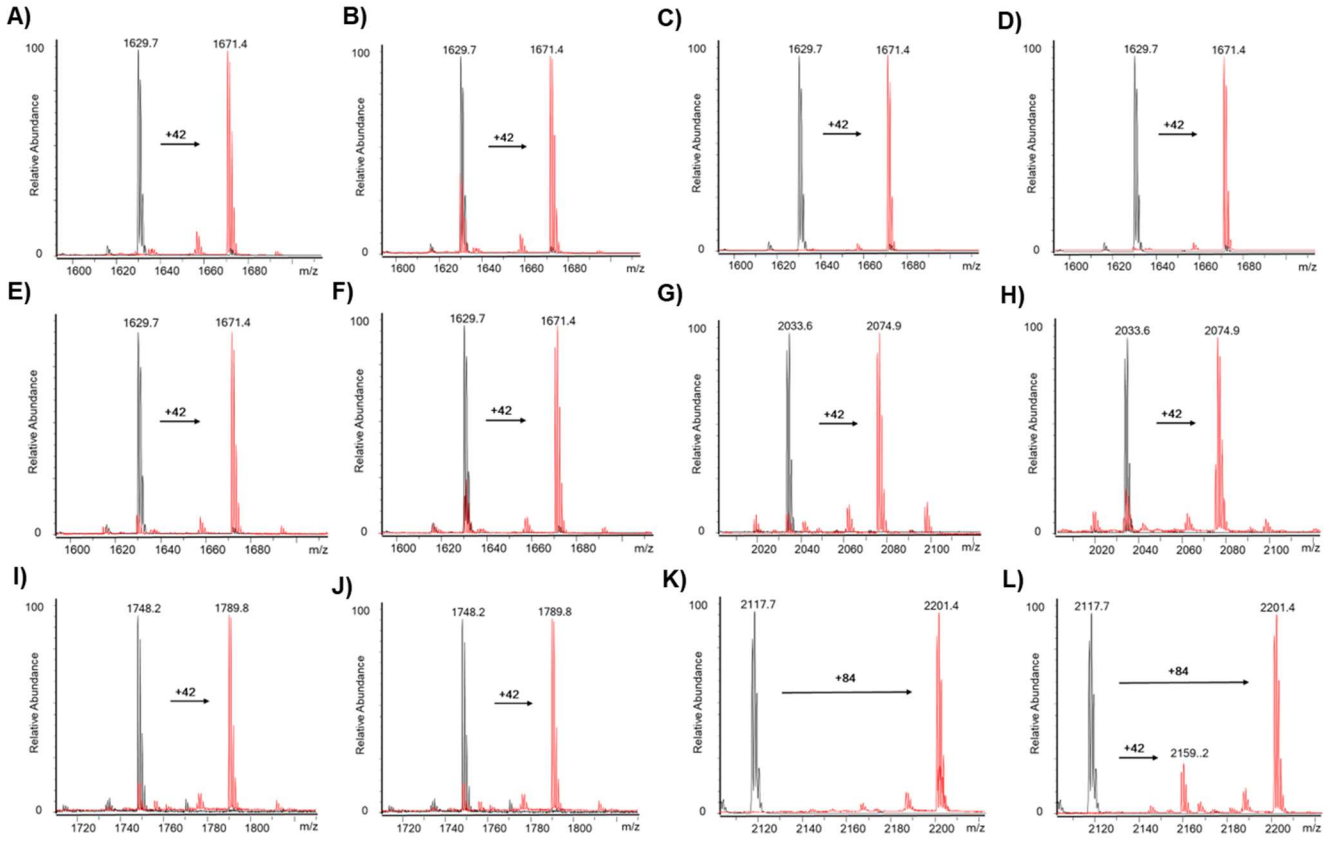

Figure S8. KAT-catalysed acetylation of histone peptide substrates in the presence of AcCoA or dpAcCoA after 2 h reaction at 37 °C. A) KAT2A (500 nM) and H3K14 peptide (50 μM) in the presence of AcCoA or B) dpAcCoA (150 μM), C) KAT2B (500 nM) and H3K14 peptide (50 μM) in the presence of AcCoA or D) dpAcCoA (150 μM), E) KAT3B (10 nM) and H3K14 peptide (20 μM) in the presence of AcCoA or F) dpAcCoA (50 μM), G) KAT1 (1 μM) and the H4K12 peptide (50 μM) in the presence of AcCoA or H) dpAcCoA (150 μM), I) KAT8 (1 μM) and the H4K16 peptide (100 μM) in the presence of AcCoA or J) dpAcCoA (300 μM), and K) KAT3B (10 nM) and H4K5K12 peptide (10 μM) in the presence of AcCoA or L) dpAcCoA (30 μM).

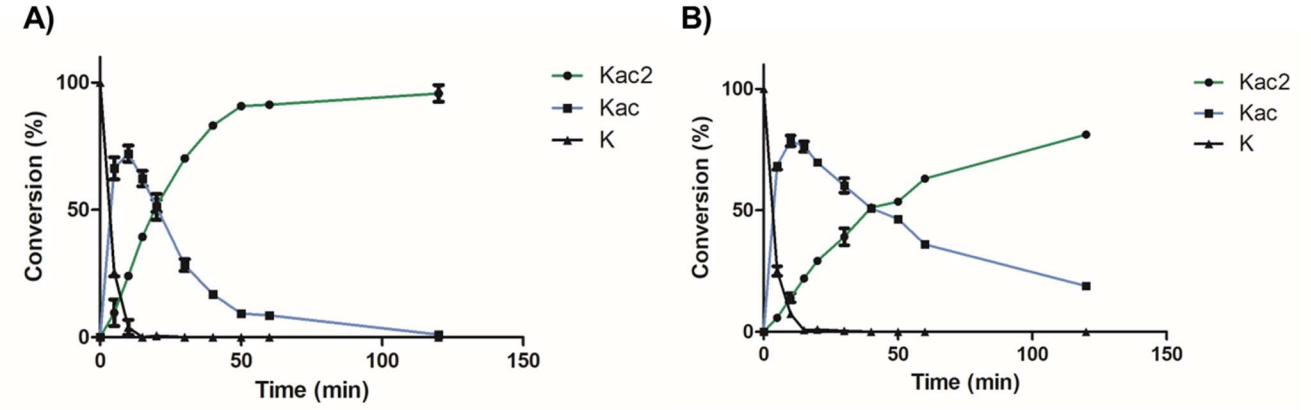

Figure S9. Time-course for the KAT3B-catalysed acetylation of the H4 peptide (H4K8acK16acK20ac) using either AcCoA (left) or dpAcCoA (right) as cosubstrate, showing the conversion for two acetylation states. A) KAT3B-catalysed (10 nM) diacetylation of H4K5K12 (10 μM) in the presence of AcCoA (30 μM) and B) KAT3B-catalysed (10 nM) diacetylation of H4K5K12 (10 μM) in the presence of dpAcCoA (30 μM).

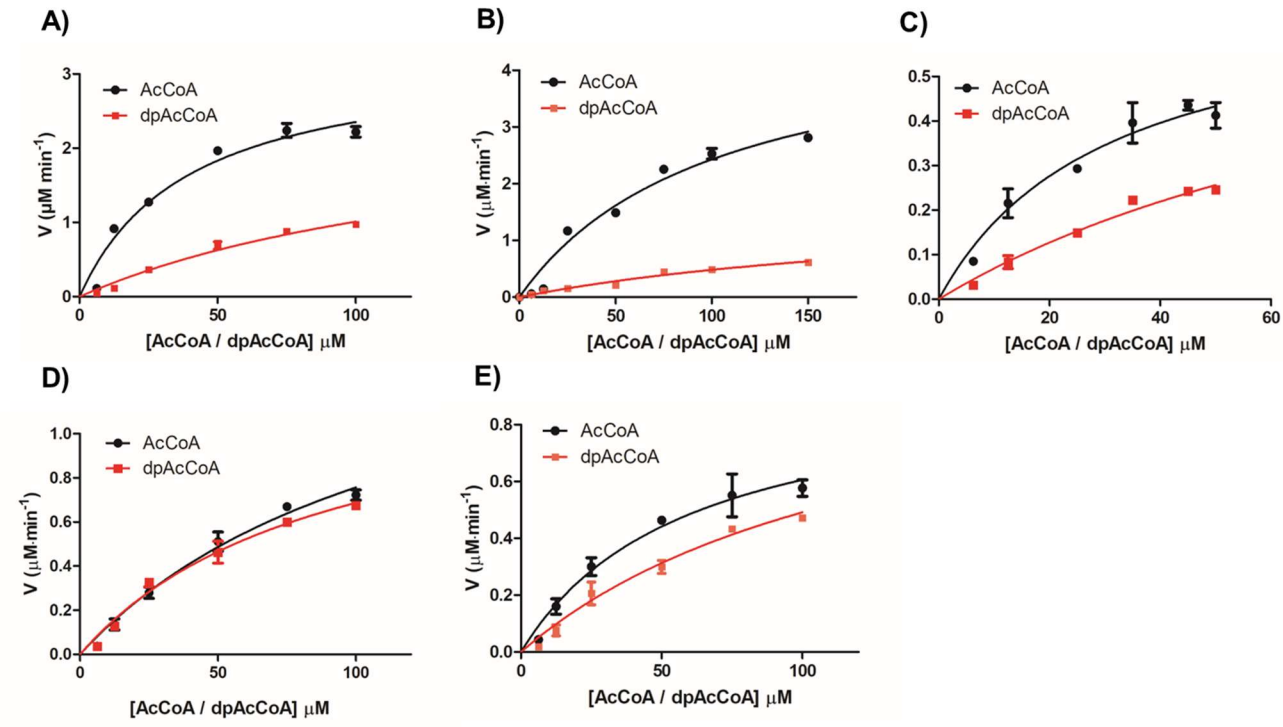

Figure S10. Steady-state kinetics of KAT-catalysed histone lysine acetylation using AcCoA (black) or dpAcCoA (red). A) Kinetics plots of KAT2A-catalysed (250 nM) acetylation of H3K14 (250 μM), B) Kinetics plots of KAT2B-catalysed (250 nM) acetylation of H3K14 (250 μM), C) Kinetics plots of KAT3B-catalysed (4 nM) acetylation of H3K14 (200 μM), D) Kinetics plots of KAT1-catalysed (500 nM) acetylation of H4K12 (200 μM), and E) Kinetics plots of KAT8-catalysed (250 nM) acetylation of H4K16 (250 μM).

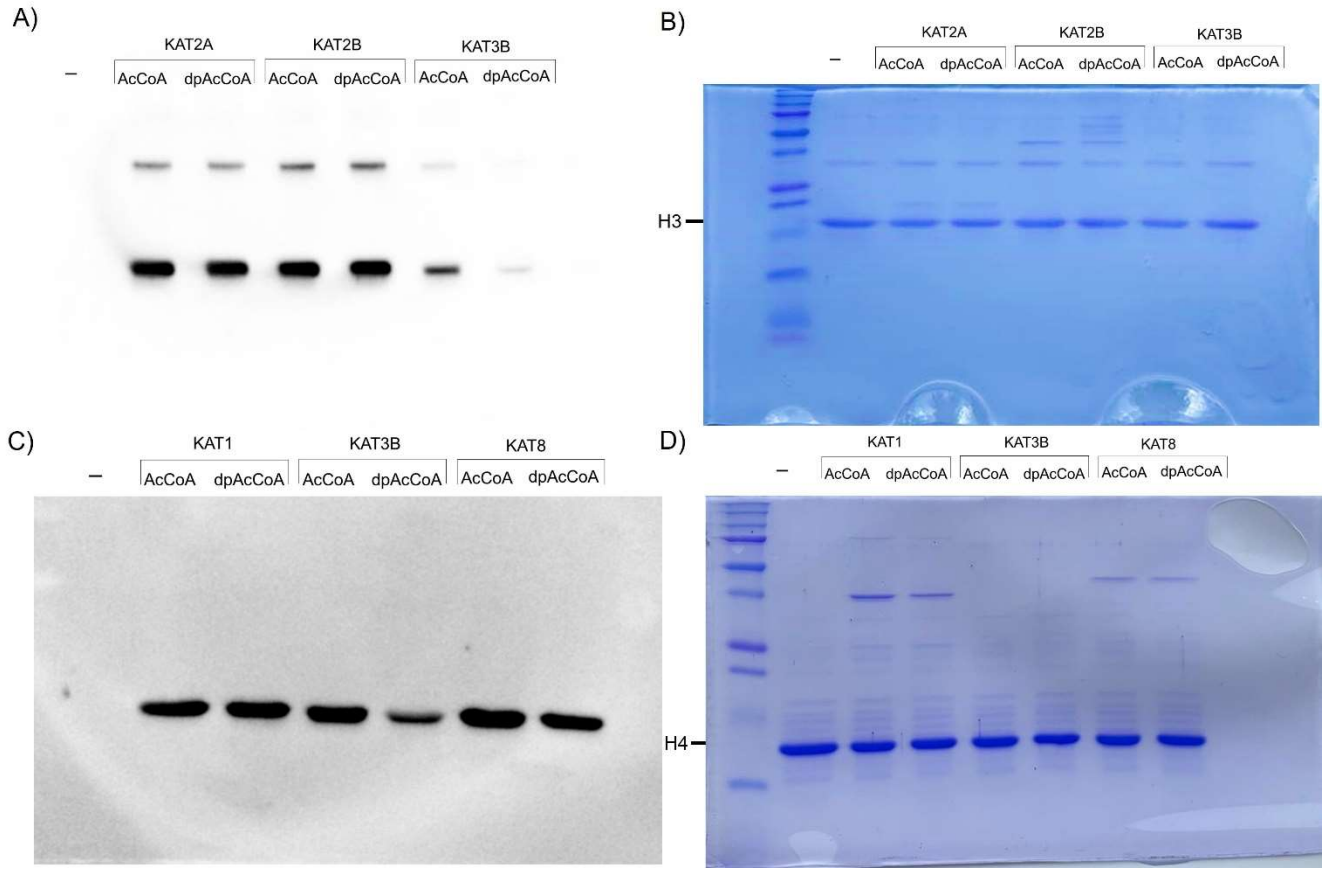

Figure S11. Western blot analyses of histone lysine acetylation by human KATs in the presence of AcCoA or dpAcCoA cosubstrates. A) Full Western blot image of H3K14ac detection levels. B) Full SDS-PAGE image of loaded H3 protein. C) Full Western blot image of H4K12ac detection levels. D) Full SDS-PAGE image of loaded H4 protein.

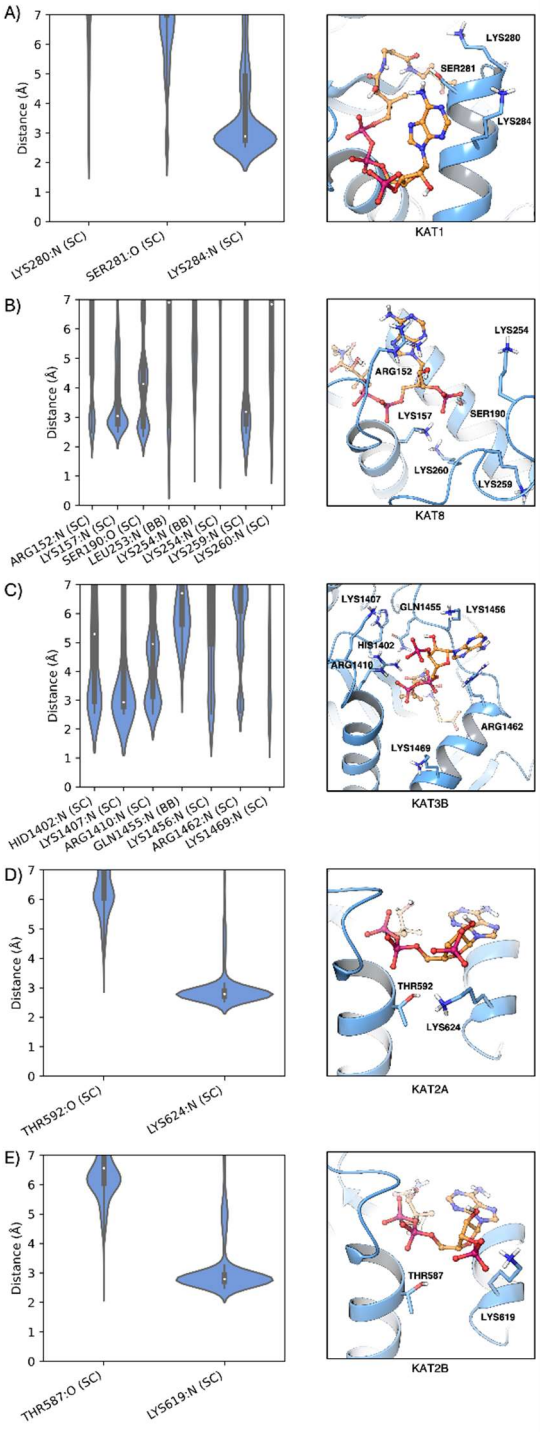

Figure S12. Primary polar interaction partners of the 3'-phosphate of AcCoA. Left column: violin plot of the distance between the 3'-phosphate and the defined atom group. SC: sidechain, BB: backbone. Right column: location of the interaction partner in the crystal structures. Primary polar interactions are defined as interactions that were found in >1% of the MD frames.

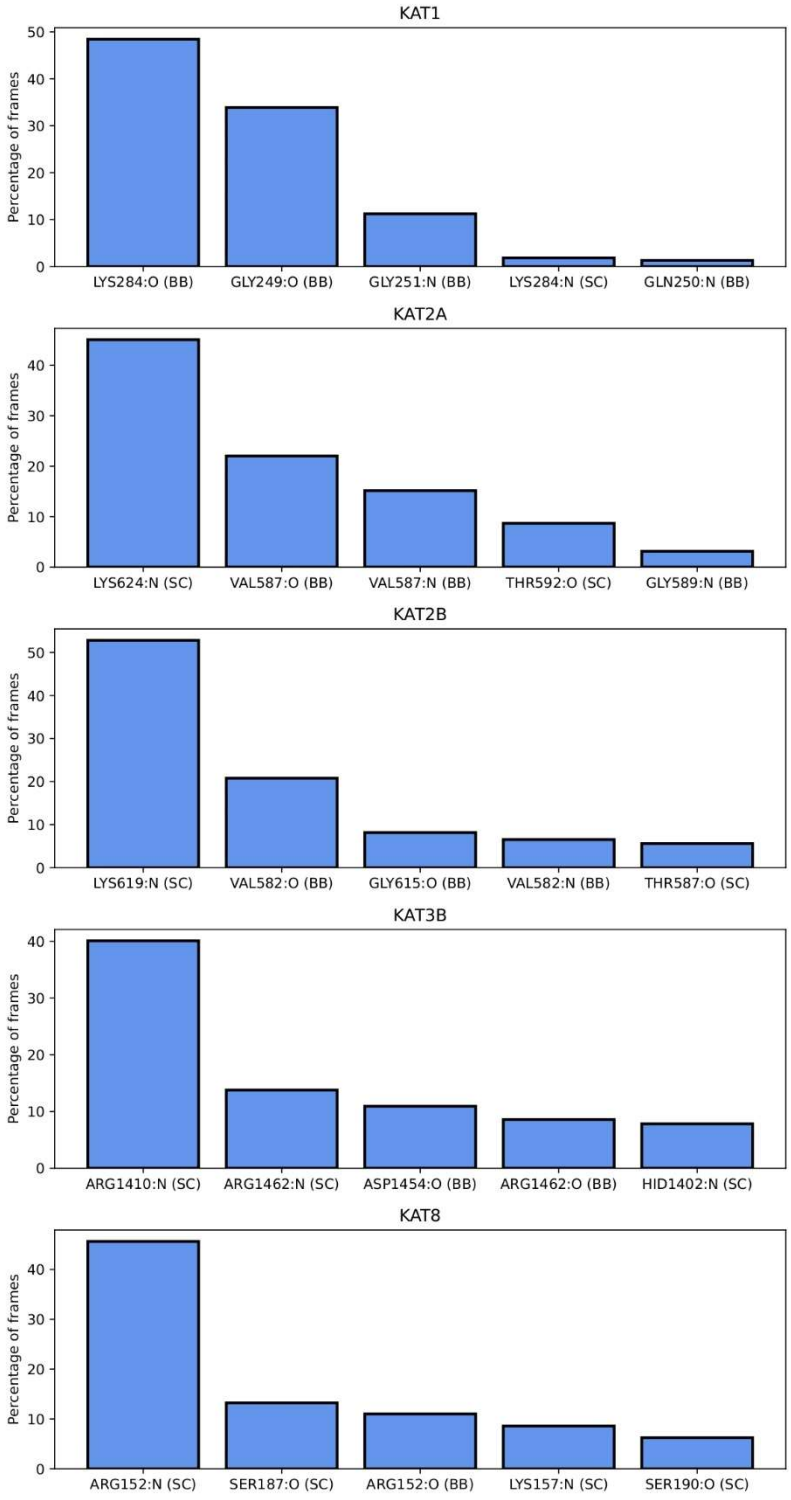

Figure S13. Frequencies at which different protein interaction partners were found to be the closest contact to the 3'-OH of dpAcCoA in the MD simulations.

9. References

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