SUPPLEMENTAL FIGURE LEGENDS

Figure S1: Pre-steady state analyses of nucleosome and H4 acetylation. To determine the time required for single turnover conditions, a rapid quench flow apparatus was used in conjunction with the filter binding assay to study early time points of nucleosome (A) and H4 (B) acetylation by picNuA4. One turnover (mono-acetylation) was determined by a stoichiometric equivalency of product formed compared to enzyme. The resulting data was plotted using Kaleidagraph software as product formed as a function of time. NCP acetylation reactions contained 1 μM picNuA4, 2 μM NCP, 100 μM ^{3}H-AcCoA; H4 reactions contained 0.5 μM picNuA4, 10 μM H4, 25 μM ^{3}H-AcCoA, both in 50 mM Tris (pH 7.5) and 1 mM DTT.

Figure S2: picNuA4 histone acetylation sites were identified and quantified using isotopic labeling and tandem mass spectrometry. Histone H4 was enzymatically acetylated by picNuA4, deuterated with acetic anhydride, and trypsin digested to yield H4 peptides of uniform length before LC-MS/MS analysis. For example, data from H4 under limiting AcCoA conditions (Figure 3) are shown. A) Chromatogram depicts the intensity versus elution time for H4 doubly charged peptides with varying amounts of enzymatic acetylation, 0 ac (m/z 725.95), 1 ac (m/z 724.44), 2 ac (m/z 722.93), 3 ac (m/z 721.42), 4 ac (m/z 719.91) peptides (see inset). Fully acetylated peptides were then identified and quantified using MS/MS fragmentation. The theoretical b and y fragment ions are shown in (B) while two example chromatograms showing intensity versus time for the y5 ion for are shown in (C), acetylated and deuterated ions respectively. The data from the y5 ion were combined with the data from the y4 ion (data not shown) to yield an average acetylation of 8 % for K16. D) The quantification of site-specific acetylation

shows similar trends between experiments, with percent acetylation versus lysine site averaged from n = 3, with error bars displaying ± one standard deviation.

Figure S3: Acetylation in substrate trapping experiments is picNuA4-mediated. To show that substrate acetylation levels were enzyme-specific, substrate-trapping reactions were conducted in which picNuA4 (no enzyme) or AcCoA (no AcCoA) were omitted. Graphs depict percent of total peptide per acetylation state (see Figure 2 for details). Data sets are representative of n = 1.

Figure S4: picNuA4 acetylates free H4 randomly under saturating conditions. picNuA4 acetylation reactions were identified and quantified by MS/MS as described in text. Graph depicts percent acetylated peptide per site, identified from the MS/MS data. Saturating reaction conditions: 100 nM picNuA4, 10 µM H4, 100 µM AcCoA, 50 mM Tris (pH 7.5) and 1 mM DTT. The data set represents a n = 1, with at least 3 time points taken under steady state conditions.

Figure S5: Multiple tail lysine residues are dispensable for efficient acetylation. Filter binding assays were conducted to determine k_{cat}/K_m values for double K→R and K→A tail mutants. All reactions contained 50 nM picNuA4, 20 µM histone substrate, 100 µM AcCoA, 50 mM Tris (pH 7.5) and 1 mM DTT. Values were derived from n = 1 data set and fit using Kaleidagraph software. Error bars for k_{cat}/K_m values reflect propagated standard error of individual k_{cat} and K_m parameters.

Figure S6: picNuA4 prefers tail lysines with glycine spacer residues. A) Free histone mutants, as shown on the left, were acetylated by picNuA4 and imaged using SYPRO-stained TAU gels. Reaction conditions were 150 nM picNuA4, 200 µM acetyl-CoA, 20 µM histone, 50 mM Tris (pH 7.5) and 1 mM DTT. Gels are representative of at least 3

replicates. B) Densitometry analyses for Figure 5 and Supplemental Figure 6 TAU gels, in which the processing efficiency coefficient, $t_{1/2}$, describes the time (in seconds) required for exactly half of the initial substrate to be converted to the fully-acetylated state by picNuA4. All values represent an average of at least 3 independent reactions for free histone and 2 independent reactions for nucleosome substrates.

Figure S7: Human picNuA4 displays random site acetylation for H4. MS/MS identification and quantification of human picNuA4 acetylation reactions, analogous to Figure 3A, in which limiting amounts of AcCoA were added to reactions and run on TAU gels. Graphs depict percent acetylation at each lysine position for the singly acetylated state. Reaction conditions: 1 µM human picNuA4, 1-2 µM AcCoA, 10 µM H4, 50 mM Tris (pH 7.5) and 1 mM DTT. Values are representative of at least 2 data sets, where experimental replicate values differed no more than 6% per site.

Table S1: H4 lysine mutation does not alter picNuA4 site specificity. picNuA4 acetylation reactions of free H4 were identified and quantified by MS/MS as described in text. Table depicts percent acetylated peptide per lysine residue from the MS/MS data. Limiting reaction conditions were 1 µM picNuA4, 10 µM substrate, 1-5 µM AcCoA, 50 mM Tris (pH 7.5) and 1 mM DTT. Values are representative of at least 2 data sets.

Figure S1

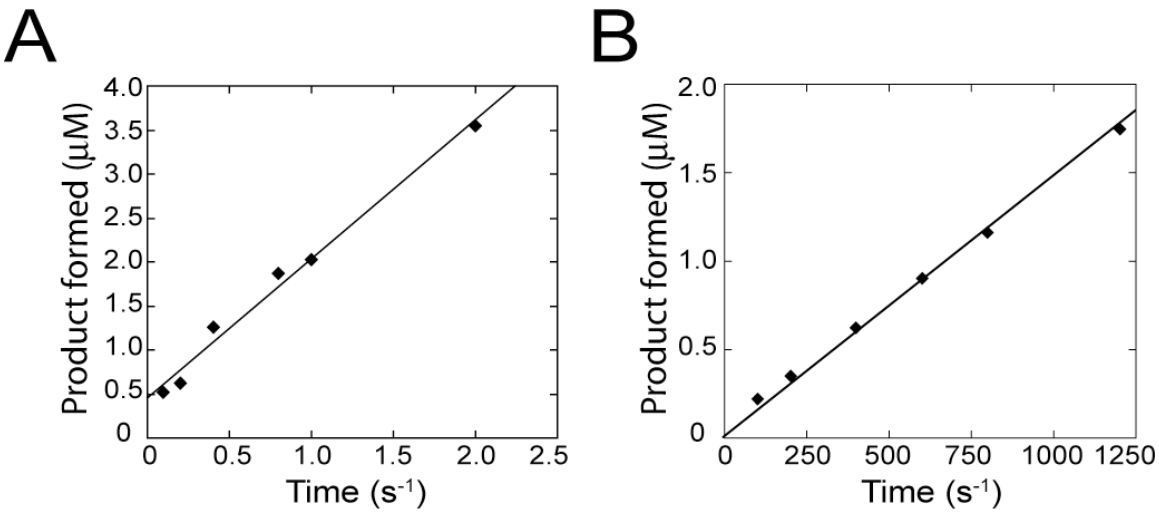

A
Product formed (μM)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
0
0.5
1.0
1.5
2.0
2.5
Time (s-1)
B
Product formed (μM)
2.0
1.5
1.0
0.5
0
0
250
500
750
1000
1250
Time (s-1)

Figure S2

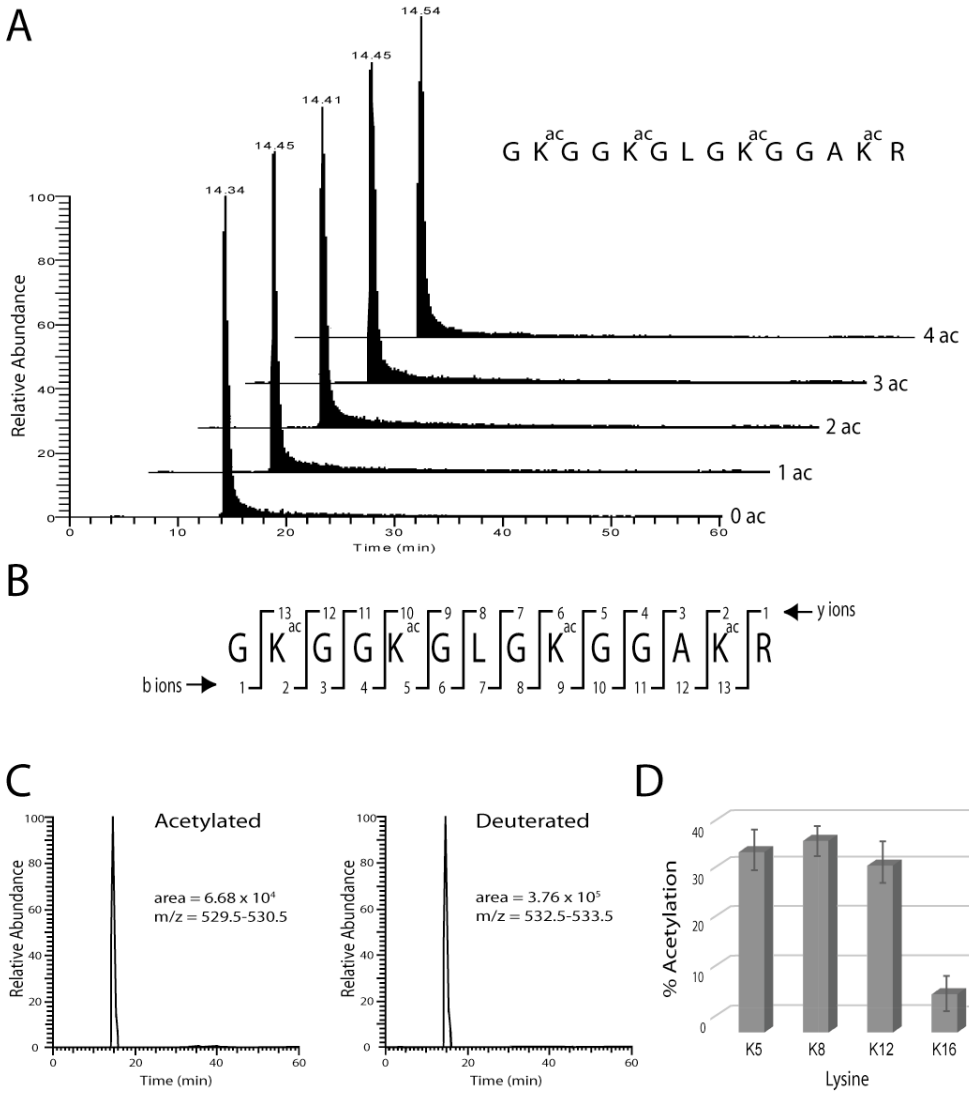

A
14.54
14.45
14.41
14.45
14.34
G K ac G G K ac G L G K ac G G A K ac R
Relative Abundance
Time (min)
4 ac
3 ac
2 ac
1 ac
0 ac
B
b ions
y ions
G K ac G G K ac G L G K ac G G A K ac R
C
Acetylated
area = 6.68 x 10⁴
m/z = 529.5-530.5
Deuterated
area = 3.76 x 10⁵
m/z = 532.5-533.5
Relative Abundance
Time (min)
D
% Acetylation
K5
K8
K12
K16
Lysine

Figure S3

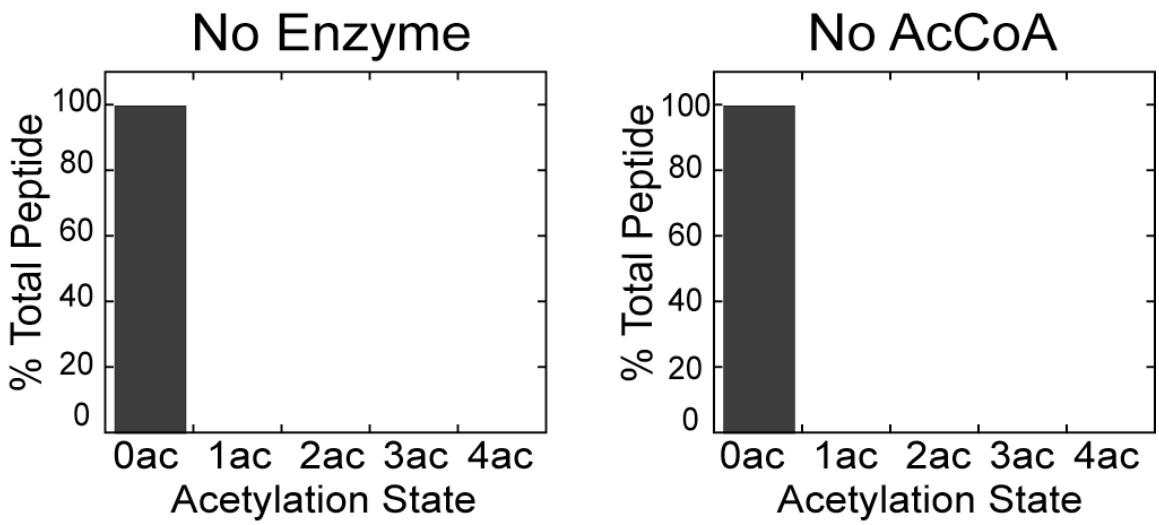

No Enzyme
% Total Peptide
100
80
60
40
20
0
0ac
1ac
2ac
3ac
4ac
Acetylation State
No AcCoA
% Total Peptide
100
80
60
40
20
0
0ac
1ac
2ac
3ac
4ac
Acetylation State

Figure S4

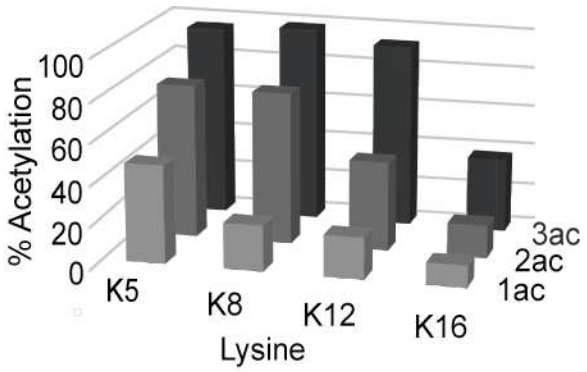


Figure S5

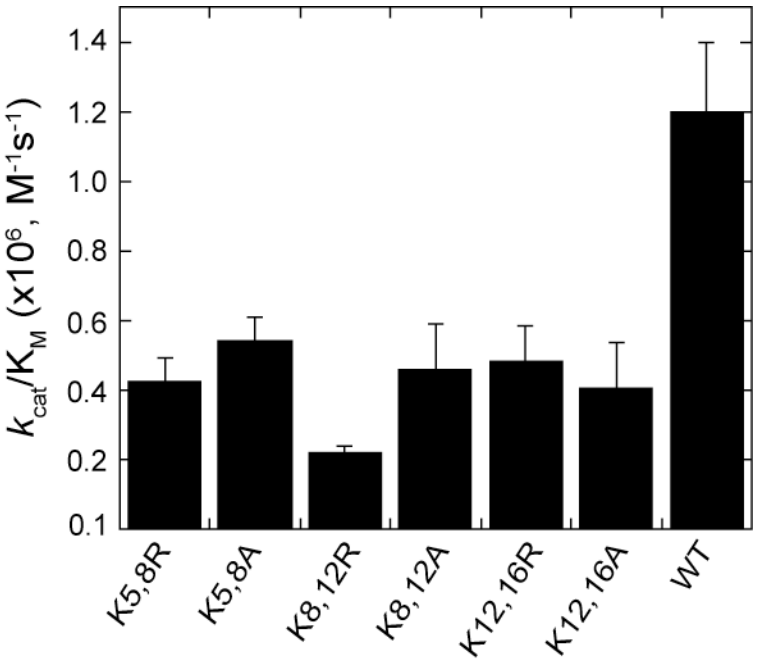
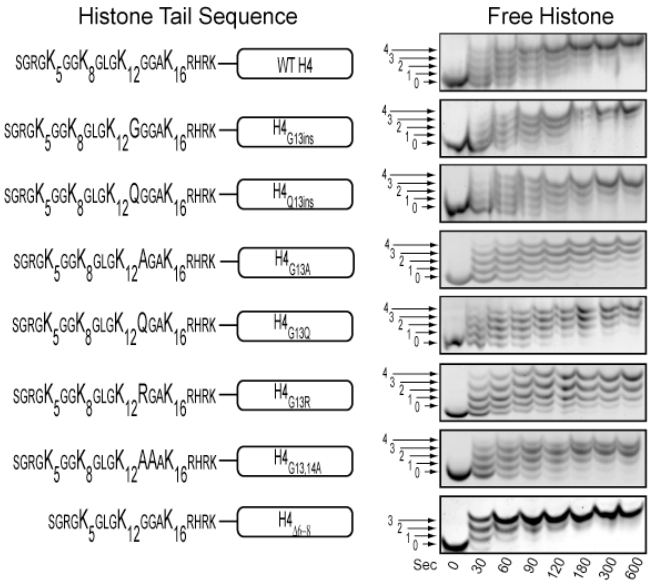

1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.1
k_{cat}/K_M (x10^6, M^{-1}s^{-1})
K5,8R
K5,8A
K8,12R
K8,12A
K12,16R
K12,16A
WT

Figure S6

A



B

TAU Densitometry ($t_{1/2}$)

Fig. 5	Free Histone (s)	Nucleosome (s)
WT H4	100.9 (±7.8)	37.4 (±4.2)
$H4_{\Delta 13\text{-}14}$	199.7 (±14.2)	177.8 (±8.7)
$H4_{GGAKins}$	126.2 (±19.1)	105.6 (±7.3)
WT H2A	> 600	128.3 (±17.9)
$H2A_{GGins}$	83.3 (±14.7)	145.7 (±23.2)
Supplemental Fig. 6		
WT H4	110.6 (±9.5)	
$H4_{G13ins}$	93.6 (±8.8)	
$H4_{Q13ins}$	86.2 (±9.3)	
$H4_{G13A}$	152.7 (±14.9)	
$H4_{G13Q}$	192.2 (±11.3)	
$H4_{G13R}$	180.2 (±12.4)	
$H4_{G13,14A}$	468.3 (±53.1)	
$H4_{\Delta 6\text{-}8}$	43.3 (±3.8)	

Figure S7

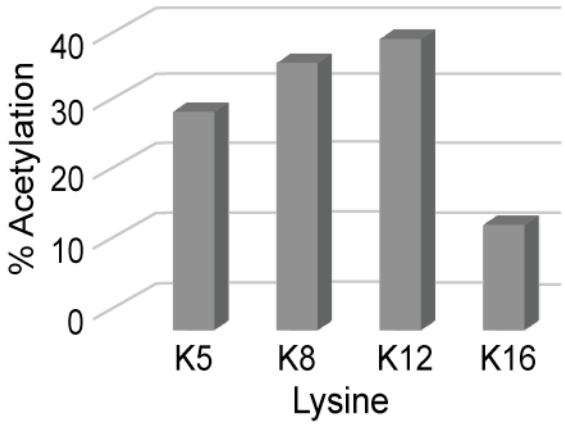


Table S1

	H4 lysine position			
	K5	K8	K12	K16
WT	41%	35%	29%	9%
5R	—	63%	32%	8%
5A	—	51%	37%	13%
8R	ND[a]	—	48%	10%
8A	73%	—	35%	5%
12R	42%	31%	—	ND[a]
12A	48%	58%	—	14%
16R	58%	25%	44%	—
16A	39%	35%	35%	—

[a] Not Detected. Peptide species could not be detected in m/z scanning range