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The Taf14 YEATS domain is a reader of histone crotonylation

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Abstract

The discovery of new histone modifications is unfolding at startling rates, however, the identification of effectors capable of interpreting these modifications has lagged behind. Here we report the YEATS domain as an effective reader of histone lysine crotonylation – an epigenetic signature associated with active transcription. We show that the Taf14 YEATS domain engages crotonyllysine via a unique π-π-π-stacking mechanism and that other YEATS domains have crotonyllysine binding activity.

Keywords

YEATS domain; crotonylated lysine; chromatin; Taf14; histone; PTM

Crotonylation of lysine residues (crotonyllysine, Kcr) has emerged as one of the fundamental histone post-translational modifications (PTMs) found in mammalian chromatin[1,2]. This epigenetic PTM is widespread and enriched at active gene promoters and potentially enhancers[1]. The crotonyllysine mark on histone H3K18 is produced by p300, a histone acetyltransferase also responsible for acetylation of histones. Owing to some differences in their genomic distribution, the crotonyllysine and acetyllysine (Kac)

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Author contributions

F.H.A., S.A.S., E.K.S., J.B.B., A.G., I.K.T and K.K. performed experiments and together with X.S., B.D.S and T.G.K. analyzed the data. F.H.A., S.A.S., B.D.S. and T.G.K. wrote the manuscript with input from all authors.

Competing Financial Interest

The authors declare no competing financial interests.

Additional information

Any supplementary information is available in the online version of this paper.

modifications have been linked to distinct functional outcomes[1,2]. p300-catalyzed histone crotonylation, which is likely metabolically regulated[3,4], stimulates transcription to a greater degree than p300-catalyzed acetylation[2]. The discovery of individual biological roles for the crotonyllysine and acetyllysine marks suggests that these PTMs can be read by distinct readers. While a number of acetyllysine readers have been identified and characterized, a specific reader of the crotonyllysine mark remains unknown (reviewed in[5,6]). A recent survey of bromodomains (BDs) demonstrates that only one BD associates very weakly with a crotonylated peptide, however it binds more tightly to acetylated peptides[7,8], inferring that bromodomains do not possess physiologically relevant crotonyllysine binding activity.

The family of acetyllysine readers has been expanded with the discovery that the YEATS (Yaf9, ENL, AF9, Taf14, Sas5)[9] domains of human AF9 and yeast Taf14 are capable of recognizing the histone mark H3K9ac[10,11]. The acetyllysine binding function of the AF9 YEATS domain is essential for the recruitment of the histone methyltransferase DOT1L to H3K9ac-containing chromatin and for DOT1L-mediated H3K79 methylation and transcription[10]. Similarly, activation of a subset of genes and DNA damage repair in yeast require the acetyllysine binding activity of the Taf14 YEATS domain[11]. Consistent with its role in gene regulation, Taf14 was identified as a core component of the transcription factor complexes TFIID and TFIIF[12]. However, Taf14 is also found in a number of chromatin-remodeling complexes (i.e., INO80, SWI/SNF and RSC) and the histone acetyltransferase complex NuA3[12–15], indicating a multifaceted role of Taf14 in transcriptional regulation and chromatin biology. In this study, we identified the Taf14 YEATS domain as a reader of crotonyllysine that binds to histone H3 crotonylated at lysine 9 (H3K9cr) via a distinctive binding mechanism. We found that H3K9cr is present in yeast and is dynamically regulated.

To elucidate the molecular basis for recognition of the H3K9cr mark, we obtained a crystal structure of the Taf14 YEATS domain in complex with $H3K9cr_{5\text{-}13}$ (residues 5–13 of H3) peptide (Fig. 1, Supplementary Results, Supplementary Fig. 1 and Supplementary Table 1). The Taf14 YEATS domain adopts an immunoglobin-like β sandwich fold containing eight anti-parallel β strands linked by short loops that form a binding site for H3K9cr (Fig. 1b). The H3K9cr peptide lays in an extended conformation in an orientation orthogonal to the β strands and is stabilized through an extensive network of direct and water-mediated hydrogen bonds and a salt bridge (Fig. 1c).

The most striking feature of the crotonyllysine recognition mechanism is the unique coordination of crotonylated lysine residue. The fully extended side chain of K9cr transverses the narrow tunnel, crossing the β sandwich at right angle in a corkscrew-like manner (Fig. 1b and Supplementary Figure 1b). The planar crotonyl group is inserted between Trp81 and Phe62 of the protein, the aromatic rings of which are positioned strictly parallel to each other and at equal distance from the crotonyl group, yielding a novel aromatic-amide/aliphatic-aromatic π-π-π-stacking system that, to our knowledge, has not been reported previously for any protein-protein interaction (Fig. 1d and Supplementary Fig. 1c). The side chain of Trp81 appears to adopt two conformations, one of which provides maximum π-stacking with the alkene functional group while the other rotamer affords maximum π-stacking with the amide π electrons (Supplementary Fig. 1c). The dual

conformation of Trp81 is likely due to the conjugated nature of the C=C and C=O π-orbitals within the crotonyl functional group.

In addition to π-π-π stacking, the crotonyl group is stabilized by a set of hydrogen bonds and electrostatic interactions. The π bond conjugation of the crotonyl group gives rise to a dipole moment of the alkene moiety, resulting in a partial positive charge on the β-carbon (Cβ) and a partial negative charge on the α-carbon (Cα). This provides the capability for the alkene moiety to form electrostatic contacts, as Cα and Cβ lay within electrostatic interaction distances of the carbonyl oxygen of Gln79 and of the hydroxyl group of Thr61, respectively. The hydroxyl group of Thr61 also participates in a hydrogen bond with the amide nitrogen of the K9cr side chain (Fig. 1d). The fixed position of the Thr61 hydroxyl group, which facilitates interactions with both the amide and Cα of K9cr, is achieved through a hydrogen bond with imidazole ring of His59. Extra stabilization of K9cr is attained by a hydrogen bond formed between its carbonyl oxygen and the backbone nitrogen of Trp81, as well as a water-mediated hydrogen bond with the backbone carbonyl group of Gly82 (Fig 1d). This distinctive mechanism was corroborated through mapping the Taf14 YEATS-H3K9cr binding interface in solution using NMR chemical shift perturbation analysis (Supplementary Fig. 2a, b).

Binding of the Taf14 YEATS domain to H3K9cr is robust. The dissociation constant (K_d) for the Taf14 YEATS-H3K9cr$_{5\text{-}13}$ complex was found to be 9.5 μM, as measured by fluorescence spectroscopy (Supplementary Fig. 2c). This value is in the range of binding affinities exhibited by the majority of histone readers, thus attesting to the physiological relevance of the H3K9cr recognition by Taf14.

To determine whether H3K9cr is present in yeast, we generated whole cell extracts from logarithmically growing yeast cells and subjected them to Western blot analysis using antibodies directed towards H3K9cr, H3K9ac and H3 (Fig. 2a, b, Supplementary Fig. 3 and Supplementary Table 2). Both H3K9cr and H3K9ac were detected in yeast histones; to our knowledge, this is the first report of H3K9cr occurring in yeast. We next asked if H3K9cr is regulated by the actions of histone acetyltransferases (HATs) and histone deacetylases (HDACs). Towards this end, we probed extracts derived from yeast cells in which major yeast HATs (HAT1, Gcn5, and Rtt109) or HDACs (Rpd3, Hos1, and Hos2) were deleted. As shown in Figure 2a, b and Supplementary Fig. 3e, H3K9cr levels were abolished or reduced considerably in the HAT deletion strains, whereas they were dramatically increased in the HDAC deletion strains. Furthermore, fluctuations in the H3K9cr levels were more substantial than fluctuations in the corresponding H3K9ac levels. Together, these results reveal that H3K9cr is a dynamic mark of chromatin in yeast and suggest an important role for this modification in transcription as it is regulated by HATs and HDACs.

We have previously shown that among acetylated histone marks, the Taf14 YEATS domain prefers acetylated H3K9[11] (also see Supplementary Fig. 3b), however it binds to H3K9cr tighter. The selectivity of Taf14 towards crotonyllysine was substantiated by $^{1}H,^{15}N$ HSQC experiments, in which either H3K9cr$_{5\text{-}13}$ or H3K9ac$_{5\text{-}13}$ peptide was titrated into the ^{15}N-labeled Taf14 YEATS domain (Fig. 2c and Supplementary Fig. 4a, b). Binding of H3K9cr induced resonance changes in slow exchange regime on the NMR time scale, indicative of

strong interaction. In contrast, binding of H3K9ac resulted in an intermediate exchange, which is characteristic of a weaker association. Furthermore, crosspeaks of Gly80 and Trp81 of the YEATS domain were uniquely perturbed by H3K9cr and H3K9ac, indicating a different chemical environment in the respective crotonyllysine and acetyllysine binding pockets (Supplementary Fig. 4a). These differences support our model that Trp81 adopts two conformations upon complex formation with the H3K9cr mark as compared to H3K9ac (Supplementary Figs. 1c, d and 4c). One of the conformations, characterized by the π stacking involving two aromatic residues and the alkene group, is observed only in the YEATS-H3K9cr complex.

To establish whether the Taf14 YEATS domain is able to recognize other recently identified acyllysine marks, we performed solution pull-down assays using H3 peptides acetylated, propionylated, butyrylated, and crotonylated at lysine 9 (residues 1–20 of H3). As shown in Figure 2d and Supplementary Fig. 5a, the Taf14 YEATS domain binds more strongly to $H3K9cr_{1\text{-}20}$, as compared to other acylated histone peptides. The preference for H3K9cr over H3K9ac, H3K9pr and H3K9bu was supported by $^{1}H,^{15}N$ HSQC titration experiments. Addition of $H3K9ac_{1\text{-}20}$, $H3K9pr_{1\text{-}20}$, and $H3K9bu_{1\text{-}20}$ peptides caused chemical shift perturbations in the Taf14 YEATS domain in intermediate exchange regime, implying that these interactions are weaker compared to the interaction with the $H3K9cr_{1\text{-}20}$ peptide (Supplementary Fig. 5b). We concluded that H3K9cr is the preferred target of this domain. From comparative structural analysis of the YEATS complexes, Gly80 emerged as candidate residue potentially responsible for the preference for crotonyllysine. In attempt to generate a mutant capable of accommodating a short acetyl moiety but discriminating against a longer, planar crotonyl moiety, we mutated Gly80 to more bulky residues, however all mutants of Gly80 lost their binding activities towards either acylated peptide, suggesting that Gly80 is absolutely required for the interaction. In contrast, mutation of Val24, a residue located on another side of Trp81, had no effect on binding (Fig. 2d and Supplementary Fig. 5a, c).

To determine if the binding to crotonyllysine is conserved, we tested human YEATS domains by pull-down experiments using singly and multiply acetylated, propionylated, butyrylated, and crotonylated histone peptides (Supplementary Fig. 6). We found that all YEATS domains tested are capable of binding to crotonyllysine peptides, though they display variable preferences for the acyl moieties. While YEATS2 and ENL showed selectivity for the crotonylated peptides, GAS41 and AF9 bound acylated peptides almost equally well.

Unlike the YEATS domain, a known acetyllysine reader, bromodomain, does not recognize crotonyllysine. We assayed a large set of BDs in pull-down experiments and found that this module is highly specific for acetyllysine and propionyllysine containing peptides (Supplementary Fig. 7). However, bromodomains did not interact (or associated very weakly) with longer acyl modifications, including crotonyllysine, as in the case of BDs of TAF1 and BRD2, supporting recent reports[7]. These results demonstrate that the YEATS domain is currently the sole reader of crotonyllysine.

In conclusion, we have identified the YEATS domain of Taf14 as the first reader of histone crotonylation. The unique and previously unobserved aromatic-amide/aliphatic-aromatic π-

π-π-stacking mechanism facilitates the specific recognition of the crotonyl moiety. We further demonstrate that H3K9cr exists in yeast and is dynamically regulated by HATs and HDACs. As we previously showed the importance of acyllysine binding by the Taf14 YEATS domain for the DNA damage response and gene transcription[11,16], it will be essential in the future to define the physiological role of crotonyllysine recognition and to differentiate the activities of Taf14 that are due to binding to crotonyllysine and acetyllysine modifications. Furthermore, the functional significance of crotonyllysine recognition by other YEATS proteins will be of great importance to elucidate and compare.

ONLINE METHODS

Protein expression and purification

The Taf14 YEATS constructs (residues 1–132 or 1–137) were expressed in *E. coli* BL21 (DE3) RIL in either Luria Broth or M19 minimal media supplemented with $^{15}NH_4Cl$ and purified as N-terminal GST fusion proteins. Cells were harvested by centrifugation and resuspended in 50 mM HEPES (pH 7.5) supplemented with 150 mM NaCl and 1 mM TCEP. Cells are lysed by freeze-thaw followed by sonication. Proteins were purified on glutathione Sepharose 4B beads and the GST tag was cleaved with PreScission protease.

X-ray data collection and structure determination

Taf14 YEATS (residues 1–137) was concentrated to 9 mg/mL in 25 mM MES (pH 6.5) and incubated with 2 molar equivalence of the $H3K9cr_{5-13}$ at RT for 30 mins prior to crystallization. Crystals were obtain via sitting drop diffusion method at 18°C by mixing 800 nL of protein/peptide solution with 800 nL of well solution composed of 44% PEG600 (*v*/*v*) and 0.2 M citric acid (pH 6.0). X-ray diffraction data was collected at a wavelength of 1.54 Å at 100 K from a single crystal on the UC Denver Biophysical Core home source composed of a Rigaku Micromax 007 high frequency microfocus X-ray generator with a Pilatus 200K 2D area detector. HKL3000 was used for indexing, scaling, and data reduction. Solution was solved via molecular replacement with Phaser[17] using the Taf14 YEATS domain (PDB 5D7E) as search model with waters, ligands, and peptide removed. Phenix[18] was used for refinement of structure and waters were manually placed by inception of difference maps in Coot[19]. Ramachandran plot indicates good stereochemistry of the three-dimensional structure with 100% of all residues falling within the favored (98%) and allowed (2%) regions. The crystallographic statistics are shown in Supplementary Table 1.

NMR spectroscopy

NMR spectroscopy was carried out on a Varian INOVA 600 MHz spectrometer outfitted with a cryogenic probe. Chemical shift perturbation (CSP) analysis was performed using uniformly ^{15}N-labeled Taf14 (1–132). $^{1}H,^{15}N$ heteronuclear single quantum coherence (HSQC) spectra of the Taf14 YEATS domain were collected in the presence of increasing concentrations of either $H3K9cr_{5-13}$, $H3K9ac_{5-13}$, $H3K9cr_{1-20}$, $H3K9ac_{1-20}$ $H3K9pr_{1-20}$, $H3K9bu_{1-20}$ or free Kcr in PBS buffer pH 6.8, 8% D_2O.

Fluorescence binding assays

Tryptophan fluorescence measurements were performed on a Fluorolog spectrofluorometer at room temperature as described[20]. The samples containing 2 μM of Taf14 YEATS in PBS (pH 7.4) and increasing concentrations of H3K9cr$_{5\text{-}13}$ were excited at 295 nm. Emission spectra were recorded from 310 to 340 nm with a 1 nm step size and a 0.5 sec integration time. The K_d value was determined using a nonlinear least-squares analysis and the equation:

$$\Delta I = \Delta I_{max} \frac{\left(([L]+[P]+K_d) - \sqrt{([L]+[P]+K_d)^2 - 4[P][L]}\right)}{2[P]}$$

where [L] is the concentration of the peptide, [P] is the concentration of the protein, ΔI is the observed change of signal intensity, and ΔI_{max} is the difference in signal intensity of the free and bound states. The K_d values were averaged over 3 separate experiments, with error calculated as the standard deviation (SD).

Peptide pull-downs

YEATS domains in pGEX vectors were expressed in SoluBL21 cells (Amsbio) by induction with 1 mM IPTG at 16–18°C overnight with shaking. Cells were lysed by freeze-thaw and sonication then purified over glutathione agarose (Pierce) in a buffer containing 50 mM Tris pH 8.0, 500 mM NaCl, 20% glycerol (*v*/*v*) and 1 mM dithiothreitol (DTT). Peptide pull-downs were performed essentially as described[21] except that the assay buffer contained 50 mM Tris pH 8.0, 500 mM NaCl, and 0.1% NP-40, and 500 pmols of biotinylated histone peptides were loaded onto streptavidin coated magnetic beads before incubation with 40 pmols of protein. Bound proteins were detected with rabbit GST antibody (Sigma, G7781). Point mutants were generated by site-directed mutagenesis and purified/assayed as described above. The YEATS domains of Taf14, AF9, ENL, and GAS41 were previously described[11].

Western blotting

Yeast cultures were grown in YPD media at 30°C to mid-log phase and extracts were prepared as previously described[22,23]. Proteins from cell lysates were separated by SDS-PAGE and transferred to a PVDF membrane. Anti-H3K9ac (Millipore, 07-352) and anti-H3K9cr (PTM Biolabs, PTM-516) were diluted to 1:2000 and 1:1000, respectively, in 1x Superblock (ThermoScientific). An HRP-conjugated anti-rabbit (GE Healthcare) was used for detection. Bands were quantified using the ImageJ program.

Dot blotting

Increasing concentrations of biotinylated histone peptides (0.06–1.5 μg) were spotted onto a PVDF membrane then probed with the anti-H3K9ac (Millipore, 07-352) or H3K9cr (PTM Biolabs, PTM-516) at 1:2000 in a 5% non-fat milk solution and detected with an HRP-conjugated anti-rabbit by enhanced chemiluminesence (ECL).

Bromodomains pull-downs

cDNAs of GST-fused bromodomains were obtained either from EpiCypher Inc. or as a kind gift from Katrin Chua (Stanford University). GST fusions were expressed as described above except that the preparation buffer contained 50 mM Tris (pH 7.5), 150 mM NaCl, 10% glycerol (*v*/*v*), and 1 mM DTT. Pull-down assays were preformed as described above except that the assay buffer contained 50 mM Tris (pH 8.0), 300 mM NaCl, and 0.1% NP-40.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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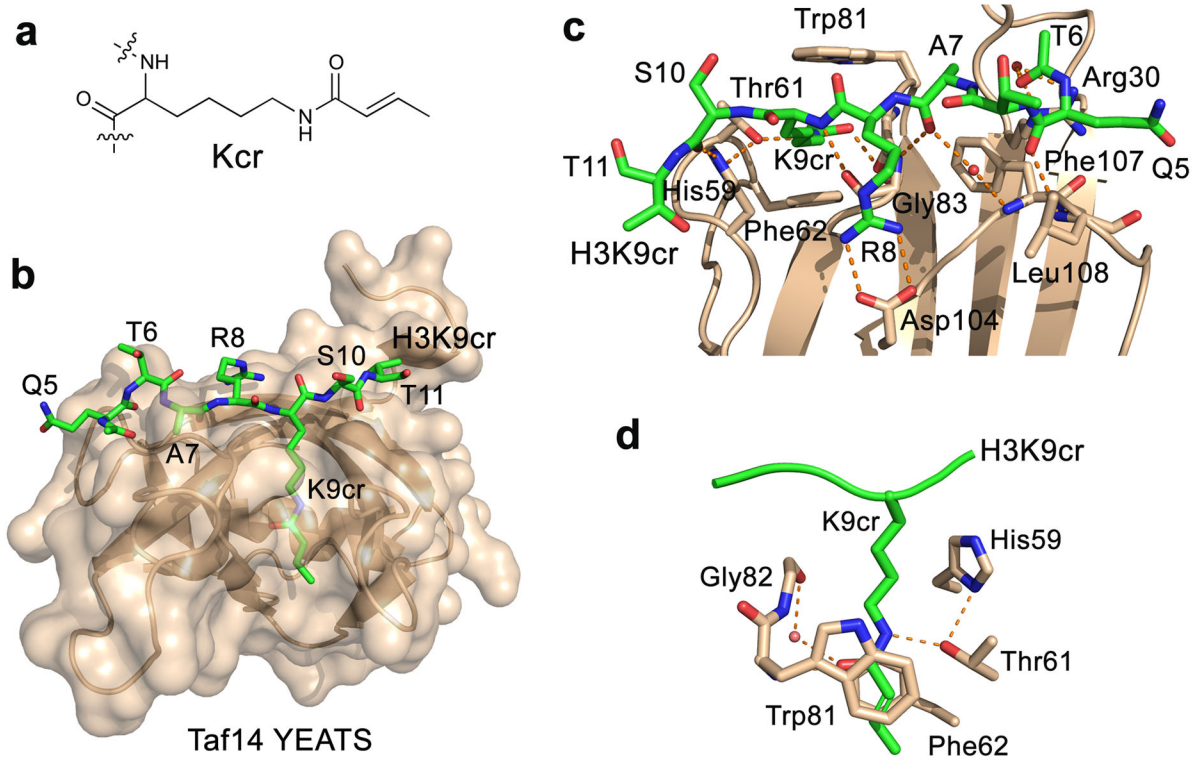

Figure 1. The structural mechanism for the recognition of H3K9cr

(**a**) Chemical structure of crotonyllysine. (**b**) The crystal structure of the Taf14 YEATS domain (wheat) in complex with the H3K9cr$_{5\text{-}13}$ peptide (green). (**c**) H3K9cr is stabilized via an extensive network of intermolecular electrostatic and polar interactions with the Taf14 YEATS domain. (**d**) The π-π-π stacking mechanism involving the alkene moiety of crotonyllysine.

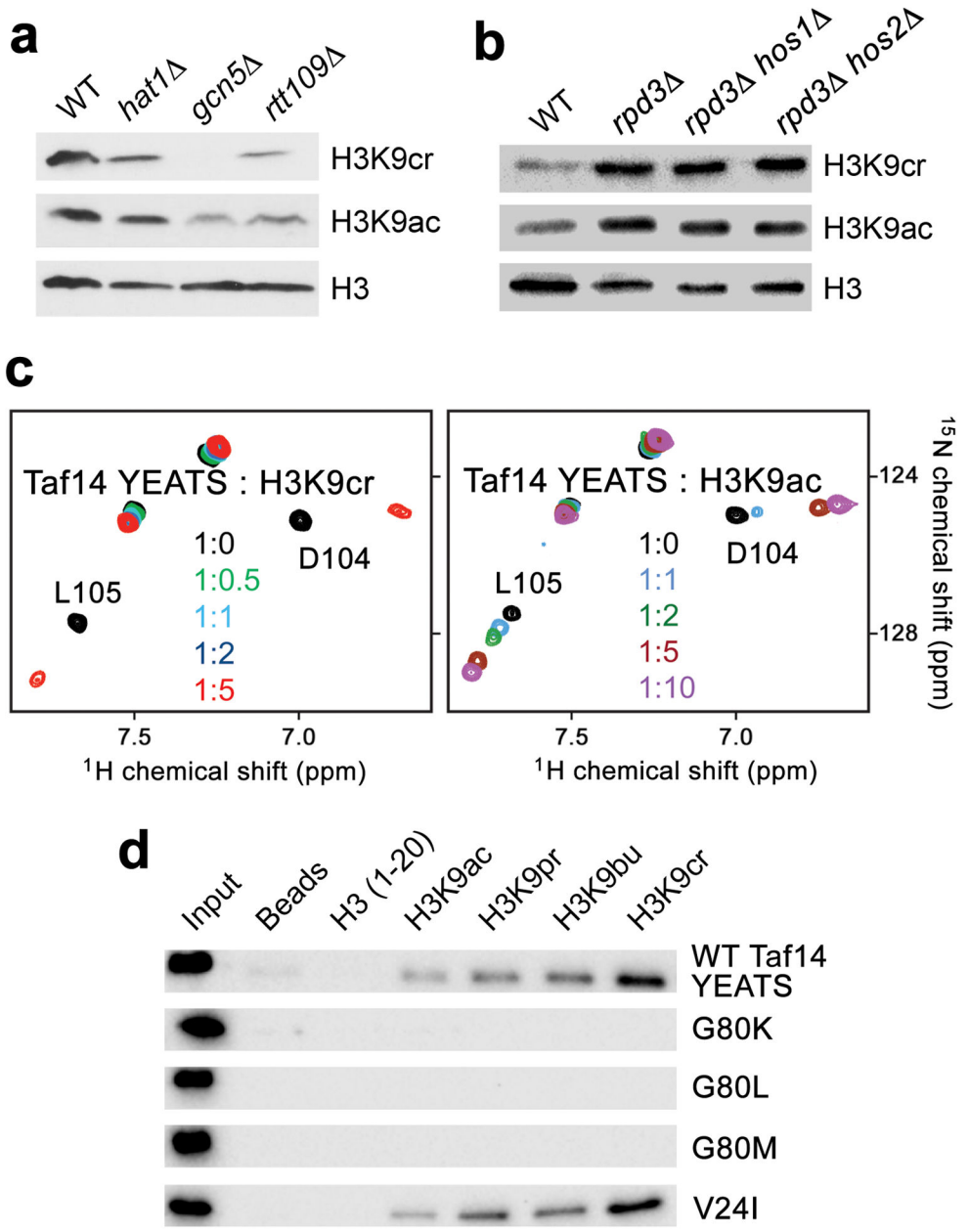

Figure 2. H3K9cr is a selective target of the Taf14 YEATS domain

(**a, b**) Western blot analysis comparing the levels of H3K9cr and H3K9ac in wild type (WT), HAT deletion, or HDAC deletion yeast strains. Total H3 was used as a loading control. (**c**) Superimposed $^{1}H,^{15}N$ HSQC spectra of Taf14 YEATS recorded as $H3K9cr_{5\text{-}13}$ and $H3K9ac_{5\text{-}13}$ peptides were titrated in. Spectra are color coded according to the protein:peptide molar ratio. (**d**) Western blot analyses of peptide pull-down assays using wild-type and mutated Taf14 YEATS domains and indicated peptides.