Transcription factor dimerization activates the p300 acetyltransferase

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The transcriptional co-activator p300 is a histone acetyltransferase (HAT) that is typically recruited to transcriptional enhancers and regulates gene expression by acetylating chromatin. Here we show that the activation of p300 directly depends on the activation and oligomerization status of transcription factor ligands. Using two model transcription factors, IRF3 and STAT1, we demonstrate that transcription factor dimerization enables the trans-autoacetylation of p300 in a highly conserved and intrinsically disordered autoinhibitory lysine–rich loop, resulting in p300 activation. We describe a crystal structure of p300 in which the autoinhibitory loop invades the active site of a neighbouring HAT domain, revealing a snapshot of a trans-autoacetylation reaction intermediate. Substrate access to the active site involves the rearrangement of an autoinhibitory RING domain. Our data explain how cellular signalling and the activation and dimerization of transcription factors control the activation of p300, and therefore explain why gene transcription is associated with chromatin acetylation.

Signals that emanate from cellular receptors ultimately lead to changes in gene expression that drive cellular change and organismal development. Gene expression is typically controlled through the coordinated activity of DNA-binding transcription factors, chromatin regulators and the general transcription machinery. For instance, in the innate immune system, pattern recognition receptors recognize and engage with various pathogen-associated molecular patterns1, and subsequently bind to adaptor proteins such as STING (stimulator of interferon genes). These adaptor proteins engage the latent DNA-binding transcription factor interferon (IFN) regulatory factor 3 (IRF3) and enable recruitment and activation of the non-canonical IκB kinase TBK11. TBK1 then phosphorylates IRF3 in a C-terminal motif, resulting in the removal of autoinhibition, dimerization and adaptor displacement2,3. Activated IRF3 dimers bind to p300/CBP (where CBP is CREB-binding protein; p300 and CBP are also known as KAT3B and KAT3A, respectively) to stimulate chromatin acetylation and gene expression of the antiviral type I IFNs IFNα and IFNβ(3,4). Type I IFNs are secreted and bind to specific cell-surface IFN receptors, which results in the activation of Janus kinase–signal transducers and activators of transcription (JAK–STAT) signalling6. The activated, tyrosine-phosphorylated STATs then dimerize, translocate to the nucleus and bind to p300/CBP to stimulate the transcription of IFN-stimulated genes7.

p300/CBP are known to interact with more than 400 binding partners including the basal transcription machinery8. The large protein interactome of p300/CBP results in near-universal recruitment of these HATs to enhancers, and p300 occupancy has been used to identify enhancers genome-wide9,10. p300/CBP catalyses the acetylation of histone H3K27 to form H3K27ac, a modification that is considered an ‘activation’ mark11. However, recruitment of p300/CBP does not always correlate with gene activation and is occasionally associated with repression12,16. A large number of chromatin regions that bind p300/CBP therefore do not contain this canonical H3K27ac modification, which indicates that HAT activity at such sites is blocked15,17.

Therefore, it is a major challenge to understand the mechanism that enables switching between inactive and active states of p300/CBP on enhancers, and to causally link cellular signalling to the recruitment of p300/CBP, the regulation of HAT activity and the establishment of repressed, poised and active chromatin. Here we have investigated how the activation and oligomerization status of p300 transcription factor ligands such as IRF3 and STAT1 affects the catalytic activity of p300. We found that the kinase-activated and dimeric, but not the inactive or monomeric, variants of these transcription factors support robust p300 HAT activation. We demonstrate that transcription factor dimerization enables p300 trans-autoacetylation in a lysine–rich, intrinsically disordered autoinhibitory loop (AIL) in the HAT domain that serves as a ‘pseudosubstrate’ and is important for regulating the HAT activity of p30018. A crystal structure of the core domain of p300 provides a snapshot of a potential trans-autoacetylation reaction intermediate in which the AIL projects into the active site of a neighbouring p300 molecule. As HAT activation is closely linked to transcription factor activation, these results causally relate cellular signalling to the activation and DNA targeting of a chromatin modifier and provide mechanistic insights into the long-standing and general correlation between an active, acetylated chromatin structure and gene transcription.

Transcription factor dimerization activates p300

To explore whether p300 is activated by signal-dependent IRF3 dimerization, we produced three recombinant IRF3 species: inactive monomers (IRF3); active, TBK1-phosphorylated IRF3 dimers (pIRF3); and a truncation mutant that lacked the C-terminal autoinhibitory element (IRF3ΔC) (Extended Data Fig. 1a, b). Truncation of the C-terminal autoinhibitory element allows for p300/CBP binding but abolishes IRF3 dimerization19. We confirmed the oligomerization status by gel filtration chromatography (Extended Data Fig. 1b), and investigated the effect of IRF3 activation and oligomerization status on the autoacetylation of p300s in the presence of [14C]acetyl coenzyme A
includes the reaction did we observe p300 activation (Fig. 1c, d). phosphorylation by TBK1 and its dimerization are required for p300 HAT IRF3 even in the absence of STING. We conclude that IRF3 phosphorylation of IRF3 on Ser396, a critical residue for IRF3 activation, and C-terminal regions (Extended Data Fig. 2a). In this configuration, the HAT dimerization and not solely interaction with the TAZ2 domain, is required for the activation of p300. Unphosphorylated, monomeric STAT1ΔN, which contains the TAD and is able to interact with the TAZ2 domain of CBP, did not stimulate p300 activity. However, stimulation with STAT1 was not as potent compared with that of IRF3, possibly because our STAT1 preparation is unphosphorylated on Ser27, which is required for maximal gene activation. Together, our data are consistent with a model in which the AIL peptide serves as an intramolecular ‘pseudosubstrate’ and a competitive HAT inhibitor. Dimeric ligands such as pIRF3 and pSTAT1 allow p300 activation by bringing two molecules together to enable trans-autoacetylation of the AIL, which in turn relieves autoinhibition and enables more efficient entry of substrates into the HAT active site.

Structure of p300 adopts an AIL–swap conformation
To further understand the role of the AIL in the regulation of these structural transitions, we crystallized the hypoacetylated form of the catalytic core of p300 comprising the bromo-RING-PHD-HAT domains (BRP-HAT) that contained the AIL. Crystals were obtained using a similar protocol as published previously. Crystals diffracted to a minimal Bragg spacing of 3.1 Å and we determined the structure by molecular replacement (Extended Data Table 1). The crystal form contained four p300 molecules in the asymmetric unit (Extended Data Fig. 2). Comparison with our previous structure showed that the bromo-PHD-HAT domains overlay well on each other with a root-mean-square deviation (r.m.s.d.) of approximately 1 Å. However, the RING domains were not visible in the initial electron density map. Anomalous difference density maps showed a density peak for the zinc atom of the RING domain, but it was not at the expected location. Manual repositioning enabled the correct placement of the RING domains into the new position and the refinement of the structure (Fig. 2a, Extended Data Fig. 3).

The p300 molecules show an antiparallel arrangement of the BRP-HAT domains (Extended Data Fig. 2a). In this configuration, the HAT

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**Fig. 1** | Transcription factor dimerization activates p300. a, p300s was incubated for the indicated times in the presence or absence of inactive, monomeric IRF3 or TBK1-phosphorylated, dimeric pIRF3. Samples were analysed by SDS–PAGE followed by Coomassie staining and autoradiography. Representative data of three independent experiments are shown. b, Quantification of the autoacetylation of p300s. c, p300 is activated by TBK1-mediated IRF3 phosphorylation. p300s was incubated with recombinant GST–STING, TBK1 and IRF3 in the presence of ATP and [14C]acetyl-CoA. Top, Coomassie-stained SDS–PAGE gel. Middle, analysis of IRF3 phosphorylation on S396 using immunoblotting. Bottom, autoradiography. Representative data of three independent experiments are shown. d, HAT scintillation proximity assay. The degree of histone H4 substrate acetylation was quantified using scintillation counting. e, As in a but using inactive, monomeric STAT1ΔN or activated, dimeric pSTAT1ΔN. Activated, dimeric pSTAT1ΔN that lacked the C-terminal TAD did not stimulate the autoacetylation of p300s. Samples were analysed as in a. Representative data of three independent experiments are shown. f, Quantification of the autoacetylation of p300s. Intensity values were normalized by dividing by the maximum autoacetylation signal obtained after 60 min. Error bars shown in b, d and f: three independent experiments were performed, data are mean ± s.d. Data analysis and plotting was performed with GraphPad Prism 7.0. For gel source data, see Supplementary Fig. 1.
The inward-rotated conformation (magenta in Fig. 3a) is stabilized by interactions between Glu1242 of the RING domain and Arg1645 and Arg1646 of helix α9 of the HAT domain. In addition, Gln1173, Thr1174 and Thr1184 of the RING domain pack against the unusual long loop (L1) in the HAT domain that covers the CoA portion of the Lys-CoA inhibitor. As a result, Leu1182 resides within about 5.5 Å of the lysine moiety of Lys-CoA (Fig. 3b). This inward conformation of the RING domain thus restricts substrate access to the HAT domain: the incoming AIL from the neighbouring p300 monomer II would clash with the RING domain in the inward conformation (Fig. 3c).

In the outward-rotated conformation, the interactions that attach the RING domain to the HAT domain are mostly disrupted (Fig. 3b). Leu1182 is positioned around 15 Å away from the substrate-binding site and the RING domain is cradled by the AIL extension of helix α6 of the neighbouring p300 molecule (monomer II residues 1524–1533; Fig. 3d). Despite shape complementarity, with a small buried surface area of about 320 Å², the interface is predominantly polar, which is uncharacteristic of a typical protein–protein interface. However, this interaction could help to stabilize an outward-rotated conformation of the RING domain and a more open active site of the HAT, apparently to enable access of the AIL and trans-acetylation.

**Regulation of HAT activity by flanking domains**

To systematically analyse the flanking domains, we generated a series of p300 constructs (Extended Data Fig. 4a) and analysed the effect on HAT activity in vitro and in cells. Overexpression of p300 generally resulted in hyperacetylated, active p300 variants (Extended Data Fig. 4b, c), which probably masks the functional role of structural elements potentially involved in autoinhibition of deacetylated p300. Deletion of the RING domain did not considerably alter autoacetylation or histone acetylation (Extended Data Fig. 5a). This deletion did
Regulation of p300 by the AIL and RING domain

We next sought to understand how the highly conserved and intrinsically disordered AIL segment contributes to the regulation of the catalytic function of p300. The AIL spans amino acid residues 1532–1567 and is positively charged in the deacetylated state, with an estimated isoelectric point (pI) of 10.9, and net charge of +7 at neutral pH. By contrast, upon autoacetylation of residues spanning Lys1542–1560, we estimate a pI of 3.5 and a net charge of −2. As the proximal substrate-binding groove of p300 is largely acidic (Fig. 2c), we proposed—consistent with earlier predictions—that a deacetylated AIL would engage the substrate binding site through electrostatic interactions, presumably to prevent access of positively charged lysine-containing substrates. Given the disordered nature of the AIL, this proposed interaction is expected to be highly dynamic.

We tested this hypothesis through all-atom Monte Carlo simulations. To make this approach tractable, our simulations held the backbone dihedral angles associated with the folded domains fixed, but all other degrees of freedom, including all backbone and side chain dihedral angles in the AIL, were fully sampled. As a result, these simulations should be seen to assess how the AIL interacts with the remainder of p300 given the observed crystal structure. Simulations were performed on the AIL in the deacetylated and acetylated states in the context of the p300 monomer. These simulations enabled us to investigate how acetylation influenced the conformation and intramolecular interactions of the AIL.

Simulations of the deacetylated AIL revealed the presence of extensive yet highly degenerate electrostatic interactions between the AIL and the RING domain and between the AIL and the HAT substrate-binding site. These interactions were quantifiable in terms of the normalized distances between pairs of amino acid residues (Fig. 5a, Supplementary Video 1). Lysine residues in the AIL dynamically associate through long-range electrostatic interactions with acidic residues (E1334, E1442, E1505, D1622, D1625 and D1628) in the p300 HAT substrate-binding pocket (Fig. 5c). The importance of these residues for substrate acetylation has been shown previously—and nuclear magnetic resonance data for CBP confirm that the AIL is intrinsically disordered in the deacetylated state.

By contrast, in the acetylated state we found no interactions between the AIL and the substrate binding site (Fig. 5b and Supplementary Video 2). The acetylated AIL essentially behaved like a self-avoiding random coil without any strong biases for interaction with itself or with the surrounding folded domains, including the bromodomain. It has been proposed that the AIL of CBP, when acetylated on Lys1596 (K1558 in p300), engages the bromodomain intramolecularly, thus competing for substrate acetylation has been shown previously—and the surrounding folded domains, including the bromodomain. It has been proposed that the AIL of CBP, when acetylated on Lys1596 (K1558 in p300), engages the bromodomain intramolecularly, thus competing with histone binding and negatively regulating substrate acetylation. Isothermal calorimetry experiments showed the highest binding affinity for multifunctional peptides, including the diacylated histone peptides H3(K14ac/K18ac) and H4(K12ac/K16ac), generally following the pattern KacNNNKac (Extended Data Table 2). Monoacetylated peptides typically had weaker binding affinity. A crystal structure of the H4(K12ac/K16ac) peptide bound to BARP (Extended Data Fig. 5c)
confirmed the acetyllysine-specific binding mode. However, a AIL peptide acetylated on the three lysines K1549, K1558 and K1560—corresponding to some of the most highly acetylated residues in the AIL—failed to bind to the BRP module. Thus our interpretation is that the multiacetylated AIL is not a substrate for the bromodomain, presumably because of suboptimal spacing or sequence environment of the acetylated lysine sites of the AIL.

To understand how the RING domain influences the ability of substrates—including the AIL—to enter the active site of an adjacent p300 molecule, we performed simulations of the AIL in the context of the loop-swapped dimer, using a harmonic potential to maintain the AIL in the active site in order to assess potential intermolecular interactions (Fig. 5d, Extended Data Fig. 6a). In the active RING conformation, the AIL is able to engage the active site residues E1442 and D1444—residues proximal to the lysine substrate binding tunnel—was reduced by 70–75% (Fig. 5d). Thus, in the inactive conformation, the RING domain at least partially reduces catalytic activity by limiting accessibility of the active site to the AIL and other substrates.

One prediction from our models is that the deacetylated form of p300 adopts a more compact conformation, owing to dynamic engagement of the AIL with the HAT substrate-binding site, whereas the acetylated form adopts a more ‘open’ conformation (Fig. 5d). To test this possibility, we produced hypo- and hyperacetylated p300 variants (Extended Data Fig. 6e–g) and analysed the preparations by SEC–MALLS. All preparations were monomeric at the concentration tested (2 mg ml$^{-1}$) (Extended Data Fig. 6b–d, Extended Data Table 3). Hyperacetylation of p300 BRP-HAT resulted in a small decrease in the elution volume, which is indicative of a larger hydrodynamic radius (Extended Data Fig. 6b). A similar result was obtained upon comparison of hyper- and hypoaecylated BRP-HAT-CH3 (Extended Data Fig. 6c). By contrast, a variant that lacks the AIL showed no change in the elution volume upon hyperacetylation (Extended Data Fig. 6c). Our data are therefore consistent with the model that the catalytic p300 ‘core’ adopts a compact conformation in the hypoacetylated state, with autoacetylation resulting in a more extended conformation.

**Discussion**

Our findings provide detailed mechanistic insights into how cellular signalling controls the activity of a chromatin regulator. We propose a multi-step process for p300 HAT activation and signal transmission to chromatin (Extended Data Fig. 7a–d). In the basal state, the deacetylated AIL is expected to maintain an overall positively charged environment in close proximity to the active site of the enzyme, thus preventing access of positively charged lysine-rich substrates. Direct access to the CoA-binding tunnel and autoacetylation of the AIL in cis appears to be prohibited, in part due to the positioning of the RING domain (Fig. 5d).

Cellular signalling initiates phosphorylation of transcription factors, such as IRF3 or STAT1, which results in their activation and dimerization. The activated, dimeric transcription factors are in their DNA-binding-competent conformation and can engage two molecules of p300 in the nucleus, thus increasing the likelihood of AIL disengagement from its inhibitory position in cis and of its capture in trans by a second p300 molecule. Association of two p300 molecules does not necessarily require precise stereospecific interactions between the structured domains, because acetylation at several lysines in the AIL indicates a series of possible conformations in such transiently associating dimers. We predict that regulated oligomerization uncouples AIL recruitment from HAT activation, which could explain why not all p300/CBP recruitment events result in chromatin acetylation and gene activation$^{12–17, 33}$.

It has been proposed that enhancer RNA interacts with the AIL to regulate CBP HAT activity$^{34}$. We have attempted to reproduce these results using Klf6, one of the most potent enhancer RNAs reported$^{34}$. We could not detect p300 HAT activation using up to equimolar amounts of Klf6 (Extended Data Fig. 7e, g). We note that, in a previous study, CBP was purified in buffer containing EDTA; this is detrimental to the structure of p300/CBP owing to the presence of multiple zinc-binding domains$^{35}$. When unfolded by incubation with EDTA, CBP and p300 have a high tendency to aggregate and to form non-specific interactions$^{35}$. Paradoxically, as the HAT domain is not affected, inclusion of EDTA can have an ‘activating’ effect in biochemical assays, apparently due to such non-specific aggregation (Extended Data Fig. 7f). The detrimental effects of EDTA on the structure and function of p300/CBP need to be taken into account in the interpretation of such data.

The ability of certain histone-modifying enzymes to bind to the post-translational modification (PTM) they generate has led to models in which such enzymes might propagate modified chromatin domains by a positive-feedback loop$^{36}$. According to this view histone PTMs and other chromatin modifications form an additional,
DNA-sequence-independent layer of the genome, which is read out by enzymes that recognize these modifications to ‘epigenetically’ regulate genomic function. An alternative view proposes that histone PTMs ultimately depend on DNA-sequence-dependent recruitment of chromatin modifiers, and so do not necessarily form an independent ‘epigenetic’ layer of the genome. The controversy has arisen because it has been difficult to disentangle, for most chromatin regulators, the relative contributions of DNA targeting and histone PTM substrate engagement to the overall chromatin-modification reaction.

We show that regulation of p300 is linked to the activation and oligomerization status of transcription factor ligands, and therefore conclude that specificity for p300-mediated chromatin acetylation arises mainly through transcription-factor-mediated and DNA-sequence-dependent genome targeting. The next question is how the bromodomain contributes to p300 function. Although it is clear that the bromodomain can engage acetylated histone peptides and bind to hyperacetylated chromatin, deletion or mutation of the bromodomain has no apparent effect on substrate acetylation; has only minimal effects in a haematopoiesis model system, and bromodomain inhibition does not adversely affect genome targeting of CBP.

We favour a model in which DNA binding provides the lead anchoring mechanism: local hyperacetylation increases the binding valency by enabling bromodomain substrate engagement, which further helps to compartmentalize the biochemical reaction and contributes to signal maintenance. p300 HAT-activating mutants form biomolecular condensates in cells when transiently overexpressed (Fig. 4c). Treatment with a HAT inhibitor or bromodomain inhibitor greatly reduces the formation of condensates, which indicates that hyperacetylation and bromodomain–substrate engagement are critical in driving assembly. The formation of condensates, possibly through phase-separation, may provide a mechanism to enable signal integration on enhancers and transcriptional control. It will be critical to disentangle cause–effect relationships of DNA targeting, chromatin modification and histone PTM substrate engagement of other chromatin regulators.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41586-018-0621-1.

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**Author contributions** E.O. designed and performed most experiments, analysed and validated the data and revised the draft with assistance from S.R., Z.I., N.H. and J.G. A.S.H. performed computational modelling and revised the draft. S.K. provided supervision, funding acquisition and commented on the draft. D.P. was involved in conceptualization, supervision, project administration, funding acquisition and wrote the original and revised drafts.

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**Additional information**

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METHODS

No statistical methods were used to predetermine sample size. The experiments were not randomized, and the investigators were not blinded to allocation during experiments and outcome assessment.

Constructs. For cell-free protein expression, cDNA of p300 (NCBI reference sequence: NM_001429.3) variants were cloned into the pIVEX2.4d vector (Roche) with a N-terminal 6× His tag and a C-terminal Flag tag. In the ΔR constructs, the RING domain was deleted. Residues 1269–1241 were replaced by a glycine acidic amino acid residue linker. In the ΔAIL constructs, loop amino acid residues comprising residues 1520–1581 were replaced by the flexible linker sequence SGGSQG. For Escherichia coli expression, cDNA encoding residues 1048–1282, for the BRP or BΔARP were cloned into the vector pETM-33 (EMBL) with a tobacco etch virus (TEV)-cleavable N-terminal glutathione S-transferase (GST) tag. p300 BRP_HAT variants were cloned into pFSTBAC1 (Thermo Fisher) and expressed in insect cells as shown previously27. p300s constructs, spanning amino acid residues 324–2094, were cloned into pFSTBAC1 vector with a N-terminal Flag tag. Haemagglutinin (HA)-tagged full-length p300 variants were cloned into pCDNA3.1 (Thermo Fisher). Point mutations were introduced by QuikChange mutagenesis (Agilent). Point mutations and nucleotide deletions carried out in p300 full-length (1–2414) or p300s (324–2094) were done through transfer vectors as described previously28. STAT1ΔN (136–748), STAT1ΔNC (136–713) and IRF3ΔC (Δ1–382) with a C-terminal intein tag were cloned into the pTXB1 vector (New England Biolabs) using the restriction enzymes Ndel (STAT1) or NcoI (IRF3) and SpeI (IRF3 (Δ427–427) with an N-terminal His-tag cleavable by TEV protease was cloned using the restriction enzymes NcoI and XhoI into the vector pETM-11 (EMBL). All constructs were confirmed by DNA sequencing.

Expression and purification. Expression and purification of Flag-tagged p300s constructs was done as described previously29. This method enables purification of p300s variants that are already pre-acetylated. Expression and purification of p300 BRP_HAT and SIRT2 were performed as described previously29. TBK1 was expressed in insect cells and purified as described previously29. Cell-free protein synthesis was done in a 50 μl reaction volume. In brief, 10 μg ml−1 of His-p300 variants in pIVEX2.4d were added to a reaction mixture containing 1 mM amino acid mix, 0.8 mM NTPs (guanosine-, uracil-, and cytidine-5′-triphosphate ribonucleotides), 1.2 mM adenosine-5′-triphosphate, 55 mM HEPES, pH 7.5, 68 μM folic acid, 0.64 mM cyclic adenosine monophosphate, 3.4 mM dithiothreitol (DTT), 27.5 mM ammonium acetate, 2 mM spermidine, 5 mM ZnCl2, 80 mM creatine phosphate, 208 mM potassium glutamate, 16 mM magnesium acetate, 250 μg ml−1 creatine kinase, 27 μg ml−1 T7 RNA polymerase, 0.175 μg ml−1 tRNA and 67 μl ml−1 300 E. coli bacterial extract. Incubation was carried out at 22°C with agitation for 16 h. Proteins were purified using Ni-NTA chromatography (IMAC Sepharose 6 FF; GE Healthcare) in buffer 1 (20 mM TRIS, pH 8.0, 300 mM NaCl, 1 mM DTT, 5 mM ZnCl2) containing Complete Protease Inhibitors EDTA-Free (Roche) and 50 mM MgCl2 and 1 mM ATP. The reaction was incubated at 30°C for 1 h and then for an additional 10 h at 21°C. Phosphorylated IRF3 was further purified by size-exclusion chromatography on a Superdex S200 16/60 column (GE Healthcare) in 20 mM HEPES, pH 7.5, 300 mM NaCl, 0.5 mM TCEP. The protein was concentrated to 16 mg ml−1 in a prewashed Amicon Ultra-15 centrifugal filter (molecular weight cut off 30 kDa; EMD Millipore), flash-frozen in liquid nitrogen and stored at −80°C.

IRF3 was expressed in E. coli BL21(DE3) at 18°C for 16 h. The cells were collected by centrifugation and resuspended in buffer 2 containing 20 mM imidazole. The cells were lysed with buffer 3 and 10 μl m M reduced Glutathione in buffer 3. The protein was further purified by gel filtration on a High Load 16/60 Superdex 200 column (GE Healthcare) equilibrated in 20 mM HEPES, pH 7.5, 300 mM NaCl, 0.5 mM TCEP. The final protein was concentrated to 16 mg ml−1 in a prewashed Amicon Ultra-15 centrifugal filter (molecular weight cut off 30 kDa; EMD Millipore), flash-frozen in liquid nitrogen and stored at −80°C.

CRystallization and structure determination. The p300 BRP_HAT construct comprising the AIL and the mutation Y167F was deacetylated as described previously30. The protein at 4.5 mg ml−1 was incubated with a triple molar excess of the bi-substrate inhibitor Lys-CoA32 before crystallization. Crystals in the P212 space group were grown by hanging-drop vapour diffusion at 4°C by mixing equal volumes of protein and crystallization solution containing 100 mM HEPES, pH 7.5, 18–22% polyethylene glycol 3350 and 0.2 M NaCl. Crystals were cryoprotected in 20–25% ethylene glycol and drop-frozen in liquid nitrogen. We collected native diffraction data to a minimum Bragg spacing of 3.1 Å resolution at the ERBF on beamline ID29 under a nitrogen gas stream at 100 K, at a wavelength of 1.282 Å. We processed the data with XDS (Extended Data Table 1). The structure of the BRP_HAT construct was solved by molecular replacement using Phaser. There were two copies in the asymmetric unit and the RING domains were initially not visible in the electron density map and are partially disordered. Inspection of an anomalous difference map indicated peak density for the zinc ions and enabled positioning of the RING domain in the outward-rotated conformation. A final model was produced by iterative rounds of manual model building in Coot and refinement using PHENIX. The final model contains residues 1045–1664 with a deletion of residues 1534–1567 and was refined to a 3.1 Å resolution with an Rwork of 19% and 26%, respectively (Extended Data Table 1). Analysis of the refined structure by MolProbity showed that there are no residues in disallowed regions of the Ramachandran plot. The MolProbity all-atom-clash score was 1.91, placing the structure in the 100th percentile among structures refined at 3.1 Å resolution (n = 2,108).

The BΔARP construct at 15 mg ml−1 was mixed with 2 mM of a 11-mer histone peptide H4 (10–20) GLGkacGAGkacRHR (only the underlined amino acid sequence is visible in the electron density map) containing two acetylated lysine residues at K12 and K16, H4(K12ac/K16ac). Crystals in the P212121 space group were grown by hanging-drop vapour diffusion at 21°C by mixing equal volumes of protein and crystallization solution containing 1.6 M ammonium sulfate and 6,960). Crystals were cryoprotected in 20% ethylene glycol and drop-frozen in liquid nitrogen. We collected native diffraction data to a minimum Bragg spacing of 2.5 Å resolution at the ERBF on beamline ID29 under a nitrogen gas stream at 100 K, at a wavelength of 1.0 Å (Extended Data Table 1). The structure of the BΔARP construct was solved by molecular replacement using Phaser. The refined structure by MolProbity showed that there are no residues in disallowed regions of the Ramachandran plot. The MolProbity all-atom-clash score was 0.97, placing the structure in the 100th percentile (n = 6,960).
A p300 HAT scintillation proximity assay was designed similar to that described previously. In brief, as a substrate we used a synthetic histone H4 peptide containing 15 amino acids derived from the N terminus of human H4 that was chemically attached to biotin with an amino hexanoic linker (Biotin-C6--GRGGGGKKLGKGGGAK) (from peptide.de). The synthetic peptide was re-suspended in water and adjusted to pH 7.0 with concentrated NaOH.

A typical reaction contained p300s (50 nM), 12.5 μM biotinylated H4 peptide, acetyl-CoA (60 μM) to 1 μM set at around 10 times the apparent K<sub>a</sub> in 20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 5 mM MgCl<sub>2</sub>, 0.01% Tween-20 and 0.1% BSA (w/v). For reactions containing pIRF3, 50 mM was added. 20 μl of a 2× reaction mixture containing p300s, H4 peptide with and without pIRF3 was pre-incubated at 30°C for 5 min. The reaction was initiated by the addition of 20 μl of 2× acetyl-CoA containing a 1:3 mix of tritiated [3H]acetyl-CoA (PerkinElmer; NET290050UC) with cold acetyl-CoA. For example, for 10 μM final acetyl-CoA concentration, a mix of 5 μM [3H]acetyl-CoA and 15 μM cold acetyl-CoA (A2056, Sigma) was used. The reaction was quenched at the indicated time points by delivering 40 μl of the reaction mix into 120 μl of 0.5 M HCl in a FlashPlate Plus Streptavidin 96-well scintillant-coated microplate (Perkin Elmer, SMP103001PK). The plate was incubated for 1 h, and light emission was counted in a Microbeta2 Scintillation Counter (Perkin Elmer) at 1 min per well in the top count mode. Counts per minute (cpm) were plotted against acetyl-CoA concentration. Typical progress curves are shown in Extended Data Fig. 1d. The initial rate was estimated by linear regression during the first 10 min of the reaction and plotted against acetyl-CoA concentration. All data were analysed using GraphPad Prism 7.0.

For the results shown in Fig. 4, acetylation reactions were performed in acetylation buffer (50 mM Hepes, pH 7.5, 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 10% glycerol and 1× complete EDTA-free protease inhibitor (Roche)) with 50 μM acetyl CoA (Sigma), 100 ng ml<sup>−1</sup> trichostatin A and 2 μg of purified histone octamer. Reactions were incubated for 30 min at 30°C and stopped by the addition of 3× SDS gel loading buffer, then used for Coomassie staining and immunoblotting.

**Multi angle laser light scattering-size exclusion chromatography.** Before SEC–MALLS runs, p300 variants were acetylated and deacetylated using p300 HAT or SIRT2 as described previously. The reactions were analysed by liquid chromatography–mass spectrometry as described previously. Size-exclusion chromatography was performed at a flow rate of 0.5 ml min<sup>−1</sup> on a Superdex 200 Increase 10/300 GL column equilibrated in SEC–MALLS buffer (20 mM HEPES, 300 mM NaCl, 5 mM ZnCl<sub>2</sub>, 0.5 mM TCEP) at 21°C. A 50 μl sample of p300 at 2 mg ml<sup>−1</sup> was injected onto the column and multi angle laser light scattering was recorded with a laser emitting at 690 nm using a DAWN-EOS detector (Wyatt Technology Corp.). The refractive index was measured using a RI2000 detector (Schambeck SFD). The molecular weight was calculated from differential refractive index measurements across the centre of the elution peaks using the Debye model for protein solutions (Veerasingham et al., 1998).

**In vitro eRNA transcription.** Klf6 eRNA corresponding to 496 nucleotides of the sense strand of human chr13:5802100-5802596 (ref. 34) was produced in vitro transcription from a pMA plasmid containing a eKlf6 insert synthesized by GeneArt Gene Synthesis (Thermo Fisher). pMA_Klf6 plasmid (50 μg) was linearized with 80 U of KpnI-HF in a final volume of 100 μl and incubated at 37°C for 14–16 h. The in vitro transcription reaction was done in a final volume of 1 μl, using 1× T7 buffer, T7 RNA Polymerase and 1 U of RNaseOUT Recombinant Ribonuclease Inhibitor (Thermo Fisher). After incubation for 2 h at 37°C, 0.5 U of TURBO DNase (2 U μl<sup>−1</sup>) (Thermo Fisher) and 1 μM CaCl<sub>2</sub> was added to the reaction and incubated for 30 min at 37°C. Following DNase treatment, 2 μl of a 30 mg ml<sup>−1</sup> stock of protease K powder (Thermo Fisher), dissolved in proteinase K buffer (10 mM TRIS pH 7.5, 1 mM CaCl<sub>2</sub> and 40% glycerol), was added and incubated for 45 min at 37°C. Buffer was exchanged into 20 mM HEPES, pH 7.5, 300 mM NaCl, 0.5 mM TCEP using Amicon Ultra-0.5 ml centrifugal filters (molecular weight cut off 3 kDa, EMD Millipore). To further purify the RNA, 3 volumes of TRizol (Thermo Fisher) was added to the RNA sample, followed by isopropanol precipitation. Purified Klf6 RNA was resuspended in 20 mM HEPES, pH 7.5, 300 mM NaCl and 0.5 mM TCEP RNA was quantified using a Nanodrop spectrophotometer (Thermo Fisher). The quality of Klf6 was assessed by agarose gel electrophoresis in 1× TBE buffer or using denaturing 6 M urea 14% PAGE (Extended Data Fig. 7c).

**Immunoblotting, immunofluorescence and antibodies.** We have used the cell lines COS and H1299. They are not on the list of commonly misidentified cell lines maintained by the International Cell Line Authentication Committee. COS cells were purchased from ATCC, product reference ATCC-CCR-1615, lot no. 4171903. H1299 cells were authenticated on December 6th 2016 by LGC Standards: Cell Line Authentication Services. COS cells were authenticated using short tandem repeat (STR) analysis as described in 2012 in ANSI Standard (ASN-0002) Human cell lines were authenticated using standardization of STR Profiling by the ATCC Standards Development Organization as described in ref. 51. Upon the receipt of COS cells
and after the authentication of H1299 cells, they were expanded. A mycoplasma contamination test (Mycoplasma Detection Kit, Lonza cat no. LT07-418) was performed and the mycoplasma-free cells were frozen and kept in liquid nitrogen. After thawing they were kept in culture for 30 passages with a mycoplasma contamination test after 15 passages. For immunoblotting, proteins were separated on 4–12% Bis-Tris SDS–PAGE gel (NuPAGE precast gel, Thermo Fisher) and transferred onto a nitrocellulose membrane (Hybond C+, GE Healthcare). Membranes were blocked with 5% skim milk in PBST buffer (PBS, 0.1% Tween-20) and incubated with rabbit polyclonal antibody (1:2,500 dilution; Cell Signaling, 4771), anti-Flag mouse monoclonal antibody (1:2,500 dilution; Sigma, F1804), and anti-HA rabbit polyclonal antibody (1:2,500 dilution; Cell Signaling, 9411). For the detection of STAT1 or IRF3 phosphorylation, the membrane was blocked with 5% milk in PBST followed by overnight incubation at 4 °C with an anti-phospho-Stat1 (Tyr701) rabbit monoclonal antibody (1:2,500 dilution; Cell Signaling no. 9171) and probed with anti-HA high-affinity monoclonal antibody (1:100 dilution; Roche Applied Science, cat. no. 11867423001) overnight at 4 °C. Cells were washed extensively with 1X PBS before and after incubation with Alexa Fluor 488-conjugated secondary antibody (1:500 dilution; Invitrogen, no. A-11006) for 1 h at 37 °C. Cells were counterstained with Hoechst (250 ng ml⁻¹) and examined under a confocal laser scanning microscope (LSM510, Zeiss).

**Data availability**

Coordinates for the p300 core structure and BΔRP bound to a diacetylated histone H4 peptide are available from the Protein Data Bank (PDB) under accession numbers 6GYR and 6GYT, respectively. Source data are available for Fig. 1b, f and Extended Data Fig. 1d. Figure 1d shows the initial velocities from reactions shown in Extended Data Fig. 1d.

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Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | The effect of IRF3 or STAT1 activation and oligomerization on p300 autoacetylation. **a**, The domain structure of IRF3. The truncation construct used is shown at the bottom. **b**, Size-exclusion chromatography of IRF3 variants. Red, unphosphorylated IRF3; blue, phosphorylated pIRF3; green, C-terminally truncated IRF3ΔC. Representative data of three independent experiments are shown. **c**, A constant amount of p300s (2 μM) was incubated alone or in the presence of C-terminally truncated IRF3ΔC (2 μM) for the indicated time points. Samples were analysed by SDS–PAGE followed by Coomassie staining and autoradiography. **d**, Progress curves of HAT scintillation proximity assay. Histone H4 substrate acetylation in the presence (green) or absence (black) of pIRF3 and varying concentrations of [3H]acetyl-CoA. The degree of histone H4 substrate acetylation at different time points and the initial velocity (cpm min⁻¹) at the indicated acetyl-CoA concentrations were determined and plotted in Fig. 1c. Three independent experiments were performed and the mean value and error bars representing the standard deviation are shown. **e**, The domain structure of STAT1. The truncation constructs used are shown at the bottom, and the Tyr701 phosphorylation site is indicated. **f**, Uncropped images of SDS–PAGE gels shown in Fig. 1d. The 14C autoacetylation signal of p300s is shown at the bottom. **g**, Size-exclusion chromatography of STAT1 variants. Black, STAT1ΔNC; green, STAT1ΔN; red, Y701-phosphorylated pSTAT1ΔNC; blue, Y701-phosphorylated pSTAT1ΔN. **h**, SDS–PAGE analysis of STAT1 variants and analysis by western blotting. Top, Coomassie staining of SDS–PAGE gel; middle, PonceauS staining; bottom, western blot using anti-Phospho-Stat1 (Tyr701). Representative data of three independent experiments are shown. For gel source data, see Supplementary Fig. 1.
Extended Data Fig. 2 | Crystal packing of the p300 core molecule.

a, There are four p300 molecules (monomers I–IV) in the asymmetric crystallographic unit. The four molecules show an antiparallel arrangement of the BRP-HAT domains. As a result, HAT domains from monomers I and II are closely apposed. Monomers III and IV engage monomer IV_{sym} and monomer III_{sym}, respectively, of a neighbouring crystallographic unit, showing that all promoters are in a AIL loop-swap conformation. Black arrows indicate the direction of the AIL. The disordered segment of the AIL is shown as a black dotted line.

b, c, Electron density of the AIL. 2F_{o} – F_{c} (b) and F_{o} – F_{c} (c) difference density omit maps contoured at 0.8 and 2.0 r.m.s.d., respectively. Coloured as in Fig. 3.
Extended Data Fig. 3  |  Structural analysis of the RING domains.

a, Superposition of the four p300 molecules (monomers I–IV) in the asymmetric crystallographic unit. Whereas the bromodomains (Bd), PHD and HAT domains superpose with a r.m.s.d. of approximately 0.9 Å, the RING domains adopt multiple conformations. b, 2Fo – Fc (blue mesh) and anomalous difference Fourier maps (orange mesh) for the four RING domains contoured around 1σ and 2.5σ, respectively.
Extended Data Fig. 4 | Regulation of HAT activity by flanking domains.

a. The domain structure of p300. Sequence conservation of the AIL is shown using WebLogo. The constructs used are shown. b. Analysis of in vitro expression of the indicated p300 variants. Purified proteins were analysed for autoacetylation by immunoblotting with anti-p300(K1499ac) antibody (left), anti-Flag antibody (middle) and Coomassie staining (right). Representative data of three independent experiments are shown. c. Representative mass spectrometric analysis of BRP_HAT_ZZ_ΔAIL after in vitro expression (red) and after SIRT2 mediated deacetylation (black).
Extended Data Fig. 5 | Regulation of HAT activity by flanking domains. a, The AIL contributes to histone substrate acetylation of activated p300. The details of the constructs used are indicated in Extended Data Fig. 4. Defined amounts of p300 variants were incubated with acetyl-CoA and the indicated histones before SDS–PAGE analysis, followed by Coomassie staining and western blotting with the indicated antibodies. b, The indicated amounts of purified p300 variants were incubated with histone octamers as in a, followed by SDS–PAGE and immunoblot analysis with the indicated antibodies. Anti-Kac, pan-acetyl-lysine antibody. Representative data of three independent experiments are shown. c, Crystal structure of the H4(K12ac/K16ac) peptide bound to the BΔRP module containing an in-frame RING deletion. Amino acid residues 1169–1241 were replaced by a single glycine residue. The deletion removes the RING domain (black arrow) and does not adversely affect the structural integrity of the BΔRP module. d, e, Indicated variants of p300 were co-expressed with p53 in H1299 cells and analysed by immunofluorescence with the indicated antibodies (d) or by western blotting (e). Representative data of three independent experiments are shown. Scale bars, 10 μm.
Extended Data Fig. 6 | Autoacetylation changes the hydrodynamic properties of p300. a, Simulations of the AIL in the context of the loop-swapped dimer. Left, cartoon of the trajectory of the AIL (dashed line). Right, representative conformations with the AIL Cα backbone atoms are coloured according to charge. b, SEC–MALLS analysis of deacetylated (blue) and acetylated (yellow) p300 core. Note the decrease in elution volume upon acetylation. c, SEC-MALLS analysis of deacetylated (blue), acetylated (red) BRP_HAT_CH3 and deacetylated (black) and acetylated (green) BRP_HAT_CH3 ΔAIL. There is no increase in elution volume upon acetylation of the ΔAIL construct. d, Comparison of acetylated and deacetylated BRP_HAT and BRP_HAT_CH3. The deacetylated BRP_HAT (green) and deacetylated BRP_HAT_CH3 (blue) elute at the same position, which is indicative of a similar hydrodynamic radius. The acetylated BRP_HAT (yellow) and BRP_HAT_CH3 (red) elute at a larger elution volume. The normalized refractive index is plotted as a function of elution volume from an S200 column coupled to a MALLS detector. Calculated molecular masses are plotted as a function of volume for each eluted peak. The experiment was carried out at least three times with similar results. One representative example of each sample is shown. e, Mass spectrometry analysis (electrospray ionization) of the BRP_HAT before (blue) and after (yellow) autoacetylation. The molecular mass and the number of acetylation events are indicated. f, Mass spectrometry analysis of BRP_HAT_CH3 before (blue) and after (red) autoacetylation. g, Mass spectrometry analysis of BRP_HAT_CH3 ΔAIL before (black) and after (green) autoacetylation.
Extended Data Fig. 7 | Molecular model and controls showing that p300 acetyltransferase activity is not stimulated by eRNA. a, p300 is maintained in the inactive state by deacetylases such as SIRT2. IRF3 is autoinhibited by a C-terminal segment in the IAD domain. b, TBK1 phosphorylation activates and dimerizes IRF3. The activated IRF3 dimer engages the IBID domain of p300. c, Recruitment of two molecules of p300 results in trans-autoacetylation in the AIL loop and HAT activation. d, Activated p300 can acetylate chromatin and engage acetylated substrates via the bromodomain. e, A constant amount of p300s (2 μM) was incubated in [14C]acetyl-CoA alone or in the presence of 2 μM Klf6 eRNA for the indicated time points. Samples were analysed by SDS–PAGE followed by Coomassie staining (top) and autoradiography (bottom). f, As in e but in the presence of 0.5 mM EDTA. The experiment was carried out at least twice with consistency. One representative example is shown. g, Quality control of Klf6 RNA. 3 μg Klf6 was deposited on a 1% agarose gel or a 14% 6 M urea PAGE gel and detected by SYBR Safe stain. M, 100-bp DNA ladder.
Extended Data Table 1 | Data collection, phasing and refinement statistics

|                         | BRP-HAT   | BΔRP          |
|-------------------------|-----------|---------------|
| **Data collection**     |           |               |
| Space group             | P2₁       | P2₁,2,2₁      |
| Cell dimensions         |           |               |
| a, b, c (Å)             | 100.7, 146.6, 116.3 | 49.6, 83.7, 165.6 |
| α, β, γ (°)             | 90, 91.7, 90   | 90, 90, 90   |
| Resolution (Å)          | 50–3.1*   | 42.7–2.50*    |
| No. reflections         | 106462    | 23996         |
| R_{sym} or R_{merge}    | 8.7 (89.7)* | 6.3 (141.3)* |
| I / σI                 | 7.48 (0.7)* | 7.6 (1.0)*    |
| Completeness (%)        | 99.0 (94.0)* | 97.29 (92.2)* |
| Redundancy              | 1.9 (1.9)* | 9.1 (3.6)*    |
| **Refinement**          |           |               |
| Resolution (Å)          | 50-3.1    | 42.7-2.5      |
| R_{work} / R_{free}     | 0.19 / 0.26 | 0.24 / 0.27  |
| No. atoms               | 19370     | 2769          |
| Protein                 | 19100     | 2693          |
| Lys-CoA ligand          | 256       | –             |
| Zinc                    | 14        | 4             |
| H4 K12AcK16Ac           | –         | 76            |
| B-factors (mean; Å²)    |           |               |
| Proteins                | 95.0      | 64.0          |
| RING domains            | 164.8     |               |
| Lys-CoA ligand          | 69.2      | –             |
| H4 K12AcK16Ac           | –         | 60.2          |
| R.m.s deviations        |           |               |
| Bond lengths (Å)        | 0.013     | 0.004         |
| Bond angles (°)         | 1.73      | 0.68          |

*Data from one crystal. Values in parentheses are for highest-resolution shell.
| Protein | Peptide (residues) | $K_d$ (µM) | N* |
|---------|-------------------|------------|----|
| BRP     | H3 unmodified (1-20) | --         | No binding |
|         | H3 K4me1 (1-20)    | --         | No binding |
|         | H3 K4me3 (1-20)    | --         | No binding |
|         | H3 K9ac (1-20)     | --         | No binding |
|         | H3 K14ac (1-20)    | 1761 ± 356 | 1.13 |
|         | H3 K12ac (1-20)    | large      | 1.01 |
|         | H3 K9acK14ac (1-20) | 578 ± 47   | 1.06 |
|         | H3 K14acK18ac (1-20) | 104 ± 27   | 1.03 |
|         | H3 K2ac (11-30)    | --         | No binding |
|         | H3 K18acK23ac (11-30) | --         | No binding |
|         | H3 S10pho (1-20)   | --         | No binding |
|         | H3 T11pho (1-20)   | --         | No binding |
| BRP     | H4 unmodified (4-24) | --         | No binding |
|         | H4 K5ac (4-24)     | --         | No binding |
|         | H4 K8ac (4-24)     | 58 ± 12    | 1.04 |
|         | H4 K5acK8ac (4-24) | 828 ± 10   | 1.03 |
|         | H4 K8acK12ac (4-24) | 90 ± 18    | 1.01 |
|         | H4 K12ac (4-24)    | 71 ± 14    | 1.05 |
|         | H4 K16ac (4-24)    | --         | No binding |
|         | H4 K12K16diac (4-24) | 25 ± 5     | 0.99 |
|         | H4 K20ac (1-24)    | 305 ± 6    | 1.00 |
|         | H4 K16acK20ac (1-24) | 205 ± 10   | 1.23 |
|         | H4 K5K8K12K16K20penta-ac (1-24) | 38 ± 4 | 1.07 |
|         | H4 S1pho K5K8K12K16K20penta-ac (1-24) | 54 ± 2 | 1.12 |
| BRP_N1132A | H4 K20ac (1-24) | --         | No binding |
|          | H4 K16acK20ac (1-24) | --         | No binding |
|          | H4 K5K8K12K16K20penta-ac (1-24) | --         | No binding |
|          | H4 S1pho K5K8K12K16K20penta-ac (1-24) | --         | No binding |
| BRP     | AIL (1545-1562)    | --         | No binding |
| BRP     | AIL K1549K1558K1560tri-ac (1545-1562) | --         | No binding |

Mean and s.d. were determined from experiments performed in triplicate. Horizontal lines separate experiments involving different histone peptides or different protein constructs. Histone peptide sequences: H3 (1-20) ARTKQTRKSTGKAPKQGL, H3 (11-30) TGGKAPKQLATHASRK3AP, H4 (4-24) GKGGKLDKGQAKRRHKLRO. AIL (Autoinhibitory loop peptide) SKNAKKNNKTSNKSS (1545-1562). No binding was detected to non-acetylated peptides, or with a construct containing a mutation that abolishes acetyllysine binding (N1132A).

* Binding stoichiometry.
Extended Data Table 3 | Summary of SEC-MALLS and mass spectrometry experiments

| Sample                  | $MM_{MS}$ Da | $MM_{th}$ Da | Acetylation level | $MM_{SLS}$ Da (2 mg·ml$^{-1}$) |
|-------------------------|--------------|--------------|------------------|---------------------------------|
| BRP_HAT                 | 73538        | 73538        | ~0               | 73380 ± 1.5%                    |
| BRP_HAT Acetyl          | 73918        | 73538        | >8               | 71690 ± 1.6%                    |
| BRP_HAT_CH3             | 92893        | 92891        | ~0               | 92810 ± 2.0%                    |
| BRP_HAT_CH3 Acetyl      | 93270        | 92891        | >9               | 90700 ± 2.0%                    |
| BRP_HAT_CH3 ∆AIL        | 86363        | 86362        | ~0               | 84650 ± 2.2%                    |
| BRP_HAT_CH3 ∆AIL Acetyl | 86446        | 86362        | >2               | 80450 ± 1.7%                    |

Column labelling is as follows: $MM_{MS}$, molar masses determined by mass spectrometry; $MM_{th}$, the theoretical molar mass calculated from the appropriate primary sequences; acetylation levels were estimated based on the mass differences as compared to the non-acetylated sample; $MM_{SLS}$, molar masses determined by SEC-MALLS at a concentration of 2 mg·ml$^{-1}$. All p300 constructs contained the mutation Y1467F. The experiment was carried out at least three times with consistency. Results from one representative example are shown. The mass and errors reported for SEC-MALLS are the weight average molar mass and residual standard deviations of the observed data from the fitted values calculated using ASTRA.
Reporting Summary

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When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

- **n/a** Confirmed
- An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. \( F \), \( t \), \( r \)) with confidence intervals, effect sizes, degrees of freedom and \( P \) value noted
- Give \( P \) values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's \( d \), Pearson's \( r \)), indicating how they were calculated
- Clearly defined error bars

State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

| Data collection | MXCuBE2, XDS (20180409), ASTRA (6.0.5.3), UNICORN 6, |
|-----------------|------------------------------------------------------|
| Data analysis   | CCP4 (7.0), ASTRA (6.0.5.3), Phenix (1.13-2998), COOT 0.8.0, Pymol (2.1.0), MolProbity (4.3), ABSINTH, CAMPARI (2), Modeller (9.19), imageJ (1.8.0_112), GraphPad Prism (7.0), |

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Data availability. Coordinates for the p300 core structure and BP\( \Delta R \) bound to a diacetylated histone H4 peptide are available from the Protein Data Bank under accession number 6GYR and 6GYT, respectively.
Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences  ☐ Behavioural & social sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences

Study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | No statistical methods were used to predetermine sample size. For Fig.1b,d,f Fig.4a,b and blots shown in Ext. Data: Three independent experiments were performed with consistency. We show one representative example. For other data, three independent technical replicates were measured and the mean value and error bars representing the standard deviation are shown. |
| Data exclusions | All attempts at replication were successful and no data was excluded |
| Replication | Three independent technical replicates were measured and the mean value and error bars representing the standard deviation are shown. |
| Randomization | Randomization is not relevant to this study, as protein and crystal samples are not required to be allocated into experimental groups. No animals or human research participants are involved in this study. |
| Blinding | Blinding is not relevant to this study, as protein and crystal samples are not required to be allocated into experimental groups in protein structural studies. No animals or human research participants are involved in this study. |

Materials & experimental systems

Policy information about availability of materials

n/a Involved in the study

☐ ☑ Unique materials

☐ ☑ Antibodies

☐ ☑ Eukaryotic cell lines

☐ ☑ Research animals

☒ ☑ Human research participants

Obtaining unique materials

All unique materials are available from the author upon request

Antibodies

Antibodies used

anti–p300 K1499ac rabbit polyclonal antibody, anti-Kac rabbit polyclonal antibody, anti-Flag mouse monoclonal antibody, anti-phospho IRF3 S396 Rabbit monoclonal antibody.

Validation

Describe the validation of each primary antibody for the species and application, noting any validation statements on the manufacturer’s website, relevant citations, antibody profiles in online databases, or data provided in the manuscript.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

COS and H1299

Authentication

COS cell line: ATCC-CR1-1651; lot N°, 4171903
H1299 cells have been authenticated on December 6th 2016 by « LGC Standards: Cell Line Authentication Service »

Mycoplasma contamination

MycoAlert Mycoplasma Detection Kit: Lonza cat N°: LT07-418

Commonly misidentified lines

(See ICLAC register)

Not on the list of commonly misidentified cell lines
| Method-specific reporting |
|---------------------------|
| n/a | Involved in the study |
| ☒ | ChIP-seq |
| ☒ | Flow cytometry |
| ☒ | Magnetic resonance imaging |