### p300 Is an Obligate Integrator of Combinatorial Transcription Factor Inputs

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# Summary

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These areas or protein complexes that mediate  $\footnotesize$  Correspondence: RT (jmlim@berkeley.edu), XD (darzacq@berkeley.edu)<br> **Summary**<br>
Transcription coactivators are proteins or protein complexes that mediat<br>(TF) function. However, they lack DNA binding capacity, prompti  $\frac{4}{1}$  (er TR) function. However, they lack DNA binding capacity, prompting the question of how they<br>engage target loci. Three non-exclusive hypotheses have been posited: coactivators are<br>recruited by complexing with TFs, by binding (TF) function. The mon-exclusive hypotheses have been posited: coactivators are<br>recruited by complexing with TFs, by binding histones through epigenetic reader domains, or by<br>partitioning into phase-separated compartments engage target local target local target local target local principal preceding to the preceding into phase-separated compartments through their extensive intrinsically disordered regions (IDRs). Using p300 as a prototypica partitioning into phase-separated compartments through their extensive intrinsically<br>disordered regions (IDRs). Using p300 as a prototypical coactivator, we systematically mutated<br>its annotated domains and show by single-m paramering into phase-separated comparationic interigor and antisome intrinsically mutated<br>its annotated domains and show by single-molecule tracking in live cells that coactivator-<br>chromatin binding depends entirely on co dison-<br>its annotated domains and show by single-molecule tracking in live cells that coactivator-<br>chromatin binding depends entirely on combinatorial binding of multiple TF-interaction<br>domains. Furthermore, we demonstrate in annoning hinding depends entirely on combinatorial binding of multiple TF-interaction<br>domains. Furthermore, we demonstrate that acetyltransferase activity negatively impacts<br>p300–chromatin association and that the N-ter domains. Furthermore, we demonstrate that acetyltransferase activity negatively impacts<br>p300–chromatin association and that the N-terminal TF-interaction domains regulate that<br>activity. Single TF-interaction domains are in p300–chromatin association and that the N-terminal TF-interaction domains regulate that<br>activity. Single TF-interaction domains are insufficient for both chromatin binding and<br>regulation of catalytic activity, implying a p partivity. Single TF-interaction domains are insufficient for both chromatin binding and<br>regulation of catalytic activity, implying a principle that could broadly inform eukaryotic gene<br>regulation: a TF must act in coordin activity, implying a principle that could broadly inform eukaryotic gene<br>regulation: a TF must act in coordination with other TFs to recruit coactivator activity.<br>**Keywords**<br>transcription, coactivator, gene regulation, sin regulation: a TF must act in coordination with other TFs to recruit coactivator activity.<br>**Keywords**<br>transcription, coactivator, gene regulation, single-molecule tracking, live-cell imaging, p300

# $\frac{1}{1}$ Keywords

regulation: a TF must act in the Must Aeywords<br>transcription, coactivator, gene regulation, single-molecule tracking, live-cell imaging, transcription, coactivation, coactivation, single-molecule tracking,  $\frac{1}{2}$ 

### Introduction

Eukaryon ators have no intrinsic ability to bind specific DNA sequences, and their recruitment to<br>target loci is instead proposed to rely on interactions with TF activation domains,<br>d histone tails, and other coactivators. specific target loci is instead proposed to rely on interactions with TF activation domains,<br>modified histone tails, and other coactivators. Accordingly, most coactivators are multi-domain<br>or multi-subunit complexes contai specified histone tails, and other coactivators. Accordingly, most coactivators are multi-domain<br>or multi-subunit complexes containing both enzymatic domains and diverse protein-protein<br>interaction modules, including epige modified histone tails, and other coataining both enzymatic domains and diverse protein-protein<br>interaction modules, including epigenetic reader domains (e.g., bromodomain, PHD domain),<br>TF-interaction domains (e.g., KIX, P interaction modules, including epigenetic reader domains (e.g., bromodomain, PHD domain),<br>TF-interaction domains (e.g., KIX, PAS), and intrinsically disordered regions (IDRs).<sup>4–6</sup> The<br>relative importance of these various TF-interaction domains (e.g., KIX, PAS), and intrinsically disordered regions (IDRs).<sup>4–6</sup> The relative importance of these various domains for target binding remains unclear. Moreover, it is puzzling how coactivators get TF-interaction domains (e.g., KIX, PAS), and intrinsically disordered regions (IDRs).<sup>4</sup> The<br>relative importance of these various domains for target binding remains unclear. Moreover, it is<br>puzzling how coactivators get d

puzzling how coactivators get distributed among target loci rapidly and reproducibly despite<br>being stoichiometrically limiting relative to TFs and even to active cis-regulatory elements.<sup>1-3</sup><br>To clarify these outstanding u being stoichiometrically limiting relative to TFs and even to active cis-regulatory elements.<sup>1-3</sup><br>To clarify these outstanding uncertainties in the field, we focused on p300—a central<br>node in gene regulation that combines being stoichiometrically limiting relative to TFs and even to active cis-regulatory elements.<sup>2</sup><br>To clarify these outstanding uncertainties in the field, we focused on p300—a cent<br>node in gene regulation that combines many To compleme in the polypeptide, making it more<br>ally tractable than multi-subunit coactivator complexes (e.g., Mediator). p300 is<br>sed of three large regions: the N-terminal region (NTR), a well-structured enzymatic<br>region ( genetically tractable than multi-subunit coactivator complexes (e.g., Mediator). p300 is<br>composed of three large regions: the N-terminal region (NTR), a well-structured enzymatic<br>central region (Core), and the C-terminal r generically tractable than multi-subulation completion (e.g.) included, procedure composed of three large regions: the N-terminal region (NTR), a well-structured enzymatic central region (Core), and the C-terminal region ( central region (Core), and the C-terminal region (CTR) (Figure 1A). In the Core are several<br>annotated chromatin-interaction domains (ChIDs), while interspersed through the highly<br>disordered NTR and CTR are various TF-inter cannotated chromatin-interaction domains (ChIDs), while interspersed through the highly<br>disordered NTR and CTR are various TF-interaction domains (TFIDs)—small helical and zinc<br>finger domains that have been shown to engage

disordered NTR and CTR are various TF-interaction domains (TFIDs)—small helical and zinc<br>finger domains that have been shown to engage in "fuzzy" binding with TF activation domains.<sup>7</sup><br>We set out to address two questions: finger domains that have been shown to engage in "fuzzy" binding with TF activation domains.<sup>7</sup><br>We set out to address two questions: First, do ChIDs, TFIDs, or IDRs—or some<br>combination thereof—mediate p300 chromatin engage finger domains that have been shown to engage in "fuzzy" binding with TF activation domains.'<br>We set out to address two questions: First, do ChIDs, TFIDs, or IDRs—or some<br>combination thereof—mediate p300 chromatin engageme e<br>S<br>D ation thereof—mediate p300 chromatin engagement? Our hypothesis was that the<br>provide sequence specificity to direct p300 to target sites, while the ChIDs bind to<br>s to stabilize p300 on chromatin. By recognizing particular TFIDs provide sequence specificity to direct p300 to target sites, while the ChIDs bind to<br>histones to stabilize p300 on chromatin. By recognizing particular histone marks, the ChIDs<br>could also contribute to directing p30 THE PERTON EXPLORED POSITED TO ANTIFY THE COMPTRED TO ANDELEMBLE THE THE CHIDS<br>
could also contribute to directing p300 to target sites bearing active marks.<sup>8</sup> Others have also<br>
argued that the IDRs of p300 cause it to pa could also contribute to directing p300 to target sites bearing active marks.<sup>8</sup> Others have also<br>argued that the IDRs of p300 cause it to partition into TF condensates.<sup>9</sup> The second question we<br>asked was whether there i could also contribute to directing p300 to target sites bearing active marks.<sup>9</sup> Others have also<br>argued that the IDRs of p300 cause it to partition into TF condensates.<sup>9</sup> The second question we<br>asked was whether there is argued that the IDRs of p300 cause it to partition into TF condensates." The second question we<br>asked was whether there is interplay between p300 chromatin binding and catalytic activity.<br>Because the p300 Core has both an Because the p300 Core has both an acetyltransferase domain and a bromodomain—which is<br>thought to bind acetylated lysines, especially on histones<sup>10</sup>—we considered that enzymatic<br>activity might enhance chromatin binding and France thought to bind acetylated lysines, especially on histones<sup>10</sup>—we considered that enzymatic<br>activity might enhance chromatin binding and stabilize active p300 at its target sites.<br>2 thought to bind acetylated lysines, especially on histones<sup>40</sup>—we considered that enzymatic<br>activity might enhance chromatin binding and stabilize active p300 at its target sites.<br>2 activity might enhance chromatin binding and stabilize active p300 at its target sites.

### Results

To address the intervalst population of observed trajectories.<sup>11</sup> The output of this analysis is a "diffusion m," or probability density for every value of diffusion coefficient (Figure 1B). The all likelihood of a molec from a population of observed trajectories.<sup>11</sup> The output of this analysis is a "diffusion spectrum," or probability density for every value of diffusion coefficient (Figure 1B). The fractional likelihood of a molecule m from a population of observed trajectories.<sup>11</sup> The output of this analysis is a "diffusion<br>spectrum," or probability density for every value of diffusion coefficient (Figure 1B). The<br>fractional likelihood of a molecule m fractional likelihood of a molecule moving with a diffusion coefficient of <0.1  $\mu$ m<sup>2</sup>s<sup>-1</sup> we call the bound fraction ( $f_{\text{bound}}$ ) because it represents the proportion of molecules diffusing at a rate indistinguishable fractional likelihood of a molecule moving with a diffusion coefficient of <0.1  $\mu$ m<sup>2</sup>s<sup>+</sup> we call the bound fraction ( $f_{\text{bound}}$ ) because it represents the proportion of molecules diffusing at a rate indistinguishable f si<br>B<br>g bound fraction (bound) because it represents the proportion of molecules diffusing at a rate<br>indistinguishable from that of chromatin motion (see Halo-H2B in Figure 1B). By measuring<br>their effects on  $f_{\text{bound}}$ , we are thu their effects on  $f_{\text{bound}}$ , we are thus able to assess the impact of various p300 mutations and<br>perturbations on chromatin interactions in the context of the live cell.<sup>11,12</sup> Although the rest of<br>the diffusion spectrum f their effects on  $J_{bound}$ , we are thus able to assess the impact of various p300 mutations and<br>perturbations on chromatin interactions in the context of the live cell.<sup>11,12</sup> Although the rest of<br>the diffusion spectrum fast perturbations on chromatin interactions in the context of the live cell.<sup>11,12</sup> Although the rest of<br>the diffusion spectrum faster than 0.1  $\mu$ m<sup>2</sup>s<sup>-1</sup> holds other valuable information, such as how<br>many distinct diffusi

the diffusion spectrum faster than 0.1  $\mu$ m<sup>2</sup><br>many distinct diffusive species (number of<br>mainly focused on changes to  $f_{\text{bound}}$  in this re<br>We first benchmarked SMT behavior<br>the endogenous *EP300* locus against stably<br>U2 i<br>P<br>H  $^+$  holds other valuable information, such as how<br>beaks) exist for a given tagged protein, we have<br>ort.<br>of the gene products of two HaloTag knock-ins at<br>integrated Halo-H2B and Halo-NLS transgenes in<br>2B was predominantly mainly focused on changes to  $f_{\text{bound}}$  in this report.<br>We first benchmarked SMT behavior of the gene products of two HaloTag knock-ins at<br>the endogenous *EP300* locus against stably integrated Halo-H2B and Halo-NLS transg Mailiny focused on changes to J<sub>bound</sub> in this report.<br>We first benchmarked SMT behavior of the<br>the endogenous *EP300* locus against stably inte<sub>{</sub><br>U2OS cells (Figure 1B-C). As expected, Halo-H2B w<br>while Halo-NLS was predo Mexicon SEP300 locus against stably integrated Halo-H2B and Halo-NLS transgenes in<br>ells (Figure 1B-C). As expected, Halo-H2B was predominantly bound ( $f_{bound} = 0.85 \pm 0.01$ )<br>alo-NLS was predominantly fast-diffusing. (We note the endogenous EP300 locus against stably integrated Halo-H2B and Halo-NCS transgenes in<br>U2OS cells (Figure 1B-C). As expected, Halo-H2B was predominantly bound ( $f_{bound} = 0.85 \pm 0.01$ )<br>while Halo-NLS was predominantly fast-While Halo-NLS was predominantly fast-diffusing. (We note that Halo-NLS has an  $f_{\text{bound}}$  of 0.11 ± 0.03, which we consider the baseline of the assay.) In this cell line, p300 had an  $f_{\text{bound}}$  of 0.11 ± 0.03, which we cons 0.03, which we consider the baseline of the assay.) In this cell line, p300 had an  $f_{\text{bound}}$  of<br>approximately 0.54, which was reproducible between two different clonal knock-in lines  $(f_{\text{bound}})$ <br>= 0.55 ± 0.02, 0.52 ± 0.03) approximately 0.54, which was reproducible between two different clonal knock-in lines  $(f_{bound} = 0.55 \pm 0.02, 0.52 \pm 0.03)$ . We also used FRAP to measure the residence time of each p300 construct on chromatin and found that approximately 0.54, which was reproducible between two different clonal knock-in lines (Jbound<br>=  $0.55 \pm 0.02$ ,  $0.52 \pm 0.03$ ). We also used FRAP to measure the residence time of each p300<br>construct on chromatin and found expond the timescale of this experiment, p300–chromatin binding events persist for<br>beyond the timescale of this experiment, p300–chromatin binding events persist for<br>approximately 26 seconds on average—on par with or sligh beyond the timescale of this experiment, p300–chromatin binding events persist for<br>approximately 26 seconds on average—on par with or slightly longer than residence times<br>typical of TFs.<sup>13</sup><br>Having established a characteri

approximately 26 seconds on average—on par with or slightly longer than residence times<br>typical of TFs.<sup>13</sup><br>Having established a characteristic profile for WT endogenous p300 diffusion, we built a<br>transgene system to facil typical of TFs.<sup>13</sup><br>Having established a characteristic profile for WT endogenous p300 diffusion, we built a<br>transgene system to facilitate mutation of p300. In order to avoid complexities arising from<br>interactions with en typical of TFs.<sup>23</sup><br>Having e<br>transgene syste<br>interactions wit<br>detectable p300<br>integration and Having established a characteristic profile in the system to facilitate mutation of p300. In order to avoid complexities arising from<br>tions with endogenous p300, we generated a clonal knock-out cell line expressing no<br>ble the system to facilitate mutation of parameterism to avoid complement mutig from<br>interactions with endogenous p300, we generated a clonal knock-out cell line expressing no<br>detectable p300 (Fig. S1A) into which various p300 detectable p300 (Fig. S1A) into which various p300 transgenes were introduced by random<br>integration and antibiotic selection. The full-length p300 transgene product behaved similarly<br>3 integration and antibiotic selection. The full-length  $p300$  transgene product behaved similarly  $\frac{3}{2}$ integration and antibiotic selection. The full-length panel  $\sim$ 

to the tagged endogenous protein (fbound = 0.51 ± 0.02—Figure 1B e), vandating this assay<br>system. The stable transgene was considerably less expressed than the endogenous protein,<br>but we verified that in our system  $f_{bound}$ 

### p300 Core is neither necessary nor sufficient for chromatin binding

but we verified that in our system  $f_{bound}$  is not sensitive to concentration of protein (Fig. S2).<br> **p300** Core is neither necessary nor sufficient for chromatin binding<br>
To address which domains mediate p300 chromatin eng **p300 Core is neither necessary nor sufficient for chromatin binding**<br>To address which domains mediate p300 chromatin engagement, we first asses<br>which of its three regions (NTR, Core, CTR) is *sufficient* for binding (Fig of its three regions (NTR, Core, CTR) is *sufficient* for binding (Figure 2B). Strikingly, the<br>ad essentially no ability to bind on its own ( $f_{\text{bound}} = 0.09 \pm 0.01$ ). The NTR exhibited a<br>capacity to bind ( $f_{\text{bound}} = 0.22 \pm$ Core had essentially no ability to bind on its own ( $f_{bound} = 0.09 \pm 0.01$ ). The NTR exhibited a modest capacity to bind ( $f_{bound} = 0.22 \pm 0.02$ ), while CTR was sufficient to reach full-length p300 levels of binding ( $f_{bound} = 0.5$ core had essentially no ability to bind on its own (bound = 0.09 ± 0.01). The NTR exhibited a<br>modest capacity to bind ( $f_{\text{bound}}$  = 0.22 ± 0.02), while CTR was sufficient to reach full-length p300<br>levels of binding ( $f_{\text{$ 

modest capacity to bind (Jbound = 0.22 ± 0.02), while CTR was sufficient to reach full length p300<br>levels of binding ( $f_{\text{bound}}$  = 0.50 ± 0.02). Both NTR and Core constructs had significantly reduced<br>residence times ( $t$  = levels of binding (Jbound = 0.50 ± 0.02). Both NTR and Core constructs had significantly reduced<br>residence times ( $t = 16$ , 14 s) compared to CTR and p300 ( $t = 26$ , 26 s) (Fig. S3).<br>We then asked which of the domains is *n* For the state times (t = 16, 14 s) compared to CTR and p300 (t = 26, 26 s) (Fig. 33).<br>We then asked which of the domains is *necessary* for binding by tracki<br>p300: NTR-Core (ACTR), Core-CTR (ANTR), and NTR-CTR (ACore) (Fi ITR-Core ( $\Delta CTR$ ), Core-CTR ( $\Delta NTR$ ), and NTR-CTR ( $\Delta Core$ ) (Figure 2D). Remarkably,  $\Delta Core$  onewhat enhanced  $f_{bound}$  of 0.62 ± 0.03—a surprising result we return to later.  $\Delta NTR$ <br>TR showed significantly decreased ability to b had a somewhat enhanced  $f_{\text{bound}}$  of 0.62 ± 0.03—a surprising result we return to later. ΔNTR and ΔCTR showed significantly decreased ability to bind chromatin  $(f_{\text{bound}} = 0.36 \pm 0.03, 0.22 \pm 0.02)$  compared to WT p300  $(f_{$ and  $\Delta$ CTR showed significantly decreased ability to bind chromatin ( $f_{\text{bound}} = 0.36 \pm 0.03$ , 0.22  $\pm$  0.02) compared to WT p300 ( $f_{\text{bound}} = 0.51 \pm 0.02$ ). Together, these results suggest that the ChID-containing Core is  $0.02$ ) compared to WT p300 ( $f_{\text{bound}} = 0.51 \pm 0.02$ ). Together, these results suggest that the ChID-containing Core is dispensable for p300-chromatin binding in vivo while the NTR and CTR are necessary and sufficient (alb 0.02) compared to WT p300 (fbound = 0.31 ± 0.02). Together, these results suggest that the ChID-<br>containing Core is dispensable for p300-chromatin binding in vivo while the NTR and CTR are<br>necessary and sufficient (albeit necessary and sufficient (albeit to different extents). To confirm this, we performed SMT on a<br>series of Core mutants which had affected p300 binding and activity in vitro (including a<br>bromodomain mutant) and saw no substa necessary for the matrix of albeit to different extents). The summanity are performed sum in this abromodomain mutant) and saw no substantial changes in our in vivo assay (Fig. S4).<br> **p300 TFIDs are necessary for chromatin** 

### p300 TFIDs are necessary for chromatin binding

series of Core mutant and saw no substantial changes in our in vivo assay (Fig. S4).<br> **p300 TFIDs are necessary for chromatin binding**<br>
The finding that p300's chromatin-binding capacity lies outside its Core domain does n p300 TFIDs are necessary for chromatin binding<br>The finding that p300's chromatin-binding capacity lies outside its Core doma<br>necessarily mean that the TFIDs are what mediate binding—it could be the I<br>compose the vast major The finality mean that the TFIDs are what mediate binding—it could be the IDRs, which<br>se the vast majority of the NTR and CTR. (Whereas each TFID is 50-80 aa, there is<br>imately 1,400 aa of IDR.) We therefore measured the su compose the vast majority of the NTR and CTR. (Whereas each TFID is 50-80 aa, there is<br>approximately 1,400 aa of IDR.) We therefore measured the sufficiency of the TFIDs for<br>chromatin binding by expressing them as HaloTag compressing the vast majority of the variable variable many contribute the variable is approximately 1,400 aa of IDR.) We therefore measured the sufficiency of the TFIDs for chromatin binding by expressing them as HaloTag chromatin binding by expressing them as HaloTag fusions and observed that each TFID has only<br>a modest capacity to bind when acting alone (Figure 3B). Next, we tested the necessity of these<br>domains by deleting each of the a modest capacity to bind when acting alone (Figure 3B). Next, we tested the necessity of these<br>domains by deleting each of the TFIDs as well as all the TFIDs in the otherwise full-length<br>protein and performed SMT (Figure domains by deleting each of the TFIDs as well as all the TFIDs in the otherwise full-length<br>protein and performed SMT (Figure 3D). Although each TFID deletion only partially impaired<br>chromatin binding, when all TFIDs were protein and performed SMT (Figure 3D). Although each TFID deletion only partially impaired<br>chromatin binding, when all TFIDs were deleted ( $\Delta ALL$ ) there was a drastic reduction in  $f_{\text{bound}}$  to<br>4 chromatin binding, when all TFIDs were deleted ( $\Delta$ ALL) there was a drastic reduction in  $f_{\text{bound}}$  to<br>4 chromatin binding, when all TFIDs were deleted (ΔALL) there was a drastic reduction in fbound to<br>4

TFIDs is required to bring p300 to chromatin. The finding that deleting all TFIDs essentially<br>incapacitated chromatin binding also suggests that the IDRs are not sufficient for p300-<br>chromatin association. Additionally, t THE INTERNATA TO BENDAL TO BENDALM THE MANG MATERING AN INTERNATALY<br>Incapacitated chromatin binding also suggests that the IDRs are not sufficient for p300-<br>chromatin association. Additionally, the lack of binding by the chromatin association. Additionally, the lack of binding by the  $\Delta$ ALL construct is another<br>indication that the Core is not sufficient for p300-chromatin binding.<br>Acetyltransferase activity opposes p300–chromatin binding

### Acetyltransferase activity opposes p300–chromatin binding

indication that the Core is not sufficient for p300-chromatin binding.<br> **Acetyltransferase activity opposes p300-chromatin binding**<br>
Although it seems that the Core does not contribute appreciably to p300-chromatin<br>
bindi indication that the Core is not sufficient for p300-chromatin binding.<br> **Acetyltransferase activity opposes p300-chromatin binding**<br>
Although it seems that the Core does not contribute appreciably to p300-chromatin<br>
bindi p300 after addition of the potent and specific catalytic inhibitor A485.<sup>15</sup> Both perturbations<br>increased p300  $f_{\text{bound}}$  similar to complete loss of the Core domain (Figure 4A), which tallies with binding, it is intriguing that its deletion increased p300  $J_{bound}$  from 0.51 ± 0.02 to 0.02 ± 0.05.<br>We wondered whether this was the consequence of the loss of its acetyltransferase activity and<br>tested this in two ways: by tested this in two ways: by imaging a catalytically dead mutant  $(Y1467F)^{14}$  and tracking WT<br>p300 after addition of the potent and specific catalytic inhibitor A485.<sup>15</sup> Both perturbations<br>increased p300  $f_{\text{bound}}$  simila tested this in two ways: by imaging a catalytically dead mutant  $(Y1467F)^{-4}$  and tracking WT<br>p300 after addition of the potent and specific catalytic inhibitor A485.<sup>15</sup> Both perturbations<br>increased p300  $f_{bound}$  similar to p300 after addition of the potent and specific catalytic inhibitor A485.<sup>25</sup> Both perturbations<br>increased p300  $f_{\text{bound}}$  similar to complete loss of the Core domain (Figure 4A), which tallies with<br>previous ChIP-seq data sh increased p300 Jbound similar to complete loss of the Core domain (Figure 4A), which tallies with<br>previous ChIP-seq data showing an increase of p300 peak heights upon A485 treatment.<sup>16</sup> To<br>demonstrate that the response to previous ChIP-seq data showing an increase of p300 peak heights upon A485 treatment.<sup>25</sup> To<br>demonstrate that the response to A485 was a direct effect of p300 activity and not an indirect<br>consequence of some off-target cell consequence of some off-target cellular response, we designed a p300 point mutant that<br>remains catalytically active in the presence of A485 (H1451K) (manuscript in preparation) and<br>as expected, saw no change by FRAP (Figu

remains catalytically active in the presence of A485 (H1451K) (manuscript in preparation) and<br>as expected, saw no change by FRAP (Figure 4B) or SMT (Fig. S5) upon A485 addition.<br>The finding that catalytic activity opposes as expected, saw no change by FRAP (Figure 4B) or SMT (Fig. S5) upon A485 addition.<br>The finding that catalytic activity opposes chromatin binding provided a possible explanation for two curious observations:  $\triangle NTR$  (Coreas expected, saw no change by FRAP (Figure 4b) or SMT (Fig. 35) upon A485 addition.<br>The finding that catalytic activity opposes chromatin binding provided a<br>explanation for two curious observations:  $\triangle NTR$  (Core-CTR) had r The finding compared to<br>
dividend for two curious observations:  $\triangle NTR$  (Core-CTR) had reduced binding compared to<br>
dividend of the two N-terminal TFIDs had a greater impact on  $f_{\text{bound}}$  than deletion of the<br>
inal TFIDs—bo CTR and deletion of the two N-terminal TFIDs had a greater impact on  $f_{bound}$  than deletion of the C-terminal TFIDs—both of which are strange because the CTR is both necessary and sufficient for full chromatin association. C-terminal TFIDs—both of which are strange because the CTR is both necessary and sufficient<br>for full chromatin association. We therefore reasoned that the N-terminus may function<br>(directly or indirectly) to inhibit Core c For full chromatin association. We therefore reasoned that the N-terminus may function (directly or indirectly) to inhibit Core catalytic activity and thereby counteract the Core's effect to reduce CTR binding. Indeed, th (directly or indirectly) to inhibit Core catalytic activity and thereby counteract the Core's effect<br>to reduce CTR binding. Indeed, the reduction in  $f_{\text{bound}}$  from adding the Core to the CTR could be<br>rescued by treatment (an easy) or indirectly) to inhibit Core cataly, are intered on the Core to the CTR could be<br>rescued by treatment with A485 (Fig. 4C). Furthermore,  $\Delta TAZ1$  and  $\Delta KIX$  were both hyper-<br>sensitive to the drug compared to the to reduce CTR binding. Indeed, the reduction in J<sub>bound</sub> from adding the Core to the CTR could be<br>rescued by treatment with A485 (Fig. 4C). Furthermore,  $\Delta$ TAZ1 and  $\Delta$ KIX were both hyper-<br>sensitive to the drug compared sensitive to the drug compared to the FL with respect to fbound (Fig. 4D), indicating that the N-<br>terminal TFIDs participate in negative regulation of core catalytic activity.  $t_{\rm F}$  , and  $t_{\rm F}$  are given a regulation of core catalytic activity.

### **Discussion**

Combining high-speed SMT with the domain-mapping strategy classically used in in vitro<br>biochemistry allowed us to determine which domains of a modular coactivator determine its<br>chromatin binding in living cells. The result biomatin binding in living cells. The results indicate that p300 binds chromatin primarily<br>through multivalent TF-TFID interactions, not by its ChIDs or IDRs (Figure 5A).<br>**SMT** discriminates between models of p300-chromati

### SMT discriminates between models of p300–chromatin engagement

chrough multivalent TF-TFID interactions, not by its ChIDs or IDRs (Figure 5A).<br> **SMT discriminates between models of p300-chromatin engagement**<br>
Two measurements we made are particularly clarifying: Deleting the Core incr SMT discriminates between models of p300-chromatin engagement<br>Two measurements we made are particularly clarifying: Deleting the<br>f<sub>bound</sub> to 0.62, while removal of the five small TFIDs essentially eliminated<br>binding, bring  $\sim$  0.62, while removal of the five small TFIDs essentially eliminated p300 chromatin<br>binging  $f_{\text{bound}}$  down to 0.14 (Figure 3). Thus, the collective action of the small helical<br>s we call TFIDs is both necessary and suff binding, bringing  $f_{bound}$  down to 0.14 (Figure 3). Thus, the collective action of the small helical<br>peptides we call TFIDs is both necessary and sufficient for p300-chromatin interactions. While<br>the Core and the IDRs undou binding, bringing Joound down to 0.14 (Figure 3). Thus, the conective action of the small helical<br>peptides we call TFIDs is both necessary and sufficient for p300–chromatin interactions. While<br>the Core and the IDRs undoubt perties and the IDRs undoubtedly contribute to other functions, our data consistently show<br>that the way p300 associates with chromatin is not through the Core domains binding histones<br>or IDR-mediated interactions but throu the Core and the IDRs undoubtedly contribute to other functions, our data consistently show<br>that the way p300 associates with chromatin is not through the Core domains binding histones<br>or IDR-mediated interactions but thro or IDR-mediated interactions but through TFID-mediated interactions with sequence-specific<br>TFs. (Though it is possible, of course, that factors other than sequence-specific TFs also engage<br>p300 TFIDs.) These findings comp TFs. (Though it is possible, of course, that factors other than sequence-specific TFs also engage<br>p300 TFIDs.) These findings comport with in vitro binding assays showing that bromodomains<br>have micromolar affinity for ace p300 TFIDs.) These findings comport with in vitro binding assays showing that bromodomains<br>have micromolar affinity for acetylated histone peptides<sup>10</sup> while TFIDs have nanomolar affinity<br>for TF peptides.<sup>17,18</sup> Furthermo p300 TF<br>
have micromolar affinity for acetylated histone peptides<sup>10</sup> while TFIDs have nanomolar affinity<br>
for TF peptides.<sup>17,18</sup> Furthermore, the increased  $f_{\text{bound}}$  of  $\Delta \text{Core}$  relative to WT suggests that<br>
catalytic have micromolar affinity for acetylated histone peptides<sup>20</sup> while TFIDs have nanomolar affinity<br>for TF peptides.<sup>17,18</sup> Furthermore, the increased  $f_{\text{bound}}$  of  $\Delta \text{Core}$  relative to WT suggests that<br>catalytic activity do for TF peptides.<sup>27,20</sup> Furthermore, the increased *f*<sub>bound</sub> of ΔCore relative to WT suggests that<br>catalytic activity does not cause p300–chromatin association through its bromodomain, which<br>is confirmed by the finding t catalytically dead mutant, or the p300 bromodomain mutant (Fig.<br>18. Fig. S4).<br>18. Fig. S4).<br>**Multiple TFID interactions enable avid-like binding of p300 to chromatin**<br>18. Beyond addressing our initial questions, our invest

### Multiple TFID interactions enable avid-like binding of p300 to chromatin

mechanism of p300 recruitment. The finding that each TFID on its own largely occupies the The A48, Fig. S4).<br>
Multiple TFID interactions enable avid-like binding of p300 to chromatin<br>
Beyond addressing our initial questions, our investigation yielded insights into the<br>
mechanism of p300 recruitment. The finding Multiple TFI<br>Beyo<br>mechanism<br>unbound st<br>unbound, a Film of p300 recruitment. The finding that each TFID on its own largely occupies the<br>d state suggests that the majority of each individual TFID's binding partners are<br>d, and that a single TFID has little or no preference f mechanism of pack consider that the majority of each individual TFID's binding partners are<br>unbound, and that a single TFID has little or no preference for chromatin-bound TFs. (We<br>consider it unlikely that the TFID is mer unbound, and that a single TFID has little or no preference for chromatin-bound TFs. (We<br>consider it unlikely that the TFID is merely unbound by a TF, given the multiplicity of TF binding<br>partners and the aforementioned na consider it unlikely that the TFID is merely unbound by a TF, given the multiplicity of TF binding<br>partners and the aforementioned nanomolar affinities measured between TFs and p300 TFIDs.)<br>Multimerization of TFIDs can str partners and the aforementioned nanomolar affinities measured between TFs and p300 TFIDs.)<br>Multimerization of TFIDs can strongly favor interactions with chromatin, as demonstrated by<br>6 partners and the aforementioned nanomolar affinities measured of the and partners,<br>Multimerization of TFIDs can strongly favor interactions with chromatin, as demonstrated by<br>6  $\frac{1}{\sqrt{2}}$ 

than either TFID alone. A simple explanation for this is an avidity effect of engaging multiple TFs<br>bound to adjacent DNA sites. Binding multiple TFs on chromatin is energetically more favorable<br>than binding them in soluti than either TFI alone. A situal emperation for the laterator, performality more favorable<br>bound to adjacent DNA sites. Binding multiple TFs on chromatin is energetically more favorable<br>than binding them in solution, as the than binding them in solution, as the entropic cost of bringing the TFs together is already paid.<br>In principle, a coactivator with multiple TFIDs could even have a higher chromatin-bound<br>fraction than any of the TFs it bin In principle, a coactivator with multiple TFIDs could even have a higher chromatin-bound<br>fraction than any of the TFs it binds. (For why we disfavor the hypothesis that additional TFIDs<br>increase p300  $f_{\text{bound}}$  by increasin In principle, a coaction than any of the TFs it binds. (For why we disfavor the hypothesis that additional TFIDs<br>
increase p300  $f_{\text{bound}}$  by increasing the number of possible binding sites on chromatin, see<br>
Supplemental N Fraction than any oriental Note,  $\frac{1}{2}$ <br>supplemental Note.)<br>p300 is an obligate integrator of combinatorial TF inputs<br>The requisite for multiple TFIDs in p300 recruitment suggests that p300 functions as an<br>obligate int

### p300 is an obligate integrator of combinatorial TF inputs

increase p300 J<sub>bound</sub> by increasing the number of possible binding sites on chromatin, see<br>Supplemental Note.)<br>**p300** is an obligate integrator of combinatorial TF inputs<br>The requisite for multiple TFIDs in p300 recruitme p300 is an obligate in<br>The requisite<br>obligate integrator—<br>chromatin. Such inte<br>trans and in cis. In t The requirement and the requirement supplementation and integrator—that is, it not only can but must bind multiple TFs to associate with tin. Such integration has profound implications for p300 recruitment at two levels—in obligate integrator that is, it not only can but must bind multiple Trs to associate with<br>chromatin. Such integration has profound implications for p300 recruitment at two levels—in<br>trans and in cis. In trans, any gene-reg chromatic such integration integration in protection of pack transmisment at the existency trans and in cis. In trans, any gene-regulatory process that requires the action of p300 must deploy multiple TFs that can engage m deploy multiple TFs that can engage multiple TFIDs. In cis, any regulatory element that requires<br>p300 activity must be able to bind more than one of its TF partners, rendering isolated TF<br>binding sequences insufficient for p300 activity must be able to bind more than one of its TF partners, rendering isolated TF<br>binding sequences insufficient for recruiting p300 in a competitive environment. If this is a<br>general rule for coactivator recruitm particularly must be able to binding sequences insufficient for recruiting p300 in a competitive environment. If this is a general rule for coactivator recruitment, the high degeneracy of eukaryotic TF recognition motifs<sup>1</sup> general rule for coactivator recruitment, the high degeneracy of eukaryotic TF recognition<br>motifs<sup>19</sup> might be straightforwardly rationalized: Because a TF must act through recruiting<br>coactivators in coordination with anot motifs<sup>19</sup> might be straightforwardly rationalized: Because a TF must act through recruiting<br>coactivators in coordination with another TF, their modest individual specificities are multiplied<br>to recruit coactivators to cis motifs<sup>25</sup> might be straightforwardly rationalized: Because a TF must act through recruiting<br>coactivators in coordination with another TF, their modest individual specificities are multiplied<br>to recruit coactivators to cis coactivators in consideration with an external specificant enterpreneut in a multiplier<br>to recruit coactivators to cis regulatory elements that are capable of binding both TFs<br>simultaneously—thus solving the problem we pos simultaneously—thus solving the problem we posed of how a limiting pool of coactivators is<br>distributed among target loci. Those factors that can engage more TFIDs on their own, such as<br>p53,<sup>20</sup> may be the more context-ind distributed among target loci. Those factors that can engage more TFIDs on their own, such as<br>p53,<sup>20</sup> may be the more context-independent activators, while those that can only engage one<br>of the TFIDs are likely more depen  $\frac{1}{2}$  and the more context-independent activators, while those that can only engage one<br>of the TFIDs are likely more dependent on other proteins (i.e., additional TFs) to achieve<br>activation of target genes.<br>Last, beca

p53,<sup>20</sup> may be the more context-independent activators, while those that can only engage one<br>of the TFIDs are likely more dependent on other proteins (i.e., additional TFs) to achieve<br>activation of target genes.<br>Last, bec activation of target genes.<br>
Last, because p300 binding is sensitive to catalytic activity, our assay was able to<br>
uncover a new mode of p300 catalytic regulation by the N-terminal TFIDs (Fig. 4C-D). The Core<br>
is most acti Last, because p30<br>
uncover a new mode of p3<br>
is most active in the abse<br>
binding partners someho The values of the absence of TAZ1 and KIX, which suggests that these domains or their<br>Lastive in the absence of TAZ1 and KIX, which suggests that these domains or their<br>Lastners somehow modulate acetyltransferase activity, is most active in the absence of TAZ1 and KIX, which suggests that these domains or their<br>binding partners somehow modulate acetyltransferase activity, which activity inhibits the<br>7 is most activity in this most active in the absence of the activity of the set of the set of TAZ1 and TAZ1 and TAZ21 and TAZ21 and TAZ21 and TAZ221 and TAZ221 and TAZ22222 and TAZ22222 and TAZ2222 and TAZ2222 and TAZ2222 a binding partners somehow modulate acetyltransferase activity, which activity inhibits the

chromation of such regulation, there is an important biological consequence of the observation:<br>That p300 catalytic activity is regulable through two of its TFIDs shows another way in which it<br>is an obligate integrator of mechanism of such an important and good consequence of the observation.<br>That p300 catalytic activity is regulable through two of its TFIDs shows another way in which it<br>is an obligate integrator of TF inputs.<br>**Acknowledgme** 

### Acknowledgments

That is an obligate integrator of TF inputs.<br> **Acknowledgments**<br>
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discussions and helpful suggestions over the years, and Vinson Fan in particul **Acknowledgments**<br>We would like to thank all<br>discussions and helpful suggestions on<br>data analysis. We also thank Luk<br>CRL Flow Cytometry Facility for use of ions and helpful suggestions over the years, and Vinson Fan in particular for his advice<br>
i analysis. We also thank Luke Lavis for providing fluorescent HaloTag ligands and the<br>
w Cytometry Facility for use of their instru and data analysis. We also thank Luke Lavis for providing fluorescent HaloTag ligands and the<br>CRL Flow Cytometry Facility for use of their instruments. This work was supported by National<br>Institutes of Health grants U54-CA CRL Flow Cytometry Facility for use of their instruments. This work was supported by National<br>Institutes of Health grants U54-CA231641-01659 (to XD), 5T32GM007232-42 (supporting JPK),<br>the Silicon Valley Community Foundatio Institutes of Health grants U54-CA231641-01659 (to XD), 5T32GM007232-42 (supporting JPK),<br>the Silicon Valley Community Foundation/CZI, and the Howard Hughes Medical Institute. JJF is a<br>Howard Hughes Medical Institute Award Institute of Health Guidal Institute Summunity Foundation/CZI, and the Howard Hughes Medical Institute. JJF is a<br>Howard Hughes Medical Institute Awardee of the Life Sciences Research Foundation.<br>**Author Contributions**<br>JJF

### Author Contributions

the Silicon Valley Community Foundation, 22, and the Hortard Hughes Medicin In the Silicon Valley Howard Hughes Medical Institute Awardee of the Life Sciences Research Foundation.<br>Author Contributions<br>JJF and JPK devised a Author Contributions<br>
JJF and JPK devised and executed experiments with support from GZ and GD<br>
guidance of RT and XD. JJF and JPK analyzed the data with help from TWG. JPK drafted<br>
text of the original manuscript. XD and The Matrix deviced and Mixture experiments with help from TWG. JPK drafted the main<br>the original manuscript. XD and RT supervised the project.<br>**ation of Interests**<br>RT and XD are co-founders of Eikon Therapeutics. guidance of the original manuscript. XD and RT supervised the project.<br> **Declaration of Interests**<br>
RT and XD are co-founders of Eikon Therapeutics.

### Declaration of Interests

Declaration of Interests<br>RT and XD are co-founders of Eikon Therapeutics. RT and XD are co-founders of Eikon Therapeutics.

### Figure Legends

Figure 1 Dentimarking rials pood amasive behavior.<br>
(A) Schematic of domain organization of p300 showing in the Core. The black line indicates IDRs. Relative lengt<br>
(left)—probability density function (top) and cumulative (A) Schematic of p300 regions. (B) SMT of isolated regions of p300 (left) with summary bar plot (A) Schematic of p300 regions. (B) SmT of isolated regions of p300 (left) with summary bar plot of  $f_{bound}$  (right). Bars repre (left)—probability density function (top) and cumulative distribution function (bottom)—with<br>plot of  $f_{bound}$  (right). Bars represent bootstrapping mean ± SD. (C) FRAP curves of the same (left)<br>with residence times plotted

plot of  $f_{bound}$  (right). Bars represent bootstrapping mean ± SD. (C) FRAP curves of the same (left)<br>with residence times plotted for each construct (right). Bars represent best fit ± 95% Cl.<br>Figure 2—Sufficiency and necess plot of  $f_{bound}$  (right). Bars represent bootstrapping mean ± 3D. (C) TIMT curves of the same (left)<br>with residence times plotted for each construct (right). Bars represent best fit ± 95% Cl.<br>Figure 2—Sufficiency and necess Figure 2—Sufficiency and necessity of p300 regions for chromatin binding.<br>(A) Schematic of p300 regions. (B) SMT of isolated regions of p300 (left) with summary<br>of  $f_{\text{bound}}$  (right). (C) Schematic of p300 truncations. (D) Figure 2—Sufficiency and necessity of p300 regions for chromatin binding.<br>(A) Schematic of p300 regions. (B) SMT of isolated regions of p300 (left) with summary bar plot

(A)  $f_{\text{bound}}$  (right). (C) Schematic of p300 truncations. (D) SMT of truncations of p300 (left) with summary bar plot of  $f_{\text{bound}}$  (right). Bars represent bootstrapping mean  $\pm$  SD. See also Fig. S3.<br>Figure 3—Sufficiency or *J*<br>sum<br>**Figu**<br>A) S<br>sum<br>dele bound (right). C) Schematic of particular in particular (e) states in a manufacture of passes (right). Bars are a separate to both truncations of p300 TFIDs for chromatin binding.<br>
Schematic of p300 TF interaction domains Figure 3—Sufficiency and necessity of p300 TFIDs for chromatin binding.<br>
A) Schematic of p300 TF interaction domains (core region in grey). (B) SMT of TFIDs (left)<br>
summary bar plot of  $f_{bound}$  (right). (C) Schematic of p30 Figure 3—Sufficiency and necessity of p300 TFIDs for chromatin binding.<br>A) Schematic of p300 TF interaction domains (core region in grey). (B) SMT of TFIDs (left) with Summary bar plot of  $f_{\text{bound}}$  (right). (C) Schematic of p300 TFID deletions. (D) SMT of TFID<br>deletions of p300 (left) with summary bar plot of  $f_{\text{bound}}$  (right). Bars represent bootstrapping<br>mean ± SD.<br>Figure 4—Effects of

Summary bar plot of  $f_{bound}$  (right). (c) Schematic of p300 TFID deletions. (D) SMT of TFID<br>deletions of p300 (left) with summary bar plot of  $f_{bound}$  (right). Bars represent bootstrapping<br>mean ± SD.<br>(A) Cartoon representatio deletions of p300 (left) with summary bar plot of Jbound (right). Bars represent bootstrapping<br>mean ± SD.<br>Figure 4—Effects of the catalytic core on p300-chromatin interactions.<br>(A) Cartoon representation (left) of p300 ac Figure 4—E<br>
Figure 4—E<br>
(A) Cartoon<br>
(yellow), su<br>
(center) of<br>
A485, active<br>
(right). (B) F Figure 4—Effects of the catalytic core on p300–chromatin interactions.<br>(A) Cartoon representation (left) of p300 active site showing residues H1451 (pink) and Y1467 (yellow), substrate mimetic (grey), and A485 inhibitor (teal). Plots comparing diffusion spectra<br>(center) of WT p300 and three perturbations of p300 catalytic activity: addition of inhibitor<br>A485, active site mutation Y14 (center) of WT p300 and three perturbations of p300 catalytic activity: addition of inhibitor A485, active site mutation Y1467F, and deletion of the catalytic core, with bar plot of  $f_{\text{bound}}$  (right). (B) FRAP plots of WT A485, active site mutation Y1467F, and deletion of the catalytic core, with bar plot of  $f_{\text{bound}}$  (right). (B) FRAP plots of WT (left) and mutant (center) in response to A485 with residence times plotted for each construc (right). (B) FRAP plots of WT (left) and mutant (center) in response to A485 with residence times plotted for each construct (right). (C) SMT of NTR-containing (left) and CTR-containing (center) constructs in the presence (right). The about the presence (light). (C) SMT of NTR-containing (left) and CTR-containing (center) constructs in the presence (lighter hue) or absence (darker hue) of A485, with bar plot of  $f_{\text{bound}}$  (right). \*Data not plants in the presence (lighter hue) or absence (darker hue) of A485, with bar plot of  $f_{\text{bound}}$ <br>(right). \*Data not acquired. (D) SMT of N-terminal  $\Delta \text{TFID}$  (left) and C-terminal  $\Delta \text{TFID}$  (center)<br>constructs in the pr (right). \*Data not acquired. (D) SMT of N-terminal  $\Delta TFD$  (left) and C-terminal  $\Delta TFD$  (center) constructs in the presence (lighter hue) or absence (darker hue) of A485, with bar plot of  $f_{\text{bound}}$  (right). SMT: bars repre constructs in the presence (lighter hue) or absence (darker hue) of A485, with bar plot of  $f_{\text{bound}}$ <br>(right). SMT: bars represent bootstrapping mean ± SD. FRAP: Bars represent best fit ± 95% Cl.<br>See also Fig. S5.<br>Figure 5

(right). SMT: bars represent bootstrapping mean ± SD. FRAP: Bars represent best fit ± 95% Cl.<br>See also Fig. S5.<br>Figure 5—Graphical model of p300-chromatin interactions.<br>(A) Three existing models of how p300 engages with ch See also Fig. S5.<br>
Figure 5—Graphical model of p300–chromatin interactions.<br>
(A) Three existing models of how p300 engages with chromatin, the second of which is strongly<br>
supported by in vivo SMT data. (B) Multivalent TFI Figure 5—Graph<br>(A) Three existin<br>supported by in<br>state. (C) The cor Figure 5—Graphical model of p300–chromatin interactions.<br>(A) Three existing models of how p300 engages with chromatin, the second of which is strongly supported by in vivo SMT data. (B) Multivalent TFID-TF interactions favor the chromatin-bound<br>state. (C) The contributions of each p300 domain to p300–chromatin interactions.<br>The second of which is strongly interactions. state. (C) The contributions of each p300 domain to p300–chromatin interactions.<br>
State. (C) The contributions of each p300 domain to p300–chromatin-interactions. state. (C) The contributions of each p300 domain to p300–chromatin interactions.

## <u>METHODS</u>

### RESOURCE AVAILABILITY

### EXPERIMENTAL MODEL

Human U-2 osteosarcoma cells (U2OS) (RRID: CVCL\_0042) were a gift from David Spector's lab,<br>Cold Spring Harbor Laboratory. Cells were cultured at 37°C with 5% CO<sub>2</sub> in homemade phenol<br>red containing DMEM (Thermo Fisher #12 Cold Spring Harbor Laboratory. Cells at the Caldman Board of Mandele phenology and the 10% fetal bovine serum (HyClone, Logan, UT, Cat. #SH30396.03, lot #AE28209315), 1 mM sodium pyruvate (Thermo Fisher 11360070), L-glutam Fetal bovine serum (HyClone, Logan, UT, Cat. #SH30396.03, lot #AE28209315), 1 mM sodium<br>pyruvate (Thermo Fisher 11360070), L-glutamine (Sigma # G3126-100G), GlutaMax (Thermo<br>Fisher #35050061), and 100 U/mL penicillin-stre fetal difference of the fisher 11360070), L-glutamine (Sigma # G3126-100G), GlutaMax (Thermo<br>Fisher #35050061), and 100 U/mL penicillin-streptomycin (Thermo Fisher #15140122). Cells<br>were subcultured at a ratio of 1:4-1:10 Fisher #35050061), and 100 U/mL penicillin-streptomycin (Thermo Fisher #15140122). Cells<br>were subcultured at a ratio of 1:4-1:10 every 2-4 days. Regular mycoplasma testing was<br>performed by PCR. Phenol red-free DMEM (Thermo Were subcultured at a ratio of 1:4-1:10 every 2-4 days. Regular mycoplasma testing was<br>performed by PCR. Phenol red-free DMEM (Thermo Fisher #21063029) supplemented with 10%<br>fetal bovine serum, 1 mM sodium pyruvate (Lonza were subcultured at a ratio of 1:4-1:10 every 2-4 anyst magnetic mycoplane testing the<br>performed by PCR. Phenol red-free DMEM (Thermo Fisher #21063029) supplemented with 10%<br>fetal bovine serum, 1 mM sodium pyruvate (Lonza performation of the looking fetal bovine serum, 1 mM sodium pyruvate (Lonza 13-115E), and 100 U/mL penicillin-<br>streptomycin was used for imaging.<br>**METHOD DETAILS**<br>Cell culture and stable cell line construction<br>Stable cells

### METHOD DETAILS

### Cell culture and stable cell line construction

transposition and antibiotic selection. Gibson Assembly was used to clone full-length p300 and METHOD DETAILS<br>Cell culture and stable cell line cons<br>Stable cells lines expressing exoger<br>transposition and antibiotic selectio<br>p300 fragments/mutants into a Pigg transposition and antibiotic selection. Gibson Assembly was used to clone full-length p300 and<br>p300 fragments/mutants into a PiggyBac vector containing a puromycin resistant gene and 3X-<br>FLAG-Halo upstream of a multiple cl man permantal antibiotic selection. The containing a puromycin resistant gene and 3X-<br>FLAG-Halo upstream of a multiple cloning site. All cloning was confirmed by Sanger sequencing.<br>Cells were lipofected using Lipofectamine SuperPiggyBac transposase vector which was subsequently mixed with 125 uL of Opti-MEM FLAG-Halo upstream of a multiple cloning and cloning and cloning and cloning.<br>Cells were lipofected using Lipofectamine 3000 (Thermo Fisher #L3000015). Following<br>manufacturer instructions, 125 uL of Opti-MEM medium (Thermo manufacturer instructions, 125 uL of Opti-MEM medium (Thermo Fisher #31985062) was<br>combined with 5 uL of P3000 Reagent, 3-5 ug of gene containing PiggyBac vector, and 1.5 ug of<br>SuperPiggyBac transposase vector which was su combined with 5 uL of P3000 Reagent, 3-5 ug of gene containing PiggyBac vector, and 1.5 ug of<br>SuperPiggyBac transposase vector which was subsequently mixed with 125 uL of Opti-MEM<br>Medium following addition of 5 uL of Lipof SuperPiggyBac transposase vector which was subsequently mixed with 125 uL of Opti-MEM<br>Medium following addition of 5 uL of Lipofectamine 3000 Reagent. After brief vortexing the<br>combined media was added directly to a 6-well Medium following addition of 5 uL of Lipofectamine 3000 Reagent. After brief vortexing the<br>combined media was added directly to a 6-well containing 70-90% confluent cells. Transfected<br>cells were cultured for 1-2 days befor combined media was added directly to a 6-well containing 70-90% confluent cells. Transfected<br>cells were cultured for 1-2 days before returning the cells to normal DMEM growth media.<br>Once confluent, cells were passaged 1:8 cells were cultured for 1-2 days before returning the cells to normal DMEM growth media.<br>Once confluent, cells were passaged 1:8 and placed in selection media—DMEM growth media<br> Once confluent, cells were passaged 1:8 and placed in selection media—DMEM growth media.<br>10 Once confluent, cells were passaged 1:8 and placed in selection media—DMEM growth media

### CRISPR/Cas9-mediated genome editing

Polyclonal stable cell lines were maintained in selection media following selection.<br> **CRISPR/Cas9-mediated genome editing**<br>
Knock-in and knock-out cell lines were created as published previously.<sup>21</sup> We transfected U2OS<br> **CRISPR/Cas9-mediated genome editing**<br>
Knock-in and knock-out cell lines were created as published previously.<sup>21</sup> We trans<br>
cells using Lipofectamine 3000 (ThermoFisher L3000015) according to the main<br>
protocol. For knoc Knock-in and knock-out cell lines were created as published previously.<sup>22</sup> We transfected U2OS<br>cells using Lipofectamine 3000 (ThermoFisher L3000015) according to the manufacturer's<br>protocol. For knock-out generation, 1 µ protocol. For knock-out generation, 1 µg of each Cas9 vector was transfected per well in a 6-<br>well plate; for knock-in generation, 2 µg repair vector and 1 µg Cas9 vector per well in a 6-well<br>plate.<br>For Halo-p300 knock-in,

protein) and the US of the US of the US of the US well plate; for knock-in generation, 2 μg repair vector and 1 μg Cas9 vector per well in a 6-well<br>plate.<br>For Halo-p300 knock-in, sgRNA sequences were designed with the hel well plate.<br>
For Halo-p300 knock-in, sgRNA sequences were designed with the help of the CRISPOR web<br>
tool to find and evaluate guides in 200bp window centered on insertion site. Guides with high<br>
specificity close to site r<br>For Ha<br>tool to<br>specifi<br>backb<br>mutat For Halo-pair and evaluate guides in 200bp window centered on insertion site. Guides with high<br>specificity close to site of insertion were chosen. Repair vectors were cloned into basic<br>backbone (pENTR) with tag with 500bp specificity close to site of insertion were chosen. Repair vectors were cloned into basic<br>backbone (pENTR) with tag with 500bp left and right homology arms. PAMs for the guides were<br>mutated in pHDR by site-directed mutagen specificity contributions. Panalogy arms and specifically backbone (pENTR) with tag with 500bp left and right homology arms. PAMs for the guides were mutated in pHDR by site-directed mutagenesis. sgRNAs were cloned into th mutated in pHDR by site-directed mutagenesis. sgRNAs were cloned into the Cas9 plasmid (a<br>gift from Frank Xie) under the U6 promoter (Zhang Lab) with an mVenus reporter gene under<br>the PGK promoter.<sup>22</sup> Two guide/repair-vec gift from Frank Xie) under the U6 promoter (Zhang Lab) with an mVenus reporter gene under<br>the PGK promoter.<sup>22</sup> Two guide/repair-vector pairs were attempted at each terminus; only N-<br>terminal clones were ultimately recover gift from Frank Xie Universaly the PGK promoter.<sup>22</sup> Two guide/repair-vector pairs were attempted at each terminus; only N-<br>terminal clones were ultimately recovered. 24-48 hours after transfection, a pool of mVenus<br>positi the PGK promoter.<sup>22</sup> Two guide/repair-vector pairs were attempted at each terminus; only N-<br>terminal clones were ultimately recovered. 24-48 hours after transfection, a pool of mVenus<br>positive cells were FACS-sorted and e positive cells were FACS-sorted and expanded for approximately a week, after which single<br>Halo-positive cells (stained with JF549) were sorted into 96-well plates. Clones were expanded<br>for ~3 weeks and genotyped with immun

Halo-positive cells (stained with JF549) were sorted into 96-well plates. Clones were expanded<br>for ~3 weeks and genotyped with immunostaining to confirm.<br>For p300 knock-out, sgRNA sequences from knock-ins were used in com For  $\approx$ 3 weeks and genotyped with immunostaining to confirm.<br>For p300 knock-out, sgRNA sequences from knock-ins were used in combination—one from<br>either terminus of *EP300*. 24-48 hours after transfection, single mVenus For p300 knock-out, sgRNA sequences from knock-ins were<br>either terminus of *EP300*. 24-48 hours after transfection, si<br>FACS-sorted into 96-well plates. Clones were expanded for ~3<br>immunostaining to confirm.<br>Cell preparatio For terminus of *EP300*. 24-48 hours after transfection, single mVenus positive cells were<br>FACS-sorted into 96-well plates. Clones were expanded for ~3 weeks and then genotyped with<br>immunostaining to confirm.<br>Cell preparat either terminus of EP300. 2446 hours after transfection, single invertis positive cells were<br>FACS-sorted into 96-well plates. Clones were expanded for ~3 weeks and then genotyped with<br>immunostaining to confirm.<br>Cell prepar

### Cell preparation and dye labeling for imaging

Fact immunostaining to confirm.<br>Fact into 1960-well preparation and dye labeling for imaging<br>For both SMT and FRAP, ~200,000 cells were plated in a 35 mm dish containing either a 20 mm<br>diameter or 14 mm wide uncoated cover cell preparation and dye lat<br>For both SMT and FRAP, ~20<br>diameter or 14 mm wide un<br>1.5-14-C) one day prior to im<br>or JFX549 dye at 5 nM, for S For both SMT and FRAP, 2007 cells were plated in a 20 mm and containing sumple a 20 mm<br>diameter or 14 mm wide uncoated coverslip (MatTek Corporation #P35G-1.5-20-C or #P35G-<br>1.5-14-C) one day prior to imaging. Before imagi 1.5-14-2, the day prior to imaging. Before imaging the traditional multiplier into the organization<br>or JFX549 dye at 5 nM, for SMT, or 50 nM, for FRAP. After 20 min of incubation, free dye was<br>removed by replacing media wi Framoved by replacing media with 2 mL of phenol-red free media and incubating for 15 minutes.<br>
11 removed by replacing media with 2 mL of phenological media and including for 15 minutes.<br>11

 $d$ 

### Flow cytometry

Folls were imaged.<br>Follow cytometry<br>Cells were grown in 12-well dishes, labeled with ~50 nM JF549 for 45 minutes, and washed<br>three times in dye-free DMEM fifteen minutes each before trypsinization, filtration, and flow.<br>Us Flow cytometry<br>Cells were grown<br>three times in dye-<br>Used PE filter on a<br>Antibodies CHE MEE BERN MELT WAT MELT, MELT CHAPTER WITH CHAPTER WITH MINDES, AND MENTER<br>Three times in dye-free DMEM fifteen minutes each before trypsinization, filtration, and flow.<br>Used PE filter on a BD Bioscience LSR Fortessa.<br>A

### Antibodies

Used PE filter on a BD Bioscience LSR Fortessa.<br>
Antibodies<br>
Endogenous p300 was detected using Abcam 48343, while transgenes were detected using<br>
Millipore Sigma F3165 (anti-FLAG)—both of which were followed by Invitrogen Antibodies<br>Endogenous p300 was detected using Abcam<br>Millipore Sigma F3165 (anti-FLAG)—both of v<br>mouse-HRP).<br>Western blotting Millipore Sigma F3165 (anti-FLAG)—both of which were followed by Invitrogen 31430 (anti-<br>mouse-HRP).<br>Western blotting<br>Samples for Western blots were prepared by either nuclear extraction or direct lysis. Nuclear<br>extraction

### Western blotting

NaCl, 25 mM HEPES, 1 mM MgCl<sub>2</sub>, 0.2 mM EDTA, 0.5% NP-40) containing protease inhibitors Mouse-HRP).<br>
Western blot<br>
Samples for \<br>
extraction wa<br>
NaCl, 25 mM<br>
(Roche) on extraction was performed by resuspending ~8 million cells in 100 uL hypotonic buffer (100 mM<br>NaCl, 25 mM HEPES, 1 mM MgCl<sub>2</sub>, 0.2 mM EDTA, 0.5% NP-40) containing protease inhibitors<br>(Roche) on ice with gentle pipetting. Fo NaCl, 25 mM HEPES, 1 mM MgCl<sub>2</sub>, 0.2 mM EDTA, 0.5% NP-40) containing protease inhibitors<br>(Roche) on ice with gentle pipetting. Following resuspension, nuclei were isolated by<br>centrifugation (5 min, 4000 g). Subsequently, (Roche) on ice with gentle pipetting. Following resuspension, nuclei were isolated by<br>centrifugation (5 min, 4000 g). Subsequently, nuclei were resuspended in 100 uL hypotonic<br>buffer containing protease inhibitors and benz (Roche) on the time general piperting. Following results and the time is that the piperting contrifugation (5 min, 4000 g). Subsequently, nuclei were resuspended in 100 uL hypotonic buffer containing protease inhibitors an buffer containing protease inhibitors and benzonase (THERMO, 0.2 uL per 100 uL) to digest<br>DNA and incubated at 25 C with gentle agitation for 20 mins. Following incubation 4X LDS (16%<br>BME, 200 mM Tris-HCl, 8% SDS, 40% glyc DNA and incubated at 25 C with gentle agitation for 20 mins. Following incubation 4X LDS (16% BME, 200 mM Tris-HCl, 8% SDS, 40% glycerol, 0.4% bromophenol blue) was added to each sample and samples were boiled for >5 mins BME, 200 mM Tris-HCl, 8% SDS, 40% glycerol, 0.4% bromophenol blue) was added to each<br>sample and samples were boiled for >5 mins. Direct lysis was performed by pelleting ~1E6 cells,<br>resuspending cell pellets in 100 uL of L BME, 200 mM TRIS-HCL, 8 MP B, 700 MP B, 100 MP B TRIS-HOM TRIS-HOM SAMED TO MAIND TRIS-HOM SAMPLE 1.16 SAMPLE 3.<br>
Fesuspending cell pellets in 100 uL of LDS, and boiling for > 5 mins. Constructs which showed a<br>
high fract resuspending cell pellets in 100 uL of LDS, and boiling for > 5 mins. Constructs which showed a<br>high fraction bound (WT, NTR, CTR, ACore, ANRID, ATAZ1, AKIX, ATAZ2, AIBID, 1132, 1451,<br>1467, AIL, 1645) were prepared as nucl resuspending cell pends in 100 u.m. CTR, ΔCore, ΔNRID, ΔTAZ1, ΔKIX, ΔTAZ2, ΔIBID, 1132, 1451,<br>1467, AIL, 1645) were prepared as nuclear extracts as they could not be visualized using direct<br>lysis. Wet transfer was perform https://www.astical fraction bound in the visualized using direct<br>1467, AIL, 1645) were prepared as nuclear extracts as they could not be visualized using direct<br>1981. Wet transfer was performed overnight at constant curre 1467, The prepared as nuclear entermentary content in these as they are they are<br>1981, Net transfer was performed overnight at constant current (90 mA) in transfer buffer (15<br>1467 mM Tris-HCl, 20 mM glycine, 20% methanol, mM Tris-HCl, 20 mM glycine, 20% methanol, 0.0375% SDS). Following Ponceau staining,<br>membrane was blocked with 10% non-fat milk in TBS for 1 hour at room temperature with<br>agitation. After removal of blocking solution, 5% no membrane was blocked with 10% non-fat milk in TBS for 1 hour at room temperature with<br>agitation. After removal of blocking solution, 5% non-fat milk in TBS containing primary<br>antibody (~20 ug) was added and incubated at ro agitation. After removal of blocking solution, 5% non-fat milk in TBS containing primary<br>antibody (~20 ug) was added and incubated at room temperature with agitation for at least 1<br>hour. Membrane was briefly washed 3 time antibody (~20 ug) was added and incubated at room temperature with agitation for at least 1<br>hour. Membrane was briefly washed 3 times with TBS and 5% non-fat milk in TBS containing<br>12 and 5% non-fat milk in TBS containing<br>hour. Membrane was briefly washed 3 times with TBS and 5% non-fat milk in TBS containing<br>12 hour. Membrane was briefly washed 3 times with TBS and 5 times with TBS containing the Second 3 times wit

From temperature for 1 hour with agitation. Finally, secondary antibody containing solution<br>was removed, and membrane was washed 3 time with TBST with short (~2 min) incubations<br>with agitation. Western blots were visualiz was removed, and membrane was washed 3 time with TBST with short (~2 min) incubations<br>with agitation. Western blots were visualized with SuperSignal  $^{TM}$  West Femto (Thermo<br>Scientific #34094).<br>Single-molecule tracking<br>Al with agitation. Western blots were visualized with SuperSignal  $^{TM}$  West Femto (Thermo<br>Scientific #34094).<br>Single-molecule tracking<br>All SMT experiments were carried out on a custom-built microscope as previously describe

Single-molecule tracking<br>All SMT experiments were carried out on a custom-built microscope as previously described.<sup>21</sup><br>In brief, a Nikon TI microscope is equipped with a 100X/NA 1.49 oil-immersion TIRF objective, a<br>motori Single-molecule tra<br>All SMT experimen<br>In brief, a Nikon TI<br>motorized mirror,<br>maintained with h Single-molecule tracking<br>All SMT experiments wer<br>In brief, a Nikon TI micros<br>motorized mirror, a perf<br>maintained with humidif<br>hardware components we All SMT experiments were carried out on a custom-built microscope as previously described.<sup>24</sup><br>In brief, a Nikon TI microscope is equipped with a 100X/NA 1.49 oil-immersion TIRF objective, a<br>motorized mirror, a perfect Foc motorized mirror, a perfect Focus system, an EM-CCD camera, and an incubation chamber<br>maintained with humidified atmosphere with 5% CO<sub>2</sub> at 37 °C. All microscope camera and<br>hardware components were controlled through the

maintained with humidified atmosphere with 5%  $CO_2$  at 37 °C. All microscope camera and<br>hardware components were controlled through the NIS-Elements software (Nikon).<br>Dye-labeled cells were excited with 561 nm laser at 11 mantalized with humidials samelphore with 362 at 37 °C. The interesting this hardware components were controlled through the NIS-Elements software (Nikon).<br>Dye-labeled cells were excited with 561 nm laser at 1100 mW (Genes hardware excited with 561 nm laser at 1100 mW (Genesis Coherent,<br>CA) and imaged through an Semrock 593/40 nm bandpass filter. All imaging was<br>using highly inclined laminated optical sheet (HiLo) illumination (Tokunaga 2008 CA) and imaged through an Semrock 593/40 nm bandpass filter. All imaging was performed<br>using highly inclined laminated optical sheet (HiLo) illumination (Tokunaga 2008). Low laser<br>power (2-5 %) was used to locate and focus The using highly inclined laminated optical sheet (HiLo) illumination (Tokunaga 2008). Low laser<br>power (2-5 %) was used to locate and focus cell nuclei, and an ROI (region of interest) of<br>100X100 pixels was positioned such power (2-5 %) was used to locate and focus cell nuclei, and an ROI (region of interest) of<br>100X100 pixels was positioned such that as much of a single nucleus as possible was captured.<br>Subsequently samples were pre-bleache power (2008) was most to locate and focus can find for a matter (region of musicing) 100X100 pixels was positioned such that as much of a single nucleus as possible was captured.<br>Subsequently samples were pre-bleached at h Subsequently samples were pre-bleached at high laser power (100%) until a limited number (1-<br>4) single particles were visible per frame. Movies were taken with 1 ms pulses of full power of<br>561 nm illumination with a camera A) single particles were visible per frame. Movies were taken with 1 ms pulses of full power of 561 nm illumination with a camera exposure of 4 ms per frame for 5,000-10,000 frames. At least 10 (but often no more than 25) 561 nm illumination with a camera exposure of 4 ms per frame for 5,000-10,000 frames. At<br>least 10 (but often no more than 25) movies were collected for each sample as one tehcnical<br>replicate on a given day. Two technical For any manufator with a camera exposure  $\mu$  per frame for 5,000-2,000 frames. The least 10 (but often no more than 25) movies were collected for each sample as one tehcnical replicate on a given day. Two technical replic replicate on a given day. Two technical replicates on two separate days were collected to<br>produce the reported results. As observed by others<sup>23</sup> the variance within collected data is<br>largely due to cell-to-cell variance, produce the reported results. As observed by others<sup>23</sup> the variance within collected data is<br>largely due to cell-to-cell variance, with day-to-day variance being relatively minimal.<br>**SMT data processing**<br>Raw SMT movies we

### SMT data processing

produce the reported results. As observed by others<sup>23</sup> the variance within collected data is<br>largely due to cell-to-cell variance, with day-to-day variance being relatively minimal.<br>**SMT data processing**<br>Raw SMT movies we **SMT** data processing<br>
Raw SMT movies were processed using the open-source software pack<br>
(https://github.com/alecheckert/quot)<sup>11</sup> to extract single molecule trajectories.<br>
package was run on each SMT movie collected usi (https://github.com/alecheckert/quot)<sup>11</sup> to extract single molecule trajectories. The quot package was run on each SMT movie collected using the following settings: [filter] start = 0; method = 'identity'; chunk\_size = 1 (https://github.com/alecheckert/quot)<sup>11</sup> to extract single molecule trajectories. The quot<br>package was run on each SMT movie collected using the following settings: [filter] start = 0;<br>method = 'identity'; chunk\_size = 1 method = 'identity'; chunk\_size = 100; [detect] method = 'llr'; k = 1.2; w = 18; t = 18; [localize]<br>method = 'ls\_int\_gaussian'; method = 'ls\_int\_gaussian'; window\_size = 9; sigma = 1.2; ridge =<br>13 method = 'ls\_int\_gaussian'; method = 'ls\_int\_gaussian'; window\_size = 9; sigma = 1.2; ridge =<br>13 method = 'ls\_int\_gaussian'; method = 'ls\_int\_gaussian'; window\_size = 9; sigma = 1.2; ridge =<br>13

 $\frac{1}{2}$   $\frac{1}{2}$  The conservative'; frame\_interval exception, consider the conserval and the conservative of the software exchange SASPT<br>(https://github.com/alecheckert/saspt).<sup>11</sup> The first 200 frames of each movie were removed<br>due to hig SMT<br>(htt<sub>i</sub><br>due<br>misc<br>(tho (https://github.com/alecheckert/saspt).<sup>11</sup> The first 200 frames of each movie were removed<br>due to high localization density. To confirm that all movies were sufficiently sparse to avoid<br>misconnections, all movies maintai (https://github.com/alecheckert/saspt).<sup>11</sup> The first 200 frames of each movie were removed<br>due to high localization density. To confirm that all movies were sufficiently sparse to avoid<br>misconnections, all movies maintain misconnections, all movies maintained a maximum number of localizations per frame of six<br>
(though most frames had 1 or less). The StateArray method was used with the following<br>
settings: likelihood\_type = 'rbme'; pixel\_siz (though most frames had 1 or less). The StateArray method was used with the following<br>settings: likelihood\_type = 'rbme'; pixel\_size\_um = 0.16; frame\_interval = 0.00448; focal\_depth<br>= 0.7; start\_frame = 200; sample\_size = (though settings: likelihood\_type = 'rbme'; pixel\_size\_um = 0.16; frame\_interval = 0.00448; focal\_depth<br>
= 0.7; start\_frame = 200; sample\_size = 1000000; splitsize = 10.<br>
Fluorescence Recovery After Photobleaching<br>
FRAP ex

### Fluorescence Recovery After Photobleaching

 $S = \frac{1}{2}$ <br>= 0.7; start\_frame = 200; sample\_size = 1000000; splitsize = 10.<br>Fluorescence Recovery After Photobleaching<br>FRAP experiments were conducted on a Zeiss LSM 900. Dye-labeled cells were excited with 561<br>nm laser Fluorescence Recovery After Photobleaching<br>FRAP experiments were conducted on a Zeiss LSM 900. Dye-lab<br>nm laser with a 150 µM pinhole. Laser power (0.7 - 7.5%), dete<br>offset (-60 - -13), and digital gain (1.0 - 1.5%) was ad FRAP experiments were conducted on a Zeite Zeinertere years tells where dones annot a numerical pair  $\frac{1}{2}$  on a digital gain (1.0 - 1.5%) was adjusted between samples and cells, due to high variations in expression le offset (-60 - -13), and digital gain (1.0 - 1.5%) was adjusted between samples and cells, due to<br>high variations in expression level between p300 constructs. For each cell, the ROI was adjusted<br>to contain the whole nucleus high variations in expression level between p300 constructs. For each cell, the ROI was adjusted<br>to contain the whole nucleus and the scan speed was set to the maximum allowable for the<br>frame size. Movies were collected us hold contain the whole nucleus and the scan speed was set to the maximum allowable for the frame size. Movies were collected using the max scan speed for the given ROI with bidirectional scanning, an 8-bit depth, and 300 m frame size. Movies were collected using the max scan speed for the given ROI with bidirectional<br>scanning, an 8-bit depth, and 300 msec delay between frames for 100 frames resulting in a 30-<br>second-long movie. Bleaching was frame size. Movies were collected asing the material replaces to 100 frames resulting in a 30-<br>second-long movie. Bleaching was performed on frame 21 of the movie, allowing the first 20<br>frames to be used to normalize the f second-long movie. Bleaching was performed on frame 21 of the movie, allowing the first 20<br>frames to be used to normalize the fractional recovery. At least 20 (but often no more than 35)<br>movies were collected for each samp second-long movie. Bleading was performed on frame 21 of the movie, and movies<br>frames to be used to normalize the fractional recovery. At least 20 (but often no more than 35)<br>movies were collected for each sample as one te movies were collected for each sample as one technical replicate on a given day. Two technical<br>replicates on two separate days were collected to produce the final results.<br>**QUANTIFICATION AND STATISTICAL ANALYSIS**<br>To deriv

### QUANTIFICATION AND STATISTICAL ANALYSIS

### Statistical analysis of SMT

movies were collected to produce the final results.<br> **QUANTIFICATION AND STATISTICAL ANALYSIS**<br> **Statistical analysis of SMT**<br>
To derive a measure of error, we performed bootstrapping analysis on all SMT datasets. For<br>
eac **QUANTIFICATION AND STATISTICAL ANALYSIS**<br>Statistical analysis of SMT<br>To derive a measure of error, we performed bootstrapping analysis on al<br>each dataset, a random sample of size *n*, where *n* is the total number of<br>was each dataset, a random sample of size *n*, where *n* is the total number of cells in the dataset,<br>was taken 100 times. The mean and standard deviation of SASPT done on the 100 samples are<br>reported. each dataset, a random sample of size *n*, where *n* is the total number of cells in the dataset,<br>was taken 100 times. The mean and standard deviation of SASPT done on the 100 samples are<br>reported.  $\mathsf{reported}.$   $\hphantom{\mathsf{1}}$ reported.

### FRAP data processing

Whose recovery could not be delineated from noise were discarded from further analysis.<br>Average fractional recovery curves and fits to both average and single cell curves were<br>performed within the same MATLAB script. Fract Average fractional recovery curves and fits to both average and single cell curves were<br>performed within the same MATLAB script. Fractional recovery curves were computed and fit<br>for each cell and sample through the followi Proformed within the same MATLAB script. Fractional recovery curves were computed and fit<br>for each cell and sample through the following procedure:<br>1. *Nuclear masking.* A 5-pixel width Gaussian filter is applied to smooth

- 
- performed and sample through the following procedure:<br>
1. **Nuclear masking.** A 5-pixel width Gaussian filter is applied to smooth the image. Nuclear<br>
1. **Nuclear masking.** A 5-pixel width Gaussian filter is applied to smoo **Formulation** 1. **Nuclear masking.** A 5-pixel width Gaussian filter is a masking is performed by thresholding (70% of the r<br>2. **Masking the Bleach Spot.** The bleach spot mask is information imported from the metadata withi 1. *Nuclear masking.* A 5-pixel width Gaussian filter is applied to smooth the image. Nuclear<br>masking is performed by thresholding (70% of the maximum intensity).<br>2. *Masking the Bleach Spot.* The bleach spot mask is gener Masking the Bleach Spot. The bleach spot mask is generated from the<br>information imported from the metadata within the image. Bleaching<br>as a control to determine the actual bleach spot size relative to the size<br>data acquisi 2. *Masking the Bleach Spot.* The bleach spot mask is generated from the location and size<br>information imported from the metadata within the image. Bleaching of H2B was used<br>as a control to determine the actual bleach spo
	- as a control to determine the actual bleach spot size relative to the size specified during<br>data acquisition.<br>**Segmented Intensity Calculation**. The sum intensity was computed for each frame<br>within the nucleus outside of data acquisition.<br>**Segmented Intensity Calculation.** The sum intensity was computed for each frame<br>within the nucleus outside of the bleach spot (I<sub>nudeus</sub>) and within the bleach spot (I<sub>bleach</sub>).<br>Additionally, the mean in Mathemathem<br>Segmented Internet<br>Within the nuclet<br>Additionally, the<br>frame.<br>Background Subt 3. Segmented *Intensity* Calculation. The sum intensity was computed for each frame<br>within the nucleus outside of the bleach spot  $(l_{nucleus})$  and within the bleach spot  $(l_{bleach})$ .<br>Additionally, the mean intensity outside of th
	- Multim the nucleus outside of the bleach spot (*Inudeus*) and within the bleach spot (*Ibleach*).<br>Additionally, the mean intensity outside of the nucleus (*l<sub>outside</sub>*) was computed for each<br>frame.<br>Background Subtraction. Additionally, the mean intensity outside of the nucleus (*loutside*) was computed for each<br>frame.<br>**Background Subtraction**. The background for each frame was computed by multiplying<br>the mean pixel intensity outside of the *Backgr*<br>the me<br>the nuo<br>nucleus<br>subtrac 4. *Background Subtraction*. The background for each frame was computed by multiplying<br>the mean pixel intensity outside of the nucleus  $(l_{outside})$  by the number of pixels inside<br>the nucleus and inside the bleach spot to obtain the nucleus and inside the bleach spot to obtain the background intensity inside the nucleus and inside the bleach spot. The resultant per frame background intensities were subtracted from the respective sum intensity valu the nucleus and inside the bleach spot. The resultant per frame background intensities were<br>subtracted from the respective sum intensity values.<br>Fractional Recovery. The fractional recovery is computed as:<br> $\frac{I_{bleach}}{I_{bleach}}$ subtracted from the respective sum intensity values.<br>
	Fractional Recovery. The fractional recovery is computed as:<br>  $fractional recovery = \frac{I_{bleach}}{I_{nucleus}}$
	-

5. **Fractional Recovery.** The fractional recovery is computed as:  
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$$
fractional recovery = \frac{\frac{I_{bleach}}{I_{nucleus}}}{avg(\left[\frac{I_{bleach}}{I_{nucleus}}\right]_{1-20})}
$$

- First 20 frames.<br> **Manual Curation.** Sum intensity projections of the first 20 frames of each acquired<br>
movie were inspected along with the intensity difference between the frames before<br>
and after bleaching that had been Manual Curation<br>Manual Curation<br>movie were ins<br>and after bleac<br>appeared dead<br>puncta, etc.) in 6. Manual Curation. Sum intensity projections of the first 20 frames of each acquired movie were inspected along with the intensity difference between the frames before and after bleaching that had been gaussian filtered i move interesting that had been gaussian filtered in Fourier space. Cells that either<br>appeared dead or showed other issues of cell health (nuclear blebbing, numerous large<br>puncta, etc.) in sum intensity projections, who's n appeared dead or showed other issues of cell health (nuclear blebbing, numerous large<br>puncta, etc.) in sum intensity projections, who's nuclear mask did not correctly contain<br>nuclear intensity, or that had a significant po appeared or since the intensity projections, who's nuclear mask did not correctly contain<br>nuclear intensity, or that had a significant portion of intensity differences outside of the<br>bleach region were removed from analysi
- puncta, etc.) in sum master, projections, the statical mask has no serveed, sensitively and<br>nuclear intensity, or that had a significant portion of intensity differences outside of the<br>bleach region were removed from analy ntheach region were removed from analysis.<br>
Average Recovery Curves for Samples. Due to the high degree of variability in signal<br>
intensity from cell to cell, which required optimization of the imaging conditions for<br>
each Average Recovery Curves for Samples. Du<br>intensity from cell to cell, which required<br>each cell, the average recovery curve was co<br>cell recovery curves based on their signal to<br>of the variance of the intensity of the first The intensity from cell to cell, which required optimization of the imaging conditions for each cell, the average recovery curve was computed as a weighted average of the single cell recovery curves based on their signal t interach cell, the average recovery curve was computed as a weighted average of the single<br>cell recovery curves based on their signal to noise. Curves were weighted by the inverse<br>of the variance of the intensity of the fi each cell recovery curves based on their signal to noise. Curves were weighted by the inverse<br>of the variance of the intensity of the first 20 frames of the movie, resulting in higher<br>contributions to the average recovery
- of the variance of the intensity of the first 20 frames of the movie, resulting in higher<br>contributions to the average recovery from cells with higher signal to noise.<br>Fitting Fractional Recovery Curves. We assessed the im contributions to the average recovery from cells with higher signal to noise.<br> **Fitting Fractional Recovery Curves.** We assessed the impact of having a different bleach<br>
spot radius on the recovery profile of WT p300, prio Fitting Fractional Recovery Curves. We assessed the impact of having a diffispot radius on the recovery profile of WT p300, prior to selecting a model fields and FRAP data. By comparing the FRAP curves using 2 and 3 uM ble spot radius on the recovery profile of WT p300, prior to selecting a model for fitting the FRAP data. By comparing the FRAP curves using 2 and 3 uM bleach spot radii, we observed (Fig SX) that the recovery profile was not FRAP data. By comparing the FRAP curves using 2 and 3 uM bleach spot radii, we observed (Fig SX) that the recovery profile was not dependent on the bleach spot size indicating that we are not observing diffusion and are in FRAP determined that the recovery profile was not dependent on the bleach spot size<br>indicating that we are not observing diffusion and are in a binding reaction dominant<br>regime.<sup>24</sup> Therefore, fractional recovery profiles indicating that we are not observing diffusion and are in a binding reaction dominant<br>regime.<sup>24</sup> Therefore, fractional recovery profiles were fit two two-exponential model:<br>fractional recovery =  $1 - Ae^{-k_a x} - (1 - B - A)e^{-k_b x}$ regime.<sup>24</sup> Therefore, fractional recovery profiles were fit two two-exponential model:<br>
fractional recovery =  $1 - Ae^{-k_a x} - (1 - B - A)e^{-k_b x}$ <br>
where A is the fraction of molecules that recover quickly, B is bleach depth (and

$$
fractional recovery = 1 - Ae^{-k_a x} - (1 - B - A)e^{-k_b x}
$$

regime.<sup>24</sup> Therefore, fractional recovery profiles were fit two two-exponential model:<br>  $fractional recovery = 1 - Ae^{-k_a x} - (1 - B - A)e^{-k_b x}$ <br>
where A is the fraction of molecules that recover quickly, B is bleach depth (a<br>
therefore 1-B-A is th  $\sqrt{t}$ ac<br>ne<br>es<br>iVi いしく しょうしょう こうしょう こうしょう こうしゃ しゅうしょう こうしゃ こうしゃ こうしゃ いちのとり しゅうしゃ しゅうしゃ しゅうしゃ しゅうしゃ しゅうしゃ しゅうしゃ しゅうしゃ therefore 1-B-A is the fraction of molecules that recover quickly, B is bleach depth (and<br>therefore 1-B-A is the fraction of molecules that recover slowly),  $k_a$  is the recovery rate<br>of molecules that recover quickly, and of molecules that recover quickly, and  $k_b$  is the recovery rate of molecules that recovery<br>slowly. For all fits, the bleach depth (*B*) was fixed to the fractional recovery of the first<br>frame following bleaching. All sam slowly. For all fits, the bleach depth (*B*) was fixed to the fractional recovery of the first<br>frame following bleaching. All samples were initially fit allowing *A*,  $k_a$ , and  $k_b$  to vary.<br>16 slowly. For all fits, the bleach depth (B) was fixed to the fractional recovery of the first frame following bleaching. All samples were initially fit allowing A,  $k_a$ , and  $k_b$  to vary.<br>16 frame following bleaching. All samples were initially fit allowing  $A$ ,  $k_a$ , and  $k_b$  to vary.

Single a second exponential recovery term. All fitting was performed using a weighted fitting<br>algorithm where the weight of any given datapoint was equal to the absolute difference<br>in intensity between a given frame and th algorithm where the weight of any given datapoint was equal to the absolute difference<br>in intensity between a given frame and the subsequent frame. This biased the fitting<br>towards the data rich, initial recovery portion o in intensity between a given frame and the subsequent frame. This biased the fitting<br>towards the data rich, initial recovery portion of the fractional recovery curves.<br>Subsequently, to further limiting overfitting, fittin towards the data rich, initial recovery portion of the fractional recovery curves.<br>Subsequently, to further limiting overfitting, fitting for all p300 samples was constrained<br>by fixing the fast recovery rate  $(k_{\alpha})$  at 0. Subsequently, to further limiting overfitting, fitting for all p300 samples was constrained<br>by fixing the fast recovery rate  $(k_a)$  at 0.95 s<sup>-1</sup> which was close the average observed<br>value and was optimized to produce high by fixing the fast recovery rate  $(k_a)$  at 0.95 s<sup>-1</sup> which was close the average observed<br>value and was optimized to produce high quality fits of both relatively fast and slow<br>recovering constructs (Table S3). Fit quality value and was optimized to produce high quality fits of both relatively fast and slow<br>recovering constructs (Table S3). Fit quality was nearly identical allowing only A and  $k_a$ <br>to vary. value are:  $\frac{1}{2}$  in the same of  $\frac{1}{2}$  in the produce of and  $\frac{1}{2}$  in the value of  $\frac{1}{2}$  and  $\frac{1}{2}$  is the vary.<br>
to vary. recovering constructs (Table S3). Fit quality was nearly identical allowing only A and  $k_a$ <br>to vary.  $t_{\rm t}$ 

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**Fig. 3**





**A)**

**TFIDs are necessary and sufficient for p300–chromatin interactions.**

