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CAF-1 promotes HIV-1 latency by leading the formation of phase-separated suppressive nuclear bodies

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1st Editorial Decision 29th Sep 2020

Dear Prof. Zhang,

Thank you for submitting your manuscript to The EMBO Journal. Your study has now been seen by three referees and their comments are provided below.

As you can see from the comments, the referees find the analysis interesting. However, they also raise a number of important concerns that should be resolved in a revised version. Should you be able to address the raised issues then we would be interested in considering a revised version. I am happy to discuss the raised points further and maybe it would be most helpful to do so via phone or video call. Let me know what would work best for you.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website:
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We generally allow three months as standard revision time. As a matter of policy, competing manuscripts published during this period will not negatively impact on our assessment of the conceptual advance presented by your study. However, we request that you contact the editor as soon as possible upon publication of any related work, to discuss how to proceed. Should you foresee a problem in meeting this three-month deadline, please let me know in advance and I can grant an extension.

Thank you for the opportunity to consider your work for publication. I look forward to discussing your revision further with you.

Yours sincerely,

Karin Dumstrei, PhD
Senior Editor
The EMBO Journal

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Please make sure you upload a letter of response to the referees' comments together with the revised manuscript.

Please also check that the title and abstract of the manuscript are brief, yet explicit, even to non-specialists.

When assembling figures, please refer to our figure preparation guideline in order to ensure proper formatting and readability in print as well as on screen:
https://bit.ly/EMBOPressFigurePreparationGuideline

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- a point-by-point response to the referees' comments, with a detailed description of the changes made (as a word file).
- a word file of the manuscript text.
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Referee #1:

In their manuscript titled, "CAF-1 promotes HIV-1 latency by leading the formation of phase-separated suppressive nuclear bodies", Ma and colleagues investigate the role of the nucleosome assembly complex, CAF-1, on HIV latency. Using both HIV reporter virus clones and primary cell experiments, they dissect how CAF-1, namely the CHAF-1A subunit, is a HIV restrictive factor acting through mechanisms involving recruitment of suppressive epigenetic complexes into nuclear condensates with implications for regulating HIV LTR dynamics. Their work also provides interesting questions into alternative functions of CAF-1. This is a mature body of work with very interesting implications for HIV transcription. Some concerns are listed below.

Major Concerns:

1. Throughout their work the authors use various methods for knockdown of CHAF-1A. However, authors should provide validation by mRNA/protein levels to confirm knockdowns via sgRNA, shRNA, and siRNA. The authors should also provide viability data about their knockdown lines used throughout experiments.

2. In Figure 1D, the authors demonstrate where CHAF-1A enriches along the HIV-1 genome. In their graph, all amplicons are normalized to siNC Ig G5'. Authors should provide graphs where the data is represented as percent input or data that is normalized to Ig pull-downs of each amplicon.

3. In Figure 1K, examination of methyl-CpG at the HIV LTR is performed. Authors should provide more details on where on LTR and how many CpG sites are targeted.

4. ChIP-qPCR on various histone marks is provided throughout the manuscript. A major concern is the lack of total histone data, particularly given that knockdown of CHAF-1A leads to increased chromatin accessibility (Fig 1L). Authors should provide data for total histone H3 and H4 for these experiments.

5. In Figure 1L, ATAC-seq shows that knockdown of CHAF-1A leads to increased DNA accessibility. In the methods, more descriptive text should be included to describe how the 5' and 3' LTRs were discriminated against when aligning given the large degree of homology between the 5' and 3' LTRs.

6. In Figure 3 and Figure 5, microscopy data is provided to investigate CHAF-1A as a major factor promoting phase separation of the CAF-1 complex into nuclear condensates. However, no quantification data is provided to demonstrate these conclusions across many cells. Authors should provide quantification data that would provide more broadly applicable results. Additionally, localization of CHAF-1A and some members of CAF-1 bodies have been previously described in Houlard et al PLoS Genetics 2006. Authors should cite this paper.

7. Latently infected cell populations of HIV patients is predominantly within memory CD4+ T-cells that are quiescent and do not underdo DNA replication. In their manuscript, the authors identify a functionality to the nucleosome assembly complex CAF-1 at the HIV-1 LTR, whose canonical function is to load nucleosomes onto newly replicated DNA. The authors should provide more in-depth discussions as to how they see CAF-1 activity fitting into the context of HIV latency - do they believe it is connected to the nucleosome assembly function or to a novel activity of the complex and is this activity linked to cell division and DNA replication?

Referee #2:

In present study, Ma et al. provide evidence that the histone chaperone chromatin assembly factor 1 (CAF-1) plays an important role in HIV-1 latency. The authors first used a global approach of RNA-sequencing and Mass Spectrometry to identify candidate factors involved in virus-silencing in a Jurkat T cell line (J-Lat 10.6) containing a full-length integrated HIV-1 genome expressing GFP upon activation. They show that CHAF1A expression as well as other suppressive factors are up-modulated in latent and downmodulated in TNF stimulated J-Lat 10.6 cells. They further show that depletion of CHAF1A increase viral gene expression in CD4+ T cells transduced with a pseudotyped-GFP reporter virus. In addition, CHAF1A depletion promoted reactivation of HIV by treatment with SAHA in either in vitro infected CD4+ T cells or in clinical samples, suggesting a role of CHAF1A in virus silencing. The authors further show that CHAF1A depletion facilitated accessibility of the HIV-5' LTR chromatin to transcriptional activators in J-Lat cell lines and identified several factors recruited by the histone chaperone CAF-1 that may suppress HIV-1 LTR activity in the TZM-bl cell line. Finally, the authors show that CAF-1 forms big nuclear condensates that could coalesce distal genes and DNA elements to manipulate the expression of multiple genes and performed mutational and functional analyses to map critical domains.

The study addresses a very important issue and contains a large quantity of data. Strengths are the global unbiased screening of gene/protein candidates involved in HIV-1 latency, the functional analysis of CHAF1A in primary HIV-infected CD4+ T cells and the molecular characterization on the CAF-1 sub-units forming a novel suppressive complex to potentially manipulate the expression of HIV-LTR. Weaknesses include the lack of an experimental system that directly address the impact of CHAF1A in the establishment and maintenance of HIV latency in CD4+ T cells to support the author conclusions. In addition, the English is frequently flawed and some statements and conclusions should be cautioned.

Specific points.

1. The majority of this work was performed using HIV-infected cell lines that differ in the mechanisms governing latent HIV-1 infection in infected individuals on long-term ART. The authors conclude that CAF1 promotes the establishment and maintenance of HIV-latency in CD4+ T cells but do not directly demonstrate this by determining the frequencies of latently-infected cells. The results on productively HIV-1 infected (GFP+) cells are suggestive but without additional data the conclusion that CAF1 promotes latency in primary cells should be cautioned.

2. The results shown regarding CHAF1A contribution to HIV-mediated suppression in J-Lat 10.6 cells are convincing. However, the impact of CHAF1A on virus reactivation and potential mechanism(s) for their promotion of virus persistence in CD4+ T cells are less clear. In Figure 7, considering that αCD3/CD28/IL-2 are wild-type CHAF1A cells, wouldn't the authors expect that depleted-CHAF1A cells (shCHAF1A) should reactivate higher GFP levels than the wild-type phenotype (αCD3/CD28/IL-2)?

3. To support their claim that CHAF1A knockdown in CD4+ T cells prevents suppression of GFP cells, the authors must provide primary qPCR and western blot data to allow quantitative assessment of knockdown efficiencies.

4. The authors should show representative primary FACS data for the results presented in Figure 7 (i.e. CD3, CD4, live-dead staining) that includes an uninfected control used to set the GFP positive and negative gates.

5. The manuscript contains numerous typos and errors that distract from the science. It has to be carefully edited and corrected.

6. line 42: "could be an important strategy to massively reactivate latent HIV-1". "Massively" should be omitted to avoid overstatement.

Referee #3:

The present manuscript identifies a function for the CAF-1 complex in repressing HIV-1 LTR using several cellular models including primary CD4 T cells and CD4 T cells isolated from HIV infected individuals under suppressive antiretroviral therapy. The study also reveals that CHAF-1A, a subunit of the CAF-1 complex, catalyzes the formation of phase-separated suppressive nuclear bodies. Finally, the authors suggest that HIV-1 transcriptional latency mediated by CAF-1 involves phase-separated suppressive nuclear bodies. The study is impressive by the amount of work presented, is of importance and bring HIV into the emergent field of Liquid-liquid phase separation (LLPS).

However, some of the experiments needs to be better controlled and the most important conclusion is not supported by the data. A key experiment is to show whether HIV-1 DNA is associated with CHAF1A nuclear bodies and whether this is dependent on the transcriptional activity of the viral genome. This can be done by ImmunoFish.

Specific major concerns

Figure 1F-I should be repeated to include the histone marks distribution along the viral genome. Importantly, from the Materials and Methods section no ChIP was performed using anti-H3. This is some how problematic since Histone marks level should be normalized to total H3. This also applies for phosphorylated RNAPII ChIP experiment which should be normalized to total RNAPII (Figure 1M).

Figure2A: Were the extracts treated with DNase before complex purification? Are the interactions observed DNA-dependent?

Figure 2I: How this experiment was controlled? Knockdown efficiency etc.

Gold standard experiments to show LLPS formation are:

1- Maintain a spherical shape (measure the sphericity of the droplet)

2- Fuse after touching (done)

3- Molecular mobility (FRAP _ do recovery plot)

4- Concentration dependence (Defining the critical concentration in vitro for condensate formation)

5- Diffusion across boundary (FRAP)

- The different immunofluorescence images showing CHAF1A localization show different size of CHAF1A condensates. How can the authors explain these differences? Is it cell cycle dependent? Are CHAF1A condensates bound to chromatin?

- There is a lack in quantification and statistics in different panels.

Please provide line scans of all the immunofluorescence images showing co-localization between CHAF1A with its partners.

Fig 4A: Please show FRAP Histogram of the mean of immobile and mobile fractions per nucleus.

- Fig5B: In CHAF1A depleted cells, authors observe a disappearance of PCNA bodies. How can they explain the exclusion of PCNA from the nucleus and its cytoplasmic accumulation?

In the bottom panel, it's not clear whether DNMT1 is endogenous or a recombinant protein. The authors did not show the pattern of endogenous DNMT1 in absence of CHAF1A.

Fig5D: Please show more convincing images and statistics about the presence of SUV39H1 hijacked within the cytoplasm.

- Authors cannot conclude that CAF-1 bodies might coalesce SUV39H1, SUV39H2 and HP1 to establish the maintenance of heterochromatin based only on 3 panel of images without any quantification.

- Fig EV3 R and Fig EV6B (middle panel) are the same with different annotation. Please correct.

- For the in Vitro part, authors did not specify the concentration of the different purified proteins neither they show the corresponding western blot, to see if there is a difference in the expression of these mutants.

Authors should check whether CHAF1A phase separation occurs in presence of the crowding agent PEG.

- Treatment with 1,6-hexanediol, which has emerged as an indicator of LLPS, has caveats. Authors should test Sorbitol or sucrose known to destabilize weak electrostatic interactions involved in protein phase separation.

Point-by-point response to each comment:

Referee #1:

In their manuscript titled, "CAF-1 promotes HIV-1 latency by leading the formation of phase-separated suppressive nuclear bodies", Ma and colleagues investigate the role of the nucleosome assembly complex, CAF-1, on HIV latency. Using both HIV reporter virus clones and primary cell experiments, they dissect how CAF-1, namely the CHAF-1A subunit, is a HIV restrictive factor acting through mechanisms involving recruitment of suppressive epigenetic complexes into nuclear condensates with implications for regulating HIV LTR dynamics. Their work also provides interesting questions into alternative functions of CAF-1. This is a mature body of work with very interesting implications for HIV transcription. Some concerns are listed below.

Reply: We thank the reviewer for the support of this study.

Major Concerns:

1. Throughout their work the authors use various methods for knockdown of CHAF-1A. However, authors should provide validation by mRNA/protein levels to confirm knockdowns via sgRNA, shRNA, and siRNA. The authors should also provide viability data about their knockdown lines used throughout experiments.

Reply: We thank the reviewer for the kind suggestion. All the 13 panels of knockdown or knockout efficiencies as well as viabilities upon CHAF1A depletion have been carefully evaluated and provided in **newly-added Appendix Figure S1 and Appendix Figure S8**. For sgRNA-mediated CHAF1A knockout in various HIV-1 latency cell lines, the knockout efficiencies were confirmed by western blot (WB) (**newly-added Appendix Figure S1A-E**). The knockout efficiency of homogeneous cell line was further confirmed by DNA sequencing to ensure that both *CHAF1A* alleles were mutated with frameshift (**newly-added Appendix Figure S1A**). For siRNA-mediated CHAF1A knockdown in J-Lat 10.6 and TZM-bl cell lines, the knockdown efficiencies were confirmed by both WB to show CHAF1A protein expression level, and RT-qPCR to show *CHAF1A* mRNA expression level (**newly-added Appendix Figure S1F and S1G**). For shRNA-mediated CHAF1A knockdown in J-Lat 8.4 cell line, the knockdown efficiencies were confirmed by both WB and RT-qPCR (**newly-added Appendix Figure S1H**). All the shRNA-mediated CHAF1A knockdown efficiencies in primary $CD4^+$ T cells have been confirmed by both WB and RT-qPCR (**newly-added Appendix Figure S8B-F**). The percentages of viable cells upon CHAF1A depletion were quantitated by measuring the percentages of amine-reactive fluorescent dye non-permeant cells. The statistical analysis results of viabilities have been shown alongside depletion efficiencies (**newly-added Appendix Figure S1 and S8**).

2. In Figure 1D, the authors demonstrate where CHAF-1A enriches along the HIV-1

genome. In their graph, all amplicons are normalized to siNC Ig G5'. Authors should provide graphs where the data is represented as percent input or data that is normalized to Ig pull-downs of each amplicon.

Reply: We apologize for not showing graphs where the data was represented in our original submission. We now show the schematics of HIV-1 defective proviruses within both J-Lat 10.6 and TZM-bl cells in **newly-added Appendix Figure S2A**. Based on the integration sites sequencing results, HIV-1 genomic DNA was integrated in the intron of cellular gene *SEC16A* in J-Lat 10.6. HIV-1 mini-genomic DNA was integrated in the intron of cellular gene *RALGDS* in TZM-bl. The ChIP-qPCR primers sites were indicated below each proviral DNA. G5' represented cellular DNA and viral 5'LTR junction in J-Lat 10.6; A: Nucleosome 0 assembly site; B: Nucleosome free region; C: Nucleosome 1 assembly site; V5: Viral 5'LTR and gag leader sequence junction; E represented *envelope*; V3: Viral poly purine tract and 3'LTR junction; G3' represented viral 3'LTR and cellular DNA junction; G5 represented cellular DNA and viral 5'LTR junction in TZM-bl; L represented *luciferase* gene region; G3 represented viral 3'LTR and cellular DNA junction in TZM-bl. During each round of ChIP-qPCR, normal rabbit IgG signals against each site were evaluated and treated as negative control. All the amplicons were firstly normalized to its own input, followed by normalizing to G5' signals of siNC IgG. Thus, the data showed in Figure 1D indicated *Fold Change of ChIP-qPCR DNA Signals Normalized to siNC IgG of G5'*. Detailed ChIP-qPCR experiments and corresponding analysis strategies have also been described in '***ChIP-qPCR*' within Materials and Methods section**.

3. In Figure 1K, examination of methyl-CpG at the HIV LTR is performed. Authors should provide more details on where on LTR and how many CpG sites are targeted.

Reply: We apologize for not showing the detailed information clearly in our original manuscript. The schematic of methyl-CpG evaluation sites on HIV-1 LTR has been shown in **Figure EV2F**. HIV-1 pseudotyped virus in J-Lat 8.4 was integrated within intron of cellular gene *FUBP1* based on integration site sequencing. There were 11 CpG sites on HIV-1 LTR (10 sites within U3 region, 1 site within U5 region). Over 95% of these sites in naïve J-Lat 8.4 cells were methylated based on methylation sequencing results. The Methyl-CpGs percentages upon CHAF1A depletion and/or 5-aza-dC treatment were significantly decreased, the results of which were shown in both **Figure 1K** and **Figure EV2H**. Detailed information on DNA methylation assay and corresponding primers has also been shown in '***DNA methylation assay*' within Materials and Methods section**.

4. ChIP-qPCR on various histone marks is provided throughout the manuscript. A major concern is the lack of total histone data, particularly given that knockdown of CHAF1A leads to increased chromatin accessibility (Fig 1L). Authors should provide data for total histone H3 and H4 for these experiments.

Reply: We thank the reviewer for the kind suggestion. We re-performed all the essential ChIP-qPCR experiments along the entire HIV-1 genomic DNA. Especially, we showed the ChIP-qPCR results of Histone H3, Histone H4 and RNAP II on HIV-1

genome in **newly-added Appendix Figure S2F, S2G and S2I**. The results showed that both total histone H3 and histone H4 on HIV-1 LTR were unchanged upon CHAF1A depletion. The depletion of CHAF1A resulted in significant increase of active histone marks (H3K4me3, H3K9Acetyl and H3K36me2) and decrease of suppressive histone marks (H3K9me3, H4K20me3 and H3K27me3) (**Figure 1F-I, Figure EV2B-2E**). Our ATAC-Seq result showed that the knockdown of CHAF1A led to increased chromatin accessibility (**Figure 1L**). These results indicated that the increased chromatin accessibility upon CHAF1A depletion was significantly correlated with the accessible epigenetic environment (increased active histone marks and decreased suppressive histone marks), rather than total histones deposited on DNA.

5. In Figure 1L, ATAC-seq shows that knockdown of CHAF-1A leads to increased DNA accessibility. In the methods, more descriptive text should be included to describe how the 5' and 3' LTRs were discriminated against when aligning given the large degree of homology between the 5' and 3' LTRs.

Reply: We are sorry for the incomplete explanation of the analysis strategy for ATAC-Seq data which we have carefully modified in the '***ATAC-Seq***' **within Materials and Methods section**. Both the HIV-1 genome and human genome were analyzed for accessibility. The reads were aligned to HIV-1 reference genome K03455, M38432 (Version K03455.1) by Bowtie2, followed by rearranging with Samtools. The human genome was aligned to human reference genome GRCh38. The integration site of HIV-1 provirus in J-Lat 10.6 has been identified by genome walking strategy. Cellular DNA and viral 5'LTR junction was different from viral 3'LTR and cellular DNA junction, although 5'LTR and 3'LTR showed significant similarity. Only the reads containing both HIV-1 5'LTR and human integration junction fragments were sorted. Thus, the read density centered HIV-1 5'LTR could be calculated and discriminated from 3'LTR.

6. In Figure 3 and Figure 5, microscopy data is provided to investigate CHAF-1A as a major factor promoting phase separation of the CAF-1 complex into nuclear condensates. However, no quantification data is provided to demonstrate these conclusions across many cells. Authors should provide quantification data that would provide more broadly applicable results. Additionally, localization of CHAF1A and some members of CAF-1 bodies have been previously described in Houlard et al PLoS Genetics 2006. Authors should cite this paper.

Reply: We thank the reviewer for the kind suggestions. In our revised manuscript, we have added over 47 panels of quantitative and statistical analysis data for all the super-resolution images showed in Figure 3 and Figure 5. All the quantification data have been shown in **newly-added Appendix Figure S3, S4 and S7**. Briefly, Line scan profiles were provided for each co-localization image. The distributions of CAF-1 bodies size, number and sphericity were measured. The percentages of target protein above threshold co-localized, the Pearson's coefficients of each protein pairs as well as the percentages of cells with co-localized cellular bodies were extensively

calculated and analyzed.

Besides, we have cited the closely related paper named '***CAF-1 Is Essential for Heterochromatin Organization in Pluripotent Embryonic Cells' (Houlard et al PLoS Genetics 2006; PMID: 17083276)*** in several proper positions. In 'CAF-1 forms nuclear bodies' result section, we found H3K9me3 maintainer HP1α was co-localized with CHAF1A, which was also discovered in Houlard et al paper. In 'CHAF1A is the leading factor of CAF-1 body' result section, we found that HP1α molecules were diffused within nuclei in the absence of CHAF1A. The similar phenomenon of CHAF1A depletion-mediated HP1α bodies alteration was also found in mouse embryonic stem cells, which was reported in Houlard et al paper. In discussion section, both our study and Houlard et al paper agreed that CAF-1 was able to act as a chromatin silencing complex to orchestrate heterochromatin formation and maintenance.

7. Latently infected cell populations of HIV patients is predominantly within memory CD4+ T-cells that are quiescent and do not underdo DNA replication. In their manuscript, the authors identify a functionality to the nucleosome assembly complex CAF-1 at the HIV-1 LTR, whose canonical function is to load nucleosomes onto newly replicated DNA. The authors should provide more in-depth discussions as to how they see CAF-1 activity fitting into the context of HIV latency - do they believe it is connected to the nucleosome assembly function or to a novel activity of the complex and is this activity linked to cell division and DNA replication?

Reply: We sincerely thank the reviewer for the insightful comment. Based on our study, we believed that CAF-1-mediated HIV-1 latency reflected a novel activity of the complex, rather than connecting to the nucleosome assembly function. The novel function of CAF-1-mediated HIV-1 latency predominantly happened in resting memory $CD4^+$ T cells. Thus, the function was not likely linked to cell division and DNA replication, either. Based on our comprehensive proteomics, imaging, biophysical and biochemical assays results, we believe that CAF-1 is a leading factor of nucleus suppressive environment, which orchestrates multi-layers of suppressive proteins to form suppressive nucleus bodies with liquid-liquid phase separation (LLPS) properties. CAF-1 bodies mediate the establishment and maintenance of heterochromatin, which also reflect its role as a suppressive epigenetic memory 'writer', 'reader' and 'maintainer'.

The question raised by the reviewer is quite important that we have re-arranged our discussion section with more in-depth discussions. **In the first paragraph, we summarized the traditional functions of CAF-1 complex**, which involved in DNA replication, cell division, heterochromatin maintenance and safeguarding cell identity. Our work on CAF-1-mediated HIV-1 latency revealed that CAF-1 functioned as versatile suppressive nuclear bodies with LLPS properties. **In more details, we elucidated the multi-functions of multi-components CAF-1 bodies in the following paragraph of discussion.** We used HIV-1 latency as research model and identified that CAF-1 recruited many suppressive epigenetic modifiers and maintainers, which 'write' and 'read' suppressive epigenetic modifications, 'read' and

‘erase’ active epigenetic modifications. Besides, CAF-1 recruited TRIM28 and SUMO paralogs. We previously have found that TRIM28 mediates HIV-1 latency by SUMOylating P-TEFb complex subunit CDK9, resulting in the transcriptional pausing of RNAP II on HIV-1 LTR (PMID: 30652970). CAF-1 hijacked TRIM28-mediated SUMOylation system to inactive CDK9, which prevented HIV-1 transcription. In addition, we believe that the multi-suppressive functions of CAF-1 could be applied to various chromatin biological processes. Thus, **we discussed its delicate role in biological processes in the next paragraph of discussion.** Previous work has shown that CAF-1 promotes heterochromatin formation (PMID: 17083276; PMID: 19498464; PMID: 15306854; PMID: 29686265). Our work indicates that CAF-1 bodies contribute to the formation of both constitutive heterochromatin and facultative heterochromatin. Phase-separated nuclear condensates guided by CAF-1 could facilitate the formation of 3D genomic architecture by condensing the chromatin and promoting the compartmentalization of chromatin and synergistically regulate multiple genes, which greatly increases the efficiency of gene regulation. Based on all the functions found previously and novel functions presented here, **we propose that CAF-1 bodies are kinds of multi-layer complexes which include both canonical and non-canonical CAF-1 bodies, the theory of which was expanded in the following paragraph of discussion.** CAF-1 forms versatile cellular bodies involve heterochromatin formation, DNA methylation and DNA replication, which reflects the canonical functions of CAF-1. CAF-1 bodies also crosstalk with PRC components and maintain H3K27me3-mediated facultative heterochromatin. Some NuRD complex components are also within CAF-1 bodies and facilitate the deacetylation of histone lysines. Our work also shows that CAF-1 hijacks TRIM28-mediated CDK9 SUMOylation, resulting in the transcriptional pausing of RNAP II on HIV-1 LTR. These functions of CAF-1 reflect its non-canonical roles in cellular biological processes. All the functions of CAF-1 involve different proteins and different biological processes. Although these processes crosstalk with each other somehow, they are relatively independent and rely on the different functions of multi-domain CAF-1 complexes. We believe that CAF-1-guided body is a core factor contributing to HIV-1 latency, which merits further evaluation to develop more effective latency-reversing agents (LRAs). **We proposed our above prospect in the last paragraph of discussion**.

Referee #2:

In present study, Ma et al. provide evidence that the histone chaperone chromatin assembly factor 1 (CAF-1) plays an important role in HIV-1 latency. The authors first used a global approach of RNA-sequencing and Mass Spectrometry to identify candidate factors involved in virus-silencing in a Jurkat T cell line (J-Lat 10.6) containing a full-length integrated HIV-1 genome expressing GFP upon activation. They show that CHAF1A expression as well as other suppressive factors are up-modulated in latent and downmodulated in TNF□ stimulated J-Lat 10.6 cells. They further show that depletion of CHAF1A increase viral gene expression in CD4+

T cells transduced with a pseudotyped-GFP reporter virus. In addition, CHAF1A depletion promoted reactivation of HIV by treatment with SAHA in either in vitro infected CD4+ T cells or in clinical samples, suggesting a role of CHAF1A in virus silencing. The authors further show that CHAF1A depletion facilitated accessibility of the HIV-5' LTR chromatin to transcriptional activators in J-Lat cell lines and identified several factors recruited by the histone chaperone CAF-1 that may suppress HIV-1 LTR activity in the TZM-bl cell line. Finally, the authors show that CAF-1 forms big nuclear condensates that could coalesce distal genes and DNA elements to manipulate the expression of multiple genes and performed mutational and functional analyses to map critical domains.

Reply: We appreciate for reviewer's positive feedback and comprehensive summaries.

The study addresses a very important issue and contains a large quantity of data. Strengths are the global unbiased screening of gene/protein candidates involved in HIV-1 latency, the functional analysis of CHAF1A in primary HIV-infected CD4+ T cells and the molecular characterization on the CAF-1 subunits forming a novel suppressive complex to potentially manipulate the expression of HIV-LTR. Weaknesses include the lack of an experimental system that directly address the impact of CHAF1A in the establishment and maintenance of HIV latency in CD4+ T cells to support the author conclusions. In addition, the English is frequently flawed and some statements and conclusions should be cautioned.

Reply: We thank the reviewer for the support of this study. We have addressed each comment according to the reviewer's suggestion. Especially, we further elucidated the impact of CHAF1A on the establishment and maintenance of HIV-1 latency in primary $CD4^+$ T cells. We also carefully checked the entire manuscript, corrected all the typos and errors, revised some statements and conclusions.

Specific points.

1. The majority of this work was performed using HIV-infected cell lines that differ in the mechanisms governing latent HIV-1 infection in infected individuals on long-term ART. The authors conclude that CAF1 promotes the establishment and maintenance of HIV-latency in CD4+ T cells but do not directly demonstrate this by determining the frequencies of latently-infected cells. The results on productively HIV-1 infected (GFP+) cells are suggestive but without additional data the conclusion that CAF1 promotes latency in primary cells should be cautioned.

Reply: We sincerely apologize for not clearly demonstrating the role of CAF-1 on the establishment and maintenance of HIV-1 latency in primary $CD4^+$ T cells. We have now re-arranged our data and presented the results of several primary $CD4^+$ T cells latency models. The results have been shown in **Figure 7 and Figure EV5**. We were totally aware that it was almost impossible to demonstrate the role of CAF-1 on the establishment of HIV-1 latency in $CD4^+$ T cells isolated from infected individuals on cART, because the latency has established in these infected cells. Thus, we conducted

several experiments in lab-made primary $CD4^+$ T cell latency models to demonstrate the contribution of CAF-1 on the establishment and maintenance of HIV-1 latency. In $CD4^+$ T cells isolated from HIV-1-infected individuals, we only demonstrated the contribution of CAF-1 on the maintenance of HIV-1 latency. Detailed experimental results and conclusions have been shown in **'*CHAF1A depletion reactivates latent HIV-1 from HIV-1-infected individuals*' within results section**. Briefly, we firstly knocked down CHAF1A in primary $CD4^+$ T cells, followed by the infection of pseudotyped HIV-1. As most of infected HIV-1 would eventually enter into latency, some of which entered latency immediately upon infection. If CHAF1A contributed to the establishment of HIV-1 latency, the depletion of CHAF1A should reduce the latency reservoir, resulting in more HIV-1-expressing cells which were indicated by the percentages of GFP-positive cells. Our results well-verified the above speculation (**Figure EV5A-D**). We further demonstrated the contribution of CHAF1A on the establishment of HIV-1 latency by latency delay experiment. We firstly infected primary $CD4^+$ T cells with pseudotyped HIV-1, followed by the knockdown of CHAF1A. During the two weeks of monitoring, we found that the absence of CHAF1A significantly delayed HIV-1 entering into latency (**Figure EV5E and F**). These results indicated that the vanguard of CAF-1 body could be a prerequisite to effectively establish HIV-1 latency. Subsequently, we evaluated whether CAF-1 bodies contributed to the maintenance of HIV-1 latency. We built both pseudotyped HIV-1 and authentic HIV-1 latency model. We found that the absence of CHAF1A significantly upregulated HIV-1 expression, which meant the established HIV-1 latency reservoir was reactivated by the absence of CHAF1A (**Figure 7A-C, Figure EV5G-J**). We further demonstrated the role of CAF-1 on the maintenance of HIV-1 latency in $CD4^+$ T cells isolated from infected individuals on cART. Our results showed that the depletion of CHAF1A significantly upregulated the expression of intracellular HIV-1 RNAs (**Figure 7D**). The genetic diversities of these RNAs were higher in CHAF1A depletion group that in SAHA treatment one, which meant RNAs of reactivated HIV-1 were transcribed from diversified HIV-1 integration sites (**Figure 7E**). Based on all the results in lab-made primary $CD4^+$ T cell latency model and $CD4^+$ T cells from HIV-1 infected individuals, we concluded that CAF-1 could contribute the establishment and maintenance of HIV-1 latency.

Another concern raised by the reviewer is that our frequencies of latently-infected cells were only indirectly demonstrated by the percentages of ever GFP-positive cells. However, all our lab-made primary $CD4^+$ T cell latency models were GFP-tagged models. HIV-1 backbones including NL4-3-ΔEnv/EGFP, NL4-3-P2A-EGFP (NPG) and NL4-3-ΔEnv/ΔNef-d2EGFP were used to infect activated $CD4^+$ T cells. All the GFP expression was driven by the HV-1 LTR where lies the HIV-1 promoter. Theoretically, 5% to 15% of $CD4^+$ T cells would be GFP-positive two days post infection, which meant that these cells have been integrated with HIV-1 proviruses which underwent productive transcription. After two weeks of resting (for NPG model, AZT was added to prevent persistent HIV-1 infection), most of the infected cells were GFP-negative, which meant that these infected cells have entered into latency. If a target (such as CHAF1A) contributes to HIV-1 latency, the depletion of it would

prevent the GFP-positive HIV-1-infected cells entering into latency, or reactivate GFP-negative (ever GFP-positive) HIV-1-infected cells. Thus, we think that the percentages of ever GFP-positive cells were sufficient to indicate the frequencies of latently-infected cells. We hope to keep this measurement.

2. The results shown regarding CHAF1A contribution to HIV-mediated suppression in J-Lat 10.6 cells are convincing. However, the impact of CHAF1A on virus reactivation and potential mechanism(s) for their promotion of virus persistence in CD4+ T cells are less clear. In Figure 7, considering that αCD3/CD28/IL-2 are wild-type CHAF1A cells, wouldn't the authors expect that depleted-CHAF1A cells (shCHAF1A) should reactivate higher GFP levels than the wild-type phenotype (αCD3/CD28/IL-2)?

Reply: We sincerely apologize that we haven't demonstrate the impact of CHAF1A on virus reactivation in primary $CD4^+$ T cells clearly. We have modified some of our statements in '*CHAF1A depletion reactivates latent HIV-1 from HIV-1-infected individuals*' within results section. The reviewer questioned that cells within αCD3/CD28/IL-2 group are wild-type CHAF1A cells. Thus, shCHAF1A-mediated HIV-1 reactivation should be stronger than wildtype CHAF1A group such as αCD3/CD28/IL-2 group. The question is interesting. We also agreed and proved that shCHAF1A-mediated HIV-1 reactivation should be stronger than wildtype CHAF1A group. However, the comparison should be controlled within the same stimulation conditions, which meant that both WT-CHAF1A and shCHAF1A groups should be untreated with αCD3/CD28/IL-2. Our data in **Figure 7 and Figure EV5** showed that shCHAF1A-mediated HIV-1 reactivation was indeed stronger than shluc (negative control) group. When both shluc and shCHAF1A groups were supplemented with SAHA (traditional LRAs), the combination of CHAF1A knockdown and SAHA stimulation was able to reactivate more HIV-1 compared with individual intervention. All of the above shluc- and shCHAF1A-related groups were untreated with αCD3/CD28/IL-2. The reviewer noticed that αCD3/CD28/IL-2 group seemed to reactivate more HIV-1 than shCHAF1A-only group. The result was not surprising. The stimulation of αCD3/CD28/IL-2 was used as positive control. It was the strongest latency reversing combination which activated not only latent HIV-1 but also all the global TCR engaged signal pathways, although it was unable to be used in clinal intervention because of the side effects of proinflammatory cytokines storm. The novel latency contributor CAF-1 complex which we identified here was another candidate to develop LRAs. We cannot conclude that the depletion of CHAF1A was more powerful than the strongest αCD3/CD28/IL-2 intervention.

3. To support their claim that CHAF1A knockdown in CD4+ T cells prevents suppression of GFP cells, the authors must provide primary qPCR and western blot data to allow quantitative assessment of knockdown efficiencies.

Reply: We thank the reviewer for the insightful suggestion. We have provided all the knockdown efficiencies data within primary $CD4^+$ T cells in **newly-added Appendix Figure S8**. The knockdown efficiencies were confirmed by both western blot (WB) to show CHAF1A protein expression level, and qPCR to show *CHAF1A* mRNA

expression level.

4. The authors should show representative primary FACS data for the results presented in Figure 7 (i.e. CD3, CD4, live-dead staining) that includes an uninfected control used to set the GFP positive and negative gates.
Reply: We apologize for this omission in our original manuscript. In **newly-added Appendix Figure S8A**, we have provided representative primary FACS data for the results presented in Figure 7, which includes an uninfected control used to set the GFP positive and negative gates. Briefly, primary $CD4^+$ T cells were isolated through human $CD4^+$ T lymphocyte enrichment magnetic cell separation system (MACS). The purities of $CD4^+$ T cells were ensured at least 90%. FSC-A, SSC-A and FSC-H were used to gate live single cells. Both CD3 and CD4 markers were used to sort RFP-positive $CD4^+$ T cells by fluorescence-activated cell sorting (FACS) system. All the shRNA lentiviruses constructs harbored a PGK promoter-driven *RFP* open reading frame. Thus, RFP-positive $CD4^+$ T cells were shRNA lentiviruses-infected $CD4^+$ T cells. The purities of sorted shRNA lentiviruses-infected RFP-positive cells were ensured at least 90% by flow cytometry. The lentiviruses-infected $CD4^+$ T cells were infected with HIV-1 pseudotyped virus, or untreated (uninfected negative control). The uninfected negative controls were used to gate HIV-1 pseudotyped virus-infected GFP-positive cells.

5. The manuscript contains numerous typos and errors that distract from the science. It has to be carefully edited and corrected.
Reply: We apologize for all the typos and errors which we made in our original manuscript. We have carefully checked the entire manuscript many times and corrected all the typos and errors to ensure that it is suitable to be published.

6. line 42: "could be an important strategy to massively reactivate latent HIV-1". "Massively" should be omitted to avoid overstatement.
Reply: We sincerely apologize for our overstatement. In our revised manuscript, we have removed the statement of ‘massively’ in both Abstract and Discussion sections.

Referee #3:

The present manuscript identifies a function for the CAF-1 complex in repressing HIV-1 LTR using several cellular models including primary CD4 T cells and CD4 T cells isolated from HIV infected individuals under suppressive antiretroviral therapy. The study also reveals that CHAF-1A, a subunit of the CAF-1 complex, catalyzes the formation of phase-separated suppressive nuclear bodies. Finally, the authors suggest that HIV-1 transcriptional latency mediated by CAF-1 involves phase-separated suppressive nuclear bodies. The study is impressive by the amount of work presented, is of importance and bring HIV into the emergent field of Liquid-liquid phase separation (LLPS). However, some of the experiments needs to be better controlled and the most important conclusion is not supported by the data. A key experiment is to

show whether HIV-1 DNA is associated with CHAF1A nuclear bodies and whether this is dependent on the transcriptional activity of the viral genome. This can be done by ImmunoFish.

Reply: We appreciate the positive feedback of the reviewer. We also thank the reviewer for the kind suggestion that conducting ImmunoFISH to investigate the association of CAF-1 bodies with HIV-1 genomic DNA in different cellular states. The experiment is so tough yet important that we have tried over 8 protocols (PMID: 20809307; PMID: 23407477; PMID: 23768491; PMID: 25731161; PMID: 28250118; PMID: 31492853; PMID: 32065142; PMID: 32820408;) and prepared over 47 ImmunoFISH samples during the 3 months revision. Finally, we proved that the HIV-1 genomic DNA was co-localized with the CAF-1 body in latent status (**newly-added Figure 3C, newly-added Appendix Figure S3C and S4C**). Upon activation, the HIV-1 genomic DNA was far away from CAF-1 bodies (**newly-added Figure 3D, newly-added Appendix Figure S3D and S4D**). The above results have been shown in **'*CAF-1 forms nuclear bodies*' within results section**. The corresponding method has been detailedly described in **'*ImmunoFISH*' within Materials and Methods section**.

Specific major concerns

Figure 1F-I should be repeated to include the histone marks distribution along the viral genome. Importantly, from the Materials and Methods section no ChIP was performed using anti-H3. This is somehow problematic since Histone marks level should be normalized to total H3. This also applies for phosphorylated RNAPII ChIP experiment which should be normalized to total RNAPII (Figure 1M).

Reply: We thank the reviewer for the insightful reminding. We have repeated all the ChIP-qPCR results within Figure 1F-I and Figure 1M to indicate ChIP-qPCR signals along the entire HIV-1 genomic DNA, the results of which have been shown in **newly-added Appendix Figure S2B-E and S2H**. Besides, we also showed the ChIP-qPCR results of Histone H3, Histone H4 and RNAP II on HIV-1 genome in **newly-added Appendix Figure S2F, S2G and S2I**. The results showed that both total histone H3 and histone H4 on HIV-1 LTR were unchanged upon CHAF1A depletion. Both total RNAP II and Pho-RNAP II on HIV-1 promoter and gene body were upregulated upon CHAF1A knockdown. The depletion of CHAF1A resulted in significant increase of active histone marks (H3K4me3, H3K9Acetyl and H3K36me2) and decrease of suppressive histone marks (H3K9me3, H4K20me3 and H3K27me3) (**Figure 1F-I, Figure EV2B-2E**). These results indicated that CHAF1A depletion favored accessible epigenetic environment (increased active histone marks and decreased suppressive histone marks) and active transcription factors, rather than total histones deposited on DNA.

Figure2A: Were the extracts treated with DNase before complex purification? Are the interactions observed DNA-dependent?

Reply: Yes, the mass spectrometry (MS) samples (**Figure 2A**) and Co-IP samples (**Figure EV2J-L**) were prepared in NP-40 lysis buffer supplemented with protease

inhibitor cocktail (PIC) and DNase I. Thus, all the CAF-1-related interactions were not DNA-dependent.

Figure 2I: How this experiment was controlled? Knockdown efficiency etc.
Reply: All the siRNAs within library have been validated by the company to ensure that at least one siRNA for each gene was able to knock down target gene efficiently (over 90% knockdown efficiency). We also have conducted 53 panels of qPCR experiments to confirm the knockdown efficiencies, the results of which have been shown in **newly-added Table EV1**. Gene IDs were shown in Column B of the table. Gene names were indicated in Column C. qPCR forward and reverse primers were shown in Column D and E. Fold changes of target mRNAs of negative controls and knockdown samples were calculated and indicated in Column F to K. The knockdown efficiencies of each gene were normalized to negative control and showed in Column L. At least 80% knockdown efficiencies were ensured for each gene.

Gold standard experiments to show LLPS formation are:
1- Maintain a spherical shape (measure the sphericity of the droplet)
2- Fuse after touching (done)
3- Molecular mobility (FRAP _ do recovery plot)
4- Concentration dependence (Defining the critical concentration in vitro for condensate formation)
5- Diffusion across boundary (FRAP)
Reply: We sincerely appreciate for the reviewer's constructive summary. These gold standard experiments of LLPS guided us to provide more convincing data to indicate the LLPS properties of CHAF1A. We have performed some new experiments and re-analyzed some data to improve the integrity of our work, which were outlined below.
1. Maintain a spherical shape. We used Imaris software to analyze 579 CAF-1 bodies of five cells which were from five samples. These samples were prepared in five independent experiments. Rendered 3D shapes of CAF-1 bodies were built based on all the 3D-Stack-SIM images of CAF-1 bodies through Surface module of Imaris. The representative rendered 3D shapes of CAF-1 bodies were shown in **newly-added Appendix Figure S4E**, which displayed XY, XZ and YZ planes of the CAF-1 body. Besides, we measured the sphericities of these 579 CAF-1 bodies. The distribution of CAF-1 bodies sphericities was shown as scatter plot in **newly-added Appendix Figure S4E**. The distribution data showed that the mean sphericity of CAF-1 bodies was 0.8. The sphericities ranged from 0.55 to 0.98. These data indicated that CAF-1 bodies maintained spherical shapes with high sphericities.
2. Fuse after touching. The corresponding data has been shown in **Figure 4B** in our original manuscript. Besides, we also provided data that one CAF-1 body was able to split into two smaller ones, which has been shown **Figure 4C** in our original manuscript.
3. Molecular mobility. The corresponding FRAP experiment has been done in **Figure 4A** in our original manuscript. The reviewer kindly suggested us to provide

the recovery plot, the data of which has been shown in **newly-added Appendix Figure S4F**. The FRAP quantitative recovery plot showed that bleached GFP-tagged CAF-1 bodies gradually recovered their fluorescence intensities. Whereas, the fluorescence intensities of unbleached CAF-1 bodies were unchanged during the 6 minutes observation.

4. Concentration dependence. This experiment was conducted by evaluating the condensate formation ability of *in vitro* purified CHAF1A proteins. As we have shown in **Figure 4G** in our original manuscript, the critical concentration of NaCl within droplet formation buffer was 125 mM. Thus, we defined the critical concentration of CHAF1A proteins to form droplets in 125 mM NaCl buffer. We found that CHAF1A was able to form droplet in various protein concentrations. Even in 0.78 μM, CHAF1A still formed small condensates. The numbers and sizes of CHAF1A droplets increased linearly with the increase of protein concentrations. This data has been shown in **newly-added Figure 4H**.

5. Diffusion across boundary. This phenomenon was also verified by the FRAP experiment, the results of which have been shown in **Figure 4A** and **newly-added Appendix Figure S4F**.

We thank the reviewer again for summarizing the above five gold standard experiments to demonstrate LLPS formation.

- *The different immunofluorescence images showing CHAF1A localization show different size of CHAF1A condensates. How can the authors explain these differences? Is it cell cycle dependent? Are CHAF1A condensates bound to chromatin?*

Reply: The reviewer raised a very interesting question, which also provoked great interest during our study. We have searched all the CAF-1-related papers, especially Prof. Bruce Stillman's work. He discovered CAF-1 in 1989 and worked on the functions of CAF-1 for 30 years. Both his work and ours showed that CHAF1A formed many condensates with different sizes. Prof. Stillman's work as well as many others showed that the sizes of CHAF1A condensates were indeed cell cycle dependent (PMID: 2546672; PMID: 15059888; PMID: 14519857; PMID: 10052459; PMID: 26908650). In non-S phase, CAF-1 bodies were very big and few. In early S phase, nuclei were densely scattered with small CAF-1 bodies. In middle S phase, CAF-1 bodies started to aggregate at the nuclear periphery and formed big nuclear puncta. In later S phase, various CAF-1 bodies were appeared simultaneously within nuclear. We also found that CAF-1 bodies were disappeared during the whole mitotic period (data not shown). Two researches showed that CAF-1 could bind to chromatin DNA directly (PMID: 26908650; PMID: 28315525). Our work also showed that CAF-1 could bind to HIV-1 LTR (**Figure 1D and Figure EV2A**). We speculated that the cell cycle dependence of CAF-1 bodies sizes might correlated with their chromatin-binding ability. However, this suppose has been out of our research scope of HIV-1 latency. We do apologize for not investigating that further. As a supplement, we showed the distributions of CAF-1 bodies diameters and numbers in **newly-added Appendix Figure S3A and B**. These statistical data were collected from 30 cells of 10 samples which were prepared in 10 independent experiments.

- *There is a lack in quantification and statistics in different panels.*

Reply: We apologize for these omissions of quantification and statistics. In our revised manuscript, we have added over 47 quantification data for all the images. These data were briefly outlined below.

1. We showed the distributions of CAF-1 bodies diameters, numbers and sphericities in **newly-added Appendix Figure S3A, S3B and S4E**.
2. We showed line scan profiles for all the co-localization images in **newly-added Appendix Figure S3C-P and S7A-J**.
3. We showed the quantification of co-localization, Pearson's coefficient of each protein pairs and percentages of cells with co-localized cellular bodies in **newly-added Appendix Figure S3Q-S and S7K-M**.
4. We also provided quantitative data for HP1α bodies, SUV39H1 bodies and SUV39H2 bodies within different cellular conditions. These data have been shown in **newly-added Appendix Figure S7N-Q**.
5. We also provided quantitative data for the distributions of droplets areas in **newly-added Figure 4G-I and Appendix Figure S6D-I**.

Please provide line scans of all the immunofluorescence images showing co-localization between CHAF1A with its partners.

Reply: We thank the reviewer for this kind reminding. We have shown line scan profiles for all the co-localization images in **newly-added Appendix Figure S3 and S7**. From the line scans, we could easily tell the co-localization between CHAF1A with its partners.

Fig 4A: Please show FRAP Histogram of the mean of immobile and mobile fractions per nucleus.

Reply: Sorry for this omission. We have provided the FRAP histogram of unbleached and bleached CAF-1 bodies in **newly-added Appendix Figure S4F**. Six unbleached and six bleached CAF-1 bodies from six different cells were observed to record fluorescence intensities for 404 seconds (over 6 minutes).

- *Fig5B: In CHAF1A depleted cells, authors observe a disappearance of PCNA bodies. How can they explain the exclusion of PCNA from the nucleus and its cytoplasmic accumulation?*

In the bottom panel, it's not clear whether DNMT1 is endogenous or a recombinant protein. The authors did not show the pattern of endogenous DNMT1 in absence of CHAF1A.

Reply: We apologize for not explaining these images clearly. If we understand correctly, the reviewer raised three questions in this comment. We would like to response to these questions point-by-point. Firstly, the reviewer concerned about the exclusion of PCNA from the nucleus upon CHAF1A knockdown. From our SIM images, we were only able to tell that PCNA bodies disappeared upon CHAF1A knockdown. We cannot state and haven't stated that PCNA proteins were excluded

from nucleus. Actually, there were still considerable PCNA proteins within nucleus upon CHAF1A knockdown. The reason why almost all the PCNA proteins seemed to be hijacked within cytoplasm was that the super-resolution SIM imaging technology treated weak fluorescence as background signal. During reconstruction from 15 angles widefield images, these weak signals would be removed automatically. We do find that large quantities of PCNA proteins were accumulated within cytoplasm upon CHAF1A knockdown. As CHAF1A harbored PCNA-interacting motif (PIP), we believed that the presence of CHAF1A within nucleus might tightly bind PCNA through PIP, which prevented the export of PCNA from nucleus to cytoplasm. Without CHAF1A, more PCNA proteins were exported to cytoplasm, resulting in the imbalance of PCNA distribution.

Secondly, in the bottom panel of Figure 5B, DNMT1 were actually exogenously expressed by GFP-tagged DNMT1-expressing plasmids. We found that only when overexpressing DNMT1 could we observe obvious DNMT1 bodies. DNMT1 also harbored PIP which tightly bound PCNA (PMID: 31235252; PMID: 12354094; PMID: 17576694). That was also the reason why DNMT1 bodies were able to stabilize PCNA within nucleus.

Thirdly, we do apologize that we didn't show the pattern of endogenous DNMT1 in the absence of CHAF1A. As we mentioned above, we could hardly tell distinct endogenous DNMT1 bodies. That was also the reason why we could not observe PCNA bodies upon CHAF1A knockdown, although the endogenous DNMT1 was able to bind PCNA tightly by PIP. When overexpressing DNMT1, we could observe distinct DNMT1 bodies, although the CHAF1A has been depleted (**Figure 5B, bottom panel**). We also have noticed that CHAF1A bodies were not influenced by the depletion of DNMT1 (**Table EV3, Row 12**). However, the potential alternative role of DNMT1 to the stabilization of PCNA bodies has beyond our research scope of CHAF1A-mediated bodies formation. We do apologize for not investigating the relationship between DNMT1 and PCNA further.

Fig5D: Please show more convincing images and statistics about the presence of SUV39H1 hijacked within the cytoplasm.

Reply: We apologize for this omission. We have specifically provided several statistical analysis data to indicate the influence of CHAF1A on the distribution of SUV39H1. We found that over 80% of SUV39H1 proteins within cell were co-localized with CHAF1A proteins, whereas only 25% of CHAF1A proteins within cell were co-localized with SUV39H1 (**newly-added Appendix Figure S7K**). The Pearson's coefficient of co-localized CHAF1A and SUV39H1 was over 0.6, which meant significant co-localization (**newly-added Appendix Figure S7L**). The percentages of cells with co-localized cellular bodies were more than 80% (**newly-added Appendix Figure S7M**). These quantification of co-localization data indicated that most of SUV39H1 proteins were within CHAF1A bodies. Besides, over 95% SUV39H1 proteins were within nucleus and formed almost 200 SUV39H1 bodies per 100 μm^2, among which 190 SUV39H1 bodies were co-localized with CHAF1A bodies (**newly-added Appendix Figure S7O-P**). However, upon CHAF1A

knockdown, we found that almost 50% of SUV39H1 proteins were hijacked within cytoplasm (**newly-added Appendix Figure S7P**). The body sizes were also significantly changed and the SUV39H1 bodies densities were decreased to only 30 bodies per 100 μm^2 (**newly-added Appendix Figure S7O**). Thus, we believed that CHAF1A might influence the distribution and body size of SUV39H1.

- Authors cannot conclude that CAF-1 bodies might coalesce SUV39H1, SUV39H2 and HP1 to establish the maintenance of heterochromatin based only on 3 panel of images without any quantification.

Reply: We apologize for these omissions. We have conducted several statistical analysis for **Figure 5C-E**, the data of which have been shown in **newly-added Appendix Figure S7K-Q**. The potential role of CAF-1 bodies on the distribution and body size of SUV39H1 has been elucidated in the above response to reviewer's comment. Like SUV39H1, over 80% of SUV39H2 proteins were co-localized with CHAF1A with Pearson's coefficient of 0.7, which meant significant co-localization (**newly-added Appendix Figure S7K and L**). The percentages of cells with co-localized CHAF1A and SUV39H2 bodies were more than 80% (**newly-added Appendix Figure S7M**). The bodies densities of SUV39H2 were over 250 bodies per 100 μm^2 (**newly-added Appendix Figure S7Q**). However, all the SUV39H2 proteins were dispersedly distributed within nucleus upon CHAF1A knockdown (**newly-added Appendix Figure S7Q**). We believed that CAF-1 bodies might coalesce SUV39H2 to form functional nuclear puncta. Apart from H3K9me3 'writers', we also evaluated the body formation abilities of H3K9me3 'reader' HP1α upon CHAF1A knockdown. We found that over 70% of HP1α proteins were co-localized with CHAF1A with Pearson's coefficient of 0.85 (**newly-added Appendix Figure S7K-L**). The percentages of co-localized cells were over 80% (**newly-added Appendix Figure S7M**). The HP1α bodies densities were 70 bodies per 100 μm^2 (**newly-added Appendix Figure S7N**). However, HP1α proteins were dispersedly distributed within nucleus upon CHAF1A knockdown (**newly-added Appendix Figure S7N**). As DS2 region of CHAF1A harbored an HP1-binding domain (HP1BD), we suspected that HP1BD might contribute the bodies formation of both CHAF1A and HP1α.The results showed that the deletion of HP1BD (CHAF1A-dHP1BD and CHAF1A-DS2-dHP1BD) resulted in the loss of HP1α bodies, however the CHAF1A mutants bodies still existed (**newly-added Appendix Figure S7K-N**). These results indicated that the HP1BD within CHAF1A played a key role in the formation of HP1α bodies. SUV39H1 and SUV39H2 were H3K9me3 modifiers. HP1α was H3K9me3 maintainer. SUV39H1, SUV39H2 and HP1α have played pivotal roles in the maintenance of heterochromatin. Our images data and corresponding analysis data have shown that most of SUV39H1, SUV39H2, HP1α and H3K9me3 showed significant co-localization with CAF-1 bodies with high Pearson's coefficient. SUV39H1, SUV39H2 and HP1α bodies were significantly altered upon CHAF1A knockdown. Therefore, we proposed that CAF-1 bodies might coalesce SUV39H1, SUV39H2 and HP1 to establish the maintenance of heterochromatin.

- *Fig EV3 R and Fig EV6B (middle panel) are the same with different annotation. Please correct.*

Reply: We sincerely apologize for this mistake. We have carefully checked the original data. We found that **Figure EV6B (middle panel)** in original manuscript has been mistakenly reused in **Figure EV3R**. **Figure EV6B (middle panel)**, which has been re-arranged as **Figure EV4B (middle panel)**, showed the true co-localization of CHAF1A-dDS13 with RFP-SUMO2. **Figure EV3R** has been replaced by its true images which showed the co-localization of CHAF1A with SUMO2. We also have carefully checked all the images in the entire manuscript to ensure that no image was mistakenly reused.

- *For the in Vitro part, authors did not specify the concentration of the different purified proteins neither they show the corresponding western blot, to see if there is a difference in the expression of these mutants.*

Reply: We apologize for these omissions. We have specified the concentrations of different purified proteins in the corresponding figure legends (**Figure 4G and 4I, Appendix Figure S6D-I**). Briefly, 10 micromole of each protein was incubated with gradient droplet formation buffer. For concentration dependence experiment (**newly-added Figure 4H**), the concentrations of CHAF1A-IDR proteins were indicated above each image, ranging from 0.78 μM to 50 μM. Besides, we have shown the corresponding western blot data of these CHAF1A mutants in **newly-added Appendix Figure S4K**. We found that all of the mutations did not alter the expression of corresponding mutants.

Authors should check whether CHAF1A phase separation occurs in presence of the crowding agent PEG.

- *Treatment with 1,6-hexanediol, which has emerged as an indicator of LLPS, has caveats. Authors should test Sorbitol or sucrose known to destabilize weak electrostatic interactions involved in protein phase separation.*

Reply: We thank the reviewer for these constructive suggestions. We have evaluated CAF-1 bodies phase separation upon the treatment with the crowding agent PEG, or sucrose and sorbitol, both of which were known to destabilize weak electrostatic interactions involved in proteins phase separation. Live cells images of pre-treatment, as well as 20 s, 5 min, 10 min, 30 min and 1 h post-treatment were captured. These results have been shown in **newly-added Appendix Figure S4G-J**. We found that CAF-1 bodies within cells existed in physiological condition and were unchanged upon the hyperosmotic stresses of 10% PEG8000, 10% Sucrose, 200 mM D-Sorbitol or 400 mM D-Sorbitol treatment. These results were unsurprising. The sensitivities of different proteins to PEG, sucrose or sorbitol might be diverse. Some proteins existed as cellular bodies in physiological conditions, whereas some proteins formed cellular bodies only upon osmotic stresses (PMID: 31792379; PMID: 32203417; PMID: 32203419; PMID: 29930091).

Again, we sincerely thank all the editors and reviewers for pointing out these

important details and giving us many constructive suggestions. We have corrected all the mistakes which the reviewers have reminded. We also have carefully read our revised manuscript many times to correct any typos and language issues. We hope that these changes are satisfactory.

Dear Prof. Zhang,

Thank you for submitting your revised manuscript to The EMBO Journal. Your study has now been seen by the three referees. As you can see below, the referees appreciate the added data and support publication here. I am therefore very pleased to let you know that we will accept the manuscript for publication here. Before sending the formal acceptance letter we just need to sort out a few things. You can use the link below to upload the revised version.

The data availability section should have all the accession numbers listed. Can you make sure that you deposit RNA seq and proteomics data and provide the accession numbers in this section. You also have the following sentence in there "Plasmids sequences for CHAF1A mutants and Primers sequences for qPCR will be made available upon request." Please make sure that this information is provided in the M&M section. Regarding the sentence "Purified proteins for in vitro experiments can be generated upon execution of a material transfer agreement (MTA) "double check that you describe how you did the purification well enough in M&M section.

Take a look that the reference section - I think in some places you have more that 10 authors listed

Some of the figures like Figure 5 and EV3 have many panels etc. I am in principle OK with that, but please take a careful look at the figures, see if they are understandable and if you might need to re-organize some of them. Just look at them from the perspective as a reader and see if they are clear enough as is and not too dense.

Please call out the tables in the text as Dataset EV1…

The EV table and movie legends need to be removed from the main Article file.

We encourage the publication of source data, particularly for electrophoretic gels and blots, with the aim of making primary data more accessible and transparent to the reader. It would be great if you could provide me with a PDF file per figure that contains the original, uncropped and unprocessed scans of all or key gels used in the figure? The PDF files should be labeled with the appropriate figure/panel number and should have molecular weight markers; further annotation could be useful but is not essential. The PDF files will be published online with the article as supplementary "Source Data" files.

I have asked our publisher to do their pre-publication checks on the paper. They will send me the file within the next few days. Please wait to upload the revised version until you have received their comments.

When you resubmit please provide a point-by-point to the editorial comments

That should be all let me know if you have any further questions

With best wishes

Karin

Karin Dumstrei, PhD
Senior Editor
The EMBO Journal

Instructions for preparing your revised manuscript:

Please check that the title and abstract of the manuscript are brief, yet explicit, even to non-specialists.

When assembling figures, please refer to our figure preparation guideline in order to ensure proper formatting and readability in print as well as on screen:
https://bit.ly/EMBOPressFigurePreparationGuideline

IMPORTANT: When you send the revision we will require
- a point-by-point response to the referees' comments, with a detailed description of the changes made (as a word file).
- a word file of the manuscript text.
- individual production quality figure files (one file per figure)
- a complete author checklist, which you can download from our author guidelines (https://www.embopress.org/page/journal/14602075/authorguide).
- Expanded View files (replacing Supplementary Information)
Please see out instructions to authors
https://www.embopress.org/page/journal/14602075/authorguide#expandedview

Please remember: Digital image enhancement is acceptable practice, as long as it accurately represents the original data and conforms to community standards. If a figure has been subjected to significant electronic manipulation, this must be noted in the figure legend or in the 'Materials and Methods' section. The editors reserve the right to request original versions of figures and the original images that were used to assemble the figure.

Further information is available in our Guide For Authors:
https://www.embopress.org/page/journal/14602075/authorguide

The revision must be submitted online within 90 days; please click on the link below to submit the revision online before 3rd May 2021.

https://emboj.msubmit.net/cgi-bin/main.plex

Referee #1:

We thank the author for responding to all of our concerns from the previous review. We have only one minor suggestion for the authors to replace figure 1 panels F-I, with the new material provided in supplementary figure 2 B-G which is more informative than what is presented in the main figure.

Referee #2:

The authors have addressed all my previous concerns.

Referee #3:

The authors addressed experimentally al the concerns raised. This manuscript will have an important impact on HIV field and beyond.

Point-by-point response to each comment:

Senior Editor Dr. Karin Dumstrei:

Thank you for submitting your revised manuscript to The EMBO Journal. Your study has now been seen by the three referees. As you can see below, the referees appreciate the added data and support publication here. I am therefore very pleased to let you know that we will accept the manuscript for publication here. Before sending the formal acceptance letter we just need to sort out a few things. You can use the link below to upload the revised version.

Reply: We thank the reviewers for supporting our study. We also sincerely thank the editor for accepting our manuscript for publication on *The EMBO Journal*. We have revised the manuscript according to the editors' instruction and uploaded the revised version.

The data availability section should have all the accession numbers listed. Can you make sure that you deposit RNA seq and proteomics data and provide the accession numbers in this section. You also have the following sentence in there "Plasmids sequences for CHAF1A mutants and Primers sequences for qPCR will be made available upon request." Please make sure that this information is provided in the M&M section. Regarding the sentence "Purified proteins for in vitro experiments can be generated upon execution of a material transfer agreement (MTA) "double check that you describe how you did the purification well enough in M&M section.

Reply: We thank the editor for pointing out these omissions. We have provided more information in the data availability section as follows:

1. We deposited the RNA-Seq data in the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE166337). The identifier number is **GSE166337**.
2. We deposited the MS proteomics data in the iProX database, an official member of ProteomeXchange Consortium. The URL is https://www.iprox.org/page/project.html?id=IPX0002805000. The corresponding identifier number is **IPX000805000**.
3. The editor suggested us to provide the qPCR primer sequences in the Materials and Methods section. Thus, in our revised manuscript, we have uploaded a **newly-added Dataset EV1 which listed ChIP primers used to explore the enrichment of target proteins on HIV-1**. The original Dataset EV1, Dataset EV2 and Dataset EV3 have been renamed as Dataset EV2, Dataset EV3 and Dataset EV4. We have corrected these names in the entire manuscript and supplementary information.
4. The editor also kindly suggested us to provide the plasmids sequences for CHAF1A mutants. However, we constructed over 100 different CHAF1A mutants constructs. The dataset are extremely large. Instead, we have **provided all the schematics of CHAF1A mutants in Appendix Fig S5**. Besides, we have **stated "Plasmids sequences for CHAF1A mutants will be made available upon**

request" in Protein purification method section. Thus, we hope to keep this original statement.

5. The editor also kindly suggested us to double check that we describe how we did the purification well enough in M&M section. We have carefully checked and corrected the 'Protein purification' method section. We are sure that other scientists can easily purify the corresponding proteins according to our instructions. We are also very willing to provide these in vitro purified proteins upon execution of a material transfer agreement (MTA).

Based on all the above modifications, we modified the Data availability section as follows:

The RNA-Seq data from this publication have been deposited to the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE166337) and assigned the identifier (GSE166337). The MS proteomics data from this publication have been deposited to the iProX database (https://www.iprox.org/page/project.html?id=IPX0002805000) and assigned the identifier (IPX000805000). Further information and requests for resources and reagents should be directed to and will be fulfilled by the corresponding author, Dr. Hui Zhang (zhangh92@mail.sysu.edu.cn). Plasmids sequences for CHAF1A mutants will be made available upon request. Purified proteins for *in vitro* experiments can be provided upon execution of a material transfer agreement (MTA) with inquiries directed to Dr. Hui Zhang.

In addition, we have provided the above information in the author checklist.

Take a look that the reference section - I think in some places you have more than 10 authors listed.

Reply: We apologize for these mistakes. We have downloaded the new version of endnote reference style for *The EMBO Journal*. We have updated the style accordingly. The modified references have ensured no more than 10 authors listed.

Some of the figures like Figure 5 and EV3 have many panels etc. I am in principle OK with that, but please take a careful look at the figures, see if they are understandable and if you might need to re-organize some of them. Just look at them from the perspective as a reader and see if they are clear enough as is and not too dense.

Reply: We thank the editor for pointing our this disadvantage. Actually, all the figures seem too dense. We have tried our best to remove nearly two-thirds of the data. We also have moved 13 large figures to the Expanded View and Appendix section. We only showed the essential and vital data in main figures. As we have displayed too many figures, we have carefully clarified the logic flow and written the results and legend sections. We are sure that these figures are understandable and clear enough for readers. We sincerely thank the editor for pointing out this weakness.

Please call out the tables in the text as Dataset EV1...

Reply: We thank the editor for this kind reminding. We have renamed all the tables in

the text and supplementary information as Dataset EV1, Dataset EV2, Dataset EV3 and Dataset EV4.

The EV table and movie legends need to be removed from the main Article file.
Reply: Thanks for pointing out this mistake. We have removed the EV table and movie legends in the revised main article file.

We encourage the publication of source data, particularly for electrophoretic gels and blots, with the aim of making primary data more accessible and transparent to the reader. It would be great if you could provide me with a PDF file per figure that contains the original, uncropped and unprocessed scans of all or key gels used in the figure? The PDF files should be labeled with the appropriate figure/panel number and should have molecular weight markers; further annotation could be useful but is not essential. The PDF files will be published online with the article as supplementary "Source Data" files.
Reply: We thank the editor for pointing our these omissions. We have **provided the original western blot data for all the 18 western blot results which showed in Fig EV2J, EV2K, EV2L and Appendix Fig S1A, S1B, S1C, S1D, S1E, S1F, S1G, S1H, S4K, S6B, S8B, S8C, S8D, S8E, S8F**. Within each source data, we provided not only the original blots but also the parameters used to image and analyze the results. All the source data have been provided as individual PDF files and packaged in a ZIP file named **EMBOJ-2020-106632R1_SourceDataForExpandedViewAndAppendix**.
We haven't provided the source data for Figure 2A. Because the two silver staining gels are indeed the source data. We think that it is not necessary to provide again.
We also have stated '**Source data are available online for this figure**' in proper figure legends.

Referee #1:

We thank the author for responding to all of our concerns from the previous review. We have only one minor suggestion for the authors to replace figure 1 panels F-I, with the new material provided in supplementary figure 2 B-G which is more informative than what is presented in the main figure.
Reply: We are very pleased that the reviewer is satisfied with our responses. We also agree with the reviewer's suggestion that replacing the original Fig 1F-I with more informative Appendix Fig 2B-G. However, our original Figure 1 has included too many essential panels, we are afraid that replacing the original Fig 1F-I with more informative Appendix Fig 2B-G would be extremely crowding for Figure 1. Thus, we hope to keep our original arrangement.

Referee #2:

The authors have addressed all my previous concerns.
Reply: We thank the reviewer for supporting our study.

Referee #3:

The authors addressed experimentally al the concerns raised. This manuscript will have an important impact on HIV field and beyond.
Reply: We are very happy that the reviewer is satisfied with our responses. We also thank the reviewer for supporting our study and speaking highly of our work.

Again, we sincerely thank all the editors and reviewers for pointing out the above vital details and giving us many constructive suggestions. We have formatted our manuscript as the editors kindly suggested. We also have carefully read our revised manuscript many times to correct any typos and language issues. We hope that these changes are satisfactory.

2nd Revision - Editorial Decision 19th Feb 2021

Dear Prof. Zhang,

Thanks for sending us your revised manuscript. I have now had a chance to take a careful look at it and I appreciate the introduced changes.

I am therefore very pleased to accept the revised version for publication here.

Congratulations on a nice study!

Best Karin

Karin Dumstrei, PhD
Senior Editor
The EMBO Journal

Please note that it is EMBO Journal policy for the transcript of the editorial process (containing referee reports and your response letter) to be published as an online supplement to each paper. If you do NOT want this, you will need to inform the Editorial Office via email immediately. More information is available here: https://emboj.embopress.org/about#Transparent_Process

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YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Hui Zhang
Journal Submitted to: The EMBO Journal
Manuscript Number: EMBOJ-2020-106632R1

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	The sample size was chosen based on pilot studies and other work done in our lab. We are sure that the sample size we chose is able to ensure adequate power.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	NA
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No sample has been excluded from the analysis specifically. Thus, no inclusion or exclusion criteria was included within methods.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	Samples were allocated randomly.
For animal studies, include a statement about randomization even if no randomization was used.	NA
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	Investigators blinded to raw data.
4.b. For animal studies, include a statement about blinding even if no blinding was done	NA
5. For every figure, are statistical tests justified as appropriate?	Yes. Statistical analyses were conducted utilizing Graphpad Prism 5 or Microsoft Excel. The network analysis and clustering analysis were conducted with STRING. Triplicate, sextuplicate and other replicate data were presented as mean ± SEM. A value of $p < 0.05$ was considered to be statistically significant and represented as asterisk (*). Value of $p < 0.01$ was considered to be more statistically significant and represented as double asterisks (**). Value of $p < 0.001$ was considered to be the most statistically significant and represented as triple asterisks (***). For comparison between two treatments, a Student's t-test was used. For the comparison of highly heterogeneous data including HIV-1 reactivation of clinical samples and genetic diversity index experiment, a Mann-Whitney U-test was used.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	To assess whether the data can meet our assumptions, Student's t test was used to compare independent groups under the assumptions of normal distribution and standard deviation.
Is there an estimate of variation within each group of data?	Variation within each group of data were calculated by Graphpad Prism 5 or Microsoft Excel. Standard deviation was used to estimate the variance compared to the average of the numbers in each group.

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http://www.antibodypedia.com
http://1degreebio.org
http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repor

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http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm
http://ClinicalTrials.gov
http://www.consort-statement.org
http://www.consort-statement.org/checklists/view/32-consort/66-title

http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tumo

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http://figshare.com

http://www.ncbi.nlm.nih.gov/gap

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http://jjj.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

Is the variance similar between the groups that are being statistically compared?	Yes. The variances are similar between the groups. We have assessed the variance by Student's t test to compare independent groups under the assumptions of normal distribution and standard deviation.

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	Immunoprecipitation antibodies against normal rabbit IgG (CST, 2729), CHAF1A (Proteintech, 17037-1-AP), H3K9me (Abcam, ab9045), H3K9me2 (Abcam, ab1220), H3K9me3 (Abcam, ab8898), H4K20me3 (Active Motif, 39671), H3K4me3 (Abcam, ab8580), H3K9Acetyl (Abcam, ab4441), CDK9, Pho-RNAP II (Abcam, ab5095), H3K27me3 (Abcam, ab6002), H3K36me2 (Abcam, ab9049), Histone H3 (CST, 4620), Histone H4 (Abcam, ab177840) and RNAP II (Abcam, ab26721) were used for ChIP-qPCR. IF antibodies included Donkey Anti-Mouse IgG H&L (Alexa Fluor 647) Antibody (Abcam, ab150107), Donkey Anti-Rabbit IgG H&L (CF 568) Antibody (Biotium, 20803-500µl), Donkey Anti-Rabbit IgG H&L (Alexa Fluor 647) Antibody (Abcam, ab150075), and Donkey Anti-Mouse IgG H&L (CF 568) Antibody (Biotium, 20802-500µl).
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	HEK293T, HeLa and Jurkat cells were obtained from ATCC. TZM-bl cells were obtained from NIH AIDS Reagent Program. J-Lat 6.3, 8.4, 9.2, 10.6 and 15.4 cells, which were originally constructed from Dr. Eric Verdin (The Buck Institute for Research on Aging, Novato, CA, USA) Laboratory, were obtained from Dr. Robert F. Siliciano ((Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA) Laboratory. All cells have been tested for mycoplasma utilizing PCR assay and confirmed to be mycoplasma-free.

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	NA
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	NA
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	NA

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	NA
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	NA
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	NA
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	NA

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	The RNA-Seq data from this publication have been deposited to the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE166337) and assigned the identifier (GSE166337). The MS proteomics data from this publication have been deposited to the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org/cgi/GetDataset?ID=PXD024172) via the iProX partner repository (https://www.iprox.org/page/project.html?id=IPX0002805000) and assigned the identifier (PXD024172). Further information and requests for resources and reagents should be directed to and will be fulfilled by the corresponding author, Dr. Hui Zhang (zhangh92@mail.sysu.edu.cn). Plasmids sequences for CHAF1A mutants will be made available upon request. Purified proteins for in vitro experiments can be provided upon execution of a material transfer agreement (MTA) with inquiries directed to Dr. Hui Zhang.
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	NA
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	NA

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	NA