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Cell cycle dynamics of lamina associated DNA

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Bas van Steensel

"Cell cycle dynamics of lamina associated DNA"

Reviewer #1 (Evidence, reproducibility and clarity (Required)):

In this report, van Schaik et al., modified an established CUT and RUN method and combined it with previously used DamID to identify Lamin Associated Domains (LADs) with better temporal resolution. Previous DamID experiments labeled locations where lamin proteins were present within a 5-25 hour window while the new technique, pA-DamID, labels DNA within a 30 minute window providing better temporal resolution. The authors used this technique to identify LADs at multiple stages of the cell cycle and applied this protocol to different cell types. The authors FIND differences when comparing data sets between cell cycle time points and cell lines.

Major points:

1) The data sets generated and displayed in this manuscript seem incomplete. In Figure 1G, the authors compare lamin B2 vs. lamin B1 generated LADs in HAP-1 cells and lamin A/C vs lamin B2 LADs in hTERT-RPE cells. In figure S4, panel C compares lamin B1 and lamin B2 in K562 cells and lamin B2 and lamin A/C in hTERT-RPE cells.
It would have been informative to have a complete dataset for lamin B1, lamin B2, and lamin A/C identified LADs in all cell lines analyzed. The information provided from these datasets would be useful to the scientific community.

2) The authors discovered that LADs reposition during progression through the cell cycle. It would have been interesting to know whether these changes have transcriptional consequences? One could perform RNA-SEQ experiments to discover if LAD occupancy results in transcriptional changes and choose a few genes to confirm the findings with RT-PCR. Is this the same for lamin B1, lamin B2, and lamin A/C occupied LADs? Analyze if there are any genomic features such as CTCF or transcription factor binding sites that correlate with the loss of LADs.

3) The authors state that using H3K27me3/H3K9me3 in pa-DamID showed no enrichment. This is surprising considering that both modifications are enriched in heterochromatin and at the nuclear periphery. It appears that the peripheral enrichment is masked by the larger overall internal pool. The authors should discuss this observation and comment on the sensitivity of the method to detect local enrichment versus the global levels of a protein or modification in pa-

DamID.

Minor points:

Figure 1: Change colors for Figure 1F and Figure 2D. The colors are hard to discern.

Figure 2B: Please mark which antibody was used for this analysis.

Figure 2C: Please also overlay data from pA-DamID lamin A/C experiments.

Figure 4: Please mention which antibody was used for the pA-DamID experiments used to generate this dataset.

Figure 5: Please mention which antibody was used for the pA-DamID experiments used to generate this dataset.

Figure S5 C and D: Please mention which antibody was used for the pA-DamID experiments.

Reviewer #1 (Significance (Required)):

The major contribution of this manuscript is the description of an improved method to map LADs. This is a valuable contribution. By using this new method, the findings of this paper provide some new insight in LAD dynamics throughout the cell cycle although the experiments are largely phenomenological. This is a technically sound study.

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The paper describes a new method for detecting Lamin associated DNA domains, which allows better time resolution than classical DamId. It is a good idea and its functionality is demonstrated in tissue culture cells. There are minor insights but it is important that we advance the field with new and better technologies, thus this version amply suffices to give evidence of that.

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The audience is all persons working on chromatin organization in the nucleus, which is a large audience. The data are clear as they basically are proof of principle for a new technique. There is nothing major to request as revision. They might cite papers on damID in worms and tissue specific applications of this in living organisms, as this is likely to be the situation that is most interesting in the long run. The resolution (in bp) would be interesting to know and validate.

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In the manuscript by Schaik et al (Van Steensel laboratory) the authors describe a very clever approach to identifying Lamina Associated Domains (LADs) using the principles of the 'cut-n-run' strategy. Specifically, they engineer the Dam methyltransferase used in canonical DamID in frame with a protein A moiety capable of interacting with an antibody (in this case lamins--B1, B2 and A/C). After permeabilization, cells are incubated with antibodies, then pA-Dam purified protein--for a brief time window--is added to mark associated DNA with GmATC. This technique is a valuable contribution to the field, particularly since, as the authors point out,an advantage of pA-DamID is that the labeled DNA can also be visualized in situ using the m6A-Tracer, before this DNA is sequenced. This allows for validation of findings and is highly amenable to cell sorting technologies. In addition, this technology allows for a time-resolved measure of LADs not currently available by standard DamID. The authors apply this technology to four different cell types. They noted that the 'maps' generated by the is technology differed from canonical DamID at very specific regions (small LADs in very localized regions) . They then embark on a series of experiments to show that these differences arise from cell cycle -related differences that are differentially picked up by the methods--with the pA-DamID allowing for dissection of more discrete cell cycle stages/configurations. In general they find an initial preference for sub-telomeric LADs to associate with the nuclear lamina fist, then more centromeric. There is some data suggesting loss/gain of LADs in specific regions/with specific features. The manuscript is well written and the data well presented. However, there are some points that need to be addressed . Overall, there is some oversimplification or omission of previous data in the field, a lack of clarity in how some of the data was interpreted, and some areas where clarification and/or additional analyses would be helpful. I sincerely hope the authors find the following critiques to be useful. Thank you for the opportunity to review your very nice work.

Introduction:

Microscopy studies found that telomeres are enriched near the NL in early G1 phase, leading to the hypothesis that telomeres may assist in NL reassembly onto chromatin [13].

● There have been numerous studies identifying the timing and disposition of INM proteins and Lamins at m the end of mitosis (during NE reformation). Why are you citing just this one? (e.g. Thomas Dechat et al., 2004; T. Dechat et al., 2000; Ellenberg et al., 1997; Haraguchi et al., 2001)

Furthermore, during S-phase B-type lamins have been found to transiently overlap with replication foci in the nuclear interior, at least in some cell types [20]

● While, technically, this has indeed been reported, this study is from 1994 and has not been repeated. The cells used in this study (3T3 fibroblasts) are widely used and others have not noted this phenomenon. Soften this.

Other studies have indicated that lamins are important for DNA replication [reviewed in 21].

● Likewise, direct roles for lamins in replication are controversial (acknowledged in the small section of the cited review on the role of lamins in replication).

● Perhaps combine the two sentences above to soften the implication that this is a "known" role of B-type lamins. e.g. "A handful of studies have implicated a role for B-type lamins in replication, but the direct role of the lamina in this process remains unclear. Nonetheless, ..."

Results:

So far, the cell cycle dynamics of genome - NL interactions have primarily been studied by microscopy. While these studies have been highly informative, they were often limited to a few selected loci.

● Please cite your own study (Kind et al.) and other recent papers (Luperchio et al.-https://www.biorxiv.org/content/10.1101/481598v1; Zhang et al., Nature-https://www.nature.com/articles/s41586-019-1778-y) in which they were either 1) not limited to a few selected loci and/or 2) not microscopy-directed studies? There is an argument to be made here for the resolution (time and b.p.) you have achieved through your studies that these studies did not.

● How does this data correlate with TSA-seq, another antibody-based method developed by the Belmont lab, but collaboratively developed for use in identifying LADs (ie Dam alternative) with the Van Steensel group? I can imagine there are numerous advantages to this approach (radius of "labeling" being one).

**When Dam-Lamin B1 is expressed in vivo for 5-25 hours during interphase, LADs that interact with the NL become progressively labeled, eventually resulting in a layer of labeled chromatin of up to ~1 μm thick [8]. This is because LADs are in dynamic contact with the NL. We expected that in pA-DamID this layer would be thinner, because the NL-tethered Dam is only activated for 30 minutes. In addition, permeabilization depletes small molecules including ATP and thus prevents active DNA remodeling in the nucleus [26]. Indeed, pA-DamID yields a m6A layer that is ~2.5 fold thinner than the layer in cells that express Dam-Lamin B1 in vivo (Fig. S2A-C). This is not an artifact due to collapse of chromatin onto

the NL caused by the permeabilization, because permeabilization of cells expressing Dam-Lamin B1 in vivo did not significantly reduce the thickness of the m6A layer compared to directly fixed cells (Fig. S2C). The thin layer of labeled DNA obtained by pA-DamID points to an improved temporal resolution of pA-DamID compared to conventional DamID.**

● I think this requires a bit more care. Your previous work clearly demonstrates LADs are dynamic. Others in the field have shown that these domains are also constrained within the larger sub-chromosomal compartment (self-interaction) of LADs (e.g. Luperchio 2018) within a chromosome. So, this is truly a temporal "snapshot" that may miss some regions of LADs that are less directly (or more dynamically) associated with the lamina, but still compartmentalized into the larger LAD sub-chromosomal compartment. It is unclear if the treatment used for this study perturbs these LAD-lamina dynamic interactions--one can imagine that the LADs are much less mobile generally under the protocol described in your supplemental information. In other words, LADs don't collapse, nor do they behave in the same way they would after permeabilization. The technique has compromised some of that --which is actually fine for most of the purposes in this manuscript, but this needs to be discussed.

● In addtion, imaging data showing dam-LaminB1/2 plus m6A-tracer is missing (figure S2). This should be included. Is the intensity of the "tracer" similar between conditions? If so, were the exposures kept constant in all images? This is important since the decay rate is highly related to intensity of signal.

In some cell types, especially in HCT116 and hTERT-RPE cells, we noted local discrepancies between the two methods (Fig. 2A,bottom panel). These differences involve mostly regions with low signals in DamID that have higher signals in pA-DamID. However, such differences are not obvious in HAP-1 and K562 cells.

● Only HCT116 data is shown in the indicated figure. hTERT-RPE cells are shown in the accompanying supplemental figure and use a different antibody (lamin B2) as the target for the pA-Dam.

● This brings up another point: the data (log2 ratio schema) shown in figure 2 is for HCT116 lamin B1 pA-Dam. Yet, the subsequent studies for transient/building interactions during G1 and into S (Figure 3) are done in hTERT-RPE cells using lamin B2. To be consistent, data from lamin B2 should be used in both figures (it seems lamin B2 data is available for all cell types). The comparison of Dam-Lamin B1 can be addressed in the Venn overlays (as they are now) and in the supplements. The hTERT-RPE data should be in Figure 2 since it is followed up on in the subsequent figure (ie it fails to meet the definition of being relegated to 'supplemental' data).

**suggesting that the separation of LADs and inter-LADs becomes progressively

more pronounced after mitosis. Nevertheless....**

● This is overstated, especially given the previously mentioned work (Luperchio, Zhang). More accurate to say LADs association with the nuclear lamina becomes more pronounced. LADs (predominantly B-compartment) and inter-LADs (predominantly A-compartment) show much earlier separation from each other. This may be distinct from association with the lamina. This is an important distinction as it may lead to different hypotheses regarding mechanisms of LAD targeting/association with the lamina.

Progression from prometaphase to late telophase in HeLa cells takes about 1 hour [33], suggesting that this timepoint captures the initial interactions with the reforming NL. Remarkably, the majority of these interactions is shared with later time points, indicating that most LADs can interact with the NL throughout interphase and are defined (and positioned at the NL) very soon after mitosis.

● There is wide variability in this number, some cells rapidly exit, others take significantly longer. This number is an average (and, for what it's worth, based on a very compromised cancer cell line). The "interactions' mapped are likely reflecting the ensembe measurements of the many cells that have transited into G1. Also, this statement seemingly directly contradicts the premise of many of your following data/interpretations of a sort of step-wise wave or prefered interactions from telomere proximal toward centromeric regions. This also disagrees with your previous work (Kind et al) and more recent work regarding positioning to the NL very soon after mitosis. Again, this is BULK (many cells of a continuum of configurations) versus single cell observations. This is overstated.

We next looked into characteristics of the dynamic LADs. At early time points, LADs with decreasing interactions do not have lower pA-DamID scores than stable LADs, suggesting that their detachment from the NL is not simply due to weak initial binding

● The methods used here are dynamic proximity measures. Words like "binding" and "attachment" should be avoided (use interacting, associated, etc)

LAD dynamics are linked to telomere distance and LAD size in multiple cell types

● Perhaps I am missing something, but I find relatively little data showing centromere-proximal LADs across cell cycle stages (referring here to Log2 ratio plots similar to what is shown for telomere-proximal LADs, Supplemental figure 6 is the only place where this is obvious.).

● In addtion, it seems to me that you are arguing in this and the preceding section for the following parameters: intensity of the LAD region. ie small, telomere-proximal, more euchromatic, AND less "intensely" associated.

● What is a "small" LAD? 100 kb or less? In Figure 2 (HCT1016, log 2 ratios), the original observation that leads into a discovery of changing NL associations through the cell cycle, the LAD that changes appears to be at least average size. Perhaps a "small" LAD adjacent to an "average" LAD. Nor do the signals appear to be all that low. There are regions within this sub-chromosomal plot that do appear to be "small" "low intensity" LADs. I am uncertain what parameters are defining these attributes. Are the cut-offs the same between cell types (ie is there a rule here?).

● The rules outlined above seem to break down across the different cell types. In particular, the number of active genes per Mb seems to have very little correlation overall with LADs that change. In addition, it is very unclear if "LAD size" is really a readout of both size AND intensity of interactions (understanding that this is not necessarily a direct quantitative measure of interactions).

Correlation of pA- determined LADs that change into G1/S with B-compartment sub-types

● There is certainly Hi-C data on most (all?) of the cell types analyzed in this manuscript. It would be very useful for the authors to parse out how the gain/loss LADs correlate with the B1, B2. A1, A2 (etc) compartment classifications. This may help to address the point above.

Nucleosomal pattern of pA-DamID digestion/amplification (figure S3)

● Onset of apoptosis needs to be ruled out. The nucleosomal (laddering) pattern could be due to DNA getting cleaved through programmed cell death pathways after permeabilization. These fragments could easily be amplified by the subsequent DamID protocol.

Definition of 'bulk' assays

● All of the assays were done in bulk. Some were synchronized, some were not. This is important since the implication is that anything not 'bulk' is single-cell. Throughout the manuscript and in the figures, please refer to the conditions as 'synchronized' versus 'unsynchronized'

Much of supplemental Figure 6 should be in a main figure

● It is puzzling why the first (and most easily seen/interpreted) description of LAD organization relative to telomeres/centromeres after exit from mitosis is relegated to supplemental figures. It is a foundational experiment(s) for the paper.

pA-Dam is possibly influenced by cell-cycle related chromatin accessibility (particularly at mitotic exit)

● During the transition from mitosis to early G1, there are dynamic changes to chromatin state that are directly coupled to the cell cycle. A recent report, for instance, highlights that interactions of antibodies (or other proteins) with H3K9me2/3 modifications is likely influenced by phosphorylation of histone tails. The dynamics of histone modification/chromatin state possibly occluding or interfering with the interpretation of the results must be discussed.

Reviewer #3 (Significance (Required)):

N/A

1st Editorial Decision 23rd Apr 2020

Dear Bas,

Thank you for the submission of your peer-reviewed manuscript to EMBO reports. I have now discussed the manuscript and referee reports with my colleagues here, and we would like to invite you to revise your study along the lines you suggest for publication by EMBO reports.

Please address all referee concerns in a complete point-by-point response. Acceptance of the manuscript will depend on a positive outcome of a second round of review. It is EMBO reports policy to allow a single round of major revision only and acceptance or rejection of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript.

Revised manuscripts should be submitted within three months of a request for revision; however, please contact us if a 3-months time frame is not sufficient for the revisions so that we can discuss this further. You can either publish the study as a short report or as a full article. For short reports, the revised manuscript should not exceed 27,000 characters (including spaces but excluding materials & methods and references) and 5 main plus 5 expanded view figures. The results and discussion sections must further be combined, which will help to shorten the manuscript text by eliminating some redundancy that is inevitable when discussing the same experiments twice. For a normal article there are no length limitations, but it should have more than 5 main figures and the results and discussion sections must be separate. In both cases, the entire materials and methods should be included in the main manuscript file.

Regarding data quantification, please specify the number "n" for how many independent experiments were performed, the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values in the respective figure legends. This information must be provided in the figure legends. Please also include scale bars in all microscopy images.

IMPORTANT NOTE: we perform an initial quality control of all revised manuscripts before re-review. Your manuscript will FAIL this control and the handling will be DELAYED if the following APPLIES:
1) A data availability section providing access to data deposited in public databases is missing. If you have not deposited any data, please add a sentence to the data availability section that explains that.
2) Your manuscript contains statistics and error bars based on n=2 or on technical replicates. Please use scatter blots in these cases. No statistics can be calculated if n=2.

When submitting your revised manuscript, please carefully review the instructions that follow below. Failure to include requested items will delay the evaluation of your revision.

1) a .docx formatted version of the manuscript text (including legends for main figures, EV figures and tables). Please make sure that the changes are highlighted to be clearly visible.

2) individual production quality figure files as .eps, .tif, .jpg (one file per figure).
See https://wol-prod-cdn.literatumonline.com/pb-assets/embo-site/EMBOPress_Figure_Guidelines_061115-1561436025777.pdf for more info on how to prepare your figures.

3) We replaced Supplementary Information with Expanded View (EV) Figures and Tables that are

collapsible/expandable online. A maximum of 5 EV Figures can be typeset. EV Figures should be cited as 'Figure EV1, Figure EV2" etc... in the text and their respective legends should be included in the main text after the legends of regular figures.

- For the figures that you do NOT wish to display as Expanded View figures, they should be bundled together with their legends in a single PDF file called *Appendix*, which should start with a short Table of Content. Appendix figures should be referred to in the main text as: "Appendix Figure S1, Appendix Figure S2" etc. See detailed instructions regarding expanded view here:

- Additional Tables/Datasets should be labeled and referred to as Table EV1, Dataset EV1, etc. Legends have to be provided in a separate tab in case of .xls files. Alternatively, the legend can be supplied as a separate text file (README) and zipped together with the Table/Dataset file.

4) a .docx formatted letter INCLUDING the reviewers' reports and your detailed point-by-point responses to their comments. As part of the EMBO Press transparent editorial process, the point-by-point response is part of the Review Process File (RPF), which will be published alongside your paper.

5) a complete author checklist, which you can download from our author guidelines . Please insert information in the checklist that is also reflected in the manuscript. The completed author checklist will also be part of the RPF.

6) Please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript (). Please find instructions on how to link your ORCID ID to your account in our manuscript tracking system in our Author guidelines

7) Before submitting your revision, primary datasets produced in this study need to be deposited in an appropriate public database (see https://www.embopress.org/page/journal/14693178/authorguide#datadeposition). Please remember to provide a reviewer password if the datasets are not yet public. The accession numbers and database should be listed in a formal "Data Availability" section placed after Materials & Method (see also https://www.embopress.org/page/journal/14693178/authorguide#datadeposition). Please note that the Data Availability Section is restricted to new primary data that are part of this study. * Note - All links should resolve to a page where the data can be accessed. *
If your study has not produced novel datasets, please mention this fact in the Data Availability Section.

8) We would also encourage you to include the source data for figure panels that show essential data. Numerical data should be provided as individual .xls or .csv files (including a tab describing the data). For blots or microscopy, uncropped images should be submitted (using a zip archive if multiple images need to be supplied for one panel). Additional information on source data and instruction on how to label the files are available at .

9) Our journal also encourages inclusion of *data citations in the reference list* to directly cite datasets that were re-used and obtained from public databases. Data citations in the article text are distinct from normal bibliographical citations and should directly link to the database records from which the data can be accessed. In the main text, data citations are formatted as follows: "Data ref: Smith et al, 2001" or "Data ref: NCBI Sequence Read Archive PRJNA342805, 2017". In the Reference list, data citations must be labeled with "[DATASET]". A data reference must provide the

database name, accession number/identifiers and a resolvable link to the landing page from which the data can be accessed at the end of the reference. Further instructions are available at https://www.embopress.org/page/journal/14693178/authorguide#referencesformat

We would also welcome the submission of cover suggestions, or motifs to be used by our Graphics Illustrator in designing a cover.

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I look forward to seeing a revised version of your manuscript when it is ready. Please let me know if you have questions or comments regarding the revision.

Kind regards,
Esther

Esther Schnapp, PhD
Senior Editor
EMBO reports

***
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Corr_author: van Steensel
Title: Cell cycle dynamics of lamina associated DNA

van Schaik et al: Point-by-point response

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We thank the reviewer for the helpful comments.

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It would have been informative to have a complete dataset for lamin B1, lamin B2, and lamin A/C identified LADs in all cell lines analyzed. The information provided from these datasets would be useful to the scientific community.

We have now generated Lamin B1 pA-DamID in hTERT-RPE cells. Thus, we now have a complete lamin data set in hTERT-RPE cells. We now confirm that all lamins yield very similar data (Figure 2D), and we compare Lamin B1 DamID data to the corresponding Lamin B1 pA-DamID data across all four cell lines (Figure 3, EV2).

2) The authors discovered that LADs reposition during progression through the cell cycle. It would have been interesting to know whether these changes have transcriptional consequences? One could perform RNA-SEQ experiments to discover if LAD occupancy results in transcriptional changes and choose a few genes to confirm the findings with RT-PCR. Is this the same for lamin B1, lamin B2, and lamin A/C occupied LADs? Analyze if there are any genomic features such as CTCF or transcription factor binding sites that correlate with the loss of LADs.

In the first part of this point, the reviewer suggests to look at transcriptional consequences of changes in NL interactions. To address this point, we require some measure of *nascent* transcription during the cell cycle, which is not available in any of the studied cell lines. A potential experiment would be to map polymerase occupancy with pA-DamID / CUT&RUN or run-on transcription with any other method at the synchronized time points. However, this experiment is not trivial and we feel that this goes beyond the scope of this manuscript, which focuses on the development of pA-DamID and the ^{m6}A-Tracer with a proof-of-principle example of NL binding dynamics during the cell cycle.

In the second part of this point, the reviewer asks whether changes in NL binding correlate with genomic features such as CTCF binding sites or transcription factor binding sites. In the manuscript, we already include correlations with various active features (active gene density / replication timing) (Fig. 4E-G, 5C-E), that generally correlate well with transcription factor binding. **We have added CTCF peaks as comparison (Fig. EV4F).**

3) The authors state that using H3K27me3/H3K9me3 in pa-DamID showed no enrichment. This is surprising considering that both modifications are enriched in heterochromatin and at the nuclear periphery. It appears that the peripheral enrichment is masked by the larger overall internal pool. The authors should discuss this observation and comment on the sensitivity of the method to detect local enrichment versus the global levels of a protein or modification in pa-DamID.

We believe that H3K27me3 and H3K9me3 histone modifications show the expected pattern in their distribution in the nucleus. However, due to the peripheral mask slightly extending beyond the cell boundaries, the calculated peripheral enrichment is underestimated. **This has been better described in the figure legend.** There is a small enrichment at the nuclear periphery compared to diffuse Dam and untargeted pA-Dam for H3K9me3 (Fig. 1B/1C/2C). **To further support the pA-DamID data quality of these histone modifications, we have added a comparison with ENCODE ChIP-seq data tracks in K562 cells (Appendix Fig. S1C).**

Minor points:

Figure 1: Change colors for Figure 1F and Figure 2D. The colors are hard to discern.

Figure 2B: Please mark which antibody was used for this analysis.

Figure 2C: Please also overlay data from pA-DamID lamin A/C experiments.

Figure 4: Please mention which antibody was used for the pA-DamID experiments used to generate this dataset.

Figure 5: Please mention which antibody was used for the pA-DamID experiments used to generate this dataset.

Figure S5 C and D: Please mention which antibody was used for the pA-DamID experiments.

We have made edits to address the minor comments above. However, we do not have Lamin A/C data in HAP-1 and K562 cells to add to Fig. 3C, EV2C.

Reviewer #1 (Significance (Required)):

The major contribution of this manuscript is the description of an improved method to map LADs. This is a valuable contribution. By using this new method, the findings of this paper provide some new insight in LAD dynamics throughout the cell cycle although the experiments are largely phenomenological. This is a technically sound study.

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We thank the reviewer for the positive feedback.

Reviewer #2 (Significance (Required)):

The audience is all persons working on chromatin organization in the nucleus, which is a large audience. The data are clear as they basically are proof of principle for a new technique. There is nothing major to request as revision. They might cite papers on damID in worms and tissue specific applications of this in living organisms, as this is likely to be the situation that is most interesting in the long run. The resolution (in bp) would be interesting to know and validate.

We have extended the discussion on new applications of pA-DamID.

We now compare data quality and resolution between DamID and pA-DamID, focusing on the mapping of NL interactions (Fig. EV2D-E). These plots indicate similar data quality and resolution between the two methods.

I have no other major revisions to request.

Reviewer #3 (Evidence, reproducibility and clarity (Required)):

In the manuscript by Schaik et al (Van Steensel laboratory) the authors describe a very clever approach to identifying Lamina Associated Domains (LADs) using the principles of the 'cut-n-run' strategy. Specifically, they engineer the Dam methyltransferase used in canonical DamID in frame with a protein A moiety capable of interacting with an antibody (in this case lamins--B1, B2 and A/C). After permeabilization, cells are incubated with antibodies, then pA-Dam purified protein--for a brief time window--is added to mark associated DNA with GmATC. This technique is a valuable contribution to the field, particularly since, as the authors point out,an advantage of pA-DamID is that the labeled DNA can also be visualized in situ using the m6A-Tracer, before this DNA is sequenced. This allows for validation of findings and is highly amenable to cell sorting technologies. In addition, this technology allows for a time-resolved measure of LADs not currently available by standard DamID. The authors apply this technology to four different cell types. They noted that the 'maps' generated by the is technology differed from canonical DamID at very specific regions (small LADs in very localized regions) . They then embark on a series of experiments to show that these differences arise from cell cycle -related differences that are differentially picked up by the methods--with the pA-DamID allowing for dissection of more discrete cell cycle stages/configurations. In general they find an initial preference for sub-telomeric LADs to associate with the nuclear lamina fist, then more centromeric. There is some data suggesting loss/gain of LADs in specific regions/with specific features. The manuscript is well written and the data well presented. However, there are some points that need to be addressed . Overall, there is some oversimplification or omission of previous data in the field, a lack of clarity in how some of the data was interpreted, and some areas where clarification and/or additional analyses would be helpful. I sincerely hope the authors find the following critiques to be useful. Thank you for the opportunity to review your very nice work.

We thank the reviewer for the constructive and very detailed comments, these have been extremely helpful in improving the manuscript.

Introduction:

Microscopy studies found that telomeres are enriched near the NL in early G1 phase, leading to the hypothesis that telomeres may assist in NL reassembly onto chromatin [13].

● There have been numerous studies identifying the timing and disposition of INM proteins and Lamins at m the end of mitosis (during NE reformation). Why are you citing just this one? (e.g. Thomas Dechat et al., 2004; T. Dechat et al., 2000; Ellenberg et al., 1997; Haraguchi et al., 2001)

We have expanded the introduction to better cover previous work on the reforming NL (paragraph 2) and initial genomic interactions with the NL (paragraph 3).

Furthermore, during S-phase B-type lamins have been found to transiently overlap with replication foci in the nuclear interior, at least in some cell types [20]

● While, technically, this has indeed been reported, this study is from 1994 and has not been repeated. The cells used in this study (3T3 fibroblasts) are widely used and others have not noted this phenomenon. Soften this.

Other studies have indicated that lamins are important for DNA replication [reviewed in 21].

● Likewise, direct roles for lamins in replication are controversial (acknowledged in the small section of the cited review on the role of lamins in replication).

● Perhaps combine the two sentences above to soften the implication that this is a "known" role of B-type lamins. e.g. "A handful of studies have implicated a role for B-type lamins in replication, but the direct role of the lamina in this process remains unclear. Nonetheless, ..."

This is a very good suggestion by the reviewer. We agree that literature has been controversial and should be approached with care. **We have followed the advice and changed this.**

Results:

So far, the cell cycle dynamics of genome - NL interactions have primarily been studied by microscopy. While these studies have been highly informative, they were often limited to a few selected loci.

● Please cite your own study (Kind et al.) and other recent papers (Luperchio et al.- https://www.biorxiv.org/content/10.1101/481598v1; Zhang et al., Nature- https://www.nature.com/articles/s41586-019-1778-y) in which they were either 1) not limited to a few selected loci and/or 2) not microscopy-directed studies? There is an argument to be made here for the resolution (time and b.p.) you have achieved through your studies that these studies did not.

To our knowledge, there have been no high-throughput microscopy studies of many individual loci performed studying this. Microscopy has been performed of collective sequences (i.e. all LADs (Kind, 2013 and indeed Luperchio, 2018)), which provide additional insights but lack sequence information in the images. **We have expanded the introduction to better acknowledge these microscopy studies that are not limited to single loci.** We feel that observations on LAD domain clustering (Luperchio) and B compartment formation (Zhang) are better suited for the Discussion, given that these observations are not directly related to genome – NL contact dynamics. **We already discussed B compartment formation in the discussion, but now also include the observed LAD domain clustering. Also, we have discussed data resolution in more detail in the results *(see reviewer #2)*.**

● How does this data correlate with TSA-seq, another antibody-based method developed by the Belmont lab, but collaboratively developed for use in identifying LADs (ie Dam alternative) with the Van Steensel group? I can imagine there are numerous advantages to this approach (radius of "labeling" being one).

TSA-seq provides a different perspective on genome – NL interactions, given its distance dependence rather than contact. **We have added a comparison with TSA-seq to the Discussion.**

When Dam-Lamin B1 is expressed in vivo for 5-25 hours during interphase, LADs that interact with the NL become progressively labeled, eventually resulting in a layer of labeled chromatin of up to ~1 µm thick [8]. This is because LADs are in dynamic contact with the NL. We expected that in pA-DamID this layer would be thinner, because the NL-tethered Dam is only activated for 30 minutes. In addition, permeabilization depletes small molecules including ATP and thus prevents active DNA remodeling in the nucleus [26]. Indeed, pA-DamID yields a m6A layer that is ~2.5 fold thinner than the layer in cells that express Dam-Lamin B1 in vivo (Fig. S2A-C). This is not an artifact due to collapse of chromatin onto the NL caused by the permeabilization, because permeabilization of cells expressing Dam-Lamin B1 in vivo did not significantly reduce the thickness of the m6A layer compared to directly fixed cells (Fig. S2C). The thin layer of labeled DNA obtained by pA-DamID points to an improved temporal resolution of pA-DamID compared to conventional DamID.

● I think this requires a bit more care. Your previous work clearly demonstrates LADs are dynamic. Others in the field have shown that these domains are also constrained within the larger sub-chromosomal compartment (self-interaction) of LADs (e.g. Luperchio 2018) within a chromosome. So, this is truly a temporal "snapshot" that may miss some regions of LADs that are less directly (or more dynamically) associated with the lamina, but still compartmentalized into the larger LAD sub-chromosomal compartment. It is unclear if the treatment used for this study perturbs these LAD-lamina dynamic interactions--one can imagine that the LADs are much less mobile generally under the protocol described in your supplemental information. In other words, LADs don't collapse, nor do they behave in the same way they would after permeabilization. The technique has compromised some of that --which is actually fine for most of the purposes in this manuscript, but this needs to be discussed.

As the reviewer points out, there are fundamental differences between DamID and pA-DamID in their ^{m6}A deposition that should be clear from the text. **We elaborated on this in the comparison between pA-DamID and DamID.**

● In addtion, imaging data showing dam-LaminB1/2 plus m6A-tracer is missing (figure S2). This should be included. Is the intensity of the "tracer" similar between conditions? If so, were the exposures kept constant in all images? This is important since the decay rate is highly related to intensity of signal.

We are afraid that this figure has been misinterpreted. **We have changed the figure labels and legend to explain it better.** The HT1080 Dam-Lamin B1 clonal cells (new clone kindly supplied by Jop Kind) still showed significant variation in ^{m6}A-Tracer intensity per cell, suggesting different expression levels of Dam-Lamin B1. To create optimal images for halfway decay estimation, laser settings were changed between images. **This has now been mentioned more clearly in the methods.**

In some cell types, especially in HCT116 and hTERT-RPE cells, we noted local discrepancies between the two methods (Fig. 2A,bottom panel). These differences involve mostly regions with low signals in DamID that have higher signals in pA-DamID. However, such differences are not obvious in HAP-1 and K562 cells.

● Only HCT116 data is shown in the indicated figure. hTERT-RPE cells are shown in the accompanying supplemental figure and use a different antibody (lamin B2) as the target for the pA-Dam.

We have changed the pointer to include the supplementary figure.

(See reviewer #1 for a similar comment.) We agree that the comparison between Lamin B1 DamID and Lamin B2 pA-DamID in hTERT-RPE cells leads to sense of incompleteness and confusion. **We have now generated Lamin B1 pA-DamID data in hTERT-RPE cells to solve this (Figures 2D, 3).**

● This brings up another point: the data (log2 ratio schema) shown in figure 2 is for HCT116 lamin B1 pA-Dam. Yet, the subsequent studies for transient/building interactions during G1 and into S (Figure 3) are done in hTERT-RPE cells using lamin B2. To be consistent, data from lamin B2 should be used in both figures (it seems lamin B2 data is available for all cell types). The comparison of Dam-Lamin B1 can be addressed in the Venn overlays (as they are now) and in the supplements. The hTERT-RPE data should be in Figure 2 since it is followed up on in the subsequent figure (ie it fails to meet the definition of being relegated to 'supplemental' data).

As written in the response above, we have now generate Lamin B1 pA-DamID data in hTERT-RPE data and added the other lamins to the figure. This indeed yields a more consistent story and addresses these comments.

suggesting that the separation of LADs and inter-LADs becomes progressively more pronounced after mitosis. Nevertheless....

● This is overstated, especially given the previously mentioned work (Luperchio, Zhang). More accurate to say LADs association with the nuclear lamina becomes more pronounced. LADs (predominantly B-compartment) and inter-LADs (predominantly A-compartment) show much earlier separation from each other. This may be distinct from association with the lamina. This is an important distinction as it may lead to different hypotheses regarding mechanisms of LAD targeting/association with the lamina.

We agree that this is an overinterpretation of our data. **We have changed the phrasing to make it more accurate.**

Progression from prometaphase to late telophase in HeLa cells takes about 1 hour [33], suggesting that this timepoint captures the initial interactions with the reforming NL. Remarkably, the majority of these interactions is shared with later time points, indicating that most LADs can interact with the NL throughout interphase and are defined (and positioned at the NL) very soon after mitosis.

● There is wide variability in this number, some cells rapidly exit, others take significantly longer. This number is an average (and, for what it's worth, based on a very compromised cancer cell line). The "interactions' mapped are likely reflecting the ensembe measurements of the many cells that have transited into G1. Also, this statement seemingly directly contradicts the premise of many of your following data/interpretations of a sort of step-wise wave or prefered interactions from telomere proximal toward centromeric regions. This also disagrees with your previous work (Kind et al) and more recent work regarding positioning to the NL very soon after mitosis. Again, this is BULK (many cells of a continuum of configurations) versus single cell observations. This is overstated.

We felt that there was a need to explain why we interpret the 1h time point as the initial interactions with the NL and included this reference, but the reviewer is correct that this number can vary greatly between cell types and conditions. **We have removed the reference and now include FACS and imaging data (Fig EV3 D-E) supporting this claim directly.**

We have changed the phrasing of these results to make our interpretation clearer.

We next looked into characteristics of the dynamic LADs. At early time points, LADs with decreasing interactions do not have lower pA-DamID scores than stable LADs, suggesting that their detachment from the NL is not simply due to weak initial binding

● The methods used here are dynamic proximity measures. Words like "binding" and "attachment" should be avoided (use interacting, associated, etc)

Good point. **We have replaced all occurrences of these words.**

LAD dynamics are linked to telomere distance and LAD size in multiple cell types

● Perhaps I am missing something, but I find relatively little data showing centromere-proximal LADs

across cell cycle stages (referring here to Log2 ratio plots similar to what is shown for telomere-proximal LADs, Supplemental figure 6 is the only place where this is obvious.).

To better illustrate the inverse dynamics of telomeres and centromeres in hTERT-RPE cells, we have changed Fig. 4B to a full chromosome overview.

● In addtion, it seems to me that you are arguing in this and the preceding section for the following parameters: intensity of the LAD region. ie small, telomere-proximal, more euchromatic, AND less "intensely" associated.

● What is a "small" LAD? 100 kb or less? In Figure 2 (HCT1016, log 2 ratios), the original observation that leads into a discovery of changing NL associations through the cell cycle, the LAD that changes appears to be at least average size. Perhaps a "small" LAD adjacent to an "average" LAD. Nor do the signals appear to be all that low. There are regions within this sub-chromosomal plot that do appear to be "small" "low intensity" LADs. I am uncertain what parameters are defining these attributes. Are the cut-offs the same between cell types (ie is there a rule here?).

We do not set any cut-offs for any features that we compare with. We took the strategy to define stable and dynamics LADs (Fig. 4C) and ask whether there are differences in feature distributions, including LAD size, replication timing and other features. As you can see in Fig. 4E, LADs with decreasing NL interaction are smaller than stable or increasing LADs. This strategy is consistent between cell lines. **To assist the reader in following our reasoning, we have added LAD domains and their differential status to Fig. 4B.**

● The rules outlined above seem to break down across the different cell types. In particular, the number of active genes per Mb seems to have very little correlation overall with LADs that change. In addition, it is very unclear if "LAD size" is really a readout of both size AND intensity of interactions (understanding that this is not necessarily a direct quantitative measure of interactions).

This comment reflects our reasoning why we added a comparison between cell types in Fig. 5. Indeed, we find no general trend that active gene density correlates with LADs with decreasing NL interactions in every cell type. In contrast, LADs with decreasing NL interactions are consistently close to telomeres and smaller in size than stable or increasing LADs. **We made it clearer that LAD size solely reflects the genomic size in basepairs.**

Correlation of pA- determined LADs that change into G1/S with B-compartment sub-types

● There is certainly Hi-C data on most (all?) of the cell types analyzed in this manuscript. It would be very useful for the authors to parse out how the gain/loss LADs correlate with the B1, B2. A1, A2 (etc) compartment classifications. This may help to address the point above.

We have now included a comparison with Hi-C sub-compartments (Fig. 5F).

Nucleosomal pattern of pA-DamID digestion/amplification (figure S3)

● Onset of apoptosis needs to be ruled out. The nucleosomal (laddering) pattern could be due to DNA getting cleaved through programmed cell death pathways after permeabilization. These fragments could easily be amplified by the subsequent DamID protocol.

Amplification of apoptotic fragments, if present, is visible in DamID assays using the negative controls. Every library preparation, we include one or more negative controls in which we omit DpnI.

If apoptotic fragments are present in this negative control, these can ligate to the DamID adapter and result in amplification, which we consistently do *not* see. **We have added a supplementary figure that shows this (Appendix Fig. S1A).**

Definition of 'bulk' assays

● All of the assays were done in bulk. Some were synchronized, some were not. This is important since the implication is that anything not 'bulk' is single-cell. Throughout the manuscript and in the figures, please refer to the conditions as 'synchronized' versus 'unsynchronized'

The reviewer is correct that our terminology is wrong. **We changed all occurrences of "bulk" to "unsynchronized".**

Much of supplemental Figure 6 should be in a main figure

● It is puzzling why the first (and most easily seen/interpreted) description of LAD organization relative to telomeres/centromeres after exit from mitosis is relegated to supplemental figures. It is a foundational experiment(s) for the paper.

We have replaced the zoomed-in Fig. 4B with a chromosome-wide overview that better captures this main observation. We see the remainder of Fig. EV3 as technical controls and details of the experiment that are useful to include but not necessary as main figure.

pA-Dam is possibly influenced by cell-cycle related chromatin accessibility (particularly at mitotic exit)

● During the transition from mitosis to early G1, there are dynamic changes to chromatin state that are directly coupled to the cell cycle. A recent report, for instance, highlights that interactions of antibodies (or other proteins) with H3K9me2/3 modifications is likely influenced by phosphorylation of histone tails. The dynamics of histone modification/chromatin state possibly occluding or interfering with the interpretation of the results must be discussed.

Similar to DamID, pA-DamID utilizes a Dam-control to measure DNA accessibility and control for this. We show that a change in pA-DamID score is due to changes in NL reads, while the Dam reads do not change (Fig. EV3F). In other words, we find no evidence that a change in chromatin state impacts the accessibility as measured by our Dam-control and thereby influences the results. **We now repeat this observation in the discussion.**

Reviewer #3 (Significance (Required)):

N/A

1st Revision - Editorial Decision 21st Jul 2020

Dear Bas,

Thank you for the submission of your revised manuscript. We did unfortunately not hear back from referee 3 despite several reminders, and I decided now that we can offer to publish your manuscript based on the enclosed comments by referee 1 who is satisfied with the revisions.

Only a few more minor changes are still required:

- Please add up to 5 keywords to the manuscript file, and a conflict of interest statement.

- Please add callouts to Figs 3B+C to the manuscript text.

- Please add page numbers to the table of content of the Appendix file.

- The Acknowledgements and Authors Contributions should be moved to after the Materials & Methods section.

- The Materials & Methods section should be called that.

- All Appendix information should be removed from the Article file.

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I look forward to seeing a final version of your manuscript as soon as possible.

Best regards,
Esther

Esther Schnapp, PhD
Senior Editor
EMBO reports

Referee #1:

The authors have adequately addressed all points.

Authors made the requested editorial changes.

2nd Revision - Editorial Decision 11th Aug 2020

Bas van Steensel
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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.
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- 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.
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- 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.
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  - 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?
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  - 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?	Sample sizes of pA-DamID and DamID sets are indicated in figures + legends, and are according to standards in the field. Pre-specified effect sizes were not applicable to this work. For the genomics datasets we documented the reproducibility by showing scatter plots and providing correlation coefficients.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	N/A
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	no data were excluded
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.	all data were analyzed using fully automatic computational pipelines, thus minimizing bias.
For animal studies, include a statement about randomization even if no randomization was used.	N/A
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4.b. For animal studies, include a statement about blinding even if no blinding was done	N/A
5. For every figure, are statistical tests justified as appropriate?	In most cases we conservatively use a non-parametric test. For the most important statistics (related to Up and Down LADs, Figure 4C and further) the tests are explained in detail.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	In cases of doubt about the normal distribution, we conservatively used a Wilcoxon test.

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Is there an estimate of variation within each group of data?	yes, error bars and/or individual values are shown
Is the variance similar between the groups that are being statistically compared?	In cases of doubt we conservatively used a Wilcoxon test.

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).	All antibodies are commercial. We provide catalog numbers, documentation is available on the providers' web sites.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Cell lines were directly obtained from ATCC or 4DNucleome consortium as specified in the Methods, and tested every 1-2 months for mycoplasma contamination.

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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.	N/A
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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.	N/A
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14. Report any restrictions on the availability (and/or on the use) of human data or samples.	N/A
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