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Local rewiring of genome-nuclear lamina interactions by transcription

Laura Brueckner, Peiyao A Zhao, Tom van Schaik, Christ Leemans, Jiao Sima, Daniel Peric-Hupkes, David M Gilbert, Bas van Steensel

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 27 September 2019

Thank you for submitting your study on the effect of transcription on LAD architecture for consideration by The EMBO Journal. We have now received three referee reports on your study, which are included below for your information.

As you will see, the reviewers express an interest in the study, and while they raise some concerns regarding the advance of the study, we overall find that their comments support further consideration. Therefore we would like to invite you to prepare and submit a revised manuscript. However, to make the revision a significant step forward, it will be important to take into account the referee's suggestions and to carefully address all of the issues raised by referee #1 and #3 in the revised version of the manuscript.

Please note that EMBO Journal policy allows only a single round of major revision, therefore it is important to clarify all key concerns at this stage.

REFEREE REPORTS

Referee #1:

Local rewiring of genome - nuclear lamina interactions by transcription by Brueckner et al.

This manuscript examines the effect of transcription in modifying interactions between chromatin and the nuclear lamina. To do so, the authors employ a combination of approaches to target transcriptional activation or repression to different regions in the genome and examine changes in interactions with the nuclear lamina using DamID against Lamin B1. Using this approach, the authors show that: i) transcriptional activation of genes located in lamina associated domains (LADs) leads to detachment of the nuclear lamina (Fig 1); ii) The level of detachment is associated with the length of the transcriptional unit (Fig 2) and the expression level (Fig 3); iii) Detachment of

nuclear lamina is associated with changes in replication timing (Fig 4); iv) Detachment of the nuclear lamina does not involve cooperative local mechanisms on surrounding regions (Fig 6); v) Transcriptional silencing sometimes leads to re-attachment to the nuclear lamina (Fig 7); and, vi) random integrations of strong promoters lead to modest detachment of the nuclear lamina (Fig 8).

Overall this is solid piece of work that adds confirmatory evidence to the role of transcription in disrupting chromatin to nuclear lamina interactions, for example, as reported before using orthogonal cell biology approaches (Therizols et al., Science 2014). The current study adds an additional number of loci, but falls short of finding "universal" observations or novel molecular mechanisms that would explain how these interactions are remodelled. The current manuscript also presents confirmatory results suggesting a correlation with a switch in replication timing, but falls short of characterising the molecular logic behind the partial overlap between changes in replication timing and changes in nuclear lamina association. Therefore, in my opinion the novelty of the manuscript is limited.

Major points:

1. The authors tend to examine genes that are very long (usually >500kb). However, the average mammalian gene is ~10-15kb long. Therefore, it is unclear to me whether the results found here are applicable for the majority of the genome, especially within the context of cooperative detachment.

2. In fact, upregulation of some genes (eg, PTN, Fig 2) seems to display a much more extensive reorganisation of nuclear lamina interactions than the length of the gene, including the detachment of nearby genes (eg DGKI). Are these two genes within the same LAD? If so, these results would suggest some sort of cooperativity mechanism for the detachment of the two genes, in disagreement with the results presented in Figure 6. Given the relatively low number of loci examined here, the authors should find a way of reconciling both observations.

3. In addition, for PTN, it looks like the length of detachment correlates quite closely with the change in replication timing, that shows a much higher degree of overlap than the other examples presented in the manuscript (Fig 4). Can the authors bring any insight regarding the differences observed between these loci? Are there chromatin marks or other epigenetic features that could explain the differences between the loci?

4. Related to the points above, the deletion of the promoter of Dppa2 does not seem to lead to a reattachment to the nuclear lamina, indicating that there are additional elements that determine whether a gene might interact with the nuclear lamina or not, in addition to transcription. It would be interesting to examine whether there are marked differences in chromatin properties between Dppa2 and Morc that would explain the differences in re-attachment.

5. The section on "Possible compensatory movements" (page 7; Fig 5) is underdeveloped unless the authors would be able to explain why compensatory movements happen upon detachment of MLK4, but not upon detachment of other genes.

6. Figure 8c displays the average level of detachment for integration sites at LADs. However, Figure 8d seems to indicate that most of these insertions are not transcriptionally active. Therefore, it is important to determine whether the changes in nuclear lamina detachment in Fig 8c are representative for the majority of integrations, or whether the changes are driven by outliers. To do so, the authors could include 95% confident intervals for the log2(DamID ratio) curves.

Referee #2:

The Van Steensel lab originally described lamin associated domains (LADs) about 10 years ago. Since then numerous studies have addressed their properties and have indicated there are both constitutive and facultative LADs, that seem to be related to transcription. Other than a single study (Therizols et al., 2014) which showed that movement of LADs away from the nuclear periphery was transcription dependent the relationship between LADs and transcription has not been extenensively explored. In this comprehensive study Brueckner et al investigate the relationship between transcription (or transcriptional processes) and the positioning of genomic loci in the vicinity of the

nuclear periphery. To achieve this they map using DamID the association of specific loci to the nuclear periphery under different situations (i) gene activation in mouse ES cells building on from Therizols et al. (ii) gene activation in in human RPE1 cells. Complimentary studies then use gene inactivation or transgene insertion.

Overall this is a clear well-written paper that shows a relationship between transcription (or transcriptional processes) and association to the nuclear periphery. For me one of the interesting bits of data is shown in Figure 3 where there is a clear relationship between gene expression levels and nuclear lamina (NL) association.

I have no substantive criticisms; the data is well presented and not over interpreted and provide a robust framework for future experiments. In particular it will be interesting to know what the underlying mechanistic processes are. Presumably it is not polymerase passing per se as this would be limited to gene bodies and suggesting some other activity might be responsible for the release of chromatin from the NL such as nuclear remodellers or some other change in chromatin topology.

Additional comments
Figure 1 and 2
It would be useful to show where VP64 was targeted to the locus.

It would also be interesting to show DamID and RNA-seq reads on the same plot to see the precise relationship between polymerase transcription in a locus and association with the NL.

Referee #3:

The authors of this study raise the question, which molecular mechanisms lead to the displacement of genes in facultative LADs when a gene is stimulated. A delocalization by induced activation has been observed by several studies using activation signals and DNA-FISH. As approach they have chosen targeting of VP64 by dCas9 and gRNA in mouse and human cells but also promoter deletions in mouse cells. As readout for the lamina-association they performed DamID and RNA-seq to show the transcription activation.
They observe for all targeted genes consistently a reduction of the DamID signal, more pronounced at the 5' end of the gene and not much extending beyond the gene body in most cases. They conclude that the activation of transcription is capable to displace a gene from the nuclear lamina. Further they investigate the replication timing of the artificially activated genes and observe an interesting shift affecting a wider region around the genes.
The observations presented in the manuscript are interesting, although they cannot fully explain the molecular mechanism. There are a number of points that should be considered:
The observed reduction of DamID signals triggered by gene activation seems to be restricted to the gene and does not much extend beyond, for example over the entire LAD. Can the authors rule out that the observed reduction of the DamID signal is caused by a coverage of the genes with transcribing RNA polymerases, simply blocking the access of the DNA methyltransferase to DNA?
Does the reduction of the DamID signal at the genes activated with VP64 lead to a significant displacement of the gene into the nuclear interior similar as shown by DNA-FISH in other studies (eg. Therizols, 2014)? The authors should perform DNA-FISH for some example loci to show that the gene is indeed displaced from the lamina.
The domainograms presented most of the time as top panels do not aid the understanding of the results and should be omitted. The DamID ratio is sufficient since this graph also visualizes the LAD structures. Figure 2c should be presented as DamID ratios.
In figure 2 the targeting of the human SOX6 gene is shown. It is not clear whether an alternative promoter was targeted here since the gRNA is displayed inside the gene. Also a transcript that is transcribed antisense to the 5'end of SOX6 is indicated. Neither in the Refseq genes or in the UCSC genes such a transcript is annotated.

Minor issues:
Does the observed shift in replication timing correlate with TADs?
There are some typos and ## instead of numbers in the Materials and Methods.
Figure 7 would be much clearer if the mouse strain and which gene was targeted would be displayed above the graphs.

The lettering in the figures is too small for a publication in the EMBO Journal.

1st Revision - authors' response 14 December 2019

Response to Reviewers' comments

Referee #1:

Local rewiring of genome - nuclear lamina interactions by transcription by Brueckner et al.

This manuscript examines the effect of transcription in modifying interactions between chromatin and the nuclear lamina. To do so, the authors employ a combination of approaches to target transcriptional activation or repression to different regions in the genome and examine changes in interactions with the nuclear lamina using DamID against Lamin B1. Using this approach, the authors show that: i) transcriptional activation of genes located in lamina associated domains (LADs) leads to detachment of the nuclear lamina (Fig 1); ii) The level of detachment is associated with the length of the transcriptional unit (Fig 2) and the expression level (Fig 3); iii) Detachment of nuclear lamina is associated with changes in replication timing (Fig 4); iv) Detachment of the nuclear lamina does not involve cooperative local mechanisms on surrounding regions (Fig 6); v) Transcriptional silencing sometimes leads to re-attachment to the nuclear lamina (Fig 7); and, vi) random integrations of strong promoters lead to modest detachment of the nuclear lamina (Fig 8).

Overall this is solid piece of work that adds confirmatory evidence to the role of transcription in disrupting chromatin to nuclear lamina interactions, for example, as reported before using orthogonal cell biology approaches (Therizols et al., Science 2014). The current study adds an additional number of loci, but falls short of finding "universal" observations or novel molecular mechanisms that would explain how these interactions are remodelled. The current manuscript also presents confirmatory results suggesting a correlation with a switch in replication timing, but falls short of characterising the molecular logic behind the partial overlap between changes in replication timing and changes in nuclear lamina association. Therefore, in my opinion the novelty of the manuscript is limited.

We thank the reviewer for the helpful comments. We firmly believe that this manuscript goes substantially beyond 'confirmatory'. We generated 174 genome-wide datasets (DamID, Repli-seq and RNA-seq). This is quite an advance relative to the Therizols 2014 study, which, although certainly a landmark paper, was limited to two-probe FISH studies of three genes and qPCR analysis of replication timing and expression of a handful of nearby genes. As outlined in the Introduction, the manuscript addresses several questions to which the answers were not previously known:

- What does the detachment pattern within and around a detached locus look like in detail?
- How much flanking DNA is 'dragged' along if a gene moves into the nuclear interior?
- How general is this pattern? Are there exceptions?
- What happens to the neighboring genes in terms of transcription activity?
- Is there perhaps compensatory movement of another part of the chromosome?
- Does the transcriptional activity of the gene matter for the degree of detachment?
- Can a gene also relocate from the nuclear interior to the lamina by *in*activation?
- How is the spatial relocation *exactly* linked to changes in replication timing? Is the overlap perfect, or are there differences?

To none of these questions are there clear answers in the current literature. **We have modified the abstract to better highlight the novel findings as well as the value of our extensive datasets.**

We agree that we did not solve the molecular mechanism of the detachments. This was not the scope of the manuscript; and it is a very difficult topic that is still largely unresolved despite efforts by many labs. But our observations do provide insights in the structural changes in the genome that accompany gene activation.

We find it remarkable that in most cases the detachment from the NL is very local. Clearly, our data show that a major signal for detachment must be linked to transcription elongation. Exceptions to the rule (e.g. the PTN locus) may provide important clues to additional signals. The partially uncoupled patterns of changes between lamina interactions (centered around the

transcription unit) and replication timing (more centered around the activated promoter) is also an important indication that these two processes are at least partially controlled by different signals. We believe that these insights help to advance the field, in which mechanistic relationships are notoriously difficult to unravel.

Major points:

1. The authors tend to examine genes that are very long (usually >500kb). However, the average mammalian gene is ~10-15kb long. Therefore, it is unclear to me whether the results found here are applicable for the majority of the genome, especially within the context of cooperative detachment.
This is a fair point. Our set of genes is biased towards larger genes, because the resolution of our DamID maps is approximately 10kb. Given this limitation, we focused primarily on larger genes. However, we did include *TRAM1L1* of only ~2kb (Figures 2c and EV4b) – the reviewer may have missed this. The detachment of this gene is restricted to its closely flanking regions, like most other tested genes. In addition, the transgene used in Figure 8 is also only ~2kb and shows a similar pattern. We feel that *TRAM1L1* and the transgenes sufficiently cover the shorter gene range. **We now discuss the size skew of the tested gene set in the Discussion.**

2. In fact, upregulation of some genes (eg, *PTN*, Fig 2) seems to display a much more extensive reorganisation of nuclear lamina interactions than the length of the gene, including the detachment of nearby genes (eg DGKI). Are these two genes within the same LAD? If so, these results would suggest some sort of cooperativity mechanism for the detachment of the two genes, in disagreement with the results presented in Figure 6. Given the relatively low number of loci examined here, the authors should find a way of reconciling both observations.
By 'cooperativity' we meant a synergistic effect of *two* activated genes on NL detachment. We now removed this term altogether. We do not think that the PTN locus is a case of cooperativity, because *DGKI* is not detectably activated at the mRNA level -- if anything, its expression goes down a little bit (the precise mRNA tracks are shown in revised Figure EV1g, formerly S2g). Nevertheless, *PTN* is indeed an interesting exception to the rule, and worth discussing more extensively. **This we do now in the Discussion.**

3. In addition, for PTN, it looks like the length of detachment correlates quite closely with the change in replication timing, that shows a much higher degree of overlap than the other examples presented in the manuscript (Fig 4). Can the authors bring any insight regarding the differences observed between these loci? Are there chromatin marks or other epigenetic features that could explain the differences between the loci?
We interpreted this differently. The changes in replication timing consistently span 1.5-2 Mb for all genes tested (Figure 4 and EV2), even when the changes in lamina interactions are more restricted. Hence, we proposed that there is a minimum span for changes in replication timing (Discussion, final paragraph). Under this hypothesis it is not surprising that the lamina interaction changes for the PTN (which extend over ~1Mb and gradually decay with distance, just like replication timing changes) appear to overlap better with replication timing. **We now elaborate on this interpretation in the Discussion**.

Unfortunately, no other reliable maps of chromatin marks are publicly available for RPE-1 cells. We did 'borrow' some unpublished maps of histone marks (H3K4me1, H3K4me3, H3K27me3, H3K36me3) from our colleagues in our institute to explore whether there is any interesting pattern that might explain why DGKI partially detaches along with *PTN*. This did not yield any meaningful insights. Because these epigenome maps are not publicly available (our colleagues intend to publish these data in their own paper in the future), we propose not to include these results.

However, overlay with Hi-C data shows an interesting pattern, particularly in contrast with the replication timing changes. While the changes in NL interactions appear partially correlated with TAD patterns, no such correlation is visible for replication timing. **We now include this result as a new supplementary figure EV3.**

4. Related to the points above, the deletion of the promoter of Dppa2 does not seem to lead to a reattachment to the nuclear lamina, indicating that there are additional elements that determine whether a gene might interact with the nuclear lamina or not, in addition to transcription. It would be interesting to examine whether there are marked differences in chromatin properties between Dppa2 and Morc that would explain the differences in re-attachment.

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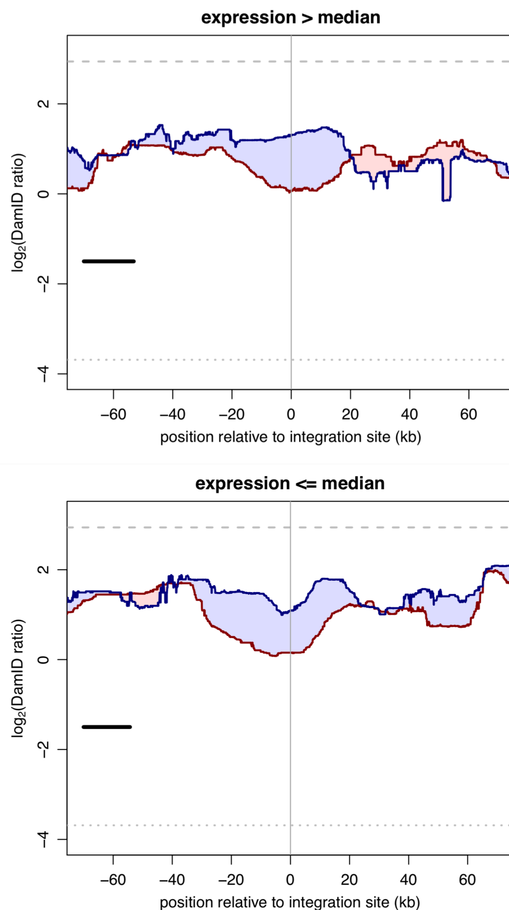
This persistent detachment of the Dppa2/4 region is indeed interesting. **We now emphasize this further in the text.** Again, we checked a series epigenome maps, but they don't provide much insight. Susan Gasser's lab recently reported that H3K27ac can counteract NL interactions in *C. elegans*. For this reason we took a particularly close look at maps of this mark. H3K27ac is abundantly present in the Dppa2/4 region, also in a fairly broad domain around the small promoter deletion. However, a similar amount is present in other regions that do show increased NL interactions in the triple mutant. It is thus difficult to say whether this mark explains the behavior of the Dppa2/4 region. We feel that this analysis would just clutter the manuscript and therefore decided not to include it. Moreover, several other papers have already extensively studied the correlations of NL interactions with histone modifications. We also analyzed the Hi-C dataset from Bonev et al, Cell 2017. Despite the claimed high resolution, we found these data for the Dppa2/4 region too noisy to be interpretable. So we also do not include this analysis in the revised manuscript.

Please note that the aim of this manuscript was to understand the impact of *transcription* on NL contacts (and replication timing). We generated 174 genome-wide datasets for this. No doubt (many) other mechanisms affect NL interactions, but to identify them is beyond the scope of this manuscript.

5. The section on "Possible compensatory movements" (page 7; Fig 5) is underdeveloped unless the authors would be able to explain why compensatory movements happen upon detachment of MLK4, but not upon detachment of other genes.
We agree that this is not the most developed part of the manuscript. Yet, we feel that we should discuss the "red" areas of the plots - ignoring them would raise questions among readers, and it is an interesting possibility that some regions of the genome undergo compensatory movements. To our knowledge this has never been proposed or reported. We would have appreciated concrete suggestions from the reviewer how we should tackle this issue further, as it is not trivial to address experimentally. **We have checked and toned down the conclusions.**

6. Figure 8c displays the average level of detachment for integration sites at LADs. However, Figure 8d seems to indicate that most of these insertions are not transcriptionally active. Therefore, it is important to determine whether the changes in nuclear lamina detachment in Fig 8c are representative for the majority of integrations, or whether the changes are driven by outliers. To do so, the authors could include 95% confident intervals for the log2(DamID ratio) curves.
We apologize for the confusion. In the original figure, the expression values in panels 8d and 8e did not have the same units, and on top of that, one was on a log10 scale and the other on a log2 scale. This may have led to the impression that several integrations have low expression. The contrary is true. **We now use the same units and scale, and show the actual distribution of the expression levels of the integrations (compared to endogenous genes) in Fig 8e.** From this direct comparison it should be clear that the expression level of *all* integrations is quite high.

To be sure, we split the LAD integrations for which we have expression data into two groups of equal size with high vs low expression. The effects on NL interactions are virtually the same in both groups, indicating that also in the "low" group the expression levels are sufficient to cause detachment from the NL:



We propose not to include this figure in the final paper, to avoid an overload of supplementary figures. Data from individual integrations are unfortunately too noisy to plot individually.

While revising figures 8d-e we spotted some minor imperfections in the integration mapping scripts; subsequent correction led to some minor changes in Figures 8d-e, but the conclusions are not affected at all.

Referee #2:

The Van Steensel lab originally described lamin associated domains (LADs) about 10 years ago. Since then numerous studies have addressed their properties and have indicated there are both constitutive and facultative LADs, that seem to be related to transcription. Other than a single study (Therizols et al., 2014) which showed that movement of LADs away from the nuclear periphery was transcription dependent the relationship between LADs and transcription has not been extenensively explored. In this comprehensive study Brueckner et al investigate the relationship between transcription (or transcriptional processes) and the positioning of genomic loci in the vicinity of the nuclear periphery. To achieve this they map using DamID the association of specific loci to the nuclear periphery under different situations (i) gene activation in mouse ES cells building on from Therizols et al. (ii) gene activation in in human RPE1 cells. Complimentary studies then use gene inactivation or transgene insertion.

Overall this is a clear well-written paper that shows a relationship between transcription (or transcriptional processes) and association to the nuclear periphery. For me one of the interesting bits of data is shown in Figure 3 where there is a clear relationship between gene expression levels and nuclear lamina (NL) association.

I have no substantive criticisms; the data is well presented and not over interpreted and provide a robust framework for future experiments. In particular it will be interesting to know what the underlying mechanistic processes are. Presumably it is not polymerase passing per se as this would be limited to gene bodies and suggesting some other activity might be responsible for the release of

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chromatin from the NL such as nuclear remodellers or some other change in chromatin topology.

Additional comments
Figure 1 and 2
It would be useful to show where VP64 was targeted to the locus.
Thank you for the positive and constructive comments. In Figure 2 (and all other CRISPRa figures) the targeting sites were indicated by the green vertical dotted lines. The precise coordinates are also listed in Table S1. The TALE-VP64 target locations were indeed missing; we now obtained the coordinates (added to the Methods section), **and mark them also by green dotted vertical lines in Figure 1**.

It would also be interesting to show DamID and RNA-seq reads on the same plot to see the precise relationship between polymerase transcription in a locus and association with the NL.
We have modified figures EV1 (formerly S2) and EV5a (formerly S5b) to show the aligned RNA-seq reads rather than the change in expression per gene. This is indeed much more informative. **For some of these plots we now added a comment in the Results section (ABCB1, PTN)**.

Referee #3:

The authors of this study raise the question, which molecular mechanisms lead to the displacement of genes in facultative LADs when a gene is stimulated. A delocalization by induced activation has been observed by several studies using activation signals and DNA-FISH. As approach they have chosen targeting of VP64 by dCas9 and gRNA in mouse and human cells but also promoter deletions in mouse cells. As readout for the lamina-association they performed DamID and RNA-seq to show the transcription activation.
They observe for all targeted genes consistently a reduction of the DamID signal, more pronounced at the 5' end of the gene and not much extending beyond the gene body in most cases. They conclude that the activation of transcription is capable to displace a gene from the nuclear lamina. Further they investigate the replication timing of the artificially activated genes and observe an interesting shift affecting a wider region around the genes.
The observations presented in the manuscript are interesting, although they cannot fully explain the molecular mechanism. There are a number of points that should be considered:

The observed reduction of DamID signals triggered by gene activation seems to be restricted to the gene and does not much extend beyond, for example over the entire LAD. Can the authors rule out that the observed reduction of the DamID signal is caused by a coverage of the genes with transcribing RNA polymerases, simply blocking the access of the DNA methyltransferase to DNA?
Yes, we can rule this out. The reviewer may have missed that we always use a Dam-only reference to which the Dam-LaminB1 data are normalized. This effectively corrects for any local variation in accessibility. This normalization is standard practice in DamID. There is previous experimental evidence that DamID can also detect interaction of proteins within active genes (most notably the H3K36me3-interacting protein MRG15, see Figure 5c in Filion et al, Cell. 2010;143:212-224). Thus, DamID is not blocked by the transcription machinery. **We now mention this in the Results**.

Does the reduction of the DamID signal at the genes activated with VP64 lead to a significant displacement of the gene into the nuclear interior similar as shown by DNA-FISH in other studies (eg. Therizols, 2014)? The authors should perform DNA-FISH for some example loci to show that the gene is indeed displaced from the lamina.
Please note that FISH had already been done (by Therizols et al) for the experiments in Figure 1 - this is why we chose this model. Thus the FISH and DamID results cross-validate. More generally, DamID of nuclear lamina interactions has been confirmed/validated by FISH or other microscopy approaches in numerous previous papers by various labs, e.g.:
- Pickersgill et al, Nat Genet. 2006 Sep;38(9):1005-14.
- Guelen et al, Nature. 2008 Jun 12;453(7197):948-51.
- Reddy et al, Nature. 2008 Mar 13;452(7184):243-7.
- Zullo et al, Cell. 2012 Jun 22;149(7):1474-87.
- Wu & Yao, BMC Genomics. 2013 Aug 30;14:591
- Kind et al, Cell. 2015 Sep 24;163(1):134-47.

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- Harr et al, J Cell Biol. 2015 Jan 5;208(1):33-52.
- Robson et al, Mol Cell. 2016 Jun 16;62(6):834-847.
- Lenain et al, Genome Res. 2017 Oct;27(10):1634-1644.
- Robson et al, Genome Res. 2017 Jul;27(7):1126-1138.
- Zheng et al, Mol Cell. 2018 Sep 6;71(5):802-815
- Borsos et al, Nature. 2019 May;569(7758):729-733.

Perhaps more compelling is that we previously directly visualized the ^{m6}A deposited by Dam-LaminB1 (Kind et al, Cell. 2013;153:178-92). It is clearly at edge of the nucleus. Furthermore, DamID maps of NL interactions closely match the independent TSA-seq method (Chen, J Cell Biol. 2018; 217:4025-4048.) and even spatial models based on single-cell Hi-C maps (Nagano et al, Nature. 2013; 502:59-64.).

With such a long list of validation experiments over a span of 13 years, it is time that DamID becomes accepted as a standard reliable method to detect lamina interactions. In, fact, there seems to be an unfair double standard in the field: FISH experiments in published papers are rarely validated by other methods (such as DamID), even though FISH is a tricky method. Given this, and the extremely low throughput and limited genomic resolution of FISH, we see little merit in once again 'validating' the DamID data. Because FISH is not a core expertise in our lab, this would take several months of work, with very little added value.

The domainograms presented most of the time as top panels do not aid the understanding of the results and should be omitted. The DamID ratio is sufficient since this graph also visualizes the LAD structures. Figure 2c should be presented as DamID ratios.

The domainograms are very important for proper interpretation of the results. Showing a browser track of DamID log-ratios alone is not sufficient, as one would not be able to distinguish real signals from random fluctuations. The domainograms provide the necessary statistical perspective. For the same reason, domainograms are standard practice in the 4C community (with very similar data structures). We appreciate that domainograms might initially be a bit complicated due to the multi-scale analysis. Multi-scale analysis is however unavoidable, because one cannot know *a priori* at which scale the changes might occur. **We now provide an explanatory diagram as new Figure S1 to clarify the domainogram principle for readers who are not familiar with 4C data**.

Regarding Figure 2c, we have tried many ways of plotting these results. Domainograms really are the most compact and effective way to visualize the comparison across all targeted loci and help to focus on statistically meaningful effects. Please note that the majority (13/14) of the corresponding DamID tracks can be found in Figures 1, 2, 4, 5, EV2, EV4, EV5, so both types of information are available.

In figure 2 the targeting of the human SOX6 gene is shown. It is not clear whether an alternative promoter was targeted here since the gRNA is displayed inside the gene. Also a transcript that is transcribed antisense to the 5'end of SOX6 is indicated. Neither in the Refseq genes or in the UCSC genes such a transcript is annotated.

Indeed, this is an alternative promoter. **We now mention this in the text. We now also plot the actual mRNA-seq tracks in figure EV1 (formerly S2).** Figure S3h shows that the SOX6 transcript indeed starts from the targeted internal promoter.

Regarding the antisense transcript annotation: Between different genome databases and builds, non-coding transcripts appear and disappear due to different criteria applied in the annotation algorithms. Our mRNA-seq data (Figure EV1h) indicate that RPE-1 cells express a shorter version of this transcript. In any case, we do not draw any conclusions regarding this transcript, as it does not appear to change expression when we target the internal *SOX6* promoter.

Minor issues:

Does the observed shift in replication timing correlate with TADs?

We have now investigated available Hi-C data. We focused on the PTN locus, where the most striking difference between DamID and Repli-seq change was observed. The replication timing does not appear to track with the TAD structure, while the NL interactions seem to correspond partially. **We now show these results as new figure EV3.**

There are some typos and ## instead of numbers in the Materials and Methods.

Thank you for pointing these out - **we have corrected them. We also added more details on the RNA-seq methods.**

Figure 7 would be much clearer if the mouse strain and which gene was targeted would be displayed above the graphs.
We have now added the mouse strain (i.e., the allele that was measured) inside the figure panels, at the top of the legends.
In Figure 7a,c the deletions are marked by the yellow bars; the genes of which the promoters were deleted are named in the figures. In Figure 7e the PAS integration site is marked by the red triangle and the vertical red dotted line. **The figure legend now explains these features more clearly**. The right-hand panels are the respective control alleles, and hence the deletions are not marked (7b, d), and the position of the (absent) PAS insertion (7f) is only marked by a grey dotted line for reference.

The lettering in the figures is too small for a publication in the EMBO Journal.
We have increased the font sizes. @Editor: please advise if this is sufficient.

2nd Editorial Decision 16 January 2020

Thank you for submitting your revised manuscript for our consideration, it has now been seen once more by the original referees (see comments below). I am pleased to say that the referees find that their concerns have been satisfactorily addressed and now support publication. I would therefore now like to ask you to address several editorial issues that are listed in detail below in a final revised version. Please make any changes to the manuscript text in the attached document only using the "track changes" option. Once these minor issues are resolved, we will be happy to formally accept the manuscript for publication.

REFEREE REPORTS
Referee #1:

The authors have satisfactorily addressed my concerns and I do not have further comments about the submission.

Referee #2:

I am satisfied with the reviewers response to my comments

Referee #3:

Revised manuscript Brueckner et al.:

The authors have addressed all points that were raised by this reviewer properly. I accept the arguments of the authors that DNA-FISH will not further strengthen this manuscript.
Altogether, the manuscript has nicely improved and can be accepted for publication.

2nd Revision - authors' response 23 January 2020

Authors made the requested editorial amendments.

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PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name:	Dr. Bas van Steensel
Journal Submitted to:	The EMBO Journal
Manuscript Number:	EMBOJ-2019-103159R1

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.

USEFUL LINKS FOR COMPLETING THIS FORM

http://www.antibodypedia.com
http://1degreebio.org
http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repo
http://grants.nih.gov/grants/olaw/olaw.htm
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-tum
http://datadryad.org
http://figshare.com
http://www.ncbi.nlm.nih.gov/gap
http://www.ebi.ac.uk/ega
http://biomodels.net/
http://biomodels.net/miriam/
http://jjj.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

B- Statistics and general methods

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

Question	Answer
1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	This was not pre-determined, but by comparing the domainograms and the DamID tracks a good impression can be obtained of effect sizes that are 'significant'.
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 samples 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.	N/A
For animal studies, include a statement about randomization even if no randomization was used.	N/A
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.	Data processing is done by computational pipelines that work irrespective of sample ID. In all DamID experiments, the Dam-Lamin and Dam-only samples were processed in parallel, so that any effects of chromatin accessibility or PCR-biases are properly corrected for.
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?	The main statistical approach used are the domainograms, which are explained in the main text and in figure S2.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Domainograms are based on ranking, so there are no assumptions regarding the underlying distribution.

Is there an estimate of variation within each group of data?	Implicitly, by means of the domainogram calculations.
Is the variance similar between the groups that are being statistically compared?	N/A

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).	no antibodies used
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	The identity of the F1 hybrid ES cells was confirmed by sequence analysis of the DamID-seq data. RPE-1 cells expressing the SunTag system were directly obtained from the source lab (R. Medema, NKI). All cell cultures were routinely subjected to a monthy mycoplasma test and found to be negative.

* 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.	N/A
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	N/A
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.	N/A

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	N/A
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
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	N/A
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	N/A
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	N/A
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.	N/A
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.	N/A

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	Data Availability section is provided and lists the accession codes for DamID-seq, Repli-Seq and RNA-seq datasets.
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).	Data processing/analysis code is provided via Github, URL is listed.
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).	N/A
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.	See 19

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.	N/A