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PML-NB-dependent type I interferon memory results in a restricted form of HSV latency

Jon Suzich, Sean Cuddy, Hiam Baidas, Sara Dochnal, Eugene Ke, Austin Schinlever, Aleksandra Babnis, Boutell Chris, and Anna Cliffe
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Corresponding author(s): Anna Cliffe (cliffe@virginia.edu)

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1st Revision - Editorial Decision 24th Feb 2021

Dear Dr. Cliffe,

Thank you for the submission of your research manuscript to EMBO reports. We have now received the reports from the three referees that were asked to evaluate your study, which can be found at the end of this email.

As you will see, the referees think that these findings are of interest. However, they have several comments, concerns and suggestions, indicating that a major revision of the manuscript is necessary to allow publication of the study in EMBO reports. As the reports are below, and all their points need to be addressed, I will not detail them here.

Given the constructive referee comments, we would like to invite you to revise your manuscript with the understanding that all referee concerns must be addressed in the revised manuscript or in the detailed point-by-point response. Acceptance of your manuscript will depend on a positive outcome of a second round of review. It is EMBO reports policy to allow a single round of revision only and acceptance 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. We are aware that many laboratories cannot function at full efficiency during the current COVID-19/SARS-CoV-2 pandemic and we have therefore extended our 'scooping protection policy' to cover the period required for full revision. Please contact me to discuss the revision should you need additional time, and also if you see a paper with related content published elsewhere.

When submitting your revised manuscript, please also carefully review the instructions that follow below.

PLEASE NOTE THAT upon resubmission revised manuscripts are subjected to an initial quality control prior to exposition to re-review. Upon failure in the initial quality control, the manuscripts are sent back to the authors, which may lead to delays. Frequent reasons for such a failure are the lack of the data availability section (please see below) and the presence of statistics based on n=2 (the authors are then asked to present scatter plots or provide more data points).

When submitting your revised manuscript, we will require:

1) a .docx formatted version of the final manuscript text (including legends for main figures, EV figures and tables), but without the figures included. Please make sure that changes are highlighted to be clearly visible. Figure legends should be compiled at the end of the manuscript text.

2) individual production quality figure files as .eps, .tif, .jpg (one file per figure), of main figures and EV figures. Please upload these as separate, individual files upon re-submission.

The Expanded View format, which will be displayed in the main HTML of the paper in a collapsible format, has replaced the Supplementary information. You can submit up to 5 images as Expanded View. Please follow the nomenclature Figure EV1, Figure EV2 etc. The figure legend for these should be included in the main manuscript document file in a section called Expanded View Figure Legends after the main Figure Legends section. Additional Supplementary material should be supplied as a single pdf file labeled Appendix. The Appendix should have page numbers and needs

to include a table of content on the first page (with page numbers) and legends for all content. Please follow the nomenclature Appendix Figure Sx, Appendix Table Sx etc. throughout the text, and also label the figures and tables according to this nomenclature.

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3) 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.

4) a complete author checklist, which you can download from our author guidelines (https://www.embopress.org/page/journal/14693178/authorguide). Please insert page numbers in the checklist to indicate where the requested information can be found in the manuscript. The completed author checklist will also be part of the RPF.

Please also follow our guidelines for the use of living organisms, and the respective reporting guidelines: http://www.embopress.org/page/journal/14693178/authorguide#livingorganisms

5) that primary datasets produced in this study (e.g. RNA-seq, ChIP-seq, structural and array data) are deposited in an appropriate public database. If no primary datasets have been deposited, please also state this a dedicated section (e.g. 'No primary datasets have been generated and deposited'), see below.

See also: http://embor.embopress.org/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 & Methods) that follows the model below. This is now mandatory (like the COI statement). Please note that the Data Availability Section is restricted to new primary data that are part of this study.

Data availability

The datasets produced in this study are available in the following databases:

- RNA-Seq data: Gene Expression Omnibus GSE46843 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE46843)
- [data type]: [name of the resource] [accession number/identifier/doi] ([URL or identifiers.org/DATABASE:ACCESSION])

*** Note - All links should resolve to a page where the data can be accessed. ***

Moreover, I have these editorial requests:

6) We strongly encourage the publication of original source data with the aim of making primary data more accessible and transparent to the reader. The source data will be published in a separate source data file online along with the accepted manuscript and will be linked to the relevant figure. If you would like to use this opportunity, please submit the source data (for example scans of entire gels or blots, data points of graphs in an excel sheet, additional images, etc.) of your key experiments together with the revised manuscript. If you want to provide source data, please include size markers for scans of entire gels, label the scans with figure and panel number, and send one PDF file per figure.

7) Our journal 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: http://www.embopress.org/page/journal/14693178/authorguide#referencesformat

8) Regarding data quantification and statistics, can you please specify, where applicable, the number "n" for how many independent experiments (biological replicates) were performed, the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values in the respective figure legends. Please provide statistical testing where applicable, and also add a paragraph detailing this to the methods section. See:
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9) Please also note our new reference format:
http://www.embopress.org/page/journal/14693178/authorguide#referencesformat

10) For microscopic images, please add scale bars of similar style and thickness to all the microscopic images, using clearly visible black or white bars (depending on the background). Please place these in the lower right corner of the images. Please do not write on or near the bars in the image but define the size in the respective figure legend.

11) Please provide the abstract written in present tense.

12) Please add an authors contributions section (near the acknowledgements) and a headline 'Conflict of interest statement' to the COI statement.

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.

Yours sincerely,

Achim Breiling
Editor
EMBO Reports

Referee #1:

PML-Dependent Memory of Type I Interferon Treatment Results in a Restricted Form of HSV Latency

The present manuscript describes that PML NBs, formed in primary peripheral neurons in response to IFN-a treatment, induce a form of HSV latency with a restricted reactivation potential. PML NBs are formed in neurons only upon IFN-a treatment and persist even when the IFN signaling fades. When type I IFN is present during initial infection, HSV-1 genome colocalize with PML NBs and cannot be fully reactivated. However, PML does not seem to be required for the establishment of latency, but does contribute to maintenance of the so called "deep latency" or the inability to reach the full reactivation.

The overall impression is that it could represent an interesting contribution to our understanding of the role that PML NBs play in HSV latency in neuronal cells. However, there are some parts that require further clarification and different controls.

Figure 1: The number of PML NBs as a measure could be misleading, and it should also be combined with the volume of NBs. Namely, it is well established that PML NBs are dynamic structures, that can change their volume in dependance of partner recruitment as well as posttranslational modifications with SUMO or Ubiquitination, which ultimately also leads to PML degradation and dismantling of NBs. If there are no PML NBs in the absence of IFN treatment, does that mean that there is also no PML protein in neurons? A PML Immuno-Blot should be added to strengthen their observations.

Minor Point Figure 3: expression data are normalized to GAPDH. It would be better to normalize them to 18S or perhaps TBP1 or actin, as GAPDH could be itself differentially regulated in response to IFN (oxidative stress response pathway)

Figure 4: From the images in this figure, it seems that the number of PML in cells infected with HSV is significantly lower than in cells treated with Type I IFN. However, in fig EV4 it can be observed that number of NBs is higher. There it can also be appreciated that PML NBs of different volumes can be found juxtaposed or completely associated with the viral genome. It would therefore be important to introduce the volume of PML as an additional measure.
Sentence in row 356 is misleading: are there any PML NBs in untreated cells? This is in contrast to what is stated at the beginning of the manuscript. If there are any PML NBs in untreated cells that should be shown.

Figure 6: This part is probably the most difficult to interpret and comprehend, due to the experimental design and lack of standard controls. First of all, as a control for depletion of PML the authors show the association of PML NBs with HSV genome, and surprisingly do not show total PML protein levels - it's depletion needs to be documented by immunoblot and PML mRNA levels, and not by using PML NBs. Furthermore, it is possible that PML removal by shRNA does not have any impact on gene expression patterns in neurons, but could nevertheless cause changes in protein composition- accelerated degradation of certain proteins inside the PML NBs.
Moreover, as PML depletion has been performed in the context of IFN treatment, it is very hard to understand its effect, as it seems that it is very weak in comparison to the effect of IFN.

Figure 8: "PML depletion post infection does not result in spontaneous reactivation of HSV" - what does this mean? What factors are require for full HSV reactivation and how does PML influence

this? This is probably the most interesting part of the manuscript and has been very poorly addressed.
Have the authors considered treating cells with arsenic trioxide, which has a very well documented mode of action on PML. This could eliminate the eventual contribution of lentiviral vectors used in their experiment, as their use in per se could influence the IFN signaling.

The authors are probably aware that IFN treatment in addition to the increased abundance of PML transcripts, increases the levels of SUMO, and leads to an enhanced partner recruitment to PML NBs. Although SUMOylation had not been explored in the manuscript at all, it might be worth exploring if PML NBs that associate with the viral genomes are enriched in SUMO - this could further help in understanding the dynamics of PML NSs associated with HSV.

A big part of discussion is dedicated to histone posttranslational modifications, while none of those possibilities were addressed here.

Referee #2:

Suzich et al. exploit a powerful neuronal cell culture model of HSV1 latency relying upon primary murine sympathetic neurons to investigate how cellular subnuclear condensates called promyelocytic leukemia-nuclear bodies (PML-NBs) impact the establishment of a latent infection and reactivation from latency. This is an important problem in the field of HSV1 latency that may also apply to other viruses whose biology is influenced by PML-NBs and roles for PML-NBs in cell intrinsic innate immune defenses (as PML is encoded by an ISG). The authors report that while PML-NBs are not required to establish latency, transient type I IFN exposure around the time of initial infection results in i) PML induction and PML-NB accumulation; ii) entrapment of viral genomes within PML-NBs; and iii) restricted reactivation. This demonstrates that type I IFN exposure solely at the time of infection is sufficient to restrict reactivation and correlates with entrapment of viral genomes within PML-NBs. Furthermore, it is consistent with type I IFN treatment establishing a more restrictive form of latency less prone to inducible reactivation, in part, through PML entrapment of viral genomes. Finally, it raises the exciting possibility that the persistence of PML-NBs post-IFN treatment could represent a form of innate immune memory in neurons. The data are convincing and rigorous, the manuscript is well written and the results of significance to a wide variety of researchers investigating virus-host interactions, virus latency, and the role of PML-NBs in cell intrinsic innate responses and their contribution to regulating latency and reactivation.

A few specific comments and editorial issues to improve the manuscript are suggested below.

Specific comments:

1) Inclusion of a control to demonstrate operationally the effectiveness of the IFNAR1 ab to block detectable IFN signaling during this limited time window under the conditions used in their cultured neuron model system would further strengthen the authors claims.

2) Lines 318-319 pls clarify - this is hard to interpret. Was there no detectable induction of ISG15 or IRF7 expression by type I interferon in these neurons?

3) Lines 377-378 what about anti-IFNAR blocking ab addition for -18h samples? please clarify

4) Line 450: why is 150U/ml IFNa used in some experiments and 600U/ml used in others? Does the amount of type I IFN influence genome entrapment by PML, latency or reactivation?

5) line 453-458: In text here and in Fig 7 legend- I assume reactivation was induced by LY application? (I assume panels C/E w/o LY inducer? and D/F + LY? Please clarify.

6) Line 461-463: "Taken together, these data demonstrate that type I IFN exposure solely at the time of infection results in entrapment of viral genomes in PML-NBs to directly promote a deeper form of latency that is restricted for reactivation."
As written, this statement (to me) seems to somewhat blur what was firmly demonstrated by data and what is interpretation (even though the interpretation that it is genome entrapment per se by PML that promotes latency and restricts reactivation is likely correct). Perhaps it could be restated to more rigorously delineate what is supported by data and what is interpretation / correlation.
For example: "...these data demonstrate that type I IFN exposure solely at the time of infection results in entrapment of viral genomes in PML-NBs and restricts reactivation. This is consistent with.... genome entrapment by PML promoting a more restrictive or deeper form of latency where reactivation is limited."

7) The authors might consider referring to a prior study [PMCID: PMC5340258 DOI: 10.1016/j.celrep.2017.01.017] investigating how HSV1 reactivation was influenced by type I IFN application during and after inducible reactivation in a related cultured neuron model. While this earlier study did not evaluate or even anticipate how latency establishment is impacted by IFN, it seems very relevant to i) presenting a balanced background / introduction on what is known regarding the impact of type I IFN on latency/reactivation, especially in cultured sympathetic neuron models of latency; ii) the identification of a limited window of reactivation where type I IFN is active (ie Phase 1 vs Phase 2 as discussed in the manuscript text); and iii) the induction of PML by IFNb and IFNg in latently-infected rat sympathetic neurons induced to reactivate (supplemental tables). This latter point relates to lines 321-322.

Minor editorial comments

Line 77: please revise to read, "...heterogeneity in latency may ultimately be reflected in part by the association of..."

Line 337: please change "synthesis" to "accumulation" to more accurately reflect what is measured by immunoblotting.

Line 372: please clarify "loss inhibition"; should "loss" be deleted?

Lines 390, 391: I assume that "-3pi" is meant to read "-3dpi". Please correct. If this is not the case please clarify.

Line 457: Instead of "significantly increased", please refer to numerical fold increase in median values directly in text. My crude approximation looks like between 4 to 4.5-fold (?)

Line 474: "significantly increased" Again, again, please quantify the extent of the increase in the text, for example here in terms of % increase.

Line 475: please change "reactivate" to "inducibly reactivate" to distinguish any impact on spontaneous reactivation

The discussion is a bit on the longer side--- the authors might consider trimming 1/2 page or so. I leave that decision to them.

Throughout the manuscript, the authors conflate the terms "absence", "devoid of" and "not present" with their inability to detect a signal. The size of PML condensates could conceivably vary and in some cases be sufficiently small so as to evade detection by microscopy (this caveat applies to all subcellular condensates). This in no way detracts from their findings, but is a more rigorous way to describe their findings (in terms of what they measure). This should be corrected throughout the manuscript.
Example of this include:
line 198 should be revised to read, "...treatment of sensory neurons did not result in DETECTABLE formation of PML-NBs.

line 201-202: "Therefore, PML-NBs are LARGELY UNDETECTABLE in primary sympathetic and sensory neurons....

line 204: "The absence of DETECTABLE PML-NBs....."

lines 224: "devoid of DETECTABLE discrete puncta of Daxx....."

line 230- 231 "Therefore, PML-NBs containing their well characterized associated proteins are not DETECTED in cultured primary neurons but form in response to type I IFN exposure. "

Similar qualifications including the term "detectable"/ "detected" need to be introduced into lines 300-301, 315, 317

Line 409-410: "....and found that THE PRESENCE OR ABSENCE OF ICP0 had no DETECTABLE impact on the ability of vDNA foci to colocalize to PML-NBs"

Line 441: " Because PML depletion did not DETECTABLY prevent....."

Line 480-481: Therefore, PML depletion post-infection does not DETECTABLY result in spontaneous reactivation of PML-NB-associated viral genomes..."

Referee #3:

Summary:
• 1. Does this manuscript report a single key finding? Yes, Viral genomes possess a memory of the IFN response during de novo infection, which results in differential subnuclear positioning and ultimately restricts the ability of genomes to reactivate.

• 2. Is the reported work of significance? Yes.

• 3. Is it of general interest to the molecular biology community? Yes. Several unanswered questions are addressed about the role of innate immune responses in the establishment and reactivation from latency.

• 4. Is the single major finding robustly documented using independent lines of experimental evidence? Yes.

Constructive criticism for the authors:

In this paper, the authors report that if primary murine neurons are infected in the presence of type I interferon, a type of latency is established that is restricted for reactivation. They also show that PML-NBs are absent from primary sympathetic and sensory neurons form with type I IFN treatment. PML-NBs persist even when IFN signaling resolves. If type I IFN is present during the initial infection, HSV-1 genomes colocalized with PML-NBs throughout a latent infection of neurons. Depletion of PML prior to or following infection did not impact the establishment latency, but could rescue the ability of HSV to reactivate from IFN-treated neurons. This study demonstrates that viral genomes possess a memory of the IFN response during de novo infection, which results in differential subnuclear positioning and ultimately restricts the ability of genomes to reactivate. These are very important findings that will be of interest to the broad scientific community interested in the pathogenesis of herpes viruses and the role of type I IFN and PML in regulating host responses to pathogens. The paper is well written and the experiments clearly presented. The images and quantification are of high quality. Specific comments below relate to some instances where the clarity could be improved.

Specific comments:

1. Line 161. In the sentence beginning with "Latency can be..." , the logic of this statement is not clear.
2. Fig.1 Although DAPI is not mentioned anywhere in the manuscript; however, several panels in Fig. 1 and Fig EV1 are labeled as DAPI. Line. 214 of the text, they refer to Hoechst staining. Please clarify.
3. Fig. 1. Whether the staining is with DAPI or Hoechst, could the authors comment on the dense foci that probably represent heterochromatin. This should be clarified for readers who aren't familiar with DAPI/Hoechst staining diffusely in the nuclei vs in dense regions. Could the authors also comment on the significance of these foci and their relationship to the PML-NBs? Some PML foci appear at the periphery of the DAPI foci. What is the significance of this pattern?
4. Line 191. Fig. EV1. The figure doesn't correspond with the Figure legend. " Type I IFN treatment using IFN-alpha (IFNα) or IFN-ß (Fig.EV1A) led to a significant induction of PML-NBs in both sensory and sympathetic " Only IFNßis shown.
5. Line 362. "Rapid colocalization of viral DNA by PML-NBs during lytic HSV-1 infection of human fibroblasts occurs independently of type I IFN exposure, and we confirmed this was also true in dermal fibroblasts isolated from postnatal mice (Fig. EV4A)". This colocalization is not very clear from this figure. The number of Edu foci is low. Are there Edu foci that are not colocalized? It is not clear where the zoomed cells are coming from.
6. Line 396-413. For those not familiar with the history of ICP0 and PML NBs, a little more context could be given to make this paragraph understandable. It is known in nonneuronal cells that ICP0 colocalizes with PML-NBs and then causes their disruption. None of this is mentioned, and in Fig EV5D it is not clear why they still see PML bodies at 9 hrs. In Fig 5D, they use an ICP0 null mutant, but they don't show that ICP0 is really absent in those infections.
7. The paragraph in the discussion beginning on line 502 and ending on 534 is confusing and rather vague in parts.
a. Can the authors clarify the sentence that begins on line 510? Explain the conditions in which PML is re-expressed in both adult mouse and human brains. It is not clear what kind of intranuclear inclusions are being referred to. How do the authors explain these observations

b. Line 513. "In our study, we could not detect PML-NBs in adult primary neurons isolated from the SCG or the TG. In contrast to our findings, PML-NBs have previously been shown to be present in adult TG neurons (Catez et al., 2012, Maroui et al., 2016). However, Catez et al. (2012) describes a subpopulation of adult TG neurons that did not display any PML signal in the nucleus." Could the authors say a little more in explanation of how this discrepancy can be explained?
c. In the sentence starting on line 519, it is not clear what is meant.

Minor edits:
1. Line 127. There seems to be something missing from this sentence. The construction is not parallel.

We thank the editor and reviewers for their time and enthusiasm for this study.

REVIEWER #1

The present manuscript describes that PML NBs, formed in primary peripheral neurons in response to IFN-a treatment, induce a form of HSV latency with a restricted reactivation potential. PML NBs are formed in neurons only upon IFN-a treatment and persist even when the IFN signaling fades. When type I IFN is present during initial infection, HSV-1 genome colocalize with PML NBs and cannot be fully reactivated. However, PML does not seem to be required for the establishment of latency, but does contribute to maintenance of the so called "deep latency" or the inability to reach the full reactivation.

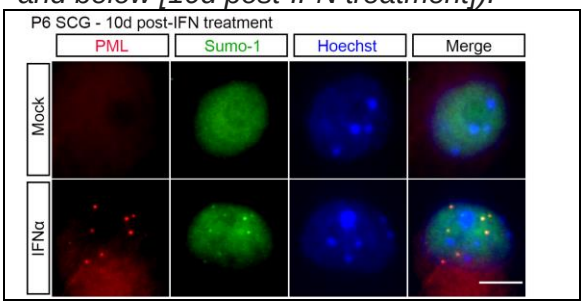
The overall impression is that it could represent an interesting contribution to our understanding of the role that PML NBs play in HSV latency in neuronal cells. However, there are some parts that require further clarification and different controls.

We thank the reviewer for their comments.

Figure 1: The number of PML NBs as a measure could be misleading, and it should also be combined with the volume of NBs. Namely, it is well established that PML NBs are dynamic structures, that can change their volume in dependance of partner recruitment as well as posttranslational modifications with SUMO or Ubiquitination, which ultimately also leads to PML degradation and dismantling of NBs. If there are no PML NBs in the absence of IFN treatment, does that mean that there is also no PML protein in neurons? A PML Immuno-Blot should be added to strengthen their observations.

We agree with the reviewer that a PML immuno-blot would add to our observations and have made multiple attempts at quantifying PML protein levels in our cultured SCG neurons. We have attempted to optimize the collection/lysis process by collecting in RIPA buffer, Laemmli buffer and Urea/Thiourea buffer. We have also tried multiple PML antibodies (EMD Millipore mab3738, Abcam ab67761) and imaged using both ECL detection reagents and LICOR. Unfortunately, we do not believe that the quality of these immune-blots is sufficient enough to make accurate conclusions on protein quantities. We appreciate that soluble PML could also be present in neurons. However, because we detect no PML-NBs prior to IFN treatment and viral genome co-localization with PML-NB, the focus of this manuscript is on the PML-NBs themselves and not soluble PML. To make this clear we have changed the title to "PML-NB dependent…." In terms of PML-NB volume, there are no detectible PML-NBs in the absence of IFN treatment so we are unable to measure the change in volume.

To confirm the presence of SUMO-1 at PML-NBs in our cultured SCG neurons, we performed immunofluorescence and found SUMO-1 colocalized with IFNα-induced PML-NBs (Figure EV1E) and persisted following cessation of IFNα signaling (Figure EV5C [3d post-IFN treatment] and below [10d post-IFN treatment]).



Minor Point Figure 3: expression data are normalized to GAPDH. It would be better to normalize them to 18S or perhaps TBP1 or actin, as GAPDH could be itself differentially regulated in response to IFN (oxidative stress response pathway)

To confirm the validity of GAPDH as a cellular control for our RT-qPCR experiments, we compared ISG15 expression in samples normalized to either GAPDH or 18S and found no difference in fold change (Please see data below). This is further supported by our RNA seq data showing no difference in GAPDH expression between untreated and IFN-treated samples (shCtrl samples: log2FoldChange = -0.12; shPML samples: log2FoldChange = -0.06)

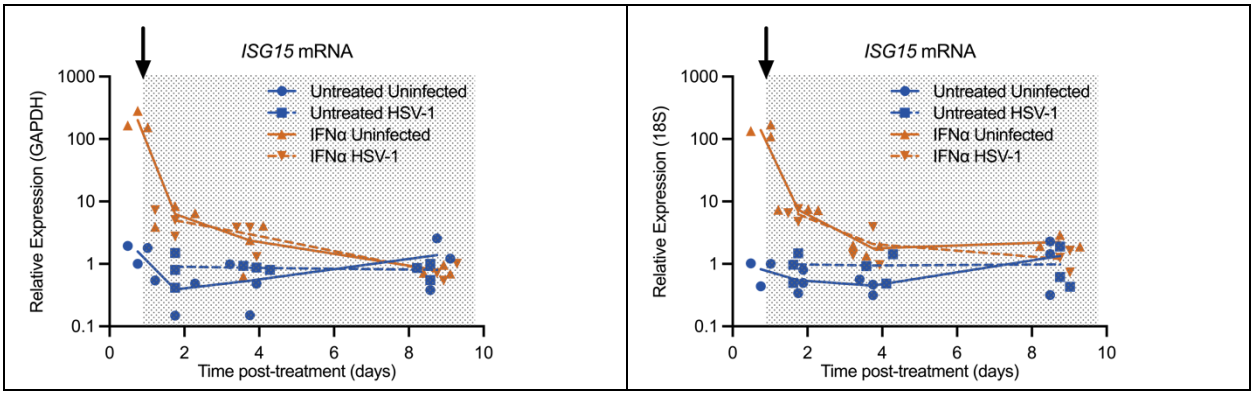


Figure 4: From the images in this figure, it seems that the number of PML in cells infected with HSV is significantly lower than in cells treated with Type I IFN. However, in fig EV4 it can be observed that number of NBs is higher. There it can also be appreciated that PML NBs of different volumes can be found juxtaposed or completely associated with the viral genome. It would therefore be important to introduce the volume of PML as an additional measure. Sentence in row 356 is misleading: are there any PML NBs in untreated cells? This is in contrast to what is stated at the beginning of the manuscript. If there are any PML NBs in untreated cells that should be shown.

For our colocalization immunofluorescence images in Figure 4A, we took z-stacks of a single genome rather than through the entire nucleus of the neuron to minimize background and improve image quality of the PML-NB and vDNA association. It is not representative of the total number of PML-NBs per nucleus. We apologize for this confusion and have clarified this in text. Additionally, we have shown that HSV-1 infection does not seem to impact the number of PML-NBs either in the presence or absence of IFNα (Figure 3D).

Given the resolution of epifluorescence imaging, it is hard to further define the level of PML-NB association with vDNA as either juxtaposed or completely associated.

We occasionally find neurons in our untreated cultures that will have PML-NBs, but the average PML-NB per nucleus is well under 1(Figure 1C-1F). Figure EV4A is an image of PML-NB and vDNA association in lytic infection of P6 dermal fibroblasts, a non-neuronal control. PML-NB can be detected in untreated dermal fibroblasts (this is what was stated in line 356) but we did not observe the same detection in untreated neurons.

Figure 6: This part is probably the most difficult to interpret and comprehend, due to the experimental design and lack of standard controls. First of all, as a control for depletion of PML the authors show the association of PML NBs with HSV genome, and surprisingly do not show total PML protein levels - it's depletion needs to be documented by immunoblot and PML mRNA levels, and not by using PML NBs. Furthermore, it is possible that PML removal by shRNA does not have any impact on gene expression patterns in neurons, but could nevertheless cause changes in protein composition- accelerated degradation of certain proteins inside the PML NBs.
Moreover, as PML depletion has been performed in the context of IFN treatment, it is very hard to understand its effect, as it seems that it is very weak in comparison to the effect of IFN.

To confirm the validity of our shRNA PML knockdown, we show depletion of PML-NBs by immunofluorescence and depletion of Pml mRNA in P6 SCG neurons by RT-qPCR. As mentioned above, we have not been able to optimize conditions to make conclusions on PML protein levels in our peripheral neurons.

We agree with the reviewer that it could be possible for PML depletion to impact protein composition; however, the goal of our RNA-seq experiment was to specifically show that ISG expression was not altered by PML depletion and subsequently impacting reactivation based on previous reports that PML can potentially alter ISG expression (Ulbricht et al, 2012; Chen et al, 2015; Kim & Ahn, 2015; Scherer et al, 2016; McFarlane et al, 2019). The RNA-seq experiment was a control to determine whether PML knock-down could be performed prior to IFN treatment without impacting ISG expression. We apologize for this confusion and for clarification have decided to move this data to the supplemental (Figure EV6A-C).

Figure 8: "PML depletion post infection does not result in spontaneous reactivation of HSV" - what does this mean? What factors are require for full HSV reactivation and how does PML influence this? This is probably the most interesting part of the manuscript and has been very poorly addressed.
Have the authors considered treating cells with arsenic trioxide, which has a very well documented mode of action on PML. This could eliminate the eventual contribution of lentiviral vectors used in their experiment, as their use in per se could influence the IFN signaling.

By 'PML depletion post infection does not result in spontaneous reactivation of HSV,' we mean that depletion of PML alone (disruption of PML-NBs in the absence of any additional known physiological stimulus of reactivation) did not lead to reactivation of latent viral genomes. It had previously been concluded that quiescent genomes associated with PML-NBs could be transcriptionally reactivated following PML-NB disruption via induced expression of ICP0 (Cohen et al, 2018), but we did not find this to be the case in our latently infected peripheral neurons.

To further address these points, we show that LY294002-mediated reactivation of latent genomes following PML depletion post-infection is DLK-dependent, as the DLK inhibitor GNE completely inhibited reactivation (Figure 7F). We have previously shown that latent HSV-1 genomes respond to both activation of cell stress signaling (treatment with LY294002) (Cliffe et al, 2015) and hyperexcitability (Cuddy et al, 2020), both known physiological stimuli of reactivation, via a common DLK/JNK-dependent pathway to result in reactivation. These data indicate that even when PML-NBs are disrupted, activation of cell stress pathways are required to induce reactivation, likely because of activation of transcription factors that are required for reactivation. This is an ongoing area of research in the lab and far beyond what can be answered in a single study.

In addition, we investigated reactivation of neurons that were latent infected in the presence of absence of IFNα following treatment with a concentration of arsenic trioxide (ATO; 1uM) that fully disrupted IFNα-induced PML-NBs in our peripheral neurons (Fig. EV7A). Interestingly, we found that ATO is a very potent stimulator of reactivation independent of IFNα-treatment, indicating that ATO is capable of triggering reactivation of genomes that are either PML-NB-associated or not (Fig. EV7B). This is likely because ATO is a potent activator of the cell stress response and can result in robust histone phosphorylation (Gehani et al, 2010), which we have previously linked to reactivation (Cliffe et al., 2015). Although ATO could also induce reactivation in the presence of PML-NBs, this reactivation was still less robust than mock treated neurons, likely reflecting the time required for disruption of PML-NB by ATO. This did not allow us to further assess whether disruption of PML-NBs alone leads to reactivation and we feel it a less specific method than depletion with three independent shRNAs.

The authors are probably aware that IFN treatment in addition to the increased abundance of PML transcripts, increases the levels of SUMO, and leads to an enhanced partner recruitment to PML NBs. Although SUMOylation had not been explored in the manuscript at all, it might be worth exploring if PML NBs that associate with the viral genomes are enriched in SUMO - this could further help in understanding the dynamics of PML NSs associated with HSV.

We agree with the reviewer that PML-NB dynamics could impact association with viral gnomes. When we looked at colocalization of SUMO-1 at IFNα-PML-NBs, we found SUMO-1 colocalized with IFNα-induced PML-NBs and persisted following cessation of IFNα signaling (Figure EV1E, EV5C). To further address this, we quantified PML-NB volume and found that PML-NBs associated with genomes had a significantly greater volume than PML-NBs that were not associated with genomes (Figure 4E). One interpretation of this result could be that there is enhanced partner recruitment. We were unable to confidently calculate volume of SUMO-1 at PML-NBs due to high background staining with the SUMO-1 antibody, but we did not see a qualitative difference in our immunofluorescence images.

A big part of discussion is dedicated to histone posttranslational modifications, while none of those possibilities were addressed here.

We have shortened the discussion. Furthermore, we are hope to continue to explore if PML-NBs regulate either the compaction or chromatin structure of latent viral genomes in the future.

REVIEWER #2

Suzich et al. exploit a powerful neuronal cell culture model of HSV1 latency relying upon primary murine sympathetic neurons to investigate how cellular subnuclear condensates called promyelocytic leukemia-nuclear bodies (PML-NBs) impact the establishment of a latent infection and reactivation from latency. This is an important problem in the field of HSV1 latency that may also apply to other viruses whose biology is influenced by PML-NBs and roles for PML-NBs in cell intrinsic innate immune defenses (as PML is encoded by an ISG). The authors report that while PML-NBs are not required to establish latency, transient type I IFN exposure around the time of initial infection results in i) PML induction and PML-NB accumulation; ii) entrapment of viral genomes within PML-NBs; and iii) restricted reactivation. This demonstrates that type I IFN exposure solely at the time of infection is sufficient to restrict reactivation and correlates with entrapment of viral genomes within PML-NBs. Furthermore, it is consistent with type I IFN treatment establishing a more restrictive form of latency less prone to inducible reactivation, in part, through PML entrapment of viral genomes. Finally, it raises the exciting possibility that the persistence of PML-NBs post-IFN treatment could represent a form of innate

immune memory in neurons. The data are convincing and rigorous, the manuscript is well written and the results of significance to a wide variety of researchers investigating virus-host interactions, virus latency, and the role of PML-NBs in cell intrinsic innate responses and their contribution to regulating latency and reactivation.

A few specific comments and editorial issues to improve the manuscript are suggested below.

We thank the reviewer for their comments.

Specific comments:

1) Inclusion of a control to demonstrate operationally the effectiveness of the IFNAR1 ab to block detectable IFN signaling during this limited time window under the conditions used in their cultured neuron model system would further strengthen the authors claims.

To confirm the effectiveness of the IFNAR1 ab to block detectable IFN signaling, we validated it by its ability to block ISG expression (ISG15) in cultured SCG neurons by RT-qPCR (Figure EV2A). In *these experiments, we pretreated neurons with the IFNAR1 ab for 2 hours, then treated neurons with 600u/ml IFNα for 18hrs in the presence of the IFNAR1 ab for 18 hours prior to RNA collection.*

2) Lines 318-319 pls clarify - this is hard to interpret. Was there no detectable induction of ISG15 or IRF7 expression by type I interferon in these neurons?

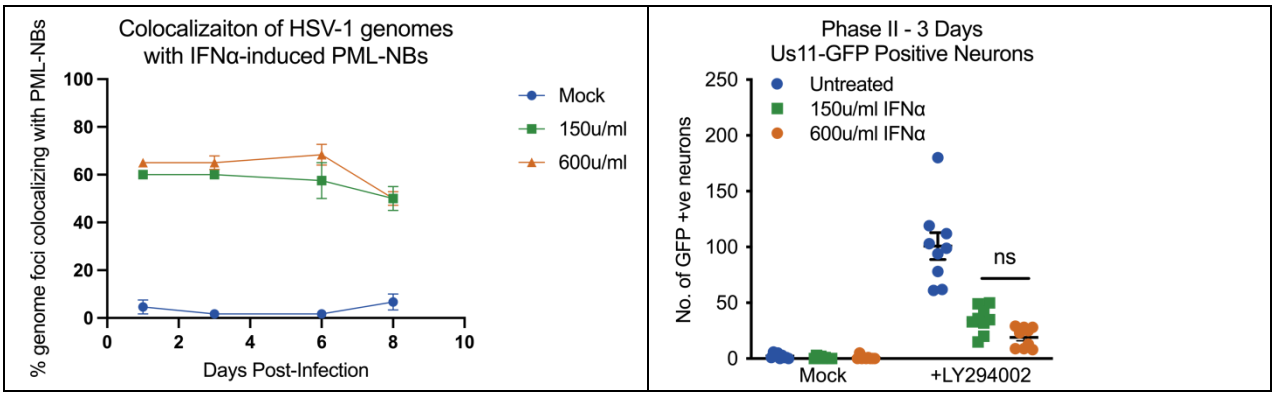
We have amended the text to better clarify this important point. We found no difference in IRF7 or ISG15 expression between Untreated Uninfected neurons and Untreated HSV-1-infected neurons nor between INFα-treated Uninfected neurons and INFα-treated HSV-1-infected neurons. This indicates that the presence of HSV-1 was not altering ISG expression in either the presence or absence of IFNα.

3) Lines 377-378 what about anti-IFNAR blocking ab addition for -18h samples? please clarify

We have amended the text to clarify the experimental conditions. Following the infection of the -18h samples, the anti-IFNAR1 blocking ab was included in the neuronal media until collection.

4) Line 450: why is 150U/ml IFNa used in some experiments and 600U/ml used in others? Does the amount of type I IFN influence genome entrapment by PML, latency or reactivation?

We found no difference in average number of PML-NBs per nucleus (Figure 1C-1F), percent of genomes colocalizing to PML-NBs or reactivation of HSV-1 in neurons treated with 150u/ml or 600u/ml IFNα. Please see data below.



5) line 453-458: In text here and in Fig 7 legend- I assume reactivation was induced by LY application? (I assume panels C/E w/o LY inducer? and D/F + LY? Please clarify.

We have amended the text and figure to indicate that reactivation was induced by LY294002.

6) Line 461-463: "Taken together, these data demonstrate that type I IFN exposure solely at the time of infection results in entrapment of viral genomes in PML-NBs to directly promote a deeper form of latency that is restricted for reactivation."
As written, this statement (to me) seems to somewhat blur what was firmly demonstrated by data and what is interpretation (even though the interpretation that it is genome entrapment per se by PML that promotes latency and restricts reactivation is likely correct). Perhaps it could be restated to more rigorously delineate what is supported by data and what is interpretation / correlation.
For example: "...these data demonstrate that type I IFN exposure solely at the time of infection results in entrapment of viral genomes in PML-NBs and restricts reactivation. This is consistent with.... genome entrapment by PML promoting a more restrictive or deeper form of latency where reactivation is limited. "

We have amended the text to better represent what our data has firmly demonstrated.

7) The authors might consider referring to a prior study [PMCID: PMC5340258 DOI: 10.1016/j.celrep.2017.01.017] investigating how HSV1 reactivation was influenced by type I IFN application during and after inducible reactivation in a related cultured neuron model. While this earlier study did not evaluate or even anticipate how latency establishment is impacted by IFN, it seems very relevant to i) presenting a balanced background / introduction on what is known regarding the impact of type I IFN on latency/reactivation, especially in cultured sympathetic neuron models of latency; ii) the identification of a limited window of reactivation where type I IFN is active (ie Phase 1 vs Phase 2 as discussed in the manuscript text); and iii) the induction of PML by IFNb and IFNg in latently-infected rat sympathetic neurons induced to reactivate (supplemental tables). This latter point relates to lines 321-322.

We agree with the reviewer about the importance and relevance of this prior study and have included the findings in our introduction and throughout the manuscript.

Minor editorial comments

Line 77: please revise to read, "...heterogeneity in latency may ultimately be reflected in part by the association of..."

We have amended the text as indicated.

Line 337: please change "synthesis" to "accumulation" to more accurately reflect what is measured by immunoblotting.

We have amended the text as indicated.

Line 372: please clarify "loss inhibition"; should "loss" be deleted?

We have amended the text as indicated.

Lines 390, 391: I assume that "-3pi" is meant to read "-3dpi". Please correct. If this is not the case please clarify.

We have amended the text as indicated.

Line 457: Instead of "significantly increased", please refer to numerical fold increase in median values directly in text. My crude approximation looks like between 4 to 4.5-fold (?)

We have amended the text as indicated.

Line 474: "significantly increased" Again, again, please quantify the extent of the increase in the text, for example here in terms of % increase.

We have amended the text as indicated.

Line 475: please change "reactivate" to "inducibly reactivate" to distinguish any impact on spontaneous reactivation

We have amended the text as indicated.

The discussion is a bit on the longer side--- the authors might consider trimming 1/2 page or so. I leave that decision to them.

Throughout the manuscript, the authors conflate the terms "absence", "devoid of" and "not present" with their inability to detect a signal. The size of PML condensates could conceivably vary and in some cases be sufficiently small so as to evade detection by microscopy (this caveat applies to all subcellular condensates). This in no way detracts from their findings, but is a more rigorous way to describe their findings (in terms of what they measure). This should be corrected throughout the manuscript.

We agree with the reviewer and have amended the text as indicated below.

Example of this include:
line 198 should be revised to read, "...treatment of sensory neurons did not result in DETECTABLE formation of PML-NBs.

line 201-202: "Therefore, PML-NBs are LARGELY UNDETECTABLE in primary sympathetic and sensory neurons....

line 204: "The absence of DETECTABLE PML-NBs....."

lines 224: "devoid of DETECTABLE discrete puncta of Daxx....."

line 230- 231 "Therefore, PML-NBs containing their well characterized associated proteins are not DETECTED in cultured primary neurons but form in response to type I IFN exposure."

Similar qualifications including the term "detectable"/ "detected" need to be introduced into lines 300-301, 315, 317

Line 409-410: "....and found that THE PRESENCE OR ABSENCE OF ICP0 had no DETECTABLE impact on the ability of vDNA foci to colocalize to PML-NBs"

Line 441: " Because PML depletion did not DETECTABLY prevent....."

Line 480-481: Therefore, PML depletion post-infection does not DETECTABLY result in spontaneous reactivation of PML-NB-associated viral genomes..."

REVIEWER #3

Summary:

• 1. Does this manuscript report a single key finding? Yes, Viral genomes possess a memory of the IFN response during de novo infection, which results in differential subnuclear positioning and ultimately restricts the ability of genomes to reactivate.

• 2. Is the reported work of significance? Yes.

• 3. Is it of general interest to the molecular biology community? Yes. Several unanswered questions are addressed about the role of innate immune responses in the establishment and reactivation from latency.

• 4. Is the single major finding robustly documented using independent lines of experimental evidence? Yes.

We thank the reviewer for their comments.

Constructive criticism for the authors:

In this paper, the authors report that if primary murine neurons are infected in the presence of type I interferon, a type of latency is established that is restricted for reactivation. They also show that PML-NBs are absent from primary sympathetic and sensory neurons form with type I IFN treatment. PML-NBs persist even when IFN signaling resolves. If type I IFN is present during the initial infection, HSV-1 genomes colocalized with PML-NBs throughout a latent infection of neurons. Depletion of PML prior to or following infection did not impact the establishment latency, but could rescue the ability of HSV to reactivate from IFN-treated neurons. This study demonstrates that viral genomes possess a memory of the IFN response during de novo infection, which results in differential subnuclear positioning and ultimately

restricts the ability of genomes to reactivate. These are very important findings that will be of interest to the broad scientific community interested in the pathogenesis of herpes viruses and the role of type I IFN and PML in regulating host responses to pathogens. The paper is well written and the experiments clearly presented. The images and quantification are of high quality. Specific comments below relate to some instances where the clarity could be improved.

Specific comments:

1. Line 161. In the sentence beginning with "Latency can be..." , the logic of this statement is not clear.

We have amended the text to better clarify this important point.

2. Fig.1 Although DAPI is not mentioned anywhere in the manuscript; however, several panels in Fig. 1 and Fig EV1 are labeled as DAPI. Line. 214 of the text, they refer to Hoechst staining. Please clarify.

We utilized Hoechst staining for staining of nuclei and have amended the figures to indicate this.

3. Fig. 1. Whether the staining is with DAPI or Hoechst, could the authors comment on the dense foci that probably represent heterochromatin. This should be clarified for readers who aren't familiar with DAPI/Hoechst staining diffusely in the nuclei vs in dense regions. Could the authors also comment on the significance of these foci and their relationship to the PML-NBs? Some PML foci appear at the periphery of the DAPI foci. What is the significance of this pattern?

We agree with the reviewer that the dense foci of Hoechst staining most likely represents heterochromatin and is an interesting observation. We included a description of this Hoechst staining and its potential importance in lines 221-226 and lines 723-725.

4. Line 191. Fig. EV1. The figure doesn't correspond with the Figure legend. " Type I IFN treatment using IFN-alpha (IFNα) or IFN-ß (Fig.EV1A) led to a significant induction of PML-NBs in both sensory and sympathetic " Only IFNß is shown.

We have amended the text to correspond with the correct figures.

5. Line 362. "Rapid colocalization of viral DNA by PML-NBs during lytic HSV-1 infection of human fibroblasts occurs independently of type I IFN exposure, and we confirmed this was also true in dermal fibroblasts isolated from postnatal mice (Fig. EV4A)". This colocalization is not very clear from this figure. The number of Edu foci is low. Are there Edu foci that are not colocalized? It is not clear where the zoomed cells are coming from.

We have amended the representative images and quantified colocalization to better represent this point.

6. Line 396-413. For those not familiar with the history of ICP0 and PML NBs, a little more context could be given to make this paragraph understandable. It is known in nonneuronal cells that ICP0 colocalizes with PML-NBs and then causes their disruption. None of this is mentioned, and in Fig EV5D it is not clear why they still see PML bodies at 9 hrs. In Fig 5D, they use an ICP0 null mutant, but they don't show that ICP0 is really absent in those infections.

We have amended the text to provide more background and context to our ICP0 experiments. In Fig EV5D, it is very interesting that we see colocalization of ICP0 at PML-NBs without disruption of PML-NBs and this could represent a neuron-specific phenotype. This is something we are following up on. In experiments presented in EV5D, we utilized low passage stocks of the ICP0-null mutant n212 (Cai & Schaffer, 1989) and the rescue virus n212R (Lee et al, 2016) provided by the laboratory of Dr. David Knipe. We furthered evaluated ICP0 by immunofluorescence of neurons infected with either n212 or n212R for 8 hours (Fig. EV5G).

7. The paragraph in the discussion beginning on line 502 and ending on 534 is confusing and rather vague in parts.

We have amended the text to better clarify these important points.

a. Can the authors clarify the sentence that begins on line 510? Explain the conditions in which PML is re-expressed in both adult mouse and human brains. It is not clear what kind of intranuclear inclusions are being referred to. How do the authors explain these observations

b. Line 513. "In our study, we could not detect PML-NBs in adult primary neurons isolated from the SCG or the TG. In contrast to our findings, PML-NBs have previously been shown to be present in adult TG neurons (Catez et al., 2012, Maroui et al., 2016). However, Catez et al. (2012) describes a subpopulation of adult TG neurons that did not display any PML signal in the nucleus." Could the authors say a little more in explanation of how this discrepancy can be explained?

c. In the sentence starting on line 519, it is not clear what is meant.

Minor edits:
1. Line 127. There seems to be something missing from this sentence. The construction is not parallel.

We have amended the text to better clarify this sentence.

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2nd Revision - Editorial Decision 24th May 2021

Dear Dr. Cliffe

Thank you for the submission of your revised manuscript to our editorial offices. We have now received the reports from the three referees that were asked to re-evaluate your study, you will find below. As you will see, the referees now fully support the publication of your study in EMBO reports.

Before we can proceed with formal acceptance, I have these editorial requests I ask you to address in a final revised manuscript:

- Could the title be compacted? I suggest:
PML-NB-dependent type I interferon memory results in a restricted form of HSV latency

- Please add up to five key words to the title page and order the manuscript sections like this:
Title page - Abstract - Introduction - Results - Discussion - Materials and Methods -Data availability section - Acknowledgements - Author contributions - Conflict of interest statement - References - Figure legends - Expanded View Figure legends. Please separate the main figure legends form the EV figure legends.

- Please remove list of ORCID identifiers from the manuscript. Make sure, though, that the ORCIDs are linked to the profiles of the respective authors in our manuscript submission system.

- We can accommodate only 5 EV figures. Please fuse some of the present EV figures to have not more than 5 EV figures in the final version. Finally, please update the callouts accordingly in the manuscript text.

- Please name the three tables 'Tables 1, 2 and 3', and change their callouts in the methods section. There is no need to call these supplementary tables.

- Please make sure that the number "n" for how many independent experiments were performed, their nature (biological versus technical replicates), the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values is indicated in the respective figure legends (also of the EV figures), and that statistical testing has been done where applicable. Please avoid the phrase 'independent experiment', but clearly state if these were biological or technical replicates.

- Could statistical testing be done for the data in Figs. 3A-D, 4B, 5A-D, 6A/B, 7E, EV4B, EV5D, EV6D and EV7A. Please make sure that in all diagrams where statistical testing has been done, but there is no significant difference, this is indicated in the diagrams (with 'ns').

- Why is there this greyish dotted area in Figs. 3A-C? Please explain this in the legend.

- For the microscopic images, please add scale bars of similar style and thickness to all the microscopic images, using clearly visible black or white bars (depending on the background). Please place these in the lower right corner of the images. Please do not write on or near the bars in the image but define the size in the respective figure legend.

- Please make sure that the references are formatted according to our journal style. See:
http://www.embopress.org/page/journal/14693178/authorguide#referencesformat

Camarena V (2011) has no journal and issue listed.

- Please also fill in section E of the author checklist (N/A).

- Finally, please find attached a word file of the manuscript text (provided by our publisher) with changes we ask you to include in your final manuscript text, and some queries, we ask you to address. Please provide your final manuscript file with track changes, in order that we can see any modifications done.

In addition, I would need from you:
- a short, two-sentence summary of the manuscript (not more than 35 words).
- two to four bullet points highlighting the key findings of your study.
- a schematic summary figure (in jpeg or tiff format with the exact width of 550 pixels and a height of not more than 400 pixels) that can be used as a visual synopsis on our website.

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

Kind regards,

Achim Breiling
Editor
EMBO Reports

Referee #1:

The authors have successfully addressed all the point that I made in the first round, and the manuscript is now suitable for publication.

Referee #2:

The authors have done a very nice job of addressing all of my comments.

Referee #3:

This study demonstrates that viral genomes possess a memory of the IFN response during de novo infection, which results in differential subnuclear positioning and ultimately restricts the ability of genomes to reactivate. These are important findings that will be of interest to the broad scientific community interested in the pathogenesis of herpes viruses and the role of type I IFN and PML in regulating host response to pathogens. The authors have been responsive to the three previous reviews, and the clarity of several sections improved over the original submission.

The authors have addressed all minor editorial requests.

3rd Revision - Editorial Decision 8th Jun 2021

Dr. Anna Cliffe
University of Virginia
United States

Dear Dr. Cliffe,

I am very pleased to accept your manuscript for publication in the next available issue of EMBO reports. Thank you for your contribution to our journal.

At the end of this email I include important information about how to proceed. Please ensure that you take the time to read the information and complete and return the necessary forms to allow us to publish your manuscript as quickly as possible.

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Yours sincerely,

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Corresponding Author Name: Anna R Cliffe
Journal Submitted to: EMBO reports
Manuscript Number: EMBOR-2021-52547

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 iustified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

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

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

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

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

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

B- Statistics and general methods

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

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	Trial experiments were performed on the following: GFP-positive cells after HSV reactivation, HSV mRNA levels and PML-NB numbers. Power analysis was the carried out using G Power.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	See above.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	There was no exclusion.
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.	Experiments were conduced on pooled neurons from multiple mice and independently replicated on muliple litters.
For animal studies, include a statement about randomization even if no randomization was used.	Primary neuonal experiments were randomized as they containted mixed neurons from multiple mice for each N and repeated on multiple independent litters.
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.	Yes. Although the phenotype being tested here made blinidng problematic as the conditions +/- interferon were striking. To ensure sciencitif rigor, we therefore performed multiple assays, for example both counting GFP positive cells and extracting RNA for RT-qPCR.
4.b. For animal studies, include a statement about blinding even if no blinding was done	When possible, blinding was performed in which samples were labelled with a key by one investigator and counted by another.
5. For every figure, are statistical tests justified as appropriate?	Yes. For all data a Kolmogorov-Smirnov test to determine normalitiy. When samples were not nromally distributed, a Mann-Whitney test or Wilcoxin test wwas performed dependeing on whether the sampes were paired. All data analysed by multi-way comparisons were normally distributed and therefoe ANOVA with a Tukey's multiple comparison test
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Yes. See above.
Is there an estimate of variation within each group of data?	No

USEFUL LINKS FOR COMPLETING THIS FORM

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

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-tumo

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/

Is the variance similar between the groups that are being statistically compared?	When appropriate. Although ANOVA was carried out on all multiple comparisons.

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).	See supplemental table 1. Antibodies against PML and ATRX were validated by knock-down and knock-out in floxed mice available in the lab. Daxx and Mx1 by knock-down. ICP0 using the ICP0 null virus.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Experiments were performed on primary neurons directly isolated from mice.

* 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.	CD-1 mice aged post-natal day 1-2 or 28. Source - in hose colony of CD-1 mice for P1-2 pups. P28s purchased from Charles River laboratories.
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.	Yes

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	Done
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).	Done
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.	N/A

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.	No