natureresearch

Peer Review Information

Journal: Nature Cell Biology
Manuscript Title: Poly(ADP-ribosylation) of P-TEFb by PARP1 disrupts phase separation to inhibit global transcription upon DNA damage
Corresponding author name(s): Xiang Gao, Huasong Lu and Qiang Zhou

Reviewer Comments & Decisions:

Decision Letter, initial version:

Subject: Decision on Nature Cell Biology submission NCB-Z45754
Message:

*Please delete the link to your author homepage if you wish to forward this email to co-authors.

Dear Professor Zhou,

Your manuscript, "Poly(ADP-ribosylation) of P-TEFb by PARP1 disrupts phase separation to inhibit global transcription upon genotoxic stress", has now been seen by 3 referees, who are experts in DNA repair and Poly(ADP-ribosylation) (referee 1); transcription and phase separation (referee 2); and Poly(ADP-ribosylation) (referee 3). As you will see from their comments (attached below) they find this work of potential interest, but have raised substantial concerns, which in our view would need to be addressed with considerable revisions before we can consider publication in Nature Cell Biology.

Nature Cell Biology editors discuss the referee reports in detail within the editorial team, including the chief editor, to identify key referee points that should be addressed with priority, and requests that are overruled as being beyond the scope of the current study. To guide the scope of the revisions, I have listed these points below. We are committed to providing a fair and constructive peer-review process, so please feel free to contact me if you would like to discuss any of the referee comments further.

In particular, it would be essential to:

a) put the findings in the context of the existing literature reporting other mechanisms underlying the inhibitory effect of PARylation on transcription after DNA damage (referee 1, point 1).

b) strengthen the connection between PARylation and CycT1 LLPS (referee 1, points 4, 5, 7).

c) better dissect the dynamics of CycT1 recruitment to DNA lesions (referee 1, point3, referee 2, point 1, referee 3, point 1).

d) All other referee concerns pertaining to strengthening existing data, providing controls, methodological details, clarifications and textual changes, should also be addressed.

e) Finally please pay close attention to our guidelines on statistical and methodological reporting (listed below) as failure to do so may delay the reconsideration of the revised manuscript. In particular please provide:

- a Supplementary Figure including unprocessed images of all gels/blots in the form of a multi-page pdf file. Please ensure that blots/gels are labeled and the sections presented in the figures are clearly indicated.

- a Supplementary Table including all numerical source data in Excel format, with data for different figures provided as different sheets within a single Excel file. The file should include source data giving rise to graphical representations and statistical descriptions in the paper and for all instances where the figures present representative experiments of multiple independent repeats, the source data of all repeats should be provided.

We would be happy to consider a revised manuscript that would satisfactorily address these points, unless a similar paper is published elsewhere, or is accepted for publication in Nature Cell Biology in the meantime.

When revising the manuscript please:

- ensure that it conforms to our format instructions and publication policies (see below and www.nature.com/nature/authors/).

- provide a point-by-point rebuttal to the full referee reports verbatim, as provided at the end of this letter.

- provide the completed Editorial Policy Checklist (found here https://www.nature.com/authors/policies/Policy.pdf), and Reporting Summary (found here https://www.nature.com/authors/policies/ReportingSummary.pdf). This is essential for reconsideration of the manuscript and these documents will be available to editors and referees in the event of peer

review. For more information see http://www.nature.com/authors/policies/availability.html or contact me.

Nature Cell Biology is committed to improving transparency in authorship. As part of our efforts in this direction, we are now requesting that all authors identified as ‘corresponding author’ on published papers create and link their Open Researcher and Contributor Identifier (ORCID) with their account on the Manuscript Tracking System (MTS), prior to acceptance. ORCID helps the scientific community achieve unambiguous attribution of all scholarly contributions. You can create and link your ORCID from the home page of the MTS by clicking on ‘Modify my Springer Nature account’. For more information please visit please visit www.springernature.com/orcid.

Please submit the revised manuscript files and the point-by-point rebuttal to the referee comments using this link:

[REDACTED]

*This url links to your confidential home page and associated information about manuscripts you may have submitted or be reviewing for us. If you wish to forward this email to co-authors, please delete the link to your homepage.

We would like to receive a revised submission within six months. We would be happy to consider a revision even after this timeframe, however if the resubmission deadline is missed and the paper is eventually published, the submission date will be the date when the revised manuscript was received.

We hope that you will find our referees' comments, and editorial guidance helpful. Please do not hesitate to contact me if there is anything you would like to discuss.

Best wishes,

Jie Wang

Jie Wang, PhD
Senior Editor
Nature Cell Biology

Tel: +44 (0) 207 843 4924
email: jie.wang@nature.com

Reviewers' Comments:

Reviewer #1:
Remarks to the Author:
Poly(ADP-ribsoyl)ation (PARylation) plays multiple roles in transcription, DNA replication and DNA repair. PAR chains serve as a recruitment platform for PAR-binding proteins, which has been proposed to induce their phase separation. On the other hand, PAR can also be attached covalently to target proteins to regulate their functions. PAR formation is strongly induced by DNA damage, and has been linked to suppression of transcription around DNA strand breaks (e.g. PMID: 20937877; 28336775). Here, the authors describe a mechanism for PAR-mediated inhibition of transcription through covalent PARylation of a histidine-rich domain in cyclin T1. This modification prevents CycT1 from undergoing phase separation, which in turn prevents its interaction partner CDK9 from hyperphosphorylating RNA Polymerase II, thereby blocking transcription elongation. The study is generally interesting, and the experiments are overall well done. However, some important points need to be addressed and clarified, primarily regarding previous work on PAR-mediated inhibition of transcription after DNA damage, regarding the sequence of events after PAR induction, and also regarding the functional significance of the proposed impaired cyclin T1 phase separation for transcription inhibition.

Main points:

1) Previous studies have linked PARP1-dependent PARylation after DNA damage to inhibition of transcription by recruiting the NuRD complex (PMID: 20937877) and negative elongation factor NELF-E (PMID: 28336775). How do these studies relate to findings in the current manuscript? Is PARylation of CycT1 specific to certain types of DNA damage? For instance, is the mechanism, which the authors propose, induced by endonuclease-triggered DNA single- and double-strand breaks (making use of CRISPR/Cas9 (double cutter and nickase) and the I-SceI system as in previous works)? Conversely, are repressive chromatin remodeling complexes and NELF-E involved in the inhibition of transcription after MNNG-induced damage? In light of the previous work on PAR-dependent transcription inhibition, which surprisingly is not discussed in the current manuscript, it seems important to carefully compare conditions and different types of DNA damage to elucidate the specificity of PAR-regulated mechanisms for transcription inhibition.

2) The DNA damage response kinases ATM and DNA-PK have also been implicated in transcription inhibition after DNA damage (PMID: 20550933, 22343725, 25066234, 31048545). Do ATM and DNA-PK get activated under the conditions used in the current study to induce DNA damage and PARylation of CycT1? If so, do they contribute to transcription inhibition?

3) For covalent PARylation to occur on a target protein, the protein to be modified first needs to be recruited to activated PARP1 at sites of DNA damage. Indeed, the authors observe PAR-dependent recruitment of CycT1 (Fig. 1I and 2A). And they identify several other phase separating proteins in their MS data (e.g. FUS, EWS, G3BP, hnRNPs). Does the initial CycT1 recruitment coincide with a PAR-induced phase separation (PMID: 26286827)? Would CycT1 later be excluded from the damaged area, as has been observed for other proteins (e.g. PMID: 26286827, 25030905, 22424773)? If so, would such an exclusion, which is consistent with inhibition of transcription elongation as the authors propose, depend on PARylation of the histidine-rich region, or on ATM and DNA-PK? Time-resolved recruitment to DNA damage should be evaluated for the different CycT1 constructs and in the absence and presence of enzyme inhibitors (PARP, PARG, ATM/DNA-PK/ATR).

4) In Fig. 2F the authors show that PARylated PARP1 is incorporated into CycT1-IDR droplets. But would PARylated PARP1 stimulate CycT1-IDR droplet formation, e.g. under sub-optimal conditions for CycT1-IDR droplet formation? And would addition of NAD+ to such droplets dissolve them due to CycT1 PARylation? These would be important experiments to elucidate the sequence of events and test predictions based on the model put forth by the authors.

5) Related to the previous points, testing the hypothesis that the histidine-rich domain interacts with PAR by electrostatic interactions (similar to what has been described for the RGG-repeats in FUS) deserves further attention. Would introducing negative charges in the HRD abolish PAR binding and CycT1 PARylation?

6) Are Mut1 and Mut2 impaired in PAR binding?

7) For PARylation-deficient CycT1 mutants, are they functional in all other aspects, i.e. do they represent clean separation-of-function mutants? Do these mutants form droplets in vitro similar to wild-type? Are the mutant droplets inert to in vitro PARylation (Fig. 5F, repeated with mutants)?

8) For the experiments shown in Fig. 5D and S5D, how can the authors exclude that MNNG/H2O2-induced damage triggers PARylation at many genomic locations, which recruits CycT1 without displaying visible CycT1 foci, and that the disappearance of CycT1 in nuclear speckles is a consequence of such damage recruitment rather than a consequence of covalent CycT1 PARylation?

9) The results shown in Fig. 5H are important, but not totally convincing. A more quantitative analysis would be needed and ideally a validation by an orthogonal technique.

10) The results shown in Fig. 6F-M are not sufficiently linked to PARylation. Does PARP inhibition result in similar phenotypes? Would there be epistasis between Mut2 expression and PARP inhibition?

Additional points:

1) Input controls seem to be missing from Co-IP experiments. And molecular weight markers should be included for Western blot data.

2) Where affinity purification (IP) has been performed prior to Western blot, it would be helpful to indicate this directly in the figure panel and not only in the figure legend.

3) Some text editing could further improve main text and the abstract.

Reviewer #2:
Remarks to the Author:
Fu et al. present evidence for a role of PARP1 in stalling Pol II elongation during DNA lesions. While it is well recognized that DNA damage results in global transcriptional silencing to preserve the integrity of the genome, the mechanism behind this process is less characterized. Here, the authors provide novel insights into this process by demonstrating that PARP1 impedes Pol II elongation by inhibiting P-TEFb - the key regulator of transitioning paused Pol II into its elongating form. More specifically, they provide evidence that PARP1 modifies the histidine-rich domain (HRD) of CycT resulting in abolished phase separation of CycT1. Finally, the functional consequences of PARP-mediated modification of CycT were analyzed and found to result in inhibition of transcription, enhanced DNA repair, and increased cell survival.
The study is well designed and nicely explains the interplay between DNA repair, transcriptional regulation, and phase-separated condensates. The paper would be of interest to a broad audience and make a substantial contribution to the field. However, the study could benefit from providing additional mechanistic details and further efforts are required before publication.

Major points:

1. The authors provide convincing evidence that PAR-PARP1 interacts with the P-TEFb complex shown in Figure 1 using mass spectrometry and co-IP experiments. They further show that CycT1 is recruited to DNA lesions in a PARP1 dependent fashion using live-cell imaging. However, limited data is provided for Figure 1 I.
-It is unclear how many replicates were performed and quantifications are missing.
-Why are there no distinct puncta of GFP-CycT in the PARP WT Pre-damage condition in a similar fashion to what can be seen in Figure 5D?
-What is the dynamic nature of the CycT1 puncta formed upon laser irradiation?

-To provide further proof for the hypothesis that PAR-PARP1 and CycT interact at the genome near DNA damaged sites, the authors might deploy Co-IF or ChIP-seq of PARP and CycT upon DNA damage.

2. Scale bars are missing from Figure 1 I and Figure 6 L. Scale bars are depicted in Figure 2F and Figure 5 C, D, E, F, but there is no explanation for what the bar represents.

3. The authors could do a better job in citing relevant original research papers rather than review papers.

4. DNA lesions are known to alter the chromatin structure for example by H2A-ubiquitination. How are previously reported chromatin changes upon DNA damage correlated to the current study? What is the order of events?

5. Throughout the manuscript, key information is missing in the in vitro droplet experiments.
- Protein concentration is a key determinant of phase separation but details on what protein concentration was used are not clear. Have the authors assayed condensate formation at different concentrations? Further, is the concentration used comparable with the concentration of the protein in a cell?
- Quantification of in vitro droplets is missing. For example, while there is a substantial difference between PARP1 vs PAR-PARP1 partition into CycT1 droplets, a partition ratio is needed to provide a quantitative measurement.
- Assays of homotypic droplet formation. The authors have previously demonstrated that CycT1 form homotypic droplets in Lu et al. Does PARP-1 or PAR-PARP1 form homotypic droplets at certain conditions?

Minor points:

-Row 54. NELF is misspelled NEFL.
-Row 179. “CycT, but not CDK9” is repeated.

Reviewer #3:
Remarks to the Author:
This study by Fu et al provides insights into the mechanisms through which DNA damage triggers genome wide transcriptional shutdown. The authors present evidence that this occurs via PARP1 dependent ADP-ribosylation of the P-TEFb subunit CycT1 HRD preventing CycT1 phase separation and Pol II phosphorylation by CDK9. The data included in this study represent a substantial body of work that

is timely and quite convincing. I have listed some missing controls and several other suggestions to improve the manuscript.

1) Figure 5D & E – The authors provide extensive quantification of nuclear GFP-CycT1 punta following treatments with DNA-damaging agents which is highly convincing but does not provide any insight into the how dynamic this process is. Would it be possible to include a time-course experiment to follow the disassembly of phase-separated CycT1 nuclear speckles in the same cell(s) before and after DNA damage to provide further evidence that disassembly of GFP-CycT1 puncta correlates timely with inhibition of global transcriptional elongation following DNA damage?

2) The authors show that CycT1 is PARylated following MNNG, H2O2 and IR but not following UV treatment concluding that PARylation of CycT1 is not involved in the UV-initiated NER pathway. Can the authors show that the UV treatment used in their experiments causes substantial DNA damage? This is currently not shown and therefore the conclusion that PARylation of CycT1 does not occur in UV-induced DNA damage is not fully supported.

3) Fig 3I & 3J & 3K – The HRD of CycT1 is required for interaction and ADP-ribosylation by PARP1. Is it possible that the HDR of CycT1 effects nuclear localisation as this would also explain the results as shown?

4) Fig 4H – Please provide a blot for HPF1.

5) Fig 4 – It would be interesting to know whether the histidine ADP-ribosylation sites in CycT1 are dependent on HPF1.

6) Size markers are included for some WBs but not all. Please provide these for all WB data.

7) Fig 5B – Please include LARP7 blot to confirm that it is knocked-down in this experiment.

8) Can the authors include sequence alignments showing all residues mutated in CycT1 Mut1 and Mut2.

9) There is a number of typos throughout the manuscript (eg. mCherry-PAR-PAPR1 on page 4).

GUIDELINES FOR MANUSCRIPT SUBMISSION TO NATURE CELL BIOLOGY

READABILITY OF MANUSCRIPTS – Nature Cell Biology is read by cell biologists from diverse backgrounds, many of whom are not native English speakers. Authors should aim to communicate their findings clearly, explaining technical jargon that might be unfamiliar to non-specialists, and avoiding non-standard abbreviations. Titles and abstracts should concisely communicate the main findings of the study, and the background, rationale, results and conclusions should be clearly explained in the

manuscript in a manner accessible to a broad cell biology audience. Nature Cell Biology uses British spelling.

MANUSCRIPT FORMAT – please follow the guidelines listed in our Guide to Authors regarding manuscript formats at Nature Cell Biology.

TITLE – should be no more than 100 characters including spaces, without punctuation and avoiding technical terms, abbreviations, and active verbs..

AUTHOR NAMES – should be given in full.

AUTHOR AFFILIATIONS – should be denoted with numerical superscripts (not symbols) preceding the names. Full addresses should be included, with US states in full and providing zip/post codes. The corresponding author is denoted by: "Correspondence should be addressed to [initials]."

ABSTRACT AND MAIN TEXT – please follow the guidelines that are specific to the format of your manuscript, as listed in our Guide to Authors (http://www.nature.com/ncb/pdf/ncb_gta.pdf) Briefly, Nature Cell Biology Articles, Resources and Technical Reports have 3500 words, including a 150 word abstract, and the main text is subdivided in Introduction, Results, and Discussion sections. Nature Cell Biology Letters have up to 2500 words, including a 180 word introductory paragraph (abstract), and the text is not subdivided in sections.

ACKNOWLEDGEMENTS – should be kept brief. Professional titles and affiliations are unnecessary. Grant numbers can be listed.

AUTHOR CONTRIBUTIONS – must be included after the Acknowledgements, detailing the contributions of each author to the paper (e.g. experimental work, project planning, data analysis etc.). Each author should be listed by his/her initials.

FINANCIAL AND NON-FINANCIAL COMPETING INTERESTS – the authors must include one of three declarations: (1) that they have no financial and non-financial competing interests; (2) that they have financial and non-financial competing interests; or (3) that they decline to respond, after the Author Contributions section. This statement will be published with the article, and in cases where financial and non-financial competing interests are declared, these will be itemized in a web supplement to the article. For further details please see https://www.nature.com/licenceforms/nrg/competing-interests.pdf.

REFERENCES – are limited to a total of 70 for Articles, Resources, Technical Reports; and 40 for Letters. This includes references in the main text and Methods combined. References must be numbered sequentially as they appear in the main text, tables and figure legends and Methods and must follow the precise style of Nature Cell Biology references. References only cited in the Methods should be numbered consecutively following the last reference cited in the main text. References only associated with Supplementary Information (e.g. in supplementary legends) do not count toward the total reference limit and do not need to be cited in numerical continuity with references in the main text. Only published papers can be cited, and each publication cited should be included in the numbered reference list, which should include the manuscript titles. Footnotes are not permitted.

METHODS – Nature Cell Biology publishes methods online. The methods section should be provided as a separate Word document, which will be copyedited and appended to the manuscript PDF, and incorporated within the HTML format of the paper.

Methods should be written concisely, but should contain all elements necessary to allow interpretation and replication of the results. As a guideline, Methods sections typically do not exceed 3,000 words. The Methods should be divided into subsections listing reagents and techniques. When citing previous methods, accurate references should be provided and any alterations should be noted. Information must be provided about: antibody dilutions, company names, catalogue numbers and clone numbers for monoclonal antibodies; sequences of RNAi and cDNA probes/primers or company names and catalogue numbers if reagents are commercial; cell line names, sources and information on cell line identity and authentication. Animal studies and experiments involving human subjects must be reported in detail, identifying the committees approving the protocols. For studies involving human subjects/samples, a statement must be included confirming that informed consent was obtained. Statistical analyses and information on the reproducibility of experimental results should be provided in a section titled "Statistics and Reproducibility".

All Nature Cell Biology manuscripts submitted on or after March 21 2016 must include a Data availability statement at the end of the Methods section. For Springer Nature policies on data availability see http://www.nature.com/authors/policies/availability.html; for more information on this particular policy see http://www.nature.com/authors/policies/data/data-availability-statements-data-citations.pdf. The Data availability statement should include:

• Accession codes for primary datasets (generated during the study under consideration and designated as "primary accessions") and secondary datasets (published datasets reanalysed during the study under consideration, designated as "referenced accessions"). For primary accessions data should be made public to coincide with publication of the manuscript. A list of data types for which submission to community-endorsed public repositories is mandated (including sequence, structure, microarray, deep sequencing data) can be found here http://www.nature.com/authors/policies/availability.html#data.

• Unique identifiers (accession codes, DOIs or other unique persistent identifier) and hyperlinks for datasets deposited in an approved repository, but for which data deposition is not mandated (see here for details http://www.nature.com/sdata/data-policies/repositories).

• At a minimum, please include a statement confirming that all relevant data are available from the authors, and/or are included with the manuscript (e.g. as source data or supplementary information), listing which data are included (e.g. by figure panels and data types) and mentioning any restrictions on availability.

• If a dataset has a Digital Object Identifier (DOI) as its unique identifier, we strongly encourage including this in the Reference list and citing the dataset in the Methods.

We recommend that you upload the step-by-step protocols used in this manuscript to the Protocol Exchange. More details can found at www.nature.com/protocolexchange/about.

DISPLAY ITEMS – main display items are limited to 6-8 main figures and/or main tables for Articles, Resources, Technical Reports; and 5 main figures and/or main tables for Letters. For Supplementary Information see below.

FIGURES – Colour figure publication costs $600 for the first, and $300 for each subsequent colour figure. All panels of a multi-panel figure must be logically connected and arranged as they would appear in the final version. Unnecessary figures and figure panels should be avoided (e.g. data presented in small tables could be stated briefly in the text instead).

All imaging data should be accompanied by scale bars, which should be defined in the legend.
Cropped images of gels/blots are acceptable, but need to be accompanied by size markers, and to retain visible background signal within the linear range (i.e. should not be saturated). The boundaries of panels with low background have to be demarked with black lines. Splicing of panels should only be considered if unavoidable, and must be clearly marked on the figure, and noted in the legend with a statement on whether the samples were obtained and processed simultaneously. Quantitative comparisons between samples on different gels/blots are discouraged; if this is unavoidable, it should only be performed for samples derived from the same experiment with gels/blots were processed in parallel, which needs to be stated in the legend.

Figures should be provided at approximately the size that they are to be printed at (single column is 86 mm, double column is 170 mm) and should not exceed an A4 page (8.5 x 11"). Reduction to the scale that will be used on the page is not necessary, but multi-panel figures should be sized so that the whole

figure can be reduced by the same amount at the smallest size at which essential details in each panel are visible. In the interest of our colour-blind readers we ask that you avoid using red and green for contrast in figures. Replacing red with magenta and green with turquoise are two possible colour-safe alternatives. Lines with widths of less than 1 point should be avoided. Sans serif typefaces, such as Helvetica (preferred) or Arial should be used. All text that forms part of a figure should be rewritable and removable.

We accept files from the following graphics packages in either PC or Macintosh format:

- For line art, graphs, charts and schematics we prefer Adobe Illustrator (.AI), Encapsulated PostScript (.EPS) or Portable Document Format (.PDF). Files should be saved or exported as such directly from the application in which they were made, to allow us to restyle them according to our journal house style.

- We accept PowerPoint (.PPT) files if they are fully editable. However, please refrain from adding PowerPoint graphical effects to objects, as this results in them outputting poor quality raster art. Text used for PowerPoint figures should be Helvetica (preferred) or Arial.

- We do not recommend using Adobe Photoshop for designing figures, but we can accept Photoshop generated (.PSD or .TIFF) files only if each element included in the figure (text, labels, pictures, graphs, arrows and scale bars) are on separate layers. All text should be editable in 'type layers' and line-art such as graphs and other simple schematics should be preserved and embedded within 'vector smart objects' - not flattened raster/bitmap graphics.

- Some programs can generate Postscript by 'printing to file' (found in the Print dialogue). If using an application not listed above, save the file in PostScript format or email our Art Editor, Allen Beattie for advice (a.beattie@nature.com).

Regardless of format, all figures must be vector graphic compatible files, not supplied in a flattened raster/bitmap graphics format, but should be fully editable, allowing us to highlight/copy/paste all text and move individual parts of the figures (i.e. arrows, lines, x and y axes, graphs, tick marks, scale bars etc.). The only parts of the figure that should be in pixel raster/bitmap format are photographic images or 3D rendered graphics/complex technical illustrations.

All placed images (i.e. a photo incorporated into a figure) should be on a separate layer and independent from any superimposed scale bars or text. Individual photographic images must be a minimum of 300+ DPI (at actual size) or kept constant from the original picture acquisition and not decreased in resolution post image acquisition. All colour artwork should be RGB format.

FIGURE LEGENDS – must not exceed 350 words for each figure to allow fit on a single printed NCB page together with the figure. They must include a brief title for the whole figure, and short descriptions of each panel with definitions of the symbols used, but without detailing methodology.

TABLES – main tables should be provided as individual Word files, together with a brief title and legend. For supplementary tables see below.

SUPPLEMENTARY INFORMATION – Supplementary information is material directly relevant to the conclusion of a paper, but which cannot be included in the printed version in order to keep the manuscript concise and accessible to the general reader. Supplementary information is an integral part of a Nature Cell Biology publication and should be prepared and presented with as much care as the main display item, but it must not include non-essential data or text, which may be removed at the editor's discretion. All supplementary material is fully peer-reviewed and published online as part of the HTML version of the manuscript. Supplementary Figures and Supplementary Notes are appended at the end of the main PDF of the published manuscript.

Supplementary items should relate to a main text figure, wherever possible, and should be mentioned sequentially in the main manuscript, designated as Supplementary Figure, Table, Video, or Note, and numbered continuously (e.g. Supplementary Figure 1, Supplementary Figure 2, Supplementary Table 1, Supplementary Table 2 etc.).

Unprocessed scans of all key data generated through electrophoretic separation techniques need to be presented in a supplementary figure that should be labelled and numbered as the final supplementary figure, and should be mentioned in every relevant figure legend. This figure does not count towards the total number of figures and is the only figure that can be displayed over multiple pages, but should be provided as a single file, in PDF or TIFF format. Data in this figure can be displayed in a relatively informal style, but size markers and the figures panels corresponding to the presented data must be indicated.

The total number of Supplementary Figures (not including the "unprocessed scans" Supplementary Figure) should not exceed the number of main display items (figures and/or tables (see our Guide to Authors and March 2012 editorial http://www.nature.com/ncb/authors/submit/index.html#suppinfo; http://www.nature.com/ncb/journal/v14/n3/index.html#ed). No restrictions apply to Supplementary Tables or Videos, but we advise authors to be selective in including supplemental data.

Each Supplementary Figure should be provided as a single page and as an individual file in one of our accepted figure formats and should be presented according to our figure guidelines (see above). Supplementary Tables should be provided as individual Excel files. Supplementary Videos should be

provided as .avi or .mov files up to 50 MB in size. Supplementary Figures, Tables and Videos much be accompanied by a separate Word document including titles and legends.

GUIDELINES FOR EXPERIMENTAL AND STATISTICAL REPORTING

REPORTING REQUIREMENTS – To improve the quality of methods and statistics reporting in our papers we have recently revised the reporting checklist we introduced in 2013. We are now asking all life sciences authors to complete two items: an Editorial Policy Checklist (found here https://www.nature.com/authors/policies/Policy.pdf) that verifies compliance with all required editorial policies and a reporting summary (found here https://www.nature.com/authors/policies/ReportingSummary.pdf) that collects information on experimental design and reagents. These documents are available to referees to aid the evaluation of the manuscript. Please note that these forms are dynamic 'smart pdfs' and must therefore be downloaded and completed in Adobe Reader. We will then flatten them for ease of use by the reviewers. If you would like to reference the guidance text as you complete the template, please access these flattened versions at http://www.nature.com/authors/policies/availability.html.

STATISTICS – Wherever statistics have been derived the legend needs to provide the n number (i.e. the sample size used to derive statistics) as a precise value (not a range), and define what this value represents. Error bars need to be defined in the legends (e.g. SD, SEM) together with a measure of centre (e.g. mean, median). Box plots need to be defined in terms of minima, maxima, centre, and percentiles. Ranges are more appropriate than standard errors for small data sets. Wherever statistical significance has been derived, precise p values need to be provided and the statistical test used needs to be stated in the legend. Statistics such as error bars must not be derived from n<3. For sample sizes of n<5 please plot the individual data points rather than providing bar graphs. Deriving statistics from technical replicate samples, rather than biological replicates is strongly discouraged. Wherever statistical significance has been derived, precise p values need to be provided and the statistical test stated in the legend.

Information on how many times each experiment was repeated independently with similar results needs to be provided in the legends and/or Methods for all experiments, and in particular wherever representative experiments are shown.

We strongly recommend the presentation of source data for graphical and statistical analyses as a separate Supplementary Table, and request that source data for all independent repeats are provided when representative experiments of multiple independent repeats, or averages of two independent experiments are presented. This supplementary table should be in Excel format, with data for different

figures provided as different sheets within a single Excel file. It should be labelled and numbered as one of the supplementary tables, titled “Statistics Source Data”, and mentioned in all relevant figure legends.

--------- Please don't hesitate to contact NCB@nature.com should you have queries about any of the above requirements ---------

Author Rebuttal to Initial comments

Our point-by-point response to the referees' comments

Reviewer #1:

Remarks to the Author:

Poly(ADP-ribsoyl)ation (PARylation) plays multiple roles in transcription, DNA replication and DNA repair. PAR chains serve as a recruitment platform for PAR-binding proteins, which has been proposed to induce their phase separation. On the other hand, PAR can also be attached covalently to target proteins to regulate their functions. PAR formation is strongly induced by DNA damage, and has been linked to suppression of transcription around DNA strand breaks (e.g. PMID: 20937877; 28336775). Here, the authors describe a mechanism for PAR-mediated inhibition of transcription through covalent PARylation of a histidine-rich domain in cyclin T1. This modification prevents CycT1 from undergoing phase separation, which in turn prevents its interaction partner CDK9 from hyperphosphorylating RNA Polymerase II, thereby blocking transcription elongation. The study is generally interesting, and the experiments are overall well done. However, some important points need to be addressed and clarified, primarily regarding previous work on PAR-mediated inhibition of transcription after DNA damage, regarding the sequence of events after PAR induction, and also regarding the functional significance of the proposed impaired cyclin T1 phase separation for transcription inhibition.

Main points:

1) Previous studies have linked PARP1-dependent PARylation after DNA damage to inhibition of transcription by recruiting the NuRD complex (PMID: 20937877) and negative elongation factor NELF-E (PMID: 28336775). How do these studies relate to findings in the current manuscript? Is PARylation of CycT1 specific to certain types of

DNA damage? For instance, is the mechanism, which the authors propose, induced by endonuclease-triggered DNA single- and double-strand breaks (making use of CRISPR/Cas9 (double cutter and nickase) and the I-SceI system as in previous works)? Conversely, are repressive chromatin remodeling complexes and NELF-E involved in the inhibition of transcription after MNNG-induced damage? In light of the previous work on PAR-dependent transcription inhibition, which surprisingly is not discussed in the current manuscript, it seems important to carefully compare conditions and different types of DNA damage to elucidate the specificity of PAR-regulated mechanisms for transcription inhibition.

We thank the reviewer for raising this critical point. Following the reviewer's suggestion, we have performed additional experiments to carefully examine the roles of NuRD and NELF-E in MNNG-induced transcriptional inhibition. Using the incorporation of 5-EU into newly synthesized RNA as a proxy to monitor transcription in situ with high efficiency and sensitivity, we observed that transcription remained largely silenced in the MTA1 (a NuRD complex subunit) or NELF-E knockdown cells after the treatment with MNNG. This suggests that the MNNG-induced transcriptional inhibition is not through invoking the action of NuRD or NELF-E. In contrast, the MNNG-induced inhibition was significantly alleviated in cells expressing CycT1-Mut2, demonstrating that CycT1 and its PARylation play a critical role in this process. We have now included these new data in revised Fig. 6g and Extended Data Fig. 8a-d. The previous work on PAR-dependent transcription inhibition mechanisms that involve NuRD and NELF-E have also been cited.

2) The DNA damage response kinases ATM and DNA-PK have also been implicated in transcription inhibition after DNA damage (PMID: 20550933, 22343725, 25066234, 31048545). Do ATM and DNA-PK get activated under the conditions used in the current study to induce DNA damage and PARylation of CycT1? If so, do they contribute to transcription inhibition?

This is an excellent question. In revised Extended Data Fig. 3f, we showed that both ATM and DNA-PK were indeed activated by MNNG, albeit with dynamics that were different from that of CycT1 PARylation (Fig. 3c and Extended Data Fig. 3f). In addition, we have provided evidence in Extended Data Fig. 3i and Extended Data Fig. 8e showing that the MMNG-induced PARylation of CycT1 and transcriptional repression were unaffected in cells pretreated with the ATM or DNA-PK inhibitor. These results

suggest that ATM and DNA-PK are unlikely to be employed by MNNG to cause transcriptional inhibition.

3) For covalent PARylation to occur on a target protein, the protein to be modified first needs to be recruited to activated PARP1 at sites of DNA damage. Indeed, the authors observe PAR-dependent recruitment of CycT1 (Fig. 1I and 2A). And they identify several other phase separating proteins in their MS data (e.g. FUS, EWS, G3BP, hnRNPs). Does the initial CycT1 recruitment coincide with a PAR-induced phase separation (PMID: 26286827)? Would CycT1 later be excluded from the damaged area, as has been observed for other proteins (e.g. PMID: 26286827, 25030905, 22424773)? If so, would such an exclusion, which is consistent with inhibition of transcription elongation as the authors propose, depend on PARylation of the histidine-rich region, or on ATM and DNA-PK? Time-resolved recruitment to DNA damage should be evaluated for the different CycT1 constructs and in the absence and presence of enzyme inhibitors (PARP, PARG, ATM/DNA-PK/ATR).

To address the reviewer's concern and act on his/her recommendation, we performed the time-resolved recruitment experiments as suggested. First, our results show that at 3 minutes after laser-irradiation, the recruitment of GFP-CycT1 to DNA lesions was abolished when PARP1 was knocked out (Fig. 1j) or its enzymatic activity was inhibited by AZD2281 (Fig. 1k), a highly selective PARP1/2 inhibitor. In contrast, treating cells with inhibitors targeting ATM, DNA-PK, or PARG did not affect CycT1's recruitment to these sites (Fig. 1k). Second, we have compared the abilities of WT and ∆HRD CycT1 to be recruited to DNA lesions at 3 minutes after laser irradiation. In stark contrast to WT CycT1 that was efficiently recruited to the sites, ∆HRD was not (Fig. 2g), consistent with our earlier observations that the HRD is important for CycT1's binding to PARP1 and recruitment to DNA lesions for PARylation. Lastly, we monitored the damage-induced CycT1 recruitment over an extended period of time. The result in Extended Data Fig.1a showed that CycT1 was rapidly recruited to DNA lesions within 1 min, peaked during the next 2-3 min, and then gradually decreased over time. In multiple experiments, we did not observe an apparent exclusion of CycT1 from the irradiated sites, suggesting that the response of CycT1 to laser irradiation is different from that of FUS/EWS/THRAP3/SAF-A as reported previously.

4) In Fig. 2F the authors show that PARylated PARP1 is incorporated into CycT1-IDR droplets. But would PARylated PARP1 stimulate CycT1-IDR droplet formation, e.g.

under sub-optimal conditions for CycT1-IDR droplet formation? And would addition of NAD+ to such droplets dissolve them due to CycT1 PARylation? These would be important experiments to elucidate the sequence of events and test predictions based on the model put forth by the authors.

We thank the reviewer for raising this important point. We have repeated the experiment by decreasing the protein concentration of CycT1-IDR used in droplet formation. Data in revised Fig. 2i showed that the PARylated PARP1 indeed enhanced phase separation by CycT1-IDR and became incorporated into the droplets (note that PAR-PARP1 alone was not able to form droplets; see Extended Data Fig. 1d). As controls, the PARP1 mutant E988Q, which is defective for PAR chain elongation and can only catalyze MARylation, was less efficient than WT PAR-PARP1 in stimulating the droplet formation. Moreover, the catalytically inactive PARP1 mutant E988A was completely inactive in this assay.

To answer the reviewer's question about the sequence of events for PAR-PARP1 binding and CycT1 PARylation, we added NAD^+ to pre-formed PARP1-CycT1 droplets as suggested. As shown in Extended Data Fig. 6i & 6j, the addition and the resultant CycT1 PARylation efficiently dissolved the pre-formed droplets, which agrees with our model that the PARylation of CycT1 inhibits its phase separation. Taken together, these new results suggest the following sequence of events: Once DNA damage is induced, the activated and PARylated PARP1 recruits CycT1 to DNA lesions and may initially promote CycT1 phase separation. The PAR chains on PARP1 can potentially serve as a scaffold to stimulate this process as reported previously (PMID: 26286827, 28336775). However, once all the conditions are met, CycT1 is also PARylated and the additional PAR chains present *in cis* neutralize the positive charges of the neighboring HRD and disrupt phase separation.

5) Related to the previous points, testing the hypothesis that the histidine-rich domain interacts with PAR by electrostatic interactions (similar to what has been described for the RGG-repeats in FUS) deserves further attention. Would introducing negative charges in the HRD abolish PAR binding and CycT1 PARylation?

Following the reviewer's suggestion, we have generated a CycT1 mutant, termed CycT1-6E, by replacing six positively charged residues (K502/R504/K507/K509/K511/K527; they were not identified as PARylation sites)

around the stretch of histidine repeats with negatively charged glutamic acid. The new result in Extended Data Fig. 3j showed that CycT1-6E showed significantly reduced PARylation upon MNNG treatment and also decreased binding to PAR-PARP1 (Fig. 2h). These results support the conclusion that the HRD of CycT1 interacts with PAR-PARP1 mainly through electrostatic interactions.

6) Are Mut1 and Mut2 impaired in PAR binding?

Following the reviewer's suggestion, we have added new PAR binding data in Extended Data Fig. 5c, which demonstrate that CycT1 Mut1 and Mut2 exhibited similar PAR binding activities compared to WT CycT1. This result indicates that the reduced PARylation of CycT1 Mut1 and Mut2 was not due to an impaired PAR binding by the two.

7) For PARylation-deficient CycT1 mutants, are they functional in all other aspects, i.e. do they represent clean separation-of-function mutants? Do these mutants form droplets in vitro similar to wild-type? Are the mutant droplets inert to in vitro PARylation (Fig. 5F, repeated with mutants)?

We thank the reviewer for raising this critical point. In the cell nucleus, P-TEFb's activity is controlled by its associations with the various positive and negative regulatory factors. As far as we know, the C-terminal intrinsically disordered region of CycT1, where all the mutated residues are located, is not involved in P-TEFb's direct binding to these regulators. Indeed, CycT1 Mut2 showed no change in binding to the known P-TEFb partners, as revealed by the co-IP experiment (Extended Data Fig. 5d). Moreover, the result in Fig. 5h demonstrated that in the absence of MNNG, Mut2 supported CDK9's autophosphorylation, as well as trans-phosphorylation of the Pol II CTD_{52} similarly as did WT CycT1.

In addition to providing evidence in support of the view that the PARylation-deficient CycT1 mutants remain functional in all other aspects, we also performed additional experiments as suggested by the reviewer to investigate the impacts of the mutations on CycT1's phase separation and PARylation-directed regulation. Our data in Extended Data Fig. 6g showed that CycT1 Mut2 underwent phase separation and formed liquid droplets similarly as did CycT1 WT. Moreover, droplet formation by CycT1 Mut2 was little affected by in vitro PARylation (Extended Data Fig. 6h), which is in sharp contrast

to the situation involving WT CycT1 (Fig. 5f) and consistent with the notion that the DNA damage-induced PARylation inhibits phase separation of WT but not mutant CycT1.

8) For the experiments shown in Fig. 5D and S5D, how can the authors exclude that MNNG/H2O2-induced damage triggers PARylation at many genomic locations, which recruits CycT1 without displaying visible CycT1 foci, and that the disappearance of CycT1 in nuclear speckles is a consequence of such damage recruitment rather than a consequence of covalent CycT1 PARylation?

This is a very challenging question to address, because, as the reviewer already mentioned, the recruitment of CycT1 by activated PARP1 to DNA damage sites is a prerequisite for its subsequent PARylation. Although at this moment we cannot completely rule out the contribution of the recruitment by PARP1 for suppressing CycT1's phase separation, we often observed in our time-resolved imaging experiments (Fig. 1i-k and Extended Data Fig. 1a) that the CycT1 speckles present in the nucleus were not disassembled during an extended period of time after laser irradiation. It is important to note that the laser irradiation experiments utilize microbeams to generate DNA damage precisely at selected locations, thus allowing us to monitor the recruitment of CycT1 by activated PARP1 on-site without eliciting DNA damage elsewhere in the nucleus. These observations, therefore, suggest that the mere recruitment of CycT1 by activated PARP1 is not the reason causing the disappearance of CycT1 speckles/condensates. Rather, the results in Fig. 5f and Extended Data Fig. 6j indicate that it is the PARylation of CycT1 that disrupted droplet formation, demonstrating that this covalent modification is necessary and sufficient to inhibit CycT1 phase separation.

9) The results shown in Fig. 5H are important, but not totally convincing. A more quantitative analysis would be needed and ideally a validation by an orthogonal technique.

Following on the reviewer's suggestion, we performed quantitative analysis of the levels of hyperphosphorylated CTD_{52} (IIo) in Fig. 5g & 5h and the results are now included at the bottom of the revised figures. Importantly, the quantification supports the original conclusions that the in vitro PARylated CycT1 did not affect CDK9's autophosphorylation but failed to support CDK9's trans-hyperphosphorylation of the CTD (Fig. 5g) that requires the phase-separated environment; and that P-TEFb (CDK9-

CycT1) containing WT but not Mut2 CycT1 displayed a decreased ability to hyperphosphorylate the CTD once isolated from MNNG-treated cells (Fig. 5h). This is consistent with our earlier demonstrations that MNNG induced PARylation of WT CycT1 to destroy phase separation but had little effect on Mut2 that lacks the modification sites.

We tried but did not succeed in using anti-phospho-CTD immunofluorescence staining as an orthogonal technique to verify these conclusions. Unfortunately, the staining with several available anti-phospho-CTD antibodies all had high background of non-specific signals and did not produce clean and unambiguous results.

Nevertheless, we would like to point out that since P-TEFb's transcriptional activity is entirely dependent on and correlates well with its kinase activity, especially its ability to hyperphosphorylate Pol II CTD, the transcription assay detecting the incorporation of 5-EU into newly synthesized RNA can be viewed as an orthogonal technique to detect P-TEFb's activity. In this regard, the new transcription result in Fig. 6g is completely consistent with the kinase result in Fig. 5h. The results from these two complementary experiments validate the conclusion that MNNG could inhibit the kinase and transcriptional activities of P-TFFb containing only WT but not Mut2 CycT1.

10) The results shown in Fig. 6F-M are not sufficiently linked to PARylation. Does PARP inhibition result in similar phenotypes? Would there be epistasis between Mut2 expression and PARP inhibition?

In response to this criticism, we have performed additional experiments using the PARP1 inhibitor AZD2281 to evaluate PARP's function in DNA damage response. Our new results showed that pretreating cells with the inhibitor mitigated transcriptional inhibition induced by MNNG (Fig. 6c), caused accumulation of more γH2AX foci and level after the IR treatment (Extended Data Fig. 9b & 9c), and decreased cell viability after DNA damage (Extended Data Fig. 9a), all of which phenocopied the response of the Mut2-expressing cells to DNA damage.

Additional points:

1) Input controls seem to be missing from Co-IP experiments. And molecular weight markers should be included for Western blot data.

We thank the reviewer for the suggestion. We have now included the input controls for all Co-IP experiments and also added the molecular weight markers for Western blot data.

2) Where affinity purification (IP) has been performed prior to Western blot, it would be helpful to indicate this directly in the figure panel and not only in the figure legend.

We thank the reviewer for the suggestion and have made the recommended change in the revised manuscript.

3) Some text editing could further improve main text and the abstract.

Thanks for the suggestion. We have made thorough text editing and all the changes are highlighted in blue color. We hope the revised manuscript is improved in this regard.

Reviewer #2:
Remarks to the Author:
Fu et al. present evidence for a role of PARP1 in stalling Pol II elongation during DNA lesions. While it is well recognized that DNA damage results in global transcriptional silencing to preserve the integrity of the genome, the mechanism behind this process is less characterized. Here, the authors provide novel insights into this process by demonstrating that PARP1 impedes Pol II elongation by inhibiting P-TEFb -the key regulator of transitioning paused Pol II into its elongating form. More specifically, they provide evidence that PARP1 modifies the histidine-rich domain (HRD) of CycT resulting in abolished phase separation of CycT1. Finally, the functional consequences of PARP-mediated modification of CycT were analyzed and found to result in inhibition of transcription, enhanced DNA repair, and increased cell survival.
The study is well designed and nicely explains the interplay between DNA repair, transcriptional regulation, and phase-separated condensates. The paper would be of interest to a broad audience and make a substantial contribution to the field. However, the study could benefit from providing additional mechanistic details and further efforts are required before publication.

Major points:

1. The authors provide convincing evidence that PAR-PARP1 interacts with the P-TEFb complex shown in Figure 1 using mass spectrometry and co-IP experiments. They further show that CycT1 is recruited to DNA lesions in a PARP1 dependent fashion using live-cell imaging. However, limited data is provided for Figure 1 I.

We thank the reviewer for raising this concern, please see our detailed response below.

-It is unclear how many replicates were performed and quantifications are missing.

The experiment mentioned by the reviewer has been repeated at least three times. The result from a repeat of the same experiment performed under improved conditions (see below), as well as quantification of the percentages of cells showing the demonstrated effect are now shown in revised Fig. 1j.

-Why are there no distinct puncta of GFP-CycT in the PARP WT Pre-damage condition in a similar fashion to what can be seen in Figure 5D?

We apologize for the confusion and would like to point out that MicroPoint (Andor, Oxford) that was used for the laser-irradiation experiment shown in original Figure 1i was installed on a widefield fluorescence microscope, which unfortunately is not optimal for visualizing the GFP-CycT1 puncta in the nucleus. Now, we have improved the experimental settings by upgrading the objective lens and adjusting the exposure time, and obtained a set of new images that do allow the visualization of the puncta to some extent in the cells (new Figure 1i and 1j). Admittedly, the puncta pattern is still not as clear and prominent as that seen in Fig. 5D, which was obtained in fixed cells using the LSM 880 confocal microscope (ZEISS) with a much better spatial resolution.

-What is the dynamic nature of the CycT1 puncta formed upon laser irradiation?

We have performed a laser irradiation followed by time-lapse imaging experiment to monitor the dynamics of GFP-CycT1 recruitment over an extended period of time, where we observed a rapid and transient recruitment and retention of GFP-CycT1 at the sites of irradiation. In particular, GFP-CycT1 was rapidly recruited to DNA lesions within 1 min, peaked during the next 2-3 min, and then gradually decreased over time. The

CycT1 puncta outside of the laser-irradiated area remained largely unaffected during the entire time, which is understandable given that the irradiated area was highly localized and very small in comparison. This new piece of data is now displayed in Extended Data Fig. 1a. In contrast, the new data in Extended Data Fig. 7f showed that the MNNG-induced disassembly of CycT1 puncta began as early as 20 min post-treatment and became very evident at 40 min.

-To provide further proof for the hypothesis that PAR-PARP1 and CycT interact at the genome near DNA damaged sites, the authors might deploy Co-IF or ChIP-seq of PARP and CycT upon DNA damage.

We have performed the Co-IF experiment suggested by the reviewer. The new result in Fig.1i showed that PARP1 and GFP-CycT1 were indeed co-localized at the sites of laser-irradiation.

2. Scale bars are missing from Figure 1 I and Figure 6 L. Scale bars are depicted in Figure 2F and Figure 5 C, D, E, F, but there is no explanation for what the bar represents.

We thank the reviewer for pointing out these issues. We have added scale bars in the revised figures and provided explanations in the relevant figure legends.

3. The authors could do a better job in citing relevant original research papers rather than review papers.

We thank the reviewer for this suggestion and have made the recommended change in the revised manuscript.

4. DNA lesions are known to alter the chromatin structure for example by H2A-ubiquitination. How are previously reported chromatin changes upon DNA damage correlated to the current study? What is the order of events?

To address the reviewer's concern, we performed Western blotting to monitor the level of H2AX ubiquitination at different time points after the MNNG treatment. As shown in Extended Data Fig. 3f, the H2AX ubiquitination was not detected until 2 hours post

treatment, which was different from the PARylation of CycT1 that was rapidly induced by MNNG and peaked at 30 min post treatment (Fig. 3c).

5. Throughout the manuscript, key information is missing in the in vitro droplet experiments.
- Protein concentration is a key determinant of phase separation but details on what protein concentration was used are not clear. Have the authors assayed condensate formation at different concentrations? Further, is the concentration used comparable with the concentration of the protein in a cell?

We thank the reviewer for raising this point and have added the information about protein concentrations used in the droplet formation experiments in relevant figure legends. It is well noted that proteins that can undergo LLPS are not evenly distributed in a cell. However, the traditional method to determine intracellular concentration of a protein usually only generate an “averaged” value that can be very different from its localized concentration inside phase-separated condensates. Furthermore, due to the presence of other protein or nucleic acid components in the condensates that may modulate phase separation, the concentration of a protein that is required to form liquid droplets in vitro in the absence of all the other modulators may be quite different from its concentration present in the condensates in vivo. In light of these considerations/limitations, we have not attempted to measure the averaged intracellular concentrations of CycT1 and PARP1 at this moment. Nevertheless, given that our droplet formation experiments produce results that closely match the observations obtained in living cells, we are confident about using this in vitro assay as a proxy for gaining mechanistic insights that are not easily obtained in vivo.

- Quantification of in vitro droplets is missing. For example, while there is a substantial difference between PARP1 vs PAR-PARP1 partition into CyCT1 droplets, a partition ratio is needed to provide a quantitative measurement.

Acting on the reviewer’s suggestion, we have provided the quantitative measurement in revised Figure 2i & 5f.

- Assays of homotypic droplet formation. The authors have previously demonstrated that CycT1 form homotypic droplets in Lu et al. Does PARP-1 or PAR-PARP1 form homotypic droplets at certain conditions?

To address this question, we performed droplet formation assay using mCherry-PARP1 and mCherry-PAR-PARP1. The result in Extended Data Fig. 1d showed that neither one was able to form homotypic droplets under the current experimental conditions. However, when the same concentration of PAR-PARP1 was mixed with WT GFP-CycT1-IDR, it promoted droplet formation by CycT1-IDR and became incorporated into the droplets (new Fig. 2i).

Minor points:

-Row 54. NELF is misspelled NEFL.
-Row 179. "CycT, but not CDK9" is repeated.

We thank the reviewer for pointing out these mistakes, which have been corrected in the revised manuscript.

Reviewer #3:
Remarks to the Author:
This study by Fu et al provides insights into the mechanisms through which DNA damage triggers genome wide transcriptional shutdown. The authors present evidence that this occurs via PARP1 dependent ADP-ribosylation of the P-TEFb subunit CycT1 HRD preventing CycT1 phase separation and Pol II phosphorylation by CDK9. The data included in this study represent a substantial body of work that is timely and quite convincing. I have listed some missing controls and several other suggestions to improve the manuscript.

1) Figure 5D & E – The authors provide extensive quantification of nuclear GFP-CycT1 punta following treatments with DNA-damaging agents which is highly convincing but does not provide any insight into the how dynamic this process is. Would it be possible to include a time-course experiment to follow the disassembly of phase-separated CycT1 nuclear speckles in the same cell(s) before and after DNA damage to provide

further evidence that disassembly of GFP-CycT1 puncta correlates timely with inhibition of global transcriptional elongation following DNA damage?

We thank the reviewer for giving us a chance to further improve our work. To facilitate the subsequent quantitative and statistical analysis, we performed a time-course immunofluorescence staining experiment to monitor the dynamic change of CycT1 puncta after MNNG treatment. The data in Extended Data Fig. 7f show that the MNNG-induced disassembly of CycT1 puncta began as early as 20 min post-treatment and became very evident at 40 min. Meanwhile, by performing individual 20-min 5-EU labeling in situ to measure RNA synthesis at different time points following the MNNG treatment, we found that the MNNG inhibition of transcription occurred in parallel with the disappearance of CycT1 puncta in cells (Extended Data Fig. 7d & 7e). These new results are consistent with our model that the MNNG-induced CycT1 PARylation disrupts P-TEFb phase separation to inhibit transcription upon DNA damage.

2) The authors show that CycT1 is PARylated following MNNG, H2O2 and IR but not following UV treatment concluding that PARylation of CycT1 is not involved in the UV-initiated NER pathway. Can the authors show that the UV treatment used in their experiments causes substantial DNA damage? This is currently not shown and therefore the conclusion that PARylation of CycT1 does not occur in UV-induced DNA damage is not fully supported.

In Extended Data Fig. 3b, we showed that the interaction between CDK9 and HEXIM1 was significantly disrupted by UV, which is a known phenomenon caused by UV irradiation. Notably, the same UV treatment condition failed to induce CycT1 PARylation as shown in Fig. 3b. To address the reviewer's concern and further demonstrate that the UV dose used in the experiment was able to cause substantial DNA damage, we performed anti-γH2AX Western blot analysis with lysates derived from the treated cells. The result in Extended Data Fig. 3c showed that the level of γH2AX, a widely used molecular marker of DNA damage, was significantly increased upon the treatment, indicating the accumulation of DNA damage in the cells.

3) Fig 3I & 3J & 3K – The HRD of CycT1 is required for interaction and ADP-ribosylation by PARP1. Is it possible that the HDR of CycT1 effects nuclear localisation as this would also explain the results as shown?

As we have reported previously, both WT CycT1 and CycT1-ΔHRD are localized in the nucleus, but the WT protein displays a punctate staining pattern, whereas the mutant does not (see Fig. 4c in Lu et al. *Nature*, 2018; **558**:318-323).

4) Fig 4H – Please provide a blot for HPF1.

We have added the HPF1 blot in revised Fig. 4h as suggested.

5) Fig 4 – It would be interesting to know whether the histidine ADP-ribosylation sites in CycT1 are dependent on HPF1.

We thank the reviewer for the suggestion. Although it is interesting to investigate whether the ADP-ribosylation of histidine residues depends on HPF1, we feel that the topic is beyond the scope of the current study. The direct PARylation of histidines is rarely reported, its regulation and implications, as well as a possible role of HPF1 in this process deserve a full-scale project that we are working on and will report the findings separately.

6) Size markers are included for some WBs but not all. Please provide these for all WB data.

We apologize for the omission and have made the recommended change in all revised figures.

7) Fig 5B – Please include LARP7 blot to confirm that it is knocked-down in this experiment.

We have included the LARP7 blot in Extended Data Fig. 6d as suggested.

8) Can the authors include sequence alignments showing all residues mutated in CycT1 Mut1 and Mut2.

Thanks for the suggestion. We have labeled the CycT1 residues altered in the two mutants in revised Extended Data Fig. 5b. In particular, CycT1-Mut1 was generated by changing the 12 MS-identified residues to alanines in the full-length CycT1 background,

with all the mutated residues shown in Figure 4f. In addition, the blue color labeled serines displayed in Extended Data Fig. 5b were further mutated to alanines, resulting in CycT1-Mut2.

9) There is a number of typos throughout the manuscript (eg. mCherry-PAR-PAPR1 on page 4).

We thank the reviewer for pointing out the mistakes, which have been corrected in the revised manuscript.

Decision Letter, first revision:

Subject: Decision on Nature Cell Biology submission NCB-Z45754A
Message:

*Please delete the link to your author homepage if you wish to forward this email to co-authors.

Dear Professor Zhou,

Your manuscript, "Poly(ADP-ribosylation) of P-TEFb by PARP1 disrupts phase separation to inhibit global transcription upon genotoxic stress", has now been seen by 3 of our original referees. As you will see from their comments (attached below) referee #1 raised some important points that we believe should be addressed before we can consider publication in Nature Cell Biology.

Nature Cell Biology editors discuss the referee reports in detail within the editorial team, including the chief editor, to identify key referee points that should be addressed with priority, and requests that are overruled as being beyond the scope of the current study. To guide the scope of the revisions, I have listed these points below. We are committed to providing a fair and constructive peer-review process, so please feel free to contact me if you would like to discuss any of the referee comments further.

In particular, it would be essential to:

a) compare conditions and different types of DNA damage to elucidate the specificity of PAR-regulated mechanisms for transcription inhibition:

1) The revised manuscript provides some evidence that NELF-E, NuRD, ATM and DNA-PK are not involved in MNNG-induced transcriptional inhibition. Rather, PARylation of CycT1 by PARP1 seems to

play a major role. In response to other types of DNA damage, however, the situation seems less clear. To avoid confusion, it would be very helpful to address whether transcriptional inhibition after base lesions, single and double strand breaks, respectively, is dependent on PARylation of CycT1 and/or on ATM, DNA-PK, NuRD and NELF-E. As previously mentioned, systems to investigate this exist (e.g. using CRISPR/Cas9 and the enzymes I-SceI, FokI, AsiSI) and could be used to test the generality of the findings and at the same time link the study better to related previous works. Such experiments should help to define the type(s) of lesions that trigger and depend on CycT1 PARylation for transcriptional inhibition. Currently, the authors use “genotoxic stress” (title) and “less bulky lesions” (abstract), which are rather vague terms.

Moreover, the survival experiments shown in Fig. 6 could easily be extended to other types of DNA damage to identify under which conditions the proposed mechanism is functionally relevant and under which ones not (see previous point).

b) conduct epistasis analysis between CycT1-Mut2 and PARP inhibition:

2) The epistasis analysis between CycT1-Mut2 and PARP loss or inhibition, suggested previously, was not conducted, but in theory has a potential to strengthen the functional aspects of the study.

c) All other referee concerns pertaining to clarifications and textual changes, should also be addressed.

d) Finally please pay close attention to our guidelines on statistical and methodological reporting (listed below) as failure to do so may delay the reconsideration of the revised manuscript. In particular please provide:

- a Supplementary Figure including unprocessed images of all gels/blots in the form of a multi-page pdf file. Please ensure that blots/gels are labeled and the sections presented in the figures are clearly indicated.

- a Supplementary Table including all numerical source data in Excel format, with data for different figures provided as different sheets within a single Excel file. The file should include source data giving rise to graphical representations and statistical descriptions in the paper and for all instances where the figures present representative experiments of multiple independent repeats, the source data of all repeats should be provided.

We therefore invite you to take these points into account when revising the manuscript. In addition, when preparing the revision please:

- ensure that it conforms to our format instructions and publication policies (see below and www.nature.com/nature/authors/).

- provide a point-by-point rebuttal to the full referee reports verbatim, as provided at the end of this letter.

- provide the completed Editorial Policy Checklist (found here https://www.nature.com/authors/policies/Policy.pdf),and Reporting Summary (found here https://www.nature.com/authors/policies/ReportingSummary.pdf). This is essential for reconsideration of the manuscript and these documents will be available to editors and referees in the event of peer review. For more information see http://www.nature.com/authors/policies/availability.html or contact me.

Nature Cell Biology is committed to improving transparency in authorship. As part of our efforts in this direction, we are now requesting that all authors identified as ‘corresponding author’ on published papers create and link their Open Researcher and Contributor Identifier (ORCID) with their account on the Manuscript Tracking System (MTS), prior to acceptance. ORCID helps the scientific community achieve unambiguous attribution of all scholarly contributions. You can create and link your ORCID from the home page of the MTS by clicking on ‘Modify my Springer Nature account’. For more information please visit please visit www.springernature.com/orcid.

Please submit the revised manuscript files and the point-by-point rebuttal to the referee comments using this link:

[REDACTED]

*This url links to your confidential home page and associated information about manuscripts you may have submitted or be reviewing for us. If you wish to forward this email to co-authors, please delete the link to your homepage.

We would like to receive the revision within four weeks. If submitted within this time period, reconsideration of the revised manuscript will not be affected by related studies published elsewhere, or accepted for publication in Nature Cell Biology in the meantime. We would be happy to consider a revision even after this timeframe, but in that case we will consider the published literature at the time of resubmission when assessing the file.

We hope that you will find our referees' comments, and editorial guidance helpful. Please do not hesitate to contact me if there is anything you would like to discuss.

Best wishes,

Jie Wang

Jie Wang, PhD
Senior Editor
Nature Cell Biology

Tel: +44 (0) 207 843 4924
email: jie.wang@nature.com

Reviewers' Comments:

Reviewer #1:
Remarks to the Author:
The authors present a thoroughly revised manuscript, in which several of my initial comments have been addressed. Previously missing controls are now included, and mechanistically it is now clearer how CycT1 gets recruited to sites of DNA damage by PARylation, and how PARylation disrupts CycT1 phase separation and inhibits transcriptional elongation. Most of the data seem unambiguous and, overall, several novel and interesting findings are presented by this work.

Remaining points:

1) The revised manuscript provides some evidence that NELF-E, NuRD, ATM and DNA-PK are not involved in MNNG-induced transcriptional inhibition. Rather, PARylation of CycT1 by PARP1 seems to play a major role. In response to other types of DNA damage, however, the situation seems less clear. To avoid confusion, it would be very helpful to address whether transcriptional inhibition after base lesions, single and double strand breaks, respectively, is dependent on PARylation of CycT1 and/or on ATM, DNA-PK, NuRD and NELF-E. As previously mentioned, systems to investigate this exist (e.g. using CRISPR/Cas9 and the enzymes I-SceI, FokI, AsiSI) and could be used to test the generality of the findings and at the same time link the study better to related previous works. Such experiments should help to define the type(s) of lesions that trigger and depend on CycT1 PARylation for transcriptional inhibition. Currently, the authors use “genotoxic stress” (title) and “less bulky lesions” (abstract), which are rather vague terms.

2) The epistasis analysis between CycT1-Mut2 and PARP loss or inhibition, suggested previously, was not conducted, but in theory has a potential to strengthen the functional aspects of the study. Moreover, the survival experiments shown in Fig. 6 could easily be extended to other types of DNA damage to identify under which conditions the proposed mechanism is functionally relevant and under which ones not (see previous point).

3) The newly added data reconcile how CycT1 is recruited to sites of DNA damage in a PAR-dependent manner (Fig. 1-2) and then covalently PARylated, which can disrupt CycT1 phase separation (Fig. 3-5 and new Extended Data Fig. 6i-j). This dual function of PARP1-mediated PARylation, which is consistent with and extends previous models on the regulation of phase separation at sites of DNA damage by PARP1 and PAR (electrostatic interaction between PAR and positively charged protein motifs, electrostatic repulsion upon modification with negatively charged PTMs), should be discussed in more depth together with the related previous literature, including PAR-regulated phase separation of FUS with its RGG repeats (seemingly playing a similar role as the CycT1 HRD).

Reviewer #2:
Remarks to the Author:
The study provides novel insights into the mechanism by which DNA damage results in global transcriptional silencing by demonstrating that PARP1 impedes Pol II elongation through condensate-associated inhibition of P-TEFb. The paper thus makes a substantial contribution to the field. The authors have fully addressed my prior concerns.

Reviewer #3:
Remarks to the Author:
The authors have addressed my concerns.

Author Rebuttal, first revision:

Our point-by-point response to the referees' comments

Reviewer #1:

Remarks to the Author:

The authors present a thoroughly revised manuscript, in which several of my initial comments have been addressed. Previously missing controls are now included, and

mechanistically it is now clearer how CycT1 gets recruited to sites of DNA damage by PARylation, and how PARylation disrupts CycT1 phase separation and inhibits transcriptional elongation. Most of the data seem unambiguous and, overall, several novel and interesting findings are presented by this work.

We thank the reviewer for carefully assessing our revised manuscript and are happy to know that his/her major concerns have been adequately addressed by our revisions and inclusion of new experiments.

Remaining points:
1) The revised manuscript provides some evidence that NELF-E, NuRD, ATM and DNA-PK are not involved in MNNG-induced transcriptional inhibition. Rather, PARylation of CycT1 by PARP1 seems to play a major role. In response to other types of DNA damage, however, the situation seems less clear. To avoid confusion, it would be very helpful to address whether transcriptional inhibition after base lesions, single and double strand breaks, respectively, is dependent on PARylation of CycT1 and/or on ATM, DNA-PK, NuRD and NELF-E. As previously mentioned, systems to investigate this exist (e.g. using CRISPR/Cas9 and the enzymes I-SceI, FokI, AsiSI) and could be used to test the generality of the findings and at the same time link the study better to related previous works. Such experiments should help to define the type(s) of lesions that trigger and depend on CycT1 PARylation for transcriptional inhibition. Currently, the authors use "genotoxic stress" (title) and "less bulky lesions" (abstract), which are rather vague terms.

Following the reviewer's suggestion, we investigated the possible involvement of the PAR-mediated mechanism in causing transcriptional inhibition at defined DSB loci in a FokI-mediated DSB reporter cell line developed by the Greenberg laboratory. We observed that the inhibition of PARP1 by AZD2281 failed to alleviate the suppression of reporter gene expression by the induced DSBs (New Extended Data Fig. 7e). As a control, the inhibition of ATM activity resulted in a significant recovery of reporter gene transcription in the presence of the DSBs, which agrees with the published reports (PMID: 20550933, 28336775, 33101843). This new result suggests that the PARP1-mediated CycT1 PARylation is probably not used by the endonuclease-mediated DSBs to inhibit transcription. This is similar to the situation involving UV irradiation as described in the current study. It is tempting to speculate that since both DSB and the major distortion of DNA double helix caused by UV can naturally block the forward

translocation of Pol II, the PARP1-CycT1 signaling probably does not play a major role in suppressing transcription by these two types of damage.

To eliminate the confusion caused by the use of vague terms in the title and abstract, we have revised the relevant texts to make the description more precise and accurate. Please see the places highlighted in red in the revised manuscript.

2) The epistasis analysis between CycT1-Mut2 and PARP loss or inhibition, suggested previously, was not conducted, but in theory has a potential to strengthen the functional aspects of the study.

Acting on the reviewer's recommendation, we performed the epistasis analysis between CycT1-Mut2 and PARP1 inhibition. The new result in Extended Data Fig. 9b & 9c indicate that in the absence of the PARP1 inhibitor AZD2281, the Mut2-expressing cells displayed an increased accumulation of γH2AX, a sensitive DNA damage marker, after the IR treatment compared to the WT CycT1-expressinng cells. However, pretreating cells with AZD2281 only allowed IR to further increase the γH2AX level in WT but not Mut2 cells, and that the γH2AX level in the Mut2 cells remained similarly high with or without the pretreatment. Together with the other data presented in the current study, this new result confirms that transcriptional inhibition after IR treatment serves to promote repair and cell survival, and this effect is primarily mediated through PARP1's modification of CycT1 and inhibition of P-TEFb phase separation.

Moreover, the survival experiments shown in Fig. 6 could easily be extended to other types of DNA damage to identify under which conditions the proposed mechanism is functionally relevant and under which ones not (see previous point).

Following the reviewer's suggestion, we examined the sensitivities of cells expressing WT or Mut2 CycT1 to UV irradiation and found that the Mut2-expressing cells displayed a similar viability upon exposure to UV compared to the WT CycT1-expressing cells (Extended Data Fig. 9a). In contrast, the Mut2 cells were more sensitive to the treatments by MNNG, H_2O_2, or IR than the WT cells (Fig. 6h-j). The new UV result is not surprising as the data in Fig. 3b already showed that the PARylation of CycT1 was not induced by UV. Together, our results suggest that the PARP1-CycT1 mechanism reported here is most likely not involved in the UV-induced DNA damage response.

3) The newly added data reconcile how CycT1 is recruited to sites of DNA damage in a PAR-dependent manner (Fig. 1-2) and then covalently PARylated, which can disrupt CycT1 phase separation (Fig. 3-5 and new Extended Data Fig. 6i-j). This dual function of PARP1-mediated PARylation, which is consistent with and extends previous models on the regulation of phase separation at sites of DNA damage by PARP1 and PAR (electrostatic interaction between PAR and positively charged protein motifs, electrostatic repulsion upon modification with negatively charged PTMs), should be discussed in more depth together with the related previous literature, including PAR-regulated phase separation of FUS with its RGG repeats (seemingly playing a similar role as the CycT1 HRD).

We thank the reviewer for the suggestion and have provided in-depth discussion about the sequence of events for PARP1-mediated PARylation of CycT1 in response to DNA damage together with the findings reported in the previous literature. These are highlighted in red in the revised manuscript.

Reviewer #2:
Remarks to the Author:
The study provides novel insights into the mechanism by which DNA damage results in global transcriptional silencing by demonstrating that PARP1 impedes Pol II elongation through condensate-associated inhibition of P-TEFb. The paper thus makes a substantial contribution to the field. The authors have fully addressed my prior concerns.

We thank the reviewer for providing constructive comments during the review and are happy to learn that all of his/her concerns have been adequately addressed by our revisions.

Reviewer #3:
Remarks to the Author:
The authors have addressed my concerns.

We thank the reviewer for providing constructive comments during the review and are happy to learn that all of his/her concerns have been adequately addressed by our revisions.

Decision Letter, second revision:

Subject: Your manuscript, NCB-Z45754B
Message: Our ref: NCB-Z45754B

6th January 2022

Dear Dr. Zhou,

Thank you for submitting your revised manuscript "Poly(ADP-ribosylation) of P-TEFb by PARP1 disrupts phase separation to inhibit global transcription upon DNA damage" (NCB-Z45754B). It has now been seen by the original referee 1 and the comments are below. The reviewer finds that the paper has improved in revision, and therefore we'll be happy in principle to publish it in Nature Cell Biology, pending minor revisions to comply with our editorial and formatting guidelines.

We are now performing detailed checks on your paper and will send you a checklist detailing our editorial and formatting requirements in about a week. Please do not upload the final materials and make any revisions until you receive this additional information from us.

Thank you again for your interest in Nature Cell Biology. Please do not hesitate to contact me if you have any questions.

Sincerely,
Jie

Jie Wang, PhD
Senior Editor
Nature Cell Biology

Tel: +44 (0) 207 843 4924
email: jie.wang@nature.com

Reviewer #1 (Remarks to the Author):

The authors have addressed my remaining concerns and the new data make it more clear upon which types of DNA damage the PARP-mediated transcriptional silencing through CycT1 PARylation and disrupted CycT1 condensation is functionally relevant. Both mechanistically and conceptually, the study makes a significant contribution to the field.

Decision Letter, final requests:

Subject: NCB: Your manuscript, NCB-Z45754B
Message: Our ref: NCB-Z45754B

16th January 2022

Dear Dr. Zhou,

Thank you for your patience as we've prepared the guidelines for final submission of your Nature Cell Biology manuscript, "Poly(ADP-ribosylation) of P-TEFb by PARP1 disrupts phase separation to inhibit global transcription upon DNA damage" (NCB-Z45754B). Please carefully follow the step-by-step instructions provided in the attached file, and add a response in each row of the table to indicate the changes that you have made. Ensuring that each point is addressed will help to ensure that your revised manuscript can be swiftly handed over to our production team.

We would like to start working on your revised paper, with all of the requested files and forms, as soon as possible (preferably within one week). Please get in contact with us if you anticipate delays.

When you upload your final materials, please include a point-by-point response to any remaining reviewer comments.

If you have not done so already, please alert us to any related manuscripts from your group that are under consideration or in press at other journals, or are being written up for submission to other journals (see: https://www.nature.com/nature-research/editorial-policies/plagiarism#policy-on-duplicate-publication for details).

In recognition of the time and expertise our reviewers provide to Nature Cell Biology's editorial process, we would like to formally acknowledge their contribution to the external peer review of your manuscript entitled "Poly(ADP-ribosylation) of P-TEFb by PARP1 disrupts phase separation to inhibit global transcription upon DNA damage". For those reviewers who give their assent, we will be publishing their names alongside the published article.

Nature Cell Biology offers a Transparent Peer Review option for new original research manuscripts submitted after December 1st, 2019. As part of this initiative, we encourage our authors to support increased transparency into the peer review process by agreeing to have the reviewer comments, author rebuttal letters, and editorial decision letters published as a Supplementary item. When you submit your final files please clearly state in your cover letter whether or not you would like to

participate in this initiative. Please note that failure to state your preference will result in delays in accepting your manuscript for publication.

Cover suggestions

As you prepare your final files we encourage you to consider whether you have any images or illustrations that may be appropriate for use on the cover of Nature Cell Biology.

Covers should be both aesthetically appealing and scientifically relevant, and should be supplied at the best quality available. Due to the prominence of these images, we do not generally select images featuring faces, children, text, graphs, schematic drawings, or collages on our covers.

We accept TIFF, JPEG, PNG or PSD file formats (a layered PSD file would be ideal), and the image should be at least 300ppi resolution (preferably 600-1200 ppi), in CMYK colour mode.

If your image is selected, we may also use it on the journal website as a banner image, and may need to make artistic alterations to fit our journal style.

Please submit your suggestions, clearly labeled, along with your final files. We'll be in touch if more information is needed.

Nature Cell Biology has now transitioned to a unified Rights Collection system which will allow our Author Services team to quickly and easily collect the rights and permissions required to publish your work. Approximately 10 days after your paper is formally accepted, you will receive an email in providing you with a link to complete the grant of rights. If your paper is eligible for Open Access, our Author Services team will also be in touch regarding any additional information that may be required to arrange payment for your article.

Please note that Nature Cell Biology is a Transformative Journal (TJ). Authors may publish their research with us through the traditional subscription access route or make their paper immediately open access through payment of an article-processing charge (APC). Authors will not be required to make a final decision about access to their article until it has been accepted. Find out more about Transformative Journals

Authors may need to take specific actions to achieve compliance with funder and institutional open access mandates. For submissions from January 2021, if your research is supported by a funder that requires immediate open access (e.g. according to Plan S principles) then you should select the gold OA route, and we will direct you to the compliant route where possible. For authors selecting the

subscription publication route our standard licensing terms will need to be accepted, including our self-archiving policies. Those standard licensing terms will supersede any other terms that the author or any third party may assert apply to any version of the manuscript.

Please note that you will not receive your proofs until the publishing agreement has been received through our system.

For information regarding our different publishing models please see our Transformative Journals page. If you have any questions about costs, Open Access requirements, or our legal forms, please contact ASJournals@springernature.com.

Please use the following link for uploading these materials:
[REDACTED]

If you have any further questions, please feel free to contact me.

Best regards,

Ziqian Li
Editorial Assistant
Nature Cell Biology

On behalf of

Jie Wang, PhD
Senior Editor
Nature Cell Biology

Tel: +44 (0) 207 843 4924
email: jie.wang@nature.com

Reviewer #1:
Remarks to the Author:
The authors have addressed my remaining concerns and the new data make it more clear upon which types of DNA damage the PARP-mediated transcriptional silencing through CycT1 PARylation and

disrupted CycT1 condensation is functionally relevant. Both mechanistically and conceptually, the study makes a significant contribution to the field.

Author Rebuttal, second revision:

Our point-by-point response to the referees' comments

Reviewer #1:

Remarks to the Author:

The authors have addressed my remaining concerns and the new data make it more clear upon which types of DNA damage the PARP-mediated transcriptional silencing through CycT1 PARylation and disrupted CycT1 condensation is functionally relevant. Both mechanistically and conceptually, the study makes a significant contribution to the field.

We thank the reviewer for providing constructive comments during the review and are happy to learn that all of his/her concerns have been adequately addressed by our revisions.

Final Decision Letter:

Subject: Decision on Nature Cell Biology submission NCB-Z45754C
Message:

Dear Dr Zhou,

I am pleased to inform you that your manuscript, "Poly(ADP-ribosylation) of P-TEFb by PARP1 disrupts phase separation to inhibit global transcription upon DNA damage", has now been accepted for publication in Nature Cell Biology.

Thank you for sending us the final manuscript files to be processed for print and online production, and for returning the manuscript checklists and other forms. Your manuscript will now be passed to our production team who will be in contact with you if there are any questions with the production quality of supplied figures and text.

Over the next few weeks, your paper will be copyedited to ensure that it conforms to Nature Cell Biology style. Once your paper is typeset, you will receive an email with a link to choose the appropriate

publishing options for your paper and our Author Services team will be in touch regarding any additional information that may be required.

After the grant of rights is completed, you will receive a link to your electronic proof via email with a request to make any corrections within 48 hours. If, when you receive your proof, you cannot meet this deadline, please inform us at rjsproduction@springernature.com immediately.

You will not receive your proofs until the publishing agreement has been received through our system.

Due to the importance of these deadlines, we ask that you please let us know now whether you will be difficult to contact over the next month. If this is the case, we ask you provide us with the contact information (email, phone and fax) of someone who will be able to check the proofs on your behalf, and who will be available to address any last-minute problems.

If you have any questions about our publishing options, costs, Open Access requirements, or our legal forms, please contact ASJournals@springernature.com

Once your paper has been scheduled for online publication, the Nature press office will be in touch to confirm the details. An online order form for reprints of your paper is available at https://www.nature.com/reprints/author-reprints.html. All co-authors, authors' institutions and authors' funding agencies can order reprints using the form appropriate to their geographical region.

Publication is conditional on the manuscript not being published elsewhere and on there being no announcement of this work to any media outlet until the online publication date in Nature Cell Biology.

Please note that Nature Cell Biology is a Transformative Journal (TJ). Authors may publish their research with us through the traditional subscription access route or make their paper immediately open access through payment of an article-processing charge (APC). Authors will not be required to make a final decision about access to their article until it has been accepted. Find out more about Transformative Journals

Authors may need to take specific actions to achieve compliance with funder and institutional open access mandates. For submissions from January 2021, if your research is supported by a funder that requires immediate open access (e.g. according to Plan S principles) then you should select the gold OA route, and we will direct you to the compliant route where possible. For authors selecting the subscription publication route our standard licensing terms will need to be accepted, including our self-archiving policies. Those standard licensing terms will supersede any other terms that the author or any third party may assert apply to any version of the manuscript.

To assist our authors in disseminating their research to the broader community, our SharedIt initiative provides you with a unique shareable link that will allow anyone (with or without a subscription) to read the published article. Recipients of the link with a subscription will also be able to download and print the PDF.

If your paper includes color figures, please be aware that in order to help cover some of the additional cost of four-color reproduction, Nature Research charges our authors a fee for the printing of their color figures. Please contact our offices for exact pricing and details.

As soon as your article is published, you will receive an automated email with your shareable link.

If you have not already done so, we strongly recommend that you upload the step-by-step protocols used in this manuscript to the Protocol Exchange (www.nature.com/protocolexchange), an open online resource established by Nature Protocols that allows researchers to share their detailed experimental know-how. All uploaded protocols are made freely available, assigned DOIs for ease of citation and are fully searchable through nature.com. Protocols and the Nature and Nature research journal papers in which they are used can be linked to one another, and this link is clearly and prominently visible in the online versions of both papers. Authors who performed the specific experiments can act as primary authors for the Protocol as they will be best placed to share the methodology details, but the Corresponding Author of the present research paper should be included as one of the authors. By uploading your Protocols to Protocol Exchange, you are enabling researchers to more readily reproduce or adapt the methodology you use, as well as increasing the visibility of your protocols and papers. You can also establish a dedicated page to collect your lab Protocols. Further information can be found at www.nature.com/protocolexchange/about

You can use a single sign-on for all your accounts, view the status of all your manuscript submissions and reviews, access usage statistics for your published articles and download a record of your refereeing activity for the Nature journals.

Please feel free to contact us if you have any questions.

With kind regards,

Jie Wang, PhD
Senior Editor
Nature Cell Biology

Tel: +44 (0) 207 843 4924
email: jie.wang@nature.com