

# The stress granule protein G3BP1 promotes pre-condensation of cGAS to allow a fast response to DNA

Ming Zhao, Tian Xia, Jia-Qing Xing, Le-Hua Yin, Xiao-Wei Li, Jie Pan, Jia-Yu Liu, Li-Ming Sun, Miao Wang, Tingting Li, Jie Mao, Qiuying Han, Wen Xue, Hong Cai, Kai Wang, Xin Xu, Teng Li, Kun He, Na Wang, Ai-Ling Li, Tao Zhou, Xuemin Zhang, Weihua Li, and Tao Li

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Corresponding author(s): Tao Li ([tli@ncba.ac.cn](mailto:tli@ncba.ac.cn)), Weihua Li ([whli@ncba.ac.cn](mailto:whli@ncba.ac.cn))

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## Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

Dear Prof. Li,

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

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

Given the constructive referee comments, we would like to invite you to revise your manuscript with the understanding that all referee concerns must be addressed in the revised manuscript or in the detailed point-by-point response. Acceptance of your manuscript will depend on a positive outcome of a second round of review. It is EMBO reports policy to allow a single round of revision only and acceptance of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript.

Revised manuscripts should be submitted within three months of a request for revision. We are aware that many laboratories cannot function at full efficiency during the current COVID-19/SARS-CoV-2 pandemic and we have therefore extended our 'scooping protection policy' to cover the period required for full revision. Please contact me to discuss the revision should you need additional time, and also if you see a paper with related content published elsewhere.

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

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

When submitting your revised manuscript, we will require:

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

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

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

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5) that primary datasets produced in this study (e.g. RNA-seq, ChIP-seq, structural and array data) are deposited in an

appropriate public database. If no primary datasets have been deposited, please also state this a dedicated section (e.g. 'No primary datasets have been generated and deposited'), see below.

See also: <http://embor.embopress.org/authorguide#datadeposition>

Please remember to provide a reviewer password if the datasets are not yet public.

The accession numbers and database should be listed in a formal "Data Availability " section (placed after Materials & Methods) that follows the model below. This is now mandatory (like the COI statement). Please note that the Data Availability Section is restricted to new primary data that are part of this study.

# Data availability

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

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

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

Moreover, I have these editorial requests:

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

7) Our journal encourages inclusion of \*data citations in the reference list\* to directly cite datasets that were re-used and obtained from public databases. Data citations in the article text are distinct from normal bibliographical citations and should directly link to the database records from which the data can be accessed. In the main text, data citations are formatted as follows: "Data ref: Smith et al, 2001" or "Data ref: NCBI Sequence Read Archive PRJNA342805, 2017". In the Reference list, data citations must be labeled with "[DATASET]". A data reference must provide the database name, accession number/identifiers and a resolvable link to the landing page from which the data can be accessed at the end of the reference. Further instructions are available at: <http://www.embopress.org/page/journal/14693178/authorguide#referencesformat>

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

9) Please also note our new reference format:  
<http://www.embopress.org/page/journal/14693178/authorguide#referencesformat>

10) Please provide the abstract written in present tense.

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

Finally, please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript. Please find instructions on how to link the ORCID ID to the account in our manuscript tracking system in our Author guidelines: <http://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines>

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

Kind regards,

Achim Breiling  
Editor  
EMBO Reports

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Referee #1:

The manuscript „G3BP1 engages cGAS in a primary condensation state to enable it for an expeditious response to DNA" describes molecular fundamentals of the interaction between the DNA sensor cGAS and its regulator G3BP1. The authors show that G3BP1's previously described positive effect on cGAS activation is based on condensation of cGAS and G3BP1 into liquid-phase separated droplets. This facilitates the subsequent DNA-induced phase separation and enzymatic activation of cGAS.

Regulation of the DNA sensor cGAS is a highly topical area of investigation. Furthermore, the physico-chemical principle of liquid-liquid phase separation has emerged recently as a key phenomenon in the regulation of a multitude of cellular signalling pathways. The work presented here is thus situated in a busy field of research and will be of interest to many cell biology researchers. cGAS is emerging in the pathophysiology of many sterile conditions and has become a pharmaceutical target. This further extends the potential readership of this manuscript.

The data in this manuscript generally well-support the claims made by the authors and experiments are well-executed. The authors show a substantial amount of high-quality data. While the findings are generally well-described to the reader, the text would benefit from grammatical proof-reading to ease understanding in certain instances.

Major points:

- The authors claim that while DNA induces the dissociation of G3BP1 from cGAS, RNA does not (Figure 3E, 3F, EV2D). They reference the seminal work by Du & Chen (Science 2018) who showed that both RNA and DNA can phase-separate with cGAS, but only DNA leads to enzymatic activation. Importantly, these experiments compared double-stranded RNA with dsDNA, while the experiments described in this manuscript used a single-stranded RNA oligo. Although single-stranded nucleic acids can per se interact with cGAS, the strength of interaction and the ability of ssDNA to activate the enzyme is drastically reduced compared to dsDNA. If the authors wish to support their claims regarding G3BP1 and RNA, the experiments need to be repeated using dsRNA. This is important in particular since dsRNA can intrinsically prevent cGAS activation (Xia et al., Immunity 2018).

Minor points:

- The methods section of this manuscript describes single-step Ni agarose purification of recombinant proteins used in this study. To avoid contamination with bacterial nucleic acids, recombinant proteins are often purified using heparin affinity chromatography or treated with a nuclease during purification. Although unlikely (since G3BP1 itself does not bind DNA (Liu et al., Nature Immunology 2019)), it is possible that the phase separation of cGAS and G3BP1 in the absence of DNA is caused by nucleic acids co-purified with G3BP1. The authors therefore should provide a more detailed description of their protein purification (if further purification and/or nuclease treatment was done) or should show a control experiment addressing this, for example phase separation in the presence of benzonase.
- Immunofluorescence analysis in Figures 2A and EV1D shows that after genetic ablation of G3BP1, cGAS appears to be predominantly localised in the nucleus (in comparison to WT cells). Intriguingly, this phenomenon seems much less pronounced in Figures 2B, EV1E. Given the ever-increasing body of research that describes significant roles of nuclear cGAS, the authors should indicate whether this observation was robust throughout their experiments and discuss possible functional implications.
- The authors should clarify the wording in the figure legends as to whether pooled results from independent repeats, or technical triplicates from one representative experiment are shown.

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Referee #2:

In this study, Zhao et al. investigate further the mechanism through which G3BP1 potentiates sensing of DNA by cGAS, which they discovered recently. The authors demonstrate that G3BP1 potentiates the formation of Liquid liquid Phase Separation (LLPS) state which is essential for cGAS activation. As such, in the presence of G3BP1, cGAS sensing of DNA was potentiated which correlated with increased cGAS droplets, and led to increased cGAMP synthesis. The engagement of DNA was associated with dissociation of the cGAS-G3BP1 complexes (and full length G3BP1 was essential for LLPS formation). The mechanism of action of G3BP1 potentiation of LLPS was distinct from that of Zn<sup>2+</sup>, and together showed an additive effect for LLPS and cGAMP formation. Finally the authors showed that EGCG, which inhibits G3BP1, blunted the effect of G3BP1 on LLPS enhanced formation with cGAS - leading to decreased cGAMP. Collectively this is a very thorough study, and only a few things need to be clarified to increase its impact.

Main points:

1- This reviewer appreciates that the authors have tried to adhere to the short length of the journal, but feels that the results

should be expanded to better describe the figures (although this should be discussed with the Editor). For some figures, it is not clear what they really bring to the paper (and since this is not detailed in the results section, their impact is lost).

2- The data shown in Figure 2A-C is not very convincing as for the number and size of the puncta. This matters as this relates to the biological significance of the findings made here.

In fact, it seems quite hard to get such a p value in t-tests in C with so much overlap between the groups (this reviewer wants further clarification on the stats used - see below). One thing which seems clear is that G3BP1 loss rather leads to a more nuclear localisation of cGAS (a similar trend is seen in HeLa cells). Since cGAS activity in the nucleus is impacted by its interaction with histones, it seems hard to explain the effect on cGAMP synthesis and signalling with the only effect of LLPS. Please discuss this. In addition, using U937 and HeLa cells is not great to look at steady state interactions since these cells may exhibit low level of basal cytoplasmic DNA - being cancer cells. It would be more logical to use primary cells like primary macrophages from G3BP1<sup>-/-</sup> mice. Alternatively the authors could use cells that have known basal levels of cytosolic DNA, such as TREG1 deficient cells, additionally lacking G3BP1 (to confirm that loss of G3BP1 impacts puncta size or number "in vivo").

3) EGCG is likely to have a lot of off-target effects beyond inhibition of G3BP1 shown in vitro. The data in Figure 7H/I should be complemented with experiments in G3BP1<sup>-/-</sup> cells treated with EGCG to confirm that the read out is not impacted independently of G3BP1 (there should still be a response visible by RTqPCR in G3BP1<sup>-/-</sup> cells based on figure 4G).

4- Could the authors provide more details in the discussion about the cell types/tissues that express G3BP1? How frequent is co-expression with cGAS? This is important to mention to underline the biological relevance of these findings.

Minor points:

1- Statistical analyses on a single experiment conducted with biological triplicate do not have any value as there is no independent sampling (it seems that ALL the data shown - in bar graphs - is only representative data, i.e. from single experiments). Please average the data from your independent experiments, where possible, rather than only showing representative data. Remove the statistics if only single experiments are shown. Also, please be consistent with the use of the "dots" in the bar graphs. This reviewer assumes they represent biological/technical replicate, but this is not clear (some bar graphs have no points...).

2- Lipofectamine 2000 is not from Invivogen (you probably meant invitrogen).

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Referee #3:

In this manuscript, Ming Zhao et al. described the gel-like condensate formed by cGAS and G3BP1. Using biochemical approaches and high-resolution microscopy, they showed that G3BP1 engaged cGAS in a pre-activation condensation state to enhance the cGAS-DNA LLPS and potentially promote the expeditious DNA sensing by cGAS. Overall, this is a very interesting study and discovers a new factor of the cGAS phase separation, which has merged as a critical regulating mechanism of cGAS activation. However, there are a few comments to be addressed by the authors to further improve the quality of this manuscript.

Major points:

(1) According to the protein purification section (line 315-317), the authors only applied Ni-NTA affinity column to purify cGAS and G3BP1 proteins. Considering both cGAS and G3BP1 were reported to have strong nucleic acid binding abilities, the cGAS and G3BP1 protein samples eluted from Ni-NTA were very likely to have DNA/RNA contaminants to influence the in vitro phase separation assay results. The authors may need to improve the purification method and double-check the sample purity (i.e., examine the A280/A260 ratio of the samples).

(2) What are the in cell concentrations of the G3BP1/cGAS to justify physiological concentration? It will be better if the phase separation diagram of cGAS-G3BP1 is provided.

(3) in line 140, the authors may not claim that the puncta observed here are the G3BP1-engaged "liquid-like structure of cGAS" due to the lack of FRAP data in vivo. I suggest that they may need to add the FRAP data of G3BP1-cGAS puncta in cells if it is technically available.

Minor points:

(1) in line 124, the authors may highlight the length of dsDNA used in the assay considering the cGAS and dsDNA trigger LLPS in a DNA length-dependent manner.

(2) in line 132, it seems to me that the cGAS-G3BP1 undergoes the gel-like instead of "LLPS-like" transitions.

(3) in figure 1C right panel, the 8  $\mu$ M G3BP1 showed a significant (8-fold?) enhancement of total area than that of 4  $\mu$ M G3BP1.

Could authors comment on this observation? In the figure caption (line 628), the "total area of droplets" could be updated to "total area of condensates".

(4) in Figure 2C, does the cGAS puncta number per cell include the nuclear cGAS puncta or just cytoplasm cGAS puncta?

(5) in line 71, "allow" should be "allows".

(6) in line 160, "amount" should be "amounts".

(7) in line 179, "in consistent with" should be "being consistent with".

## Point-by-Point Response:

### Reviewer #1:

*The manuscript "G3BP1 engages cGAS in a primary condensation state to enable it for an expeditious response to DNA" describes molecular fundamentals of the interaction between the DNA sensor cGAS and its regulator G3BP1. The authors show that G3BP1's previously described positive effect on cGAS activation is based on condensation of cGAS and G3BP1 into liquid-phase separated droplets. This facilitates the subsequent DNA-induced phase separation and enzymatic activation of cGAS.*

*Regulation of the DNA sensor cGAS is a highly topical area of investigation. Furthermore, the physical-chemical principle of liquid-liquid phase separation has emerged recently as a key phenomenon in the regulation of a multitude of cellular signaling pathways. The work presented here is thus situated in a busy field of research and will be of interest to many cell biology researchers. cGAS is emerging in the pathophysiology of many sterile conditions and has become a pharmaceutical target. This further extends the potential readership of this manuscript.*

*The data in this manuscript generally well-support the claims made by the authors and experiments are well-executed. The authors show a substantial amount of high-quality data. While the findings are generally well-described to the reader, the text would benefit from grammatical proof-reading to ease understanding in certain instances.*

**Response:** The reviewer indicated that the conclusion of our study is well supported by the data and the experiments were well-executed. We greatly appreciate the reviewer's encouraging comments. We also thank the reviewer for his/her important suggestions to further improve our work. Following these suggestions, we carried out additional experiments and revised our manuscript accordingly. As detailed below, point-by-point, we can address the concerns with new data and discussions.

### **Major points:**

*1 - The authors claim that while DNA induces the dissociation of G3BP1 from cGAS, RNA does not (Figure 3E, 3F, EV2D). They reference the seminal work by Du & Chen (Science 2018) who showed that both RNA and DNA can phase-separate with cGAS,*

*but only DNA leads to enzymatic activation. Importantly, these experiments compared double-stranded RNA with dsDNA, while the experiments described in this manuscript used a single-stranded RNA oligo. Although single-stranded nucleic acids can per se interact with cGAS, the strength of interaction and the ability of ssDNA to activate the enzyme is drastically reduced compared to dsDNA. If the authors wish to support their claims regarding G3BP1 and RNA, the experiments need to be repeated using dsRNA. This is important in particular since dsRNA can intrinsically prevent cGAS activation (Xia et al., Immunity 2018).*

**Response:** We thank the reviewer for this valuable suggestion. Following this suggestion, we performed the assay using dsRNA. We found that, similar to ssRNA, dsRNA also formed condensates with cGAS and G3BP1 and the addition of dsRNA did not trigger the disassociation of G3BP1 from cGAS (Figure 3E, 3F and Figure EV3D in our revised manuscript).

As the Reviewer mentioned, a previous report showed that dsRNA (cia-cGAS) binds and prevents the activation of cGAS in nucleus (*Immunity*, 2018, PMID: 29625897). The inhibition of cGAS activation in nucleus is an actively pursued topic in the field. Although being a cytoplasmic protein, G3BP1 may not directly regulate the cGAS activation in the nucleus, it would be interesting for future study to investigate whether LLPS is involved in the dsRNA-mediated cGAS inhibition in the nucleus. We added this discussion and referenced the above paper in our revised manuscript.

**Minor points:**

*1- The methods section of this manuscript describes single-step Ni agarose purification of recombinant proteins used in this study. To avoid contamination with bacterial nucleic acids, recombinant proteins are often purified using heparin affinity chromatography or treated with a nuclease during purification. Although unlikely (since G3BP1 itself does not bind DNA (Liu et al., Nature Immunology 2019)), it is possible that the phase separation of cGAS and G3BP1 in the absence of DNA is caused by nucleic acids co-purified with G3BP1. The authors therefore should provide a more detailed description of their protein purification (if further purification and/or nuclease treatment was done) or should show a control experiment addressing this, for example phase separation in the presence of benzonase.*

**Response:** We greatly appreciate the reviewer's suggestion. In our study, we



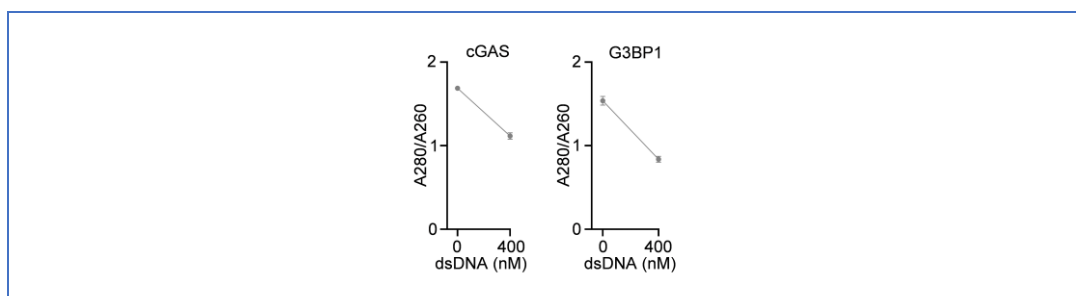
purified the proteins with a single-step Ni-agarose purification. Following to the reviewer's suggestion, we further purified the recombinant cGAS and G3BP1 proteins using heparin affinity chromatography following the Ni-agarose purification. With the newly purified proteins, we obtained similar results. Our new data consistently showed that G3BP1 formed condensates with cGAS (Figure EV1A in our revised manuscript) and promoted DNA-triggered LLPS of cGAS (Figure EV4F-G in our revised manuscript).

In addition, as Reviewer #3 also suggested, we examined the A280/A260 ratio of our one-step (Ni-agarose) purified proteins to estimate the nucleic acid contamination level. As shown in Table R\_1 below, using commercial BSA protein as control, we found that our protein samples showed similar A280/A260 ratio.

Protein	Conc. (mg/ml)	A280	A260/A280	A280/A260
BSA	12.369	12.369	0.591	1.69047377
G3BP1-mEGFP	10.796	10.796	0.633	1.579778831
cGAS-mCherry	3.845	3.845	0.578	1.730103806
G3BP1	5.17	5.17	0.684	1.461988304
cGAS	2.956	2.956	0.572	1.748251748

**Table R\_1** A280/A260 of indicated protein samples.

Further, we added dsDNAs, at a final concentration of 400 nM, into 10  $\mu$ M cGAS or 10  $\mu$ M G3BP1 solutions to mimic a nucleic acid contamination (approximately 4 DNA molecules per 100 protein molecules). By measuring the A280/A260 ratio of these samples, we found that the addition of dsDNA remarkably reduced the value of A280/A260 ratio to 1 or less than 1 (Figure R\_1). These results suggested that the nucleic acid contamination level of our original Ni-agarose-purified recombinant proteins was marginal. Actually, according to previous publications (*CRC Crit Rev Biochem* 1986, PMID: 3512164; *Nucleic Acids Res* 2010, PMID: 20497998), high-salt condition can limit the nuclei acid-protein binding, we used high-salt buffer (1 M NaCl) during our purification to reduce the residual nuclei acid in our purified recombinant proteins.



**Figure R\_1** A280/A260 of cGAS (10  $\mu$ M) or G3BP1 (10  $\mu$ M) mixed with dsDNA (400 nM).

*2- Immunofluorescence analysis in Figures 2A and EV1D shows that after genetic ablation of G3BP1, cGAS appears to be predominantly localized in the nucleus (in comparison to WT cells). Intriguingly, this phenomenon seems much less pronounced in Figures 2B, EV1E. Given the ever-increasing body of research that describes significant roles of nuclear cGAS, the authors should indicate whether this observation was robust throughout their experiments and discuss possible functional implications.*

**Response:** The reviewer indicated that our original Figures 2A and EV1D showed that in G3BP1-deficient cells, cGAS appears to be predominantly localized in the nucleus. To address this point, we first isolated the cytosolic and nuclear fractions from both WT and G3BP1-deficient cells to detect the cGAS expression in each fraction. Our data showed that G3BP1 deficiency did not obviously affect the localization of cGAS protein (Figure 2D and Figure EV1J in our revised manuscript).

We further analyzed the original Figures 2B and EV1E, in which only the merged channels were shown. When the nucleus channels and cGAS channels were shown separately, these data exhibited the similar cGAS expression pattern as original Figures 2A and EV1D. These data are shown as Figure 2B and Figure EV1H in our revised manuscript.

Thus, our data show that G3BP1 engages cGAS in a primary condensation state to enable it for an expeditious response to DNA. The ablation of G3BP1 resulted in the disorganization of cGAS primary condensation in cytoplasm and impaired cGAS activation upon DNA treatment. Because G3BP1 is a cytoplasm-localized protein, it is likely that G3BP1 mainly regulates the activation of cytosolic cGAS. As the reviewer indicated that a growing number of publications describe the significant roles of nuclear cGAS, it will be very interesting to study whether LLPS is also involve in the activity regulation of cGAS in the nucleus. We included these discussions and added several references in our revised manuscript.

*3- The authors should clarify the wording in the figure legends as to whether pooled results from independent repeats, or technical triplicates from one representative experiment are shown.*

**Response:** We apologize for our unclear description regarding our data presentation. Following the reviewer's suggestion, we clarified the figure legends for each data and clearly described biological repeats vs. technical repeats. In addition, we also revised our figures by showing 'dots' whenever possible to indicate each data point from the independent biological repeats (Figures 1 A, 1C, 1G, 1I, 2C, 2E, 2F, 4C, 4F, 5E, 6A, 6C, 6D, 6E, 7A, 7D, 7H, 7I, and Figure EV1A, EV1I, EV2C, EV3A, EV3C, EV3D, EV3H, EV4C, EV4E, EV4G, EV5C and EV5D in our revised manuscript).

## Referee #2:

*In this study, Zhao et al. investigate further the mechanism through which G3BP1 potentiates sensing of DNA by cGAS, which they discovered recently. The authors demonstrate that G3BP1 potentiates the formation of Liquid-liquid Phase Separation (LLPS) state which is essential for cGAS activation. As such, in the presence of G3BP1, cGAS sensing of DNA was potentiated which correlated with increased cGAS droplets and led to increased cGAMP synthesis. The engagement of DNA was associated with dissociation of the cGAS-G3BP1 complexes (and full length G3BP1 was essential for LLPS formation). The mechanism of action of G3BP1 potentiation of LLPS was distinct from that of Zn<sup>2+</sup>, and together showed an additive effect for LLPS and cGAMP formation. Finally, the authors showed that EGCG, which inhibits G3BP1, blunted the effect of G3BP1 on LLPS enhanced formation with cGAS - leading to decreased cGAMP.*

*Collectively this is a very thorough study, and only a few things need to be clarified to increase its impact.*

**Response:** The reviewer pointed out that our work is a very thorough study. We thank the reviewer for this encouraging comment. As detailed below, point-by-point, we performed additional experiments and revised our manuscript according to the suggestions of the reviewer.

### **Main points:**

*1- This reviewer appreciates that the authors have tried to adhere to the short length of the journal but feels that the results should be expanded to better describe the figures (although this should be discussed with the Editor). For some figures, it is not clear what they really bring to the paper (and since this is not detailed in the results section, their impact is lost).*

**Response:** We thank the reviewer for this important suggestion. In the revised manuscript, we expanded our **Results** section to better describe the figures. We also added more discussions and references according to the suggestions by all the reviewers.

*2- The data shown in Figure 2A-C is not very convincing as for the number and size of the puncta. This matters as this relates to the biological significance of the findings made here. In fact, it seems quite hard to get such a p value in t-tests in C with so much overlap between the groups (this reviewer wants further clarification on the*

stats used - see below).

One thing which seems clear is that G3BP1 loss rather leads to a more nuclear localization of cGAS (a similar trend is seen in HeLa cells). Since cGAS activity in the nucleus is impacted by its interaction with histones, it seems hard to explain the effect on cGAMP synthesis and signaling with the only effect of LLPS. Please discuss this.

In addition, using U937 and HeLa cells is not great to look at steady state interactions since these cells may exhibit low level of basal cytoplasmic DNA - being cancer cells. It would be more logical to use primary cells like primary macrophages from G3BP1<sup>-/-</sup> mice. Alternatively, the authors could use cells that have known basal levels of cytosolic DNA, such as TREX1 deficient cells, additionally lacking G3BP1 (to confirm that loss of G3BP1 impacts puncta size or number "in vivo").

**Response:** We thank the reviewer for these important points.

-- Regarding the statistical analysis for our original Figures 2A-C, the *P* values were calculated using GraphPad Prism 8.0.2. An unpaired two-tailed *t* test with Welch's correction were used to obtain the results. We provided the detailed analysis report (Figure R\_2) for Figure 2C. As indicated in the report, the *P* values for the puncta number data and the puncta volume data were both < 0.0001. We also included the raw data of all the statistical results in the revised manuscript (Source Data of our revised manuscript).

Puncta number		Puncta volume	
Table Analyzed	G3BP1 <sup>-/-</sup>	Table Analyzed	G3BP1 <sup>-/-</sup>
Column B	G3BP1 <sup>-/-</sup>	Column B	G3BP1 <sup>-/-</sup>
vs.	vs.	vs.	vs.
Column A	WT	Column A	WT
<b>Unpaired t test with Welch's correction</b>		<b>Unpaired t test with Welch's correction</b>	
P value	<0.0001	P value	<0.0001
P value summary	****	P value summary	****
Significantly different (P < 0.05)?	Yes	Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed	One- or two-tailed P value?	Two-tailed
Welch-corrected t, df	t=5.996, df=85.83	Welch-corrected t, df	t=5.270, df=79.62
<b>How big is the difference?</b>		<b>How big is the difference?</b>	
Mean of column A	369.3	Mean of column A	11.13
Mean of column B	215.5	Mean of column B	5.543
Difference between means (B - A) ± SEM	-153.8 ± 25.65	Difference between means (B - A) ± SEM	-5.591 ± 1.061
95% confidence interval	-204.7 to -102.8	95% confidence interval	-7.703 to -3.480
R squared (eta squared)	0.2952	R squared (eta squared)	0.2586
<b>F test to compare variances</b>		<b>F test to compare variances</b>	
F, DFn, Dfd	2.369, 50, 50	F, DFn, Dfd	3.048, 50, 50
P value	0.0028	P value	0.0001
P value summary	**	P value summary	***
Significantly different (P < 0.05)?	Yes	Significantly different (P < 0.05)?	Yes
<b>Data analyzed</b>		<b>Data analyzed</b>	
Sample size, column A	51	Sample size, column A	51
Sample size, column B	51	Sample size, column B	51

**Figure R\_2** Statistical analysis report of Figure 2C by GraphPad Prism 8.0.2.

-- Reviewer #1 also mentioned that G3BP1 loss rather leads to a more

nuclear localization of cGAS. To address this point, we isolated the cytosolic and nuclear fractions from both WT and G3BP1-deficient cells to detect the cGAS expression in each fraction. Our data showed that G3BP1 deficiency did not obviously affect the localization of cGAS protein (Figure 2D and Figure EV1J in our revised manuscript).

Although a number of recent publications described the inhibitory mechanisms of nucleic-localized cGAS, our data suggested that G3BP1 deficiency did not result in the translocation of cGAS to nucleus. Therefore, the inhibitory mechanism in the nucleus may not contribute to the overall cGAS inhibition when G3BP1 is deleted. We found that the ablation of G3BP1, a cytoplasm-localized protein, led to the disorganization of cGAS primary condensation state and thereby suppressing cGAS activation upon DNA treatment. These data further indicated that the G3BP1-engaged cGAS primary condensation state in cytoplasm is critical for cGAS activation. We added discussions and several references regarding this point in our revised manuscript.

-- The reviewer also raised the concern that as cancer cells, U937 and HeLa may exhibit low level of basal cytoplasmic DNA and these DNAs may affect our results. Accordingly, we detected the cytoplasmic dsDNA levels in HeLa cells and U937 cells using anti-dsDNA antibodies. We also included a human fibroblast cell line, Hs27. By performing immunofluorescence staining, we found that HeLa cells exhibited high levels of cytosolic dsDNA. In U937 cells, the cytosolic dsDNA levels were detectable but relatively lower. In contrast, we did not detect cytosolic dsDNA in our Hs27 cells (Figure EV2A in our revised manuscript).

The reviewer suggested us to use the primary macrophages from *G3bp1*<sup>-/-</sup> mice, however, the G3BP1 deficiency is lethal in mice. We therefore used the above-mentioned human fibroblast cells, Hs27, to confirm our findings. Consistent with our data from U937 and HeLa cells, we found that the deficiency of G3BP1 significantly affected cytosolic cGAS condensation formation in Hs27 cells (Figure EV2B-D in our revised manuscript).

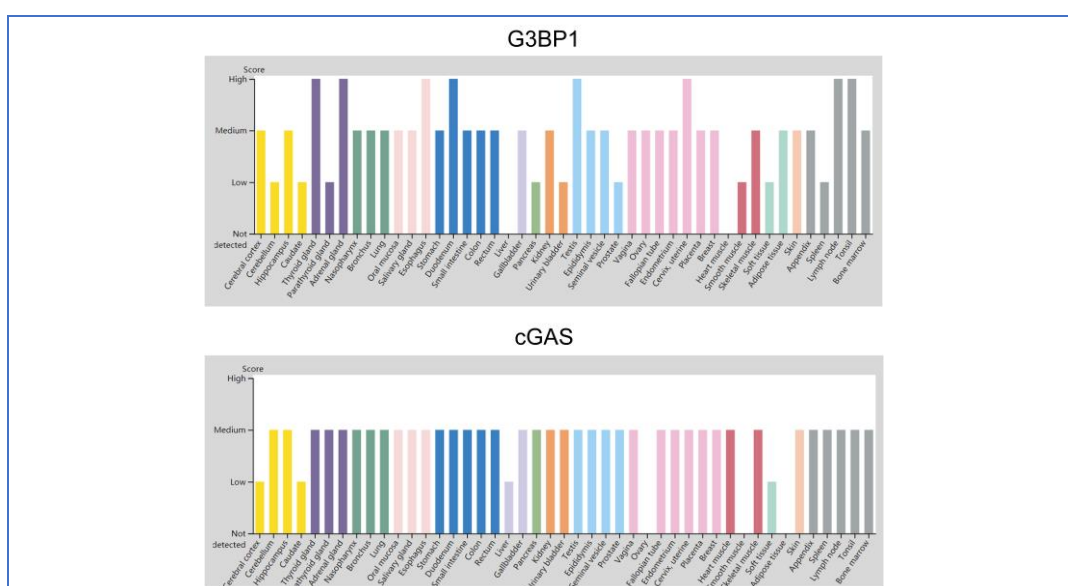
Together, these data further supported that G3BP1 engages cGAS in a primary condensation state to enable it for an expeditious response to DNA.

3- EGCG is likely to have a lot of off-target effects beyond inhibition of G3BP1 shown *in vitro*. The data in Figure 7H/I should be complemented with experiments in G3BP1<sup>-/-</sup> cells treated with EGCG to confirm that the read out is not impacted independently of G3BP1 (there should still be a response visible by RTqPCR in G3BP1<sup>-/-</sup> cells based on figure 4G).

**Response:** Following the reviewer’s suggestion, we pretreated both WT and G3BP1-deficient U937 cells with EGCG followed by HT-DNA transfection. The expression of *IFNB* mRNA was measured. We found that EGCG could no longer inhibit HT-DNA-stimulated *IFNB* expression in G3BP1-null cells (Figure 7H in our revised manuscript). We agree with the reviewer that EGCG is likely to have many targets in cells. However, in the DNA-cGAS-IFN pathway and the cell types we detected, EGCG seems to suppress cGAS activation through selectively targeting G3BP1.

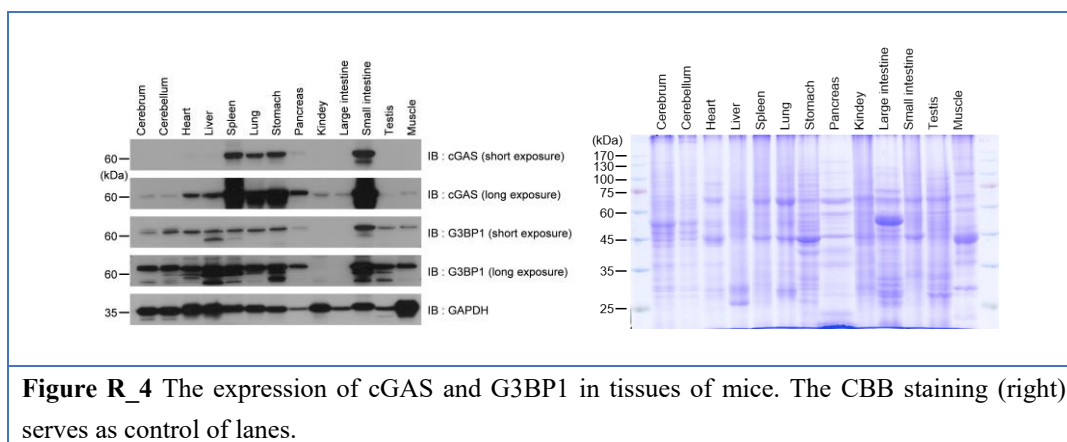
4- Could the authors provide more details in the discussion about the cell types/tissues that express G3BP1? How frequent is co-expression with cGAS? This is important to mention to underline the biological relevance of these findings.

**Response:** We appreciate the reviewer’s suggestion. In HeLa and U937 cells that we used in our study, cGAS and G3BP1 were both highly expressed. Following the reviewer’s suggestion, we also checked the human protein atlas database (<http://www.proteinatlas.org/>) and found that cGAS and G3BP1 were co-expressed in most of the tissues (Figure R\_3).



**Figure R\_3** The expression of cGAS and G3BP1.

We further detected the expression of cGAS and G3BP1 in tissues of mice. Our data show that in most tissues, cGAS and G3BP1 are co-expressed (Figure R\_4).



**Minor points:**

1- *Statistical analyses on a single experiment conducted with biological triplicate do not have any value as there is no independent sampling (it seems that ALL the data shown - in bar graphs - is only representative data, i.e. from single experiments). Please average the data from your independent experiments, where possible, rather than only showing representative data. Remove the statistics if only single experiments are shown. Also, please be consistent with the use of the "dots" in the bar graphs. This reviewer assumes they represent biological/technical replicate, but this is not clear (some bar graphs have no points...).*

**Response:** We apologize for our unclear description regarding our data presentation. Following the reviewer’s suggestion, we revised the corresponding figures. In the revised manuscript, we showed ‘dots’ (whenever possible) to indicate each data point from independent biological repeats (Figures 1 A, 1C, 1G, 1I, 2C, 2E, 2F, 4C, 4F, 5E, 6A, 6C, 6D, 6E, 7A, 7D, 7H, 7I, and Figure EV1A, EV1I, EV2C, EV3A, EV3C, EV3D, EV3H, EV4C, EV4E, EV4G, EV5C and EV5D in our revised manuscript). Only data from independent experiments (biological repeats) were subjected to statistical analysis. We also clarified the figure legends for each data and clearly described biological repeats vs. technical repeats.

2- *Lipofectamine 2000 is not from Invivogen (you probably meant invitrogen).*

**Response:** We thank the reviewer for this kind suggestion and corrected this typo in the revised manuscript.



### Referee #3:

*In this manuscript, Ming Zhao et al. described the gel-like condensate formed by cGAS and G3BP1. Using biochemical approaches and high-resolution microscopy, they showed that G3BP1 engaged cGAS in a pre-activation condensation state to enhance the cGAS-DNA LLPS and potentially promote the expeditious DNA sensing by cGAS. Overall, this is a very interesting study and discovers a new factor of the cGAS phase separation, which has merged as a critical regulating mechanism of cGAS activation. However, there are a few comments to be addressed by the authors to further improve the quality of this manuscript.*

**Response:** The reviewer thinks our work is interesting. We appreciate the reviewer's encouraging comments. We also thank the reviewer for the important suggestions to further improve the quality of our manuscript. As detailed below, point-by-point, we can address all the concerns of the reviewer with new data and discussions.

#### **Major points:**

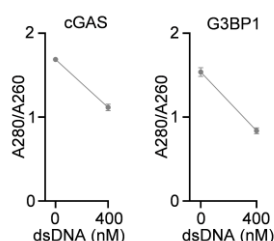
*1- According to the protein purification section (line 315-317), the authors only applied Ni-NTA affinity column to purify cGAS and G3BP1 proteins. Considering both cGAS and G3BP1 were reported to have strong nucleic acid binding abilities, the cGAS and G3BP1 protein samples eluted from Ni-NTA were very likely to have DNA/RNA contaminants to influence the in vitro phase separation assay results. The authors may need to improve the purification method and double-check the sample purity (i.e., examine the A280/A260 ratio of the samples).*

**Response:** We greatly appreciate the reviewer's suggestion. In our study, we purified the proteins with a single-step Ni-agarose purification. Following to the reviewer's suggestion, we examined the A280/A260 ratio of our one-step (Ni-agarose) purified proteins to estimate the nucleic acid contamination level. As shown in **Table R\_1** below, using commercial BSA protein as control, we found that our protein samples showed similar A280/A260 ratio.

Protein	Conc. (mg/ml)	A280	A260/A280	A280/A260
BSA	12.369	12.369	0.591	1.69047377
G3BP1-mEGFP	10.796	10.796	0.633	1.579778831
cGAS-mCherry	3.845	3.845	0.578	1.730103806
G3BP1	5.17	5.17	0.684	1.461988304
cGAS	2.956	2.956	0.572	1.748251748

**Table R\_1** A280/A260 of indicated protein samples.

Further, we added dsDNAs, at a final concentration of 400 nM, into 10  $\mu$ M cGAS or 10  $\mu$ M G3BP1 solutions to mimic a nucleic acid contamination (approximately 4 DNA molecules per 100 protein molecules). By measuring the A280/A260 ratio of these samples, we found that the addition of dsDNA remarkably reduced the value of A280/A260 ratio to 1 or less than 1 (Figure R\_1). These results suggested that the nucleic acid contamination level of our original Ni-agarose-purified recombinant proteins was marginal. Actually, according to previous publications (*CRC Crit Rev Biochem* 1986, PMID: 3512164; *Nucleic Acids Res* 2010, PMID: 20497998), high-salt condition can limit the nucleic acid-protein binding, we used high-salt buffer (1 M NaCl) during our purification to reduce the residual nucleic acid in our purified recombinant proteins.



**Figure R\_1** A280/A260 of cGAS (10  $\mu$ M) or G3BP1 (10  $\mu$ M) mixed with dsDNA (400 nM).

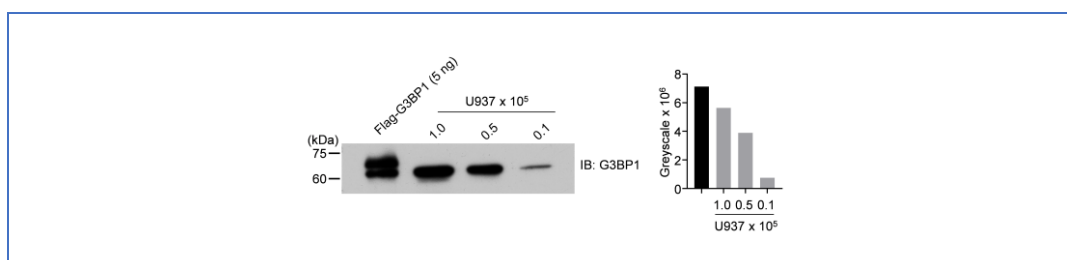
In addition, as Reviewer #1 suggested, we further purified the recombinant cGAS and G3BP1 proteins using heparin affinity chromatography following the Ni-agarose purification to remove the contamination of nucleic acid. With the newly purified proteins, we obtained similar results. Our new data consistently showed that G3BP1 formed condensates with cGAS (Figure EV1A in our revised manuscript) and promoted DNA-triggered LLPS of cGAS (Figure EV4F and G in our revised manuscript).

2- *What are the in-cell concentrations of the G3BP1/cGAS to justify physiological concentration? It will be better if the phase separation diagram of cGAS-G3BP1 is provided.*

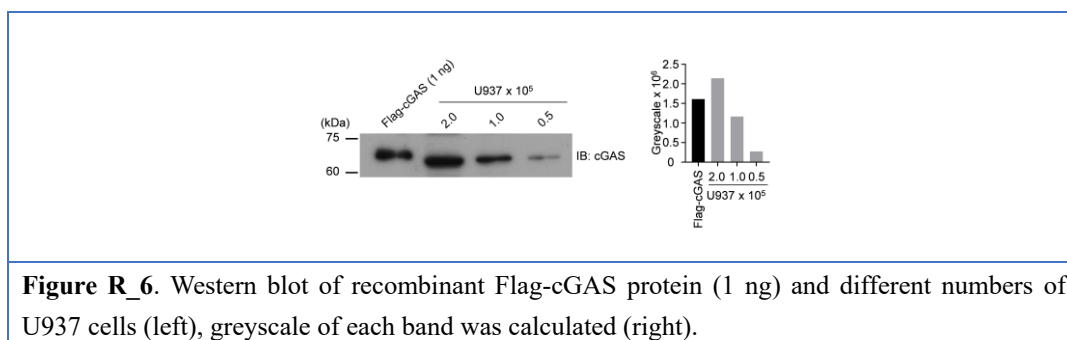
**Response:** Following the reviewer's suggestion, we measured the in-cell concentrations of cGAS and G3BP1 in U937 cells. To do so, we immunoblotted 5 ng recombinant Flag-G3BP1 or 1 ng Flag-cGAS proteins together with total cell lysates from certain numbers of cells. By measuring the

grayscale among different immunoblot bands, we estimated the amount of these proteins in each cell.

Our data show that the amounts of G3BP1 and cGAS in one cell is 0.049 pg and 0.0075 pg, respectively (Figure R\_5 and R\_6). From our imaging data, the approximate diameter of U937 is 18  $\mu\text{m}$ , thus, the cGAS concentration in cells is about 74 nM and the G3BP1 concentration is 547 nM. Interestingly, a recent study also calculated G3BP1 concentration in cells, 624 nM (*Cell*, 2020. PMID: 32302572), which is similar to our result.



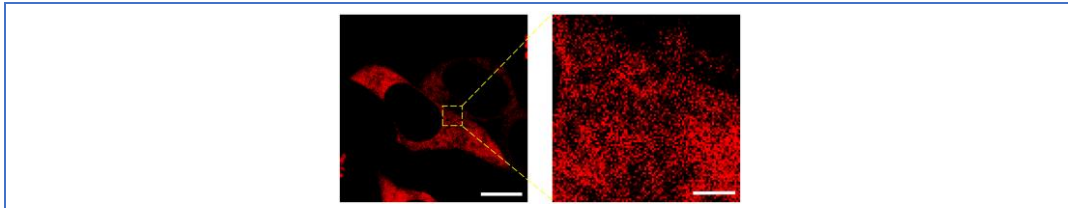
**Figure R\_5.** Western blot of recombinant Flag-G3BP1 protein (5 ng) and different numbers of U937 cells (left), greyscale of each band was calculated (right).



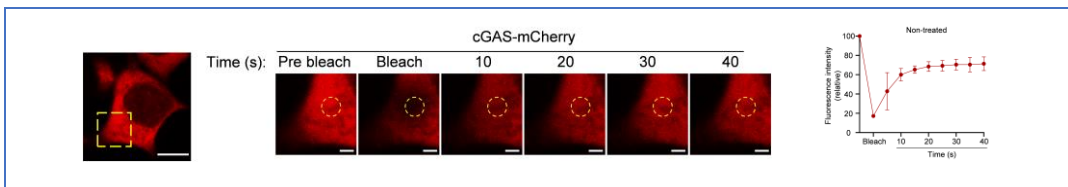
**Figure R\_6.** Western blot of recombinant Flag-cGAS protein (1 ng) and different numbers of U937 cells (left), greyscale of each band was calculated (right).

3- in line 140, the authors may not claim that the puncta observed here are the G3BP1-engaged "liquid-like structure of cGAS" due to the lack of FRAP data *in vivo*. I suggest that they may need to add the FRAP data of G3BP1-cGAS puncta in cells if it is technically available.

**Response:** Following the reviewer's suggestion, we tried performing FRAP of cGAS-G3BP1 puncta *in vivo*. To do so, we first constructed 293T cells that stably expressing cGAS-mCherry and confirmed that the cGAS exhibited puncta-like morphological characteristics (Figure R\_7). However, the individual cGAS punctum is too small to be focused by laser for FRAP experiment. We therefore bleached a region in the cytoplasm and found that the fluorescent signal of the bleached region recovered soon (Figure R\_8). This result suggested that the cGAS-G3BP1 puncta in cells are liquid-like structures.

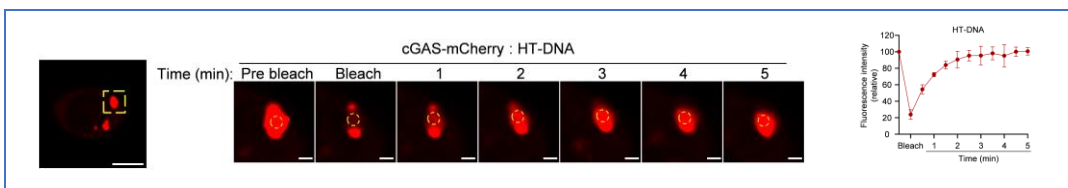


**Figure R\_7.** 293T cells stably expressing cGAS-mCherry. Scale bars, 10 µm (left), 4 µm (right).



**Figure R\_8.** FRAP of cGAS-mCherry in 293T-cGAS-mCherry cells. Scale bars, 10 µm (left), 2 µm (middle).

To further perform FRAP in cells, we treated the 293T-cGAS-mCherry cells with HT-DNA to induce the formation of cGAS-DNA condensates, which are much bigger in size (Figure R\_9). We bleached the cGAS-DNA condensates and found that the fluorescence intensity recovered soon after bleaching (Figure R\_9). These data are consistent with the previous report (*Science* 2018, PMID: 29976794).



**Figure R\_9.** FRAP of cGAS-mCherry in 293T-cGAS-mCherry cells stimulated with 2 µg/ml HT-DNA. Scale bars, 10 µm (left), 2 µm (middle).

**Minor points:**

1- in line 124, the authors may highlight the length of dsDNA used in the assay considering the cGAS and dsDNA trigger LLPS in a DNA length-dependent manner.

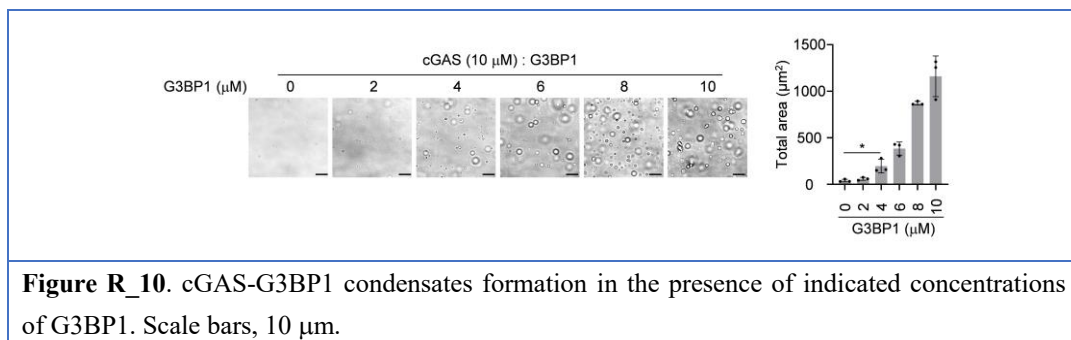
**Response:** We thank the reviewer for this point. We described the length of dsDNAs (60bp) in our revised manuscript.

2- in line 132, it seems to me that the cGAS-G3BP1 undergoes the gel-like instead of "LLPS-like" transitions.

**Response:** Following the reviewer's suggestion, we rephrased "LLPS-like" as "gel-like" in our revised manuscript.

3- in figure 1C right panel, the 8  $\mu\text{M}$  G3BP1 showed a significant (8-fold?) enhancement of total area than that of 4  $\mu\text{M}$  G3BP1. Could authors comment on this observation? In the figure caption (line 628), the "total area of droplets" could be updated to "total area of condensates".

**Response:** As the reviewer pointed out, in our original Figure 1C, 8  $\mu\text{M}$  G3BP1 showed a significant enhancement of total area than that of 4  $\mu\text{M}$  G3BP1. To address this point, we reperformed this assay with more consecutive concentrations of G3BP1, our new data showed that G3BP1 promoted the formation of the primary condensation of cGAS in a dosage-dependent manner (Figure R\_10).



**Figure R\_10.** cGAS-G3BP1 condensates formation in the presence of indicated concentrations of G3BP1. Scale bars, 10  $\mu\text{m}$ .

4- in Figure 2C, does the cGAS puncta number per cell include the nuclear cGAS puncta or just cytoplasm cGAS puncta?

**Response:** In this figure, we counted the total cGAS puncta, including the nuclear cGAS puncta.

5- in line 71, "allow" should be "allows".

6- in line 160, "amount" should be "amounts".

7- in line 179, "in consistent with" should be "being consistent with".

**Response:** We greatly appreciate these suggestions from the reviewer and corrected these points in our revised manuscript.

Dear Prof. Li,

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

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

- Please provide a more comprehensive and simpler title (of not more than 100 words including spaces). What do you mean with expeditious response to DNA? Fast? How about:  
GTPase-activating protein G3BP1 promotes primary condensation of cGAS to allow a fast response to DNA
- Please have your final manuscript carefully proof-read by a native speaker. There are several typos or grammatical errors present.
- We need more detailed legends that describe clearly what is shown in the figure panels and also explain the abbreviations used. Just one example: The legend for 7C just states 'Interaction between different G3BP1 truncated mutants and EGCG'. It remains unclear what kind of experiment is shown and what IB and WCL mean. Please carefully go through the legends and render these more comprehensible.
- Please format the references according to our journal style. We need et al. when there are more than 10 authors. See: <http://www.embopress.org/page/journal/14693178/authorguide#referencesformat>
- Regarding data quantification and statistics, please make sure that the number "n" for how many independent experiments were performed, their nature (biological versus technical replicates), the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values is indicated in the respective figure legends (also for potential EV figures and all those in the final Appendix). Please also check that all the p-values are explained in the legend, and that these fit to those shown in the figure. Please provide statistical testing where applicable. Please avoid the phrase 'independent experiment', but clearly state if these were biological or technical replicates.
- Presently some diagrams have no (e.g. 1B, 2G, 4D, 4G, 5F, 6B, 6F, 7E, 7G) or only partially statistics. Please add statistical testing to all diagrams with  $n > 2$ . Please also indicate (e.g. with n.s.) if testing was performed, but the differences are not significant.
- Please add scale bars of similar style and thickness to all the microscopic images using clearly visible black or white bars (depending on the background). Presently, some white scale bars shown are not well visible against the bright background. Please define the size of all scale bars in the respective figure legend.
- Please submit the source data (scans of entire blots) separated as one PDF file per figure (main and EV figures).
- For some movies the labelling is cut off. Movies EV10-EV19 are therefore all labelled Movie EV1, and Movies EV20-EV23 are all labelled Movie EV2. Moreover, we need a legend for each movie file. Please provide this as a text file and ZIP it together with the movie file, and upload these as one folder. Finally, please remove the movie legends from the manuscript file.
- Please enter all the funding information also into our submission system and make sure this is complete and similar to the one mentioned in the manuscript text file.
- Please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript. Please do that for co-corresponding author Weihua Li. We will not proceed with publication if this is not done. Please find instructions on how to link the ORCID ID to the account in our manuscript tracking system in our Author guidelines: <http://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines>
- Finally, please find attached a word file of the manuscript text (provided by our publisher) with changes we ask you to include in your final manuscript text, and some queries, we ask you to address. Please provide your final manuscript file with track changes, in order that we can see any modifications done.

In addition, I would need from you:

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

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

Best,

Achim Breiling  
Editor  
EMBO Reports

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Referee #1:

In their revised manuscript the authors provide a substantial amount of new data sufficiently addressing most if not all point raised by the reviewer(s). The additions to the text in results and discussion further improve the manuscript. I have no further comments.

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Referee #2:

The authors have successfully addressed my concerns - and the new data strongly supports their claims.

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Referee #3:

The authors have done an excellent job of responding to my (Reviewer 3) and other reviewers' concerns , and it can be published in the present format.





**Point-by-Point Response to Editorial Requests:**

*- Please provide a more comprehensive and simpler title (of not more than 100 words including spaces). What do you mean with expeditious response to DNA? Fast? How about: GTPase-activating protein G3BP1 promotes primary condensation of cGAS to allow a fast response to DNA*

**Response:** We thank the editor for this suggestion. Accordingly, we revised the title of our manuscript as '**The stress granule protein G3BP1 promotes primary condensation of cGAS to allow a fast response to DNA**' to seek the editor's advice.

Because we did not investigate whether the GTPase activity of G3BP1 is involved in its role in cGAS regulation, we thus used 'stress granule protein' to define G3BP1 according to previous publication.

*- Please have your final manuscript carefully proof-read by a native speaker. There are several typos or grammatical errors present.*

**Response:** Following the editor's suggestion, we had our manuscript proof-read by native English speaker, and we corrected the typos and grammatical errors.

*- We need more detailed legends that describe clearly what is shown in the figure panels and also explain the abbreviations used. Just one example: The legend for 7C just states 'Interaction between different G3BP1 truncated mutants and EGCG'. It remains unclear what kind of experiment is shown and what IB and WCL mean. Please carefully go through the legends and render these more comprehensible.*

**Response:** Following the editor's suggestion, we revised all the figure legends to describe the detailed experimental designs and procedures clearly.

- Please format the references according to our journal style. We need *et al.* when there are more than 10 authors.

See: <http://www.embopress.org/page/journal/14693178/authorguide#referencesformat>

**Response:** Following the editor's suggestion, we re-formatted the references according to the journal style of **EMBO Reports:**

(<http://www.embopress.org/page/journal/14693178/authorguide#referencesformat>).

- Regarding data quantification and statistics, please make sure that the number "n" for how many independent experiments were performed, their nature (biological versus technical replicates), the bars and error bars (e.g. SEM, SD) and the test used to calculate *p*-values is indicated in the respective figure legends (also for potential EV figures and all those in the final Appendix). Please also check that all the *p*-values are explained in the legend, and that these fit to those shown in the figure. Please provide statistical testing where applicable. Please avoid the phrase 'independent experiment', but clearly state if these were biological or technical replicates.

**Response:** Following the editor's suggestions, we stated the number of "n" in all the figure legends and clearly indicated the biological repeats versus technical replicates, instead of using 'independent experiment'.

We also defined bars and error bars in all the figure legends. In the revised manuscript, we explained all the *p*-values in the figure legends and the tests used to calculate *p*-values. We confirm that the explanations of *p*-values fit to those shown in the figures.

- Presently some diagrams have no (e.g. 1B, 2G, 4D, 4G, 5F, 6B, 6F, 7E, 7G) or only partially statistics. Please add statistical testing to all diagrams with  $n > 2$ . Please also indicate (e.g. with *n.s.*) if testing was performed, but the differences are not significant.

**Response:** We thank the editor for this suggestion and apologize for our unclear description. According to the reviewers' instructions, data generated from technical replicates are not suitable for statistical analysis. We therefore did not perform statistical analysis these data (Fig. 1B, 2G, 4D, 4G, 5F, 6B, 6F, 7E, 7G). We indicated 'technical replicates' in the corresponding figure legends.

*- Please add scale bars of similar style and thickness to all the microscopic images using clearly visible black or white bars (depending on the background). Presently, some white scale bars shown are not well visible against the bright background. Please define the size of all scale bars in the respective figure legend.*

**Response:** Following the editor's suggestions, we used scale bars with similar style and thickness in all the microscopic images and defined all the size of scale bars in the respective figure legend in our final revised manuscript.

*- Please submit the source data (scans of entire blots) separated as one PDF file per figure (main and EV figures).*

**Response:** Following the editor's suggestion, we provided the source data (scans of entire blots) separated as one PDF file per figure for main and EV figures.

*- For some movies the labelling is cut off. Movies EV10-EV19 are therefore all labelled Movie EV1, and Movies EV20-EV23 are all labelled Movie EV2. Moreover, we need a legend for each movie file. Please provide this as a text file and ZIP it together with the movie file, and upload these as one folder. Finally, please remove the movie legends from the manuscript file.*

**Response:** We appreciate the editor's suggestions. Accordingly, we prepared

movies following the editor's instructions.

*- Please enter all the funding information also into our submission system and make sure this is complete and similar to the one mentioned in the manuscript text file.*

**Response:** According to the editor's request, we provided all the funding information in the submission system and confirmed that the information is complete and consistent with those mentioned in the manuscript.

*- Please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript. Please do that for co-corresponding author Weihua Li. We will not proceed with publication if this is not done. Please find instructions on how to link the ORCID ID to the account in our manuscript tracking system in our Author guidelines: <http://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines>*

**Response:** Following the editor's instruction, Dr. Weihua Li has linked her ORCID ID (0000-0001-8030-9988) to the account in the manuscript tracking system of **EMBO Reports**.

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

**Response:** We greatly appreciate the editor's effort in editing our manuscript. Following the editor's suggestions, we provided the requested information and addressed the queries by the editors. The final manuscript file is provided in *word* format with track changes.

*In addition, I would need from you:*

*- a short, two-sentence summary of the manuscript (35 words).*

**Response:** Following the editor's suggestion, we drafted a short summary of our manuscript as '**cGAS is a critical DNA sensor for the host to detect invading pathogens. The stress-granule protein G3BP1 engages cGAS in a primary condensation state to enable it for expeditious response to DNA**'.

*- three to four short bullet points highlighting the key findings of your study.*

**Response:**

1. G3BP1 primes cGAS for its prompt activation.
2. G3BP1 engages cGAS in a primary condensation state.
3. EGCG inhibits G3BP1-promoted cGAS phase condensation and activation.

*- a schematic summary figure (synopsis image) in jpeg or tiff format with the exact width of 550 pixels and a height of not more than 400 pixels that can be used as a visual synopsis on our website.*

**Response:** We provided the synopsis image according to the editor's request.

Prof. Tao Li  
National Center of Biomedical Analysis  
27 Taiping Road  
Beijing, Beijing 100850  
China

Dear Prof. Li,

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

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

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Corresponding Author Name: Tao Li and Wei-Hua Li

Journal Submitted to: EMBO reports

Manuscript Number: EMBOR-2021-53166

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

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

#### A- Figures

##### 1. Data

The data shown in figures should satisfy the following conditions:

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

##### 2. Captions

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

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple  $\chi^2$  tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

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

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

#### B- Statistics and general methods

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

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	Representative data or 3 biological replicates were present.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	N/A
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	N/A
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	N/A
For animal studies, include a statement about randomization even if no randomization was used.	N/A
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	N/A
4.b. For animal studies, include a statement about blinding even if no blinding was done	N/A
5. For every figure, are statistical tests justified as appropriate?	In our study, we used t test to compare data from two groups. We chose the statistical test according to similar studies in the field.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	N/A
Is there an estimate of variation within each group of data?	Yes

#### USEFUL LINKS FOR COMPLETING THIS FORM

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<http://jij.biochem.sun.ac.za>  
[http://oba.od.nih.gov/biosecurity/biosecurity\\_documents.html](http://oba.od.nih.gov/biosecurity/biosecurity_documents.html)  
<http://www.selectagents.gov/>

Is the variance similar between the groups that are being statistically compared?	Yes
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### C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	In the Materials and Methods section, we provided the Cat# of our antibodies used in this study. GAPDH antibody prepared by ourselves has been verified and used in our previous publications (Proc. Natl. Acad. Sci. USA 2009, PMID: 19234109; J. Clin. Invest. 2010, PMID: 20628200; Nat. Commun. 2014, PMID: 27694884; Nat. Immunol. 2019, PMID: 30510222; Cell. 2019, PMID: 30799039). cGAS antibody prepared by ourselves has been verified and used in our previous publications (Nat. Immunol. 2019, PMID: 30510222; Cell. 2019, PMID: 30799039)
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	We included a statement in our Materials and Methods section.

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

### D- Animal Models

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

### E- Human Subjects

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

### F- Data Accessibility

18. Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'.  Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	N/A
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	N/A
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	N/A
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomedels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	N/A

### G- Dual use research of concern

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