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Poly(ADP-ribose)-binding and macroH2A mediate recruitment and functions of KDM5A at DNA lesions

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August 3, 2020

Re: JCB manuscript #202006149

Dr. Kyle Miller
The University of Texas at Austin
2506 Speedway NMS 2.104
Austin, TX 78712

Dear Dr. Miller,

Thank you for submitting your manuscript entitled "Poly(ADP-ribose)-binding and macroH2A mediate recruitment and functions of KDM5A at DNA lesions". Thank you very much for your patience with the peer review process. The manuscript was assessed by expert reviewers, whose comments are appended to this letter. We invite you to submit a revision if you can address the reviewers' key concerns, as outlined here.

You will see that the reviewers were supportive of the study but raised some concerns that require your attention:

1- Providing strong and robust evidence for the suggested new type of PAR interaction, through a coiled-coil domain, to address the comments about PAR binding and the role of PARP activity from all revs should be a focus of the revision. In particular, Reviewer #3 makes valid points in comment #2 that need to be addressed rigorously. Reviewer #3's request for evidence that the mutant PAR binding-deficient KDM5A constructs are properly expressed and stable is important and should be addressed (#3). We also agree with Reviewer #2's relevant suggestion in point #3 to strengthen the binding studies. The reviewer requests a control in their point #4 but since the outcome of the experiment is positive, adding a control may not be needed.

2- Addressing the macroH2A variant specificity of KDM5A interaction as per Revs#2-3 is less of a priority in our view. We will leave it to you to decide how to address these comments.

3- Addressing Reviewer #2's point #9 is important - we agree with the reviewer that how the KDM5A/macroH2A interaction is PAR independent in the absence of damage but PAR-dependent in the presence of damage is a puzzling question.

4- The reviewers had questions about the cell lines used throughout. We agree with Reviewer #3 point #1 that additional characterizations of the KO line need to be included. However, HCT116 were used for making a KO, 293T for biochemistry and U20S for laser micro-irradiation, so the lines are being used for different purposes depending on their strength. Therefore, we do not support the request that you would have to show all the phenotypes in all cell lines.

5- Lastly, Reviewer #2 point #2 could be discussed. Please address the reviewers' questions about the statistical significance of the data.

Please let us know if you anticipate any issues addressing these points - we would be happy to discuss the revision further as needed. Lastly, we feel that the scope of the work and its

mechanistic nature are more appropriate for our Article format than our Report format, so we invite you to resubmit the work as an Article (formatting guidelines can be found below and online: https://rupress.org/jcb/pages/submission-guidelines#manuscript-prep).

While you are revising your manuscript, please also attend to the following editorial points to help expedite the publication of your manuscript. Please direct any editorial questions to the journal office.

GENERAL GUIDELINES:

Text limits: Character count for an Article is < 40,000, not including spaces. Count includes title page, abstract, introduction, results, discussion, acknowledgments, and figure legends. Count does not include materials and methods, references, tables, or supplemental legends.

Figures: Articles may have up to 10 main text figures and up to 5 supplemental figures. Up to 10 supplemental videos or flash animations are allowed. A summary of all supplemental material should appear at the end of the Materials and methods section. Figures must be prepared according to the policies outlined in our Instructions to Authors, under Data Presentation, http://jcb.rupress.org/site/misc/ifora.xhtml. All figures in accepted manuscripts will be screened prior to publication.

IMPORTANT: It is JCB policy that if requested, original data images must be made available. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original microscopy and blot data images before submitting your revision.

As you may know, the typical timeframe for revisions is three to four months. However, we at JCB realize that the implementation of social distancing and shelter in place measures that limit spread of COVID-19 pose challenges to scientific researchers. Lab closures especially are preventing scientists from conducting experiments to further their research. Therefore, JCB has waived the revision time limit. Please feel free to reach out to the editors once your lab has reopened to decide on an appropriate time frame for resubmission. Please note that papers are generally considered through only one revision cycle, so any revised manuscript will likely be either accepted or rejected.

When submitting the revision, please include a cover letter addressing the reviewers' comments point by point. Please also highlight all changes in the text of the manuscript.

We hope that the comments below will prove constructive as your work progresses. We would be happy to discuss them further once you've had a chance to consider the points raised in this letter.

Thank you for this interesting contribution to the Journal of Cell Biology. You can contact us at the journal office with any questions, cellbio@rockefeller.edu or call (212) 327-8588.

Sincerely,

Agata Smogorzewska, MD, PhD
Monitoring Editor, Journal of Cell Biology

Melina Casadio, PhD
Senior Scientific Editor, Journal of Cell Biology

Reviewer #1 (Comments to the Authors (Required)):

I think that this manuscript is excellent it represent the state of the art in the analysis of a protein interacting with Poly(ADP-ribose). The enzyme KDM5 is extremely well characterized in terms of its interaction with Poly(ADP-ribose).
However is it possible that it is modified covalently with POLY(ADP-ribose) chains. Thus this possibility should analyzed. Finally the domains interacting with poly(ADP-ribose) are well characterized is it possible to identify the effect or at least see which
structural elements interact with poly(ADP-ribose). The manuscript would be accepted with minor revisions.

Reviewer #2 (Comments to the Authors (Required)):

This manuscript represents a follow up to Gong et. al JCB 2017, which demonstrated that KDM5A was recruited to sites of laser-induced DNA damage in a PARP1 activity-dependent manner. In the current manuscript the authors continue to characterize the role of PARP1 in mediating KDM5A's role in DDR. They begin by demonstrating a cooperative effect of KDM5A and PARP1 inhibition on the viability and DDR of HCT116 cells (Fig 1). In Fig 2 the authors use classical protein biochemistry to determine the region of KDM5A responsible for binding to PAR, identifying a region proximal to the 3rd PHD finger and demonstrate that this region is sufficient to direct recruitment to laser-induced damage in U2OS cells. In figure 3, they further narrow down this region demonstrate its necessity for promoting recruitment to sites of laser-induced damage. They also demonstrate its effect on survival after IR or PARP inhibition and implicate a role for this region in promoting HR. In figure 4 they demonstrate an interaction between KDM5A and the histone variant macroH2A and show they are epistatic in an HR reporter assay. Finally, in figure 5, the authors demonstrate that macroH2A1 is required for KDM5A recruitment to laser-induced damage and local transcriptional repression.

Overall, this is an interesting manuscript that makes two important though perhaps disparate core findings. 1) they identify the region of KDM5A responsible for the PAR-directed recruitment to sites of damage and 2) they determine that macroH2A1 plays a role in the recruitment of KDM5A to damage. However, there are a variety of specific issues which somewhat detract from the overall impact of the manuscript and places where the conclusions are not sufficiently supported by the data:

1) The concentrations of Olaparib are way beyond the reported IC50 of 6nM. This makes it quite possible that the results in figure 1 may be due to off target effects. Using a PARP1 KO or KD would address those concerns.
2) The results in figure 1 are used to support a role for KDM5A in PARP1 dependent repair pathways. However, this reasoning runs opposite to the classic "synthetic lethality" argument which suggests lethality occurs because each factor (typically BRCA1 and PARP1) functions in independent pathways, neither of which is required for viability alone, but either of which are needed for cell survival. This should be discussed.
3) Fig 2 is generally well done. However, this would be augmented by an experiment determining if the KDM5A interaction with PARP1 is dependent on PAR by treating with or without Olaparib.

4) Fig 2D is missing a positive control for the IP of a PARylated factor, such as PARP1 itself.
5) Fig 2K is missing the negative control of PARPi treatment. The fact that the current panel are labeled "DMSO", would seem to suggest the authors have this data.
6) The lack of statistical tests in Fig3J and K are concerning. All survival plots, including Fig 1A,B should have statistical test performed indicating their significance.
7) While the interaction between KDM5A and macroH2A1 is convincing, the arguments about macroH2A variant specificity for KDM5A binding are unconvincing as presented. HEK293T cells express vanishingly small levels of macroH2A1.1 compared to macroH2A1.2. The experiment shown in Fig S3G needs a control to show that one of those bands in the input is actually macroH2A1.1 (e.g. macroH2A1.1 specific KD).
8) Experiment 4B also does not provide any evidence regarding variant specificity as they express flag-tagged mH2A1.2 or mH2A2 but only probe with a macroh2A1.2 antibody. This experiment should be repeated with a flag antibody and include flag tagged versions of all three macroH2A variants. It is unclear if the Flag-mH2A2 protein is even expressed in this experiment which also has implications for the conclusions from Fig 5C,D.
9) There is insufficient explanation or discussion of how the KDM5A/macroH2A interaction is PAR independent in the absence of DDR but PAR-dependent in the presence of DDR (Fig 4C).
10) The constant switching of cell lines in different experiments (HCT116, HEK293T, U2OS) is problematic given that none of the critical experiments were performed in all three cell lines.
11) Statistical test for all of the laser-induced recruitment IF experiments (Fig 3I, Fig 5B,D, Fig S3B, I) are lacking and necessary given the low signal to noise.

Reviewer #3 (Comments to the Authors (Required)):

In the manuscript from Miller and colleagues, the authors investigate the regulation of KDM5A in response to DNA damage. This paper develops previous work from the lab, in which they show that KDM5A is recruited to DNA breaks in a PARP dependent manner and contributes to repair and to repressing transcription near the breaks. Here, they provide evidence that there is a direct interaction between KDM5A and PAR through a motif in the C-terminal region of the protein. This interaction appears to be important for the ability of KDM5A to localize to DNA breaks. They also provide evidence that macroH2A is important for KDM5A to localize to DNA breaks. Overall, this is a good advance on previous work and there are some interesting new findings. There are several areas where additional evidence or controls are needed to support the conclusions in the manuscript, detailed below.

Specific points:
1. The use of a complemented knockout (KO) cell line is an excellent addition to siRNA experiments, but additional characterization of this cell line is needed, especially since just a single clone is used here. HCT116 is annotated as already having a loss of function mutation (deletion/frameshift) in one allele of KDM5A, so it is not an ideal choice. Can the authors provide more information about the clone, such as the nature of the mutation in the remaining allele, and whether it has the annotated mutation in the parental sequence? In addition, it is important that KDM5A functions are characterised in the wt, KO and complemented KO to validate this as a model system. An easy readout would be H3 K4 methylation levels and RT-PCR of one or two known KDM5A-regulated genes.
2. In Figure 2, the authors show that there is an interaction between KDM5A and PARP1 and PAR. While the KO cell line in HCT116 might not be the ideal choice for phenotypic analysis, it would be excellent as a control for the specificity of these interactions. Using the anti-KDM5A antibody out of

the KO extract is a much more rigorous control than IgG. Also, a GFP antibody can be used with the expression construct containing cells. Addition of these controls would really help to solidify this conclusion since some of the IP results are not very convincing (particularly Figure 2C) and could be non-specific. The PARP inhibitors don't address this, since non-specific interactions would also be reduced when the amount of PAR in the input samples is massively reduced.
3. The in vitro binding assays are very nice and the identification of a new PAR binding motif is potentially important. The functional significance of this in cells appears to be supported by the cellular assays in Figure 3. However, expression analysis of the construct lacking the PAR binding region in these experiments should be provided to demonstrate that the defects are not due to problems with protein folding or stability. It would also be interesting and informative to see if the non-damage functions of KDM5A are affected by this deletion. Minor note: either use GFP or mClover, but the mix of the two terms in the legend and on the figure is potentially confusing.
4. In Figure 4, there is an apparent interaction between macroH2A1.2 and KDM5A. In Fig 4A, the KDM5A construct is overexpressed, and in 4B there is no IgG control in the cells expressing the mH2A constructs. It would again be important to include more controls for specificity to confirm this result. In Fig. 4F, there is no western provided to show depletion of mH2A1.2. On a minor note, the meaning of the colors used in Fig 4E are not defined in the legend.
5. Minor points: The statistical test is 'Tukey's' not 'Turkey's'. End of page 7 should read 'BRCA1 and BRCA2 are sensitive to' not 'sensitivity to'.

Response to reviewer's comments for Kumbhar et al. (JCB manuscript #202006149)

We would like to thank each reviewer for their time and constructive comments, which have helped us improve our study. We now provide an extensively revised manuscript with substantial amounts of new data making up a total of 24 new panels within the figures, which we believe has improved the scientific rigor, mechanistic insights and impact of our study. Please see below for our point-by-point response to each comment from all reviewers.

Reviewer #1 (Comments to the Authors (Required)):

I think that this manuscript is excellent it represent the state of the art in the analysis of a protein interacting with Poly(ADP-ribose). The enzyme KDM5 is extremely well characterized in terms of its interaction with Poly(ADP-ribose).
However is it possible that it is modified covalently with POLY(ADP-ribose) chains. Thus this possibility should analyzed.

This is a very good suggestion and an idea we had in mind when we performed an IP of KDM5A followed by western blotting to probe for PAR. We indeed found that we could detect PAR in IPed KDM5A samples. To address covalent versus non-covalent PARylation or PAR binding respectively, we performed our IP with or without 1% SDS. This experiment revealed that the PAR signal in IPed KDM5A samples was sensitive to SDS treatment, showing that this interaction was non-covalent (original **Fig. 2 D**). We have now strengthened this result by performing a similar experiment using PARP1 as a PARylated protein control as it is well established that PARP1 is PARylated extensively after DNA damage. As expected, we observe PAR signals in IPed GFP-PARP1 samples with or without SDS (new **Fig. 2 F**). Taken together, these results demonstrate that KDM5A binds PAR under these conditions and is not itself covalently modified with PAR chains, at least at a level that we can detect using these methods. These results are consistent with our identification of a PAR binding domain in KDM5A, which mediates its localization to damage sites and DDR functions (**Fig. 3-5**).

Finally the domains interacting with poly(ADP-ribose) are well characterized is it possible to identify the effect or at least see which structural elements interact with poly(ADP-ribose).

This is a very important question and one that we took a considerable amount of effort and time to address. Using our identified region of KDM5A that we showed binds to PAR chains (i.e. F9), we performed preliminary secondary structural analysis of this fragment with and without the PAR-binding region (F9 versus F9ΔPID). We established a new collaboration with Dr. Bethany Buck-Koehntop, who has extensive expertise in the structural/biophysical characterization of protein:nucleic acid complexes using multiple techniques, including solution NMR and Circular Dichroism (CD) spectroscopy. Dr. Buck-Koehntop was able to show from CD analysis that F9 contains alpha-helical propensities, consistent with its prediction as a coiled-coil domain, as well as intrinsically disordered protein (IDP) elements (new **Fig. 4 J**). Removal of the PID region, which largely abolishes PAR binding, significantly diminished the alpha-helical content, and showed the remaining protein fragment to be predominantly composed of IDP (new **Fig. 4 J**). She also performed EMSA analyses and found that KDM5A F9 preferentially binds medium to longer PAR chains and shows an apparent binding affinity in the low nM range (~100-175 nM; new **Fig. 4 K and L**). We did still observe weak μM binding for the F9ΔPID protein fragment, suggesting that some component of the IDP elements, which are known PAR interaction features within proteins, may also contribute to PAR recognition by KDM5A. Taken together, our new analysis provides strong evidence that the KDM5A PAR binding region harbors both alpha-helical and disordered regions that contribute to PAR recognition and binding. These additional data strengthen our conclusions that KDM5A engages PAR through its C-terminal PID, that contains a putative coiled-coil domain, and that these interactions are critical for promoting its localization and

function at DNA damage sites. This raises the exciting possibility that other coiled-coil domains may similarly bind PAR, which will be an area of research for future studies.

The manuscript would be accepted with minor revisions.

Reviewer #2 (Comments to the Authors (Required)):

This manuscript represents a follow up to Gong et. al JCB 2017, which demonstrated that KDM5A was recruited to sites of laser-induced DNA damage in a PARP1 activity-dependent manner. In the current manuscript the authors continue to characterize the role of PARP1 in mediating KDM5A's role in DDR. They begin by demonstrating a cooperative effect of KDM5A and PARP1 inhibition on the viability and DDR of HCT116 cells (Fig 1). In Fig 2 the authors use classical protein biochemistry to determine the region of KDM5A responsible for binding to PAR, identifying a region proximal to the 3rd PHD finger and demonstrate that this region is sufficient to direct recruitment to laser-induced damage in U2OS cells. In figure 3, they further narrow down this region demonstrate its necessity for promoting recruitment to sites of laser-induced damage. They also demonstrate its effect on survival after IR or PARP inhibition and implicate a role for this region in promoting HR. In figure 4 they demonstrate an interaction between KDM5A and the histone variant macroH2A and show they are epistatic in an HR reporter assay. Finally, in figure 5, the authors demonstrate that macroH2A1 is required for KDM5A recruitment to laser-induced damage and local transcriptional repression.

Overall, this is an interesting manuscript that makes two important though perhaps disparate core findings. 1) they identify the region of KDM5A responsible for the PAR-directed recruitment to sites of damage and 2) they determine that macroH2A1 plays a role in the recruitment of KDM5A to damage. However, there are a variety of specific issues which somewhat detract from the overall impact of the manuscript and places where the conclusions are not sufficiently supported by the data:

1) The concentrations of Olaparib are way beyond the reported IC50 of 6nM. This makes it quite possible that the results in figure 1 may be due to off target effects. Using a PARP1 KO or KD would address those concerns.

Looking in the literature, IC50 of Olaparib in HCT116 cells appear to vary, with several publications showing IC50s in HCT116 cells to be around 3 μM (Wang et al. Transl Oncol, 2017; Morii et al. Cancer Science, 2019). The concentration of Olaparib used in these published reports are in line with those used in ours. Regardless, to rule out any concerns about off target effects of the PARPi, we have repeated experiments in Figure 1 as requested using PARP1 knockdown cells by siRNA. We observed very similar results between PARPi and PARP KD cells. Specifically, PARPi deficiency by either PARPi or PARP KD resulted in increased micronuclei and DSBs in HCT116 KDM5A KO cells compared to WT HCT116 cells (new **Fig. S1 C-G**). We also performed similar experiments in WT and KDM5A KO U2OS cells and obtained the same results (new **Fig. S2 G**). We believe these new control experiments rule out any potential concerns about off-target effects with the PARPi and further support out finding that loss of KDM5A results in defects in genome integrity pathways.

2) The results in figure 1 are used to support a role for KDM5A in PARP1 dependent repair pathways. However, this reasoning runs opposite to the classic "synthetic lethality" argument which suggests lethality occurs because each factor (typically BRCA1 and PARP1) functions in independent pathways, neither of which is required for viability alone, but either of which are needed for cell survival. This should be discussed.

We agree that this subject should be further discussed. Our results obtained with PARPi were originally involved in testing the involvement of KDM5A in HR repair. However, the observed toxicity to PARPi suggests that KDM5A-deficiency impacts response to this inhibitor, including at the level of cell survival and genome integrity. Although we can only speculate, it is known that PARPi kill HR-proficient cells and that they target many pathways in addition to those involved in “synthetic lethality” of BRCA-deficient cells. We now add this to our discussion and suggest that KDM5A-deficiency is likely to contribute to cell toxicity to PARPi, as demonstrated by our extensive data in **Fig. 1 and Fig. S1 and S2**, beyond the involvement of PARylation in promoting KDM5A recruitment and function in HR repair. It will be interesting in follow up studies to identify these mechanisms. Here however, we have added the text in the discussion to address this reviewer’s question - “In addition, we observed that KDM5A-deficiency resulted in toxicity and genome instability as a result of PARPi-treatment. While this may be potentially at odds with the finding that PARP1 promotes KDM5A recruitment to DNA damage sites, PARPi broadly impact genome integrity pathways in cells, including those that are HR-proficient, during replication through PARP-trapping and DSB formation, replication fork instability and okazaki fragment processing, as well as through other cellular functions including chromatin remodeling and transcription (Azarm and Smith, 2020; Gupte et al., 2017). Indeed, while BRCA-deficient cells are exquisitely sensitive to PARPi through a concept known as “synthetic lethality”, HR-proficient cells also display cell toxicity to PARPi (Ashworth and Lord, 2018; Michelena et al., 2018). Thus, the sensitivity of KDM5A-deficient cells to PARP inhibition may not be entirely related to the involvement of PARylation in promoting KDM5A recruitment and involvement in HR repair.”

3) Fig 2 is generally well done. However, this would be augmented by an experiment determining if the KDM5A interaction with PARP1 is dependent on PAR by treating with or without Olaparib.

We had included this experiment in **Fig. 4 C** of the first submission, which showed that the interaction between KDM5A and PARP1 was reduced in Olaparib treated cells. This experiment was presented later in the text as we also used this experiment to show that the interaction between KDM5A and macroH2A1 is also PARP-dependent using Olaparib treatment. These data are now provided in new **Fig. 6 C**.

4) Fig 2D is missing a positive control for the IP of a PARylated factor, such as PARP1 itself.

This is an excellent suggestion and we now provide this additional control in new **Fig. 2 F**. As expected, IPed PARP1 is recognized by anti-PAR in both -/+ SDS treatment as it is directly PARylated unlike KDM5A which has PAR signals only in - SDS conditions since it binds to PAR and is not directly PARylated.

5) Fig 2K is missing the negative control of PARPi treatment. The fact that the current panel are labeled "DMSO", would seem to suggest the authors have this data.

This is an astute observation from the reviewer. We have now added the PARPi treatment experiment in new **Fig. 3 F and G**, which nicely shows that the early recruitment of KDM5A F8 fragment is PARP-dependent, as would be expected given its PAR-binding activities that we have identified and characterized in this manuscript.

6) The lack of statistical tests in Fig3J and K are concerning. All survival plots, including Fig 1A,B should have statistical test performed indicating their significance.

We thank the reviewer for pointing out this oversight. We have now provided statistics for all experiments as requested.

7) While the interaction between KDM5A and macroH2A1 is convincing, the arguments about macroH2A variant specificity for KDM5A binding are unconvincing as presented. HEK293T cells express vanishingly small levels of macroH2A1.1 compared to macroH2A1.2. The experiment shown in Fig S3G needs a control to show that one of those bands in the input is actually macroH2A1.1 (e.g. macroH2A1.1 specific KD).

As requested, we performed siRNA-depletion of macroH2A1.1 followed by detection with a macroH2A1.1 specific antibody. This experiment revealed that we could specifically knockdown macroH2A1.1, which was not reduced when cells were treated with macroH2A1.2 siRNAs. We now provide this control in new **Fig. S5 G** to address this point. Specificity of macroH2A1.2 antibody and siRNA-depletion efficiency are shown in new **Fig. S5 F**.

8) Experiment 4B also does not provide any evidence regarding variant specificity as they express flag-tagged mH2A1.2 or mH2A2 but only probe with a macroh2A1.2 antibody. This experiment should be repeated with a flag antibody and include flag tagged versions of all three macroH2A variants. It is unclear if the Flag-mH2A2 protein is even expressed in this experiment which also has implications for the conclusions from Fig 5C,D.

As requested, we repeated these experiments and analyzed expression of the histone variants in HEPG2 cells. Western blot analysis confirmed expression of both macroH2A1.2 and macroH2A2 (new **Fig. 6 B**). We also used macroH2A variant specific antibodies to confirm that these flag tagged macroH2A variants were indeed expressing macroH2A1.2 and macroH2A2 (new **Fig. S5 H-I**). These new results confirm expression and identity of these macroH2A variants used in our study.

9) There is insufficient explanation or discussion of how the KDM5A/macroH2A interaction is PAR independent in the absence of DDR but PAR-dependent in the presence of DDR (Fig 4C).

This is a very interesting point. Although this result was reproducible (original **Fig. 4 C** now found in new **Fig. 6 C**), the mechanistic basis for these observations is still unclear. Although we can only speculate, we believe that these results may be due to the fact that KDM5A has additional modes of interacting with chromatin in addition to PAR-dependent binding. For example, the PHD domains of KDM5A bind to unmodified H3 (PHD1) and H3K4me3 (PHD3). In our previous work (Gong et al. JCB 2017), we demonstrated that the PHD1 of KDM5A was required for its localization to DNA damage sites. In addition, KDM5A engages chromatin through H3K4me3 binding, which is decreased during DNA damage, a time that we predict PARylation becomes more relevant. Based on our previous observations and published KDM5A binding requirements to chromatin, we have now included this information in a discussion to help explain our new data presented in Fig. 6 C as requested.

10) The constant switching of cell lines in different experiments (HCT116, HEK293T, U2OS) is problematic given that none of the critical experiments were performed in all three cell lines.

For our study, we performed experiments in different cell lines to use the strengths of each for our analysis. In our first version of the manuscript, we had access only to HCT116 KDM5A KO cells. For our revision, we generated a KDM5A KO U2OS cells line using gene-editing by CRISPR-Cas9 (new **Fig. S2 A**). We have now performed several key experiments using PARPi sensitivity, micronuclei formation and DSB induction following PARPi treatment, which all gave similar results to those obtained in HCT116 cells (new **Fig. S2 C-G**). Many of the biochemical assays were performed in HEK293T cells and cell biological analyses in U2OS cells. These are well-established cell line models in the DNA damage response field, which allows us to compare our results to others in the field. Taken together,

we believe these analyses are complimentary and support our main findings from this study for the role of KDM5A in the DDR and the identification of a PAR-binding domain, which mediates these functions.

11) Statistical test for all of the laser-induced recruitment IF experiments (Fig 3I, Fig 5B,D, Fig S3B, I) are lacking and necessary given the low signal to noise.

This comment is related to this reviewer's comment 6 above. We now provide statistics for all of these experiments as requested.

Reviewer #3 (Comments to the Authors (Required)):

In the manuscript from Miller and colleagues, the authors investigate the regulation of KDM5A in response to DNA damage. This paper develops previous work from the lab, in which they show that KDM5A is recruited to DNA breaks in a PARP dependent manner and contributes to repair and to repressing transcription near the breaks. Here, they provide evidence that there is a direct interaction between KDM5A and PAR through a motif in the C-terminal region of the protein. This interaction appears to be important for the ability of KDM5A to localize to DNA breaks. They also provide evidence that macroH2A is important for KDM5A to localize to DNA breaks. Overall, this is a good advance on previous work and there are some interesting new findings. There are several areas where additional evidence or controls are needed to support the conclusions in the manuscript, detailed below.

Specific points:
1. The use of a complemented knockout (KO) cell line is an excellent addition to siRNA experiments, but additional characterization of this cell line is needed, especially since just a single clone is used here. HCT116 is annotated as already having a loss of function mutation (deletion/frameshift) in one allele of KDM5A, so it is not an ideal choice. Can the authors provide more information about the clone, such as the nature of the mutation in the remaining allele, and whether it has the annotated mutation in the parental sequence? In addition, it is important that KDM5A functions are characterised in the wt, KO and complemented KO to validate this as a model system. An easy readout would be H3 K4 methylation levels and RT-PCR of one or two known KDM5A-regulated genes.

The HCT116 KDM5A knockout cell line was generated by Horizon Discovery. For this knockout, a PGK promotor NEO cassette flanked by LoxP sites was integrated into both alleles of KDM5A at Exon 11, which removes this Exon making both KDM5A alleles non-functional. This information is now provided in the manuscript as requested. Importantly, we have performed complementation experiments using this cell line for several DNA damage phenotypes and show that we can rescue the phenotype by expression of WT KDM5A (new **Fig. 5 D-H**). These results validate the functional importance of KDM5A in the DDR as we report in this manuscript and rule out off-target effects. Furthermore, we generated a KDM5A KO U2OS cells line using gene-editing by CRISPR-Cas9 (new **Fig. S2 A**). We have now performed several key experiments using PARPi sensitivity, micronuclei formation and DSB induction following PARPi treatment, which all gave similar results to those obtained in HCT116 cells (new **Fig. S2 C-G**). As requested, we analyzed the expression of E-cadherin, a gene known to be regulated by KDM5A (Dabiri et al., 2019; Feng et al., 2017; Liang et al., 2015; Wang et al., 2013). In new **Fig. S1 B and S2 B**, we observed increased expression of *E-cadherin* mRNA by RT-qPCR in both HCT116 and U2OS KDM5A KO cell lines. We also performed western blotting of H3K4me3 and did not observe clear changes in this mark. In the literature, it has been reported that long term loss of KDM5A results in near normal levels of H3K4me3 due to adaptation and redundancy of other histone demethylases that can regulate this mark (ex. Hinohara et al. Cancer Cell, 2018). For these reasons we did not include this data. Taken together, we hope that the reviewer will agree that these additional experiments in two different cell lines, addresses any concerns about characterizing these cell lines as

KDM5A knockouts and validating these cell lines as model systems for KDM5A loss in the DNA damage response.

2. In Figure 2, the authors show that there is an interaction between KDM5A and PARP1 and PAR. While the KO cell line in HCT116 might not be the ideal choice for phenotypic analysis, it would be excellent as a control for the specificity of these interactions. Using the anti-KDM5A antibody out of the KO extract is a much more rigorous control than IgG. Also, a GFP antibody can be used with the expression construct containing cells. Addition of these controls would really help to solidify this conclusion since some of the IP results are not very convincing (particularly Figure 2C) and could be non-specific. The PARP inhibitors don't address this, since non-specific interactions would also be reduced when the amount of PAR in the input samples is massively reduced.

To address this question, we have performed an IP of KDM5A in WT and KDM5A KO cells as requested. As shown in new **Fig. S2 A**, IP of KDM5A results in co-immunoprecipitation of PARP1 in WT but not KDM5A KO cells. We had also performed a similar experiment with GFP-KDM5A using anti-GFP antibodies, which showed that KDM5A immunoprecipitated PARP1 in GFP-KDM5A expressing cells and not in cells expressing GFP only (**Fig. 6 D**). We believe these additional controls strengthen our conclusion that KDM5A interacts with PARP1.

3. The in vitro binding assays are very nice and the identification of a new PAR binding motif is potentially important. The functional significance of this in cells appears to be supported by the cellular assays in Figure 3. However, expression analysis of the construct lacking the PAR binding region in these experiments should be provided to demonstrate that the defects are not due to problems with protein folding or stability. It would also be interesting and informative to see if the non-damage functions of KDM5A are affected by this deletion. Minor note: either use GFP or mClover, but the mix of the two terms in the legend and on the figure is potentially confusing.

We agree that showing the expression level of KDM5A WT and ΔPID is important for interpreting these data. We now provide western blotting analysis of these constructs that are expressed in KDM5A KO cells. Expression of WT and ΔPID are similar, ruling out that the effects that we see upon loss of this region in KDM5A is not due to reduced expression/stability of the protein. These data are provided in new **Fig. 5 F**. Similar expression levels of GFP-KDM5A WT and ΔPID was also shown in Fig. 4D (now **Fig. 6 D**), which is consistent with these new data provided for expression in KDM5A KO cells.

It would be very interesting to study the role of the PID domain in KDM5A's function in gene expression beyond its role in genome stability and DNA repair that we have identified here. We believe this is beyond the scope of this manuscript. However, given that we observed an increase in E-cadherin mRNA in KDM5A KO HCT116 and U2OS cells compared to WT cells (new new **Fig. S1 B and S2 B**), we analyzed the expression of *E-cadherin* -/+ PARPi in undamaged conditions in U2OS cells. We reasoned that if PAR-binding was important for KDM5A repression of *E-cadherin*, we would observe an increase in *E-cadherin* in PARPi-treated cells (new **Fig. S5 C**). This result is consistent with PAR-binding being important for KDM5A in non-damaged conditions. Feature studies are warranted to understand mechanistically the potential involvement of PARylation in regulating KDM5A, and potentially other coiled-coil domain containing proteins that bind PAR, during gene expression regulation.

For GFP vs mClover, we have changed mClover on the figure to GFP to avoid any confusion as suggested. We originally had Cover as this is the variant of GFP used and is specific to the CRISPR-mClover gene-targeting assay that was utilized for this experiment. This distinguishes it from other

HDR assays that use GFP+ cells as a readout (for example the DR-GFP assay). Our clear description of this assay in the text and figure legend should now avoid the potential for any confusion.

4. In Figure 4, there is an apparent interaction between macroH2A1.2 and KDM5A. In Fig 4A, the KDM5A construct is overexpressed, and in 4B there is no IgG control in the cells expressing the mH2A constructs. It would again be important to include more controls for specificity to confirm this result. In Fig. 4F, there is no western provided to show depletion of mH2A1.2. On a minor note, the meaning of the colors used in Fig 4E are not defined in the legend.

As requested, we repeated our analysis in **Fig. 4 B** (now new **Fig. 6 B**) and now include IgG controls. These new results confirm our identification of an interaction between KDM5A and macroH2A1.2. In **Fig. S5 D**, we IPed endogenous KDM5A and similarly observed an interaction between macroH2A1.2 but not macroH2A1.1. Taken together, these results suggest an interaction between KDM5A and macroH2A1.2, which we further identified the PARP interaction domain (PID) of KDM5A as an important region in supporting this interaction (**Fig. 6 D**). We also now provide evidence for the specificity of macroH2A1.2 antibody and depletion of macroH2A1.2 by siRNA in new **Fig. S5 F and H**. We also now provide an explanation for the color coding of **Fig. 4 E** (now **Fig. 6 E**) in the legend.

5. Minor points: The statistical test is 'Tukey's' not 'Turkey's'. End of page 7 should read 'BRCA1 and BRCA2 are sensitive to' not 'sensitivity to'.

We thank this reviewer for pointing out these errors. We have now corrected them in the text.

April 5, 2021

RE: JCB Manuscript #202006149R

Dr. Kyle Miller
The University of Texas at Austin
2506 Speedway NMS 2.104
Austin, TX 78712

Dear Dr. Miller,

Thank you for submitting your revised manuscript entitled "Poly(ADP-ribose)-binding and macroH2A mediate recruitment and functions of KDM5A at DNA lesions". You will see that the reviewers praised the revision efforts and now support publication. One reviewer was not clear on the new EMSAs presented in figure 4KL. We agree it is unclear what happens with the complex since it is not in the gel or the well. But we also appreciate that you went out of your way to explain that you think that the complexes do not enter the gel so you use the loss of the band as indicative of binding. This is not the most elegant way to show it, but in combination with other data, we feel that the evidence that the binding is dependent on the small protein region is strong and that there is some evidence that longer PAR chains bind better. We do not think that you need to show more data on this. We would be happy to publish your paper in JCB pending final revisions necessary to meet our formatting guidelines (see details below).

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1) eTOC summary: A 40-word summary that describes the context and significance of the findings for a general readership should be included on the title page. The statement should be written in the present tense and refer to the work in the third person.

- Please revise the summary statement on the title page of the resubmission. **It should start with "First author name(s) et al..." to match our preferred style.**

2) Figure formatting:
Molecular weight or nucleic acid size markers must be included on all gel electrophoresis. Please check that unit labels are added to all gel panels.

3) Statistical analysis: Error bars on graphic representations of numerical data must be clearly described in the figure legend. The number of independent data points (n) represented in a graph must be indicated in the legend. Statistical methods should be explained in full in the materials and methods. For figures presenting pooled data the statistical measure should be defined in the figure legends.

4) Materials and methods: Should be comprehensive and not simply reference a previous publication for details on how an experiment was performed. Please provide full descriptions in the text for readers who may not have access to referenced manuscripts.
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vendor ID (e.g., Addgene, ATCC, etc.) or if unavailable, please briefly describe their basic genetic features *even if described in other published work or gifted to you by other investigators*
- Please include species and source for all antibodies, including secondary, as well as catalog numbers/vendor identifiers if available.
- Sequences should be provided for all oligos: primers, si/shRNA, gRNAs, etc.
- Microscope image acquisition: The following information must be provided about the acquisition and processing of images:
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b. Type, magnification, and numerical aperture of the objective lenses
c. Temperature
d. imaging medium
e. Fluorochromes
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g. Acquisition software
h. Any software used for image processing subsequent to data acquisition. Please include details and types of operations involved (e.g., type of deconvolution, 3D reconstitutions, surface or volume rendering, gamma adjustments, etc.).

5) A summary paragraph of all supplemental material should appear at the end of the Materials and methods section.
- Please include one brief descriptive sentence per item.

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Sincerely,

Agata Smogorzewska, MD, PhD
Monitoring Editor, Journal of Cell Biology

Melina Casadio, PhD
Senior Scientific Editor, Journal of Cell Biology

Reviewer #1 (Comments to the Authors (Required)):

The paper is much improved. Figure 4 K and L are hard to analyze they should improved. Alternatively another technique should be used such as SPR. The rest of manuscript is acceptable for publication.

Reviewer #2 (Comments to the Authors (Required)):

The authors have responded commendably to my concerns. I believe the manuscript is now suitable for publication and will be of interest to a wide audience.

Reviewer #3 (Comments to the Authors (Required)):

The revised manuscript has included experiments that sufficiently address the issues raised, and the conclusions are supported by the data.