NYU School of Medicine

Dear Dr. Vineeta Bajaj and Dr. Lyndsay Troyer,

Enclosed is the revised version of our manuscript (tracking number # JoVE62466) in which we have addressed all the points raised by the reviewers, as described below:

Editorial comments (*italicized in blue*)

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.
Done.

2. Do not underline any part of the text.
All underlined text has been removed.

3. Use "mL" instead of "ml" (line 136). Add a single space between the quantity and its unit. E.g. "450 nm" instead of "405nm" (line 179).
The highlighted mistakes have been corrected.

4. Include a single line space between successive protocol steps and then ensure that the highlight is no more than 3 pages including headings and spacings. Avoid the use of personal pronouns in the protocol. E.g. "we", "our" etc.
Personal pronouns have been removed from the manuscript.

5. In the JoVE Protocol format, "Notes" should be concise and used sparingly. They should only be used to provide extraneous details, optional steps, or recommendations that are not critical to a step. Any text that provides details about how to perform a particular step should be written in imperative tense (as if telling someone how to do the technique), and either be included in the step itself or added as a sub-step. Do not include "notes" as protocol steps. E.g. line 211. Consider moving some of the notes about the protocol to the discussion section.
Notes sections have been trimmed down and in some sections of the protocol, notes have been moved to the discussion.

6. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials. E.g. Nikon, Nunc, etc.
Commercial language has been removed from the text.

7. Figure 2, 3: Use "s" instead of "sec" in the labels.
"Sec" has been changed to "s" in all relevant figures.

8. Please ensure that the references appear as the following: [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Source. Volume (Issue), FirstPage – LastPage (YEAR).] For more than 6 authors, list only the first author then et al. Do not abbreviate journal names. Do not use "&/and" in the authors list of the references. Please include volume and issue numbers for all references.
Endnote style from JoVe was used to format the Reference section.

9. Please ensure that all materials used are included in the table of materials.
Table of Materials has been updated to include all materials used.

NYU School of Medicine

Reviewer #1 (*italicized in blue*)

Major Comments:

** Chapter 1. (Cultivation of U-2 OS cell) is written too vague. Authors should give either number of the cells per ml or cm2. Otherwise, the cultivation lacks the consistency, 90% of confluency and split cells 1:10th is instruction based only on subjective feeling. It could be misleading for students as well as for early-stage researchers who start with cell cultivation methods.*

Protocol for the cultivation of U-2 OS cells have been updated in the text following the reviewers' recommendations. Exact cell numbers for during splitting are now included in the protocol.

** Section 3.3. How did authors established the final concentration of olaparib (1μM and one hour prior to irradiation experiments)? It seems to be a very short time to induce any changes in the accumulation of PCNA or EXO1b. Figure 3A is not convincing either, because there are different fluorescence intensities in red channels (DMSO vs olaparib). Lower accumulation of PCNA after 1 h olaparib treatment (25 sec after irradiation) could be only a matter of higher background in the individual cell. Also, there is no change in accumulation in EXO1b protein after olaparib treatment compared to DMSO control.*

We have now included further references in the text for the routine use of PARP inhibitors at the indicated concentrations and time, prior to micro-irradiation (PMID: 29985131, PMID: 26456830, 29547717). Olaparib treatment does not cause changes in the total protein levels of PCNA or EXO1b. However, it has been reported to affect the extent of EXO1b recruitment and delay PCNA recruitment, in line with our results shown in Figure 4 (PMID: 26519824, PMID: 26400172, PMID: 29547717). Any minor differences in the total fluorescence intensity of each cell (as seen on the representative images), comes from the fact that we used a heterogenous population of cells after viral infection. Depending on the amount of viral integration events in each individual cell, cells can express different levels of the POI. Importantly, our evaluation method (PMID: 29985131, PMID: 26456830) normalizes the recruitment intensities to the unaffected areas of the nucleus, therefore the experiments are internally normalized to the overall expression level of the POI in each cell. Establishing clonal cell lines is possible, but we would not recommend them for routine use due to increased possibility of artefacts (cherry picking clones from a heterogenous genetic background), therefore we omitted that approach from our protocol. We have changed the representative images on Figure 4A to be more in line with the graph shown in Figure 4C.

** Chapter 4. Authors stated that they used Nikon LUN-F 50 mW 405nm FRAP laser. What was the final dose (laser output behind objective) that was used to induced DDR?*

In terms of the laser power, the output behind objective depends on many factors including the optical fiber, the optical properties of all the lenses and mirrors in the light path, as well as the percent power output set in the software. Usually, the power controls in NIS elements are adjusted to be linear but it isn't always perfect. The laser power reaching the cells also depends on the immersion oil and the cell culture dish used. Because there are so many variables, we do not provide estimated power output behind the objective. Recommending a power meter in the protocol to measure it directly is simply not a realistic approach for most of the laboratories. Therefore, we propose in the protocol to use well characterized repair proteins to monitor the types of lesions generated (see Figure 2 and Discussion).

** Section 4.2.2. Authors stated that they selected FOV (field of view). Did authors select the same size of FOV per each irradiation experiment or it was different? Will the different size of FOV influence the intensity of the laser/ dose and the accumulation of POI to DNA lesions?*

The same size FOV (1024x1024 pixels) and zoom (1x) was used for each irradiation experiment to ensure the same pixel size (which was 0.29 μm/pixel) throughout our experiments. Because laser dwell time is on a per pixel basis, as long as the pixel size remains the same, the relationship between the dwell time and power density will not change. If the pixels size changes due to changes in the FOV and zoom settings the pixels would represent a different size area on the sample, making comparisons not possible. We have incorporated this information into the text (section 5.1.1).

** Chapter 5. How did the authors verify the appropriate laser power setting? Did the irradiated cells proceed to the mitosis? Please, provide relevant images.*

Appropriate laser settings were determined based on the ability to induce the recruitment of PCNA, FBXL10, NTHL1 and EXO1b with the minimum necessary laser power. Cells were not followed into mitosis. Whether cells enter mitosis or eventually progress through the next G1 phase depends on several factors. Even a very limited number of DSBs can delay or stop cell cycle progression until the damage is resolved otherwise cells go through to apoptosis (PMID: 30184135). Cell fate after DNA damage is ultimately dictated by p53 levels (PMID: 27062928, PMID: 30254262, PMID: 28317845) therefore, the p53 background of the cell lines used, will dramatically affect whether they will enter mitosis or not. U-2 OS and RPE cells used in our protocol have a functional p53 response therefore being more sensitive towards the recovery from laser induced DSBs. HeLa cells, lacking functional p53, would show a more robust entry into mitosis. The focus of our protocol was to visualize repair processes in S phase, therefore studying mitotic entry was out of our scope.

** Either stable or transient transfection of proteins may bypass regular expression, did authors verify their observations by immunofluorescent labelling of endogenous accumulation PCNA and other POI after irradiation? Please, provide relevant comparative images.*

Recruitment of tagged exogenous PCNA has been well established in the literature (PMID: 25484186) therefore we did not perform immunofluorescent staining against endogenous PCNA. One of the fundamental limitations of that approach is the lack of temporal dynamics since one uses fixed cells. Therefore, to demonstrate the recruitment of endogenous PCNA in our experimental setup, we have used human retinal pigment epithelial cells (hTERT RPE-1) in which the fluorescent protein mRuby was engineered in frame with the first exon into one allele of the PCNA locus by recombinant adeno-associated virus-mediated (rAAV) homologous recombination in (the cell line was a kind gift of Jörg Mansfeld) (PMID: 28564611). Cell cycle based localization pattern was identical to our findings using exogenous PCNA. Results are shown on the new Figure 3 and updates have been made in the text accordingly.

Minor Comments:

** Section 2.1. In the manuscripts, there is no information regarding cultivation conditions (media etc.) of HEK293T cells*

Cultivation of HEK293T cells is now incorporated in the protocol. Please see section 2.1.1.

** Row 161: …plate 8.0x104 cells… per what volume?*

$8.0x10^4$ refers to the total number of cells plated into the four-well chamber. Any volume between 0.5 mL and 1 mL is sufficient for culturing in a 4 well chamber. We have updated the text to be less confusing in section 3.1.

Table of Material:

** Unify HEK293T cell or 293T cells*

293T has been changed to HEK293T in the table of materials.

** No standard DMEM Medium is mention in the Protocol*
Cultivation of HEK293T has been added to the protocol which now mentions standard DMEM Medium.

** No pre-sensitizes were used in Protocol. Why are Hoechst 33342 and BrdU mentioned in Table of Material?*
Hoechst 33342 and BrdU were discussed extensively in the Discussion and were included in the Table of Materials for the readers convenience if relevant to their experimentation.

** No Nunc™ Lab-Tek™ II Chambered Coverglass (8 well) is used in the Protocol*
Lab-Tek™ II Chambered Coverglass (8 well) has been removed from the Table of Materials.

** No Universal Mycoplasma Detection Kit is mentioned or used in the Protocol*
Use of the Universal Mycoplasma Detection Kit has been now placed into the protocol.

Reviewer #2 (*italicized in blue*)

Major Comments:
1) The manuscript contains many paragraphs unaccompanied by supporting citations. The introduction is currently too vague and lacks information about existing approaches to quantify protein recruitment to sites of DNA damage1. PCNA is a well-studied S-phase marker. It is widely used to identify S-phase cells in live cell fluorescence microscopy and to distinguish between cell-cycle specific differences of DNA damage response (DDR) protein dynamics2-4. Novelty of the method is not a pre-requisite for a JOVE publication, but I think it is particularly important to enable the scientific community to easily compare method papers by providing citations and that the introduction should provide a more through overview of existing methodology (beyond the example mentioned here). Similar to the introduction, the discussion is lacking the citation of supporting literature and a discussion of the obtained results in the context of existing literature. Additionally, the limitations imposed by the choice of slow confocal microscopy and "relative fluorescence unit" image analysis on the interpretation of recruitment kinetics results should be discussed.
We thank the reviewer for these recommendations. We have incorporated new citations throughout the manuscript accordingly. We have also added a paragraph in the discussion about the limitations of confocal microscopy. (Note: Unfortunately, we did not receive the exact recommended references of Reviewer 2.)

2) To verify that the 405 nm laser induces double-strand DNA breaks (DSBs) the laser dwell time at 50 mW power was increased until the induced damage was sufficient to observe recruitment of EGFP-FBXL10 (subunit of the FRUCC complex), a PARP1-dependent ubiquitin ligase complex previously reported to recruit to DSBs. This is a very indirect method to assess DSB induction. Instead, the cells should be fixed after laser damage and immunofluorescence with an antibody against a marker of DSBs such as γH2AX5, 6.
We performed immunofluorescence staining against γH2A.X in the micro-irradiated cells, confirming the elevated levels of DSBs when using a higher laser setting (Figure 2B). DSB induced recruitment of EGFP-FBXL10 has also been studied in detail (PMID: 29985131). We have also included a section on immunofluorescence staining this into the protocol (section 5.3) to check for γH2A.X saining.

3) Quantifying the recruitment of DDR proteins to sites of laser damage by measuring the fluorescence intensity has a major caveat that is not addressed in this manuscript but potentially affects the quality of the results shown in Figure 3A. There are bright spots close to the damage

site in Figure 3A, olaparib treated cells. These bright spots can easily skew the measurement of fluorescence intensity. In this respect the section about recruitment kinetics analysis is too vague and overall does not provide enough information to perform a reproducible quantification. For example, there is no mention of how big the analyzed regions "A", "B" and "C" should be and how to determine a suitable region "A" and "B" within the nucleus without skewing the quantification (e.g how to correct for bright foci or nucleoli within the region). This is essential for a manuscript that focuses on cells in S-phase as replication foci can provide a major source of artefacts in the quantification. The authors should further specify details regarding correcting for or preventing artefacts due to movements of the cells relative to the selected region across different timepoints. The authors should explain how ImageJ is used to perform these intensity measurements in a reproducible and automated manner and how the measurements across regions, timepoints and cells are collected and compiled to produce Figure 3B.

We have updated Section 6 of the protocol to contain more details on image analysis and important guidelines on the analyzed regions and possible artefacts (PMID: 29985131). We have also included a new representative image (Figure 4B) to aid evaluation in Section 6.

4) It would be useful for the authors to provide equations or mathematical models to extract information about the recruitment kinetics from the curves in Figure 3B. This section should be the main focus of this manuscript because the word "kinetics" implies that, at the very least, the authors will provide methods to calculate either the half time or the time when maximum recruitment is reached, and the time the intensity reaches a plateau. There are examples in the literature how to distinguish if two proteins have distinct kinetics, or merely different recruitment intensities with comparable kinetics7. I would also suggest that the authors compare the recruitment kinetics of Exo1b in S-phase cells vs G1 cells to support the statements in line 87/88 of the introduction. Furthermore, the authors say that Exo1b recruitment kinetics is changed in response to olaparib treatment. Based on the curves in Figure 3B, Exo1b reaches its maximum at around 60 seconds in both cases. According to the plots, the recruitment intensity is decreased in olaparib treated cells but based on the sample images in Figure 3A the recruitment intensity looks comparable between DMSO treatment and olaparib treatment at all three depicted timepoints.

We did not compare the recruitment profile of EXO1b in G1 vs. S-phase cells as our protocol only focuses on S phase (as part of the *Current methods to analyze DNA damage in S-phase in eukaryotic cells* JoVe methods collection). PCNA has a homogenous localization pattern in both G1 and G2 phases of the cell cycle (Figure 1) requiring additional synchronization steps to be able to evaluate just G1-phase cells. We agree with the reviewer that detailed kinetic analysis can provide valuable insights into the recruitment properties of the POI. However, in order to apply mathematical models, detailed characteristics of the recruitment has to be known (*e.g.*: contribution of multiple DNA binding domains, sensitivity towards different signaling events, *etc*.). The observed recruitment kinetics is the combined output of several factors which needs to be deconvolved into individual mechanistic contributors for proper mathematical modelling. We believe this in-depth analysis is out of the scope of this protocol and therefore we show recruitment profiles by connecting the RFU values over time, as also shown in the literature for EXO1b (PMID: 23939618, PMID: 26519824, PMID: 26884156, PMID: 26400172, PMID: 20019063). We have also corrected the text to reflect only the differences observed in the maximal extent of EXO1b accumulation (DMSO vs. olaparib), which is in agreement with the above cited literature. We removed “kinetics” from the title and the text and added additional references for optional kinetic analysis in the discussion.

5) Exo1b signal in Figure 3A is stronger in nucleoli. However, in previous literature the signal in the nucleus and the nucleoli is indistinguishable8, 9. In the cited papers Exo1b was tagged N-terminally and in this manuscript C-terminally. Could this explain the changed localization? In this

manuscript the maximum recruitment is reached after 1 minute whereas previously Exo1b was reported to reach a maximum up to 5 minutes after damage before starting to dissociate from damage sites8, 9. Could you clarify if this is due to different damaging conditions or if C-terminal tagging impairs the function and DNA damage recruitment of Exo1b? In Discussion line 376 you mention tagging can impair protein function so it would be good to check and compare the function, localization and recruitment of N- and C-terminally tagged Exo1b.

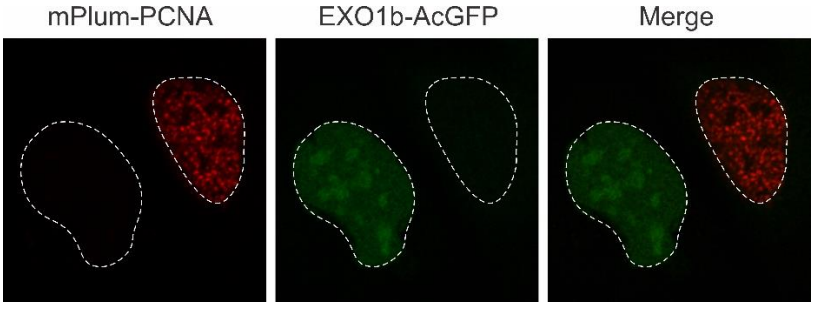
As mentioned by the reviewer the exact recruitment profile might rely on several factors, like the exact laser damage used (*e.g.:* 365 nm vs. 405 nm) or whether EXO1b is tagged either on the N or C-terminal in the studies. The PIN domain of EXO1b is on the N-terminal while the PIP motif is on the C-terminal, with both structural elements contributing to the recruitment of EXO1b. Both C-terminally: PMID: 29551515, PMID: 30622624, PMID: 26884156 as well as N-terminally: PMID: 26400172, PMID: 26519824, PMID: 20019063, PMID: 23939618 tagged EXO1b is used routinely in the literature. Localization pattern varies among these papers. In the above cited manuscripts, there are examples where EXO1b is excluded from the nucleoli, is evenly distributed in the nucleus or partially accumulates in the nucleoli (PMID: 26884156 as) we have seen it too. The localization pattern may rely on the expression levels of EXO1b, and the type of cell lines used in the different studies. Unfortunately, the exact EXO1 isoform used in the papers is not always specified. EXO1 has three isoforms, slightly differing from each other. We are using EXO1b, the longest isoform (PMID: 20019063, PMID: 29551515). It is also important to note that we were aiming to detect differences w/wo a specific treatment (in our case olaparib).

6) The authors mention in line 322-323 the importance of comparing the overexpression levels of the protein of interest to the endogenous levels. It would thus be useful to compare Exo1b overexpression and endogenous levels in U2OS cells.

We have now compared the expression levels of endogenous and exogenous EXO1b shown in Figure 4D. The expression level of EXO1b-AcGFP is similar to endogenous EXO1b.

7) In the methods the authors should mention the filters used in the microscopy setup. Especially as the authors state in line 255 that simultaneous imaging of Exo1b and PCNA was done, the authors should show a control sample to confirm no fluorescence bleed-through and cross-excitation is skewing the recruitment quantification. This will help readers to select filter sets and set up control experiments to avoid such artefacts.

We have now included in Section 4 the exact filters and lasers lines used for imaging, making a note on the importance of checking fluorescence bleed-through. We have also included here (see below) a panel showing that we did not have any fluorescence bleed-through with our imaging settings. Our protocol did not aim to cover basic confocal microscopy. We believe a laboratory routinely using fluorescent microscopy will have these appropriate controls already established before implementing our protocol.



Since infection is not 100% efficient and we did not sort for double positive cells, we used the single-colored cells (either just mPlum-PCNA or EXO1b-AcGFP positive) to show the lack of bleed-through when using simultaneous excitation during our imaging.

8) Paragraph 4.2.5 is unclear. The authors should provide specific information about how long the microirradiation takes and how much time is lost before the software starts recording data for

the recruitment curve. In order to quantitively assess protein recruitment kinetics, the first few seconds after laser damage are essential and inconsistent start of acquisition after damage can severely skew the results. Line 219 is unclear. What does 0.5-2 µm thickness mean and how is this measured?

The Limitations of micro-irradiation section of the Discussion has been updated with the exact time needed to micro-irradiate cells using our protocol and imaging system. To avoid any confusion, we removed the 0.5-2 µm thickness note from the protocol that was referring to the thickness of a typical EXO1b recruitment track.

9) 5.1.1, line 234 and 5.2.1, line 251: the scanning speed has to be provided in a more useful unit like µs/pixel. The image size and zoom has to be amended by providing the resulting pixel size (µm per pixel) of the images. Otherwise fixing the pixel dwell time of the 405 nm FRAP laser may lead to variable results. 5.1.3, line 244, please clarify if 1000-3000 µs is the dwell time per pixel or the dwell time across the whole damage line. The exact length in pixels of the line along which the 405 nm laser damage was applied has to be provided.

Section 5.1.1 and 5.2.1 has been updated to reflect the scanning speed in µs/pixel. We have also included the pixel size that results from the image size and the zoom used in the protocol. We have updated the text in section 5.1.3 to clarify that the given laser dwell time is on a per pixel basis, not across the whole damage line (ROI). We have also included information on the exact length of the ROI used for micro-irradiation in pixels (section 4.3.5).

10) In 5.1.3, line 244 the resulting FRAP 405 nm laser power at the sample should be measured and provided as a reference to replicate the described experiments. Identical 50 mW lasers will produce different effective damage depending on the amount of light lost in the optical path.

In terms of the laser power, the output behind objective depends on many factors including the optical fiber, the optical properties of all the lenses and mirrors in the light path, as well as the percent power output set in the software. Usually, the power controls in NIS elements are adjusted to be linear but it isn't always perfect. The laser power reaching the cells also depends on the immersion oil and the cell culture dish used. Because there are so many variables, we do not provide estimated power output behind the objective. Recommending a power meter in the protocol to measure it directly is simply not a realistic approach for most of the laboratories. Therefore, we propose in the protocol to use well characterized repair proteins to monitor the types of lesions generated (see Figure 2 and Discussion).

11) In 5.2.2 the exact imaging conditions (type of excitation lasers and excitation power) used for the results presented in this manuscript should be provided.

Exact imaging conditions are now included in section 5.2.2.

12) Why was EGFP used for one experiment (EGFP-FBXL10) and AcGFP in the remaining experiments? Does AcGFP dimerize like GFP? Using a dimeric form of GFP would contradict the discussion line 376-378. Please clarify this.

We have used the EGFP-FBXL10 (in a pBABE.puro backbone) construct because it is conveniently deposited at Addgene (Plasmid #126542) so everyone can easily obtain it. The recruitment properties of EGFP-FBXL10 have been extensively characterized, making it an optimal choice for DSB detection (PMID: 29985131). To achieve comparable expression levels to endogenous EXO1b we needed a retroviral expression vector with a stronger promoter. For this purpose, we chose the pRetroQ vectors sold by Takara. Takara, the manufacturer selling AcGFP based plasmids states the following on their website: "AcGFP1 (*Aequorea coerulescens* GFP) is a monomeric green fluorescent protein with spectral properties similar to those of EGFP. Although AcGFP1 and EGFP sequences have 94% homology at the amino acid level and equivalent brightness, AcGFP1 is a superior alternative for fusion applications because it is a

monomer." In our opinion both EGFP and AcGFP can be used successfully in our protocols. We have updated the text to include more information on possible monomeric fluorescent proteins that could be used.

13) A previous publication showed no recruitment of NTHL1 to a 405 nm 50 mW laser10. Please comment on this discrepancy. This is particularly important because this experiment is used as an indirect indication for the presence of oxidative damage. In line with my major comment 2): Using the recruitment, or lack of recruitment of a protein to a laser damage site should not be used as the only indicator for the presence or absence of DNA damage, especially if the results show inconsistent recruitment across different publications.
We think the reviewer was referring to the following publication PMID: 24293652. (Note: Unfortunately, we did not receive the exact recommended references of Reviewer 2.) While the paper does not show recruitment of GFP-NTHL1 with using 50 mW laser power (405 nm), it does show recruitment by using 500 mW laser power (405 nm) or by the excitation of KillerRed (generating oxidative DNA damage). The authors indicate the amount of laser power used at each scan but did not define the exact FRAP area in pixels, therefore it is uncertain how much energy was actually delivered into a pixel: "405-nm laser light for 10 scans with a rate of 5 mW/scan (total 50 mW)." Additionally, the authors used transient transfection, that can result in high protein levels when comparing it to endogenous levels, therefore requiring more lesions to see signal accumulation at the damage site due of the high signal intensity in the surrounding nucleoplasm. GFP-NTHL1 was also shown to recruit to laser stripes induced with a 780 nm NIR laser line using adequate power to induce base damage (PMID: 29443023, PMID: 21858164). In summary we believe our results are in line with the literature.

Minor Comments:
1) The NTHL1-mCherry construct is missing from the materials list and a description or reference is missing of how U2OS cells for the experiments in Figure 2 were created.
We have updated the text to include information on how the NTHL1-mCherry construct and the stable U2OS cell line was generated sec 5.1.3. Details of the plasmids used can be found in the Table of Materials.

2) Line 43: mCherry PCNA mentioned in the abstract whereas the rest of the manuscript refers to mPlum PCNA. In the list of Materials mCherry PCNA is again mentioned but as far as I understood it has not been used.
The reviewer is correct and mCherry-PCNA has been corrected to mPlum-PCNA in the abstract.

3) Line 203: Sentence grammatically wrong
We have corrected the sentence.

4) Title is misleading and I suggest the following alternative: "Laser micro-irradiation to study protein recruitment to sites of DNA damage in S-phase"
We agree with the reviewer and we removed kinetics from our title as recommended.

5) The authors mention the importance of mycoplasma contamination in the discussion (lines 318-321) but it is unclear if and how the U2OS cells used in this experiment were tested for mycoplasma
Use of the Universal Mycoplasma Detection Kit has been now placed into the protocol.

Reviewer #3 (*italicized in blue*)

Minor Comments:

NYU School of Medicine

A discussion of the statistical methods that are appropriate to use when comparing recruitment kinetics across different conditions should be added. A preferred statistical test should also be used to demonstrate the significance of the difference in exo1b kinetics in DMSO- vs olaparib-treated cells the data presented in figure 3b.

We thank the reviewer for the suggestion and have added references to statistical methods and approaches on detailed kinetic analysis of the recruitment data in the discussion. We have also included the results of a two-tailed Mann-Whitney test to compare the maximum extent of EXO1b accumulation at 1 minutes between the DMSO and olaparib treated cells (Figure 4C).

Typos and syntax:

Line 30. Undermining the structures and functions of proteins and…
Line 76. lesions and is recruited to locally
Line 185. System be thermally
Line 198. In S-phase, PCNA forms…
Line 285. Homogeneous
Line 302 are recruited demonstrating
Line 308-309 is denoted by a > 1 relative….
Line 382 homogeneously
Line 445. Figure 3. PARP1/2-dependent (olaparib inhibits both enzymes)

The following suggestions have been implemented into the text.

We appreciate the constructive suggestions made by all reviewers, and we believe that the resulting additions and changes have improved the clarity and message of our study. If you have any further questions, please feel free to contact me. I look forward to hearing from you.

Sincerely,

Gergely Rona, PhD
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02/24/2021