EMBO reports

FBL promotes cancer cell resistance to DNA damage and BRCA1 transcription via YBX1

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(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. Wu,

Thank you for the submission of your manuscript to EMBO reports. We have now received the reports from 2 referees, which are pasted below. I could unfortunately not secure a third referee for your study.

As you will see, the referees acknowledge that the findings are potentially interesting. However, they also raise important concerns and have several suggestions for how the study could be improved. Importantly, it needs to be assessed whether the reduced HR-efficiency and BRCA1 downregulation are a mere consequence of less cells being in S-phase upon FBL depletion. All other concerns and suggestions should also be addressed. Please let me know in case you disagree and we can discuss the exact revision requirements further, also in a video chat, if you wish.

I would thus like to invite you to revise your manuscript with the understanding that the referee concerns must be fully addressed and their suggestions taken on board. Please address all referee concerns in a complete point-by-point response. Acceptance of the manuscript will depend on a positive outcome of a second round of review. It is EMBO reports policy to allow a single round of major revision only and acceptance or rejection of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript.

We realize that it is difficult to revise to a specific deadline. In the interest of protecting the conceptual advance provided by the work, we recommend a revision within 3 months (11th Feb 2023). Please discuss the revision progress ahead of this time with the editor if you require more time to complete the revisions.

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1) A data availability section providing access to data deposited in public databases is missing. If you have not deposited any data, please add a sentence to the data availability section that explains that.
2) Your manuscript contains statistics and error bars based on n=2. Please use scatter blots in these cases. No statistics should be calculated if n=2.

When submitting your revised manuscript, please carefully review the instructions that follow below. Failure to include requested items will delay the evaluation of your revision.

1) a .docx formatted version of the manuscript text (including legends for main figures, EV figures and tables). Please make sure that the changes are highlighted to be clearly visible.

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3) We replaced Supplementary Information with Expanded View (EV) Figures and Tables that are collapsible/expandable online. A maximum of 5 EV Figures can be typeset. EV Figures should be cited as 'Figure EV1, Figure EV2" etc... in the text and their respective legends should be included in the main text after the legends of regular figures.

- For the figures that you do NOT wish to display as Expanded View figures, they should be bundled together with their legends in a single PDF file called *Appendix*, which should start with a short Table of Content. Appendix figures should be referred to in the main text as: "Appendix Figure S1, Appendix Figure S2" etc. See detailed instructions regarding expanded view here: <https://www.embopress.org/page/journal/14693178/authorguide#expandedview>

- Additional Tables/Datasets should be labeled and referred to as Table EV1, Dataset EV1, etc. Legends have to be provided in a separate tab in case of .xls files. Alternatively, the legend can be supplied as a separate text file (README) and zipped together with the Table/Dataset file.

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7) Before submitting your revision, primary datasets produced in this study need to be deposited in an appropriate public database (see https://www.embopress.org/page/journal/14693178/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 & Method (see also https://www.embopress.org/page/journal/14693178/authorguide#datadeposition). Please note that the Data Availability Section is restricted to new primary data that are part of this study. * Note - All links should resolve to a page where the data can be accessed. *
If your study has not produced novel datasets, please mention this fact in the Data Availability Section.

8) At EMBO Press we ask authors to provide source data for the main manuscript figures. Our source data coordinator will contact you to discuss which figure panels we would need source data for and will also provide you with helpful tips on how to upload and organize the files.

9) Our journal also 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 https://www.embopress.org/page/journal/14693178/authorguide#referencesformat

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The following points must be specified in each figure legend:

- the name of the statistical test used to generate error bars and P values,

- the number (n) of independent experiments (please specify technical or biological replicates) underlying each data point,

- the nature of the bars and error bars (s.d., s.e.m.),

- If the data are obtained from n Program fragment delivered error ``Can't locate object method "less" via package "than" (perhaps you forgot to load "than"?) at //ejpvfs23/sites23b/embor_www/letters/embor_decision_revise_and_review.txt line 56.' 2, use scatter blots showing the individual data points.

Discussion of statistical methodology can be reported in the materials and methods section, but figure legends should contain a basic description of n, P and the test applied.

- Please also include scale bars in all microscopy images.

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We would also welcome the submission of cover suggestions, or motifs to be used by our Graphics Illustrator in designing a cover.

As part of the EMBO publication's Transparent Editorial Process, EMBO reports publishes online a Review Process File (RPF) to accompany accepted manuscripts. This File will be published in conjunction with your paper and will include the referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript.

You are able to opt out of this by letting the editorial office know (emboreports@embo.org). If you do opt out, the Review Process File link will point to the following statement: "No Review Process File is available with this article, as the authors have chosen not to make the review process public in this case."

I look forward to seeing a revised form of your manuscript when it is ready. Please use this link to submit your revision: https://embor.msubmit.net/cgi-bin/main.plex

Yours sincerely,

Esther Schnapp, PhD
Senior Editor
EMBO reports

Referee #1:

This manuscript by Sun et al uncovers a role of FBL in promoting DNA damage repair by homologous recombination (HR). The data/model presented suggests FBL mediates this function by promoting YBX1 nuclear translocation. As discussed below, the authors do a great job of biochemically characterizing the interaction between FBL and YBX1. However, the mechanism proposed to account for the DNA damage sensitivity of FBL-deficient cells has multiple caveats. The mechanistic study needs extensive controls and validations to convince the readers of the proposed model.

Major strengthens of the manuscript:

1.Convincing data to show upregulation of FBL across multiple tumor types.
2. Loss of FBL triggers sensitivity to MMC and reduces HR.
3. Robust biochemical characterization of interaction between FBL and YBX1.

Major concerns:

1. Conceptual problem with the proposed mechanism: Authors propose that a key mechanism by which FBL downregulation impacts sensitivity to MMC is by downregulating HR genes, such as BRCA1. However, the authors have not considered the possibility that this could simply be an impact of the cell cycle. Data in Figure 2C and 2D demonstrate that loss of FBL results in reduction of EdU positive (S-phase cells). BRCA1 and other HR genes are predominantly expressed and are functional in S-phase cells. Hence it is likely that the reduction in HR-efficiency and BRCA1 downregulation can be a mere manifestation of reduced S-phase cells upon FBL loss. This indeed seems to be evident from the RNA-seq data (Figure 5D) where multiple S-phase genes are downregulated upon shFBL treatment.

2. In this context it is noteworthy that both HCT116 and A549 cell lines have an active p53 pathway, making them amenable to arrest in the G1/S phase of cell cycle. This reviewer believes the proposed mechanism can simply be the confounding impact of altered cell cycle. It is vital that proper cell cycle controls are included across all mechanistic experiments.

3. Reference showing that FBL-E191A/D236A is catalytically inactive is missing.

4. IF assay in Figure 4C convincingly shows localization of YBX1 to the nucleus upon DNA damage. Authors should use this assay to quantify how FBL overexpression as well as depletion impacts the nuclear translocation of YBX1. In this context, the chromatin fractionation data in Figure 4E is confusing- if FBL loss leads to decrease in nuclear translocation of YBX1, why is there not a concomitant increase in cytoplasmic fraction. Also, no controls have been included to demonstrate the purity/enrichment of nuclear and cytoplasmic fractions.

5. Data in Figure 4C demonstrates that the interaction between FBL and YBX1 is damage induced. However, it remains unclear how nuclear damage triggers the cytoplasmic association of FBL-YBX1 and their subsequent nuclear translocation.

6. Does overexpression of FBL confer resistance to MMC? This is a critical experiment to fully appreciate the importance of FBL overexpression in tumorigenesis.

7. Beyond MMC sensitivity, reduction in HR/BRCA-mediated repair confers sensitivity to multiple other genotoxic agents including camptothecin, PARP inhibitors. Are FBL-deficient cells sensitive to other DNA damaging agents?

8. Do FBL and YBX1 have nuclear localization signals or is their nuclear translocation reliant on another protein?

Minor point:

Fig4A: Authors show that overexpression of YBX1 overcomes the impact of FBL loss in the DR-HR assay. The labeling of figures needs to be fixed to indicate this. In its current form, it appears that both FBL and YBX1 were depleted.

Referee #2:

Summary:
In this study, the authors wanted to investigate the role of Fibrillarin (FBL) in DNA damage response. Indeed, there were previous studies (breast and prostate cancers) indicating that FBL may be acting as an oncogene promoting cancer cell proliferation and resistance to chemotherapy.
So, they started by expanding the analysis of FBL mRNA expression to more cancers using the TCGA database. Then, they explored in more details the molecular ways in which FBL could provide sensitization to chemotherapies using colon and lung cancer cell lines. It is very interesting that this new function of FBL is independent of its MTase activity.
Finally, they identified a new, interesting player, YBX1 and showed that YBX1 acts as a downstream effector regulating/activating BRCA1 gene transcription.

Overall impressions: This is an interesting study which reports a set of new findings suggesting a new level of regulation for BRCA1 DNA damage repair activity, involving the FBL/YBX1 pathway.
However, my there are a few important controls missing and additional experiments should be performed in order to consolidate the study and their model (see below).

Strengths: The in vivo data look well controlled and support the in vitro results.

Shortcomings: The methods and figure legends are often not detailed enough so we do not know which drug dose is used and what kind of extracts have been analyzed, making it harder to interpret the data and determine whether each experiment is adequate to answer the question. Also, the authors need to be more reserved when claiming that FBL contributes to tumorigenesis. Previous data seems to indicate it could act as an oncogene but no definite proof yet.

Comments:
The overall writing must be improved throughout the manuscript for clarity and some paragraphs in the introduction could be shortened (first one for example, since the Methyltransferase activity of FBL is not required for the new function described) so more important facts can be better articulated and expanded to allow some additional points to be mentioned in the discussion.

Figure 1: Statistics and the type of values (median, mean etc.) should be shown for panels A, B and C. We do not understand why colon and lung are chosen and why breast (and ovary, given the implication of BRCA1) or prostate are not shown. The cut off for low and High levels of FBL should also be defined.
It would be great to show an immunoblot of FBL in the cell lines chosen for the reminder of the study compared to their normal, respective tissue.
Finally, the colony formation assays are good and convincing except for the lack of control immunoblot showing the FBL levels in the rescue settings. The shRNA chosen for these representative experiments should also be mentioned.

Figure 2: My major comment in this figure is that the relevance of the sensitivity to each type of damaging agent is not discussed. These drugs do indeed create ultimately DSBs but they will be repaired by different means. So, explaining/suggesting why ICL damage is the only relevant DNA damage here is very important.
We also don't know which dose of MMC is used in the experiments shown in panels C-F. The authors should emphasize to which degree FBL KD sensitizes the cells to these drugs and correlate the colony formation assays with the EdU results.
Additionally, to support the authors' claims regarding inability to repair the g-H2AX-labeled DNA breaks, IF data showing the recruitment of factors like BRCA1, RAD51, FANCD2 should be performed...
The authors need to show results with and without MMC treatment upon FBL KD for the gH2AX staining as well as the HRR or NHEJ reporter assays. And finally, a positive control for each repair pathway (BRCA1or RAD51 and 53BP1 or Ku70, respectively) would be ideal so one can evaluate how critical, and novel is the role of FBL in the process.

Figure 3: Since the GST pull down was performed using purified GST-YBX1 added to cell lysates expressing SFB-FBL (and not purified FBL), the authors can't conclude that the interaction is direct.
Otherwise, the experiments here are well done.

Figure 4: There is one important information missing from this study which relates to what FBL/YBX1 do when FBL is in lower abundance and mostly in the nucleolus.
This figure is claiming that FBL controls/modulates YBX1 translocation to the nucleus. The data shown only partially support it. Immunoblots of FBL should be shown in panels B so we can follow what happens to FBL.
Panel D with the co-IP is confusing as we don't know in which compartment the IP is performed, and it is critical in order to support translocation of YBX1, as well as seeing the no MMC control.
Data showing a more detailed overview of what FBL and YBX1 do before and after MMC induction would benefit the study. I think a couple of simple experiments could be designed to address this concern: confocal imaging or reciprocal co-IP in the appropriate compartments. Indeed, FBL immunoblots are missing in panels B, E and F, so one can precisely follow what happens to FBL. A co-straining using the GFP-FBL and a reciprocal endogenous IP of YBX1 would clarify the message and strengthen the conclusions.

Figure 5:
The data here are well introduced and we understand the focus on HRR. However, an important notion is that BRCA1 levels

(both mRNA and proteins) are cell cycle regulated. Therefore, as shFBL induces a slowdown in proliferation, this aspect should be better controlled. And maybe if depletion of YBX1 is not inducing cell cycle arrest/slowdown, the experiments assessing the transcriptional activity on BRCA1 promoter in panels H and I, could have been done using sh/siYBX1 to reinforce the conclusions.

Some supplemental data about RPA1/RPA2 (HRR at least) and BRIP1 (DSBR and ICL) or at least a comment (here or in the discussion) should be added.

Figure 6:
Nice results. Panel E needs to show magnification of the TUNEL results because it is hard to see. The experimental design explaining why the MMC treatment started at the same time as the edited tumor cells needs to be added. Given the conclusions the authors want to make, MMC treatment would have been best after the tumors had reached a similar size in all treatment conditions (+/- shRNA and +/- MMC).

Minor comments:
• To strengthen the data, it would have been nice to see what happens to normal (colon/lung) cells upon FBL depletion.
• Table 4 should probably be Table 1.
• Figure 3: the nomenclature and color coding of panel E should be improved for an easier understanding.
• Figure 4: IF on panel C should be shown in green for a better contrast.
• Figure 5: the nomenclature and labeling of panel G needs to be simplified visually by maybe ordering the primer pairs tested from the farthest from the promoter to the closest. Also, it's mentioned and shown on the schematic that BRCA1 promoter has 4 Y-boxes but only 3 are tested (-2087 is missing). Why?
• Figure 6: Comment about BRCA1 induction after MMC.
• YBX1 is an DNA/RNA binding protein. We know BRCA1 is involved is HR, ICL-, R-loop- induced DNA damage repair. More recently, it was shown that HR requires an RNA molecule (forming an R-loop) to perform the repair appropriately. Therefore, a comment in the discussion about that notion should be added.

Response to reviewers' comments:

We are very grateful for the constructive comments from both reviewers. Following the reviewers' suggestions, we have modified the manuscript. As listed below, we have point-by-point addressed all the concerns raised by the reviewers.

Please note that page and line numbers that we mention here refer to our revised manuscript and not to the original submission. The changed and added texts and figures in the revised manuscript are indicated using red lines. Also, please note that the numbering of the figures in the revised manuscript is different from that in the original one.

Reviewer #1:

General comments:

This manuscript by Sun et al uncovers a role of FBL in promoting DNA damage repair by homologous recombination (HR). The data/model presented suggests FBL mediates this function by promoting YBX1 nuclear translocation. As discussed below, the authors do a great job of biochemically characterizing the interaction between FBL and YBX1. However, the mechanism proposed to account for the DNA damage sensitivity of FBL-deficient cells has multiple caveats. The mechanistic study needs extensive controls and validations to convince the readers of the proposed model.

Response: Thank you for the positive comments! The specific questions have been addressed below.

Major concerns:

1. *Conceptual problem with the proposed mechanism: Authors propose that a key mechanism by which FBL downregulation impacts sensitivity to MMC is by downregulating HR genes, such as BRCA1. However, the authors have not considered the possibility that this could simply be an impact of the cell cycle. Data in Figure 2C and 2D demonstrate that loss of FBL results in reduction of EdU positive (S-phase cells). BRCA1 and other HR genes are predominantly expressed and are functional in S-phase cells. Hence it is likely that the reduction in HR-efficiency and BRCA1 downregulation can be a mere manifestation of reduced S-phase cells upon FBL loss. This indeed seems to be evident from the RNA-seq data (Figure 5D) where multiple S-phase genes are downregulated upon shFBL treatment.*

Response: We appreciate your valuable comment and have added a discussion of these possibilities in the revised manuscript to acknowledge this potential confounding factor. We hope that our revisions have addressed your concerns to some extent.

We agree that the possibility of reduced S-phase cells upon FBL downregulation could downregulate BRCA1 expression. To address this concern, we conducted a cell cycle analysis on shFBL-HCT116 and control cells and observed a slight but not significant reduction in the S phase in asynchronous shFBL-HCT116 cells compared to control. Additionally, we synchronized shFBL-HCT116 and control cells at the G1/S border using a thymidine double-block protocol. Consistent with previous findings, we observed that BRCA1 expression peaked during mid to late S-phase (4-6 h) and was lowest during G1 (0 h or10 h) phases in both FBL knockdown and wild-type cells. Our results indicate that a decrease in the S phase in FBL knockdown cells compared with wild-type cells occurred at 2-4 hours release time periods. However, it is noteworthy that BRCA1 expression remained significantly decreased even when there was no significant difference in the S phase between FBL knockdown cells and wild-type cells at 6-8 hours release time periods (please refer to Fig EV5F). Therefore, our data suggest that the observed downregulation of BRCA1 is not solely due to the reduction of S-phase cells.

It was noteworthy that knockdown of either FBL or YBX1 leads to a similar phenotype, including increased cell sensitivity to DNA crosslinker agents (Fig 2A and B, Fig EV2A and B) and decreased MDR1 expression (Fig EV5B and C).

Moreover, since FBL directly interacts with YBX1, we propose that FBL may have a more direct role of in BRCA1 transcription via YBX1, which is another possible explanation for the observed phenotype in our study. Our proposed mechanism is supported by additional evidence, including results from immunofluorescence staining (Fig 4C and EV4C) and HR assay (Fig 4A), which indicate that FBL knockdown impairs DNA repair by HR, and induction of YBX1 could largely restore the HR defects induced by FBL knockdown, suggesting that YBX1 is a downstream target of FBL-mediated HR repair pathway. Therefore, we propose that FBL may be involved in repairing interstrand crosslinks (ICLs) by HR, and the FBL/YBX1/BRCA1 axis is one possible mechanism by which FBL downregulation impacts sensitivity to MMC. We have included our cell cycle analysis results in Fig EV5F to support this proposal.

Regarding the reduction of EdU positive cells in our study (Fig EV2G and H), it's worth noting that the EdU staining method is a cell labeling technique used to measure DNA synthesis, while cell cycle analysis by flow cytometry accurately reveals distribution of the proportion of cells in different cell cycle phases. In our study, it is possible that upon MMC treatment, a significant number of cells underwent DNA repair, and FBL deficiency impaired the capacity to repair damaged DNA, resulting in a decrease in EdU being incorporated into DNA during active DNA synthesis. Furthermore, Wang et al recently discovered that EdU, which is widely used in the analysis of DNA replication, DNA repair, and cell proliferation, is processed as "damage" in the human genome by the nucleotide excision repair system. This raises the possibility that such a reaction causes a futile cycle of excision and reincorporation into the repair patch, leading to eventual cell death (Wang *et al*, 2022). Thus, if we want to determine the impact of FBL on the cell cycle, flow cytometry is more accurate than analyzing S-phase cells through EdU staining. It is important to consider the limitations of each method and to interpret the results in the context of the experimental design and other supporting data. Consequently, in our revised manuscript, we removed the EdU staining results and placed them in supplementary figures.

In addition, although FBL knockdown leads to downregulation of multiple S-phase genes in RNA-seq data, we also observed that RAD51 downregulation is limited to the mRNA level and does not affect protein expression. Moreover, our results showed that RPA1/2 proteins remained unaffected in FBL knockdown cells (Figure EV5A). Taken together, these findings suggest that the mechanism underlying the downregulation of HR genes upon FBL knockdown is complex and multifaceted.

2. *In this context it is noteworthy that both HCT116 and A549 cell lines have an active p53 pathway, making them amenable to arrest in the G1/S phase of cell cycle. This reviewer believes the proposed mechanism can simply be the confounding impact of altered cell cycle. It is vital that proper cell cycle controls are included across all mechanistic experiments.*

Response: Thank you for your comment. We acknowledge the importance of proper cell cycle controls in our experiments and agree that cell cycle alterations can confound the interpretation of our results. To address this issue, as described in our

response to the first concern, we observed a slight but not significant decrease in S-phase cells upon FBL knockdown in asynchronous cells. When we synchronized shFBL-HCT116 and control cells at the G1/S border, we observed that a minor decrease in the S phase in FBL knockdown cells compared with wild-type cells occurred at 2-4 hours release time periods, but found no significant decrease in S-phase cells between synchronous FBL knockdown and control cells at 6-8 h release time period. Despite this, we still observed a significant decrease in BRCA1 expression. These findings suggest that altered cell cycle progression is not the sole contributor to the observed downregulation of BRCA1. We have performed cell cycle analysis and included additional controls to ensure that the observed downregulation of HR genes upon FBL knockdown is not solely due to altered cell cycle progression.

Furthermore, we also examined the effects of FBL knockdown on BRCA1 expression in p53-deficient HCT116 cells (HCT116-p53$^{-/-}$). Our results showed that despite the inactive p53 pathway in HCT116 cells, FBL knockdown still led to a remarkable decrease in BRCA1 expression (Fig.R1-1) [Figures for referees not shown.]

Fig.R1-1. The effect of FBL knockdown on BRCA1 expression in HCT116 wild-type cells and p53-deficient HCT116 cells (HCT116-p53$^{-/-}$) was assessed by western blot analysis.

3. *Reference showing that FBL-E191A/D236A is catalytically inactive is missing.*

Response: We apologize for missing the references. Previous studies have shown that the catalytic activity of FBL depends on two highly conserved amino acid residues (E191 and D236) in its nucleotide-binding site. This finding has been confirmed in several studies, including the following articles (Deffrasnes *et al*, 2016; Watkins & Bohnsack, 2012; Wilson & Doudna Cate, 2012; Yao *et al*, 2019). We have cited these references in our revised manuscript.

4. *IF assay in Figure 4C convincingly shows localization of YBX1 to the nucleus upon DNA damage. Authors should use this assay to quantify how FBL overexpression as well as depletion impacts the nuclear translocation of YBX1. In this context, the chromatin fractionation data in Figure 4E is confusing- if FBL loss leads to decrease in nuclear translocation of YBX1, why is there not a concomitant increase in cytoplasmic fraction. Also, no controls have been included to demonstrate the*

purity/enrichment of nuclear and cytoplasmic fractions.

Response: Thank you for your comment and suggestion. As suggested, we have redone these experiments as demonstrated in Fig 4B, C, E and F of our revised manuscript. Specifically, we investigated the impact of FBL depletion and overexpression on YBX1 nuclear translocation with or without cisplatin or MMC treatment (Figure 4C and EV4C). Consistent with our previous result, we observed that DNA damage led to YBX1 nuclear translocation. Furthermore, FBL depletion decreased the nuclear translocation of YBX1, while FBL overexpression increased nuclear YBX1 levels. We have included FBL staining in Fig 4C and EV4C in our revised manuscript.

Regarding the chromatin fractionation data in Fig 4E, we apologize for any confusion caused. We have repeated the experiments and included controls to demonstrate the purity and enrichment of nuclear and cytoplasmic fractions (Fig 4B, E and F).

5. *Data in Figure 4C demonstrates that the interaction between FBL and YBX1 is damage induced. However, it remains unclear how nuclear damage triggers the cytoplasmic association of FBL-YBX1 and their subsequent nuclear translocation.*

Response: Thank you for your comment. We acknowledge that the mechanism underlying the MMC-induced nuclear translocation of YBX1 and their subsequent association of FBL-YBX1 in the nucleus is an intriguing and significant question. Based on our current knowledge, we propose a possible explanation for this phenomenon. Previous studies have shown that YBX1 can be phosphorylated in response to DNA damage, which can influence its subcellular localization and interaction with other proteins (Koike *et al*, 1997; Kuwano *et al*, 2019; Ohga *et al*, 1996; Shibahara *et al*, 2004). Therefore, nuclear translocation of YBX1 is regulated by its phosphorylation. YBX1 has been reported to be phosphorylated at multiple sites, including S30, S34, S102, S165, and S176, leading to YBX1 translocation into the nucleus (Bargou RC *et al*, 1997; Basaki *et al*, 2007; Jayavelu *et al*, 2020; Kretov *et al*, 2019; Li *et al*, 2023; Shibata *et al*, 2020; Stratford *et al*, 2008; Sutherland *et al*, 2005). Additionally, we have observed that partial FBL is relocalized from the

nucleolus to the nucleus in response to MMC treatment, as shown in Fig 2C. This creates the spatio-temporal possibility for the FBL-YBX1 interaction. Consequently, the possible underlying mechanism is that MMC-induced phosphorylation of YBX1 mediating its nuclear translocation and the relocalization of FBL from the nucleolus to the nucleus jointly facilitates the nuclear association of FBL and YBX1. However, the exact mechanism requires further investigation. We have included a brief discussion on this possibility in our revised manuscript (Lines 30, Page 13; Lines 1-2, Page 14).

6. *Does overexpression of FBL confer resistance to MMC? This is a critical experiment to fully appreciate the importance of FBL overexpression in tumorigenesis.*

Response: Thank you for your comment. We have performed additional experiments and observed that the overexpression of FBL led to resistance against MMC (Fig EV2C and E). This finding suggests that the overexpression of FBL in various cancers may be associated with chemoresistance and FBL may play a role in promoting the capacity to repair DNA damage in tumorigenesis. We have included the results in our revised manuscript.

7. *Beyond MMC sensitivity, reduction in HR/BRCA-mediated repair confers sensitivity to multiple other genotoxic agents including camptothecin, PARP inhibitors. Are FBL-deficient cells sensitive to other DNA damaging agents?*

Response: Thank you for the question. In our previous manuscript, we demonstrated the sensitivity of FBL-deficient cells to camptothecin, cisplatin, etoposide, and H_2O_2 (please see the Fig EV2A and B). Our results showed that FBL-deficient A549 or HCT116 cells were most sensitive to MMC and cisplatin, while also exhibiting varying degrees of sensitivity to other genotoxic agents. As suggested, we have expanded our investigation to include sensitivity to Olaparib. We observed that FBL-deficient cells were also sensitive to Olaparib, due to the effect of FBL deficiency on the core DNA repair pathway HR/BRCA (Fig R1-2) [Figures for referees not shown.].

8. *Do FBL and YBX1 have nuclear localization signals or is their nuclear translocation reliant on another protein?*

Response: Thank you for the question. Both of FBL and YBX1 have nuclear localization signals (NLS) that allow them to be transported into the nucleus. FBL is an essential nucleolar protein, while YBX1 is a cytoplasmic-nuclear shuttling protein. The NLS of FBL is located in the N-terminal GAR domain of the protein. The GAR domain is involved in FBL functioning and integrates the functions of the nuclear localization signal and the nucleolar localization signal (NoLS) (Shubina *et al*, 2020). Based on the current studies, the nuclear localization of FBL seems not be reliant on another protein, but rather on the recognition of its NLS to mediate its transport into the nucleus. YBX1 contains several proposed NLS located in its C-terminal region, such as at residues 149-156, residues 185-194 and residues 276-292 (Bader & Vogt, 2023; Claudia RC van Roeyen *et al*, 2013). The exact mechanism of YBX1 translocation to the nucleus is not fully understood. Previous studies showed that YBX1 nuclear translocation is linked to response to cellular stress, interaction with other proteins (transportin-1, SRP30C1, WRN and p53) (Fujita *et al*, 2005; Guay *et al*, 2006; Mordovkina *et al*, 2016; Raffetseder *et al*, 2003; Stein *et al*, 2001) or phosphorylation of YBX1(Kretov *et al.*, 2019). YBX1 could undergo structural rearrangements in response to various stimuli or interaction, resulting in exposure of its NLS to transport proteins. Our study demonstrated that FBL promoted the nuclear accumulation of YBX1 in response to DNA damage by enhancing the interaction between FBL and YBX1, confirming that the nuclear translocation of YBX1 is dependent on damage stimulation and FBL interaction.

Minor point:

Fig4A: *Authors show that overexpression of YBX1 overcomes the impact of FBL loss in the DR-HR assay. The labeling of figures needs to be fixed to indicate this. In its current form, it appears that both FBL and YBX1 were depleted.*

Response: We apologize for any confusion caused by the labeling of the figures. To clarify, we have revised the labeling of the Fig 4A in our revised manuscript to accurately reflect the experimental conditions. Thank you for your comment.

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Reviewer #2:

Summary:

In this study, the authors wanted to investigate the role of Fibrillarin (FBL) in DNA damage response. Indeed, there were previous studies (breast and prostate cancers) indicating that FBL may be acting as an oncogene promoting cancer cell proliferation and resistance to chemotherapy. So, they started by expanding the analysis of FBL mRNA expression to more cancers using the TCGA database. Then, they explored in more details the molecular ways in which FBL could provide sensitization to chemotherapies using colon and lung cancer cell lines. It is very interesting that this new function of FBL is independent of its MTase activity.

Finally, they identified a new, interesting player, YBX1 and showed that YBX1 acts as a downstream effector regulating/activating BRCA1 gene transcription.

Overall impressions:

This is an interesting study which reports a set of new findings suggesting a new level of regulation for BRCA1 DNA damage repair activity, involving the FBL/YBX1 pathway.

However, my there are a few important controls missing and additional experiments should be performed in order to consolidate the study and their model (see below).

Strengths: The in vivo data look well controlled and support the in vitro results.

Shortcomings: The methods and figure legends are often not detailed enough so we do not know which drug dose is used and what kind of extracts have been analyzed, making it harder to interpret the data and determine whether each experiment is adequate to answer the question. Also, the authors need to be more reserved when claiming that FBL contributes to tumorigenesis. Previous data seems to indicate it could act as an oncogene but no definite proof yet.

Response: Thank you for the positive comments and interest in our study. We agree that additional controls and experiments would strengthen our findings and apologize for any confusion caused by the lack of details in our methods and figure legends. In our revision, we have included more specific information about the drug doses and extracts used in each experiment to improve the clarity of our results. We have addressed the specific concerns point-by-point.

In addition, we also appreciate your comment regarding our claims about FBL's contribution to tumorigenesis. As the reviewer mentioned, there are studies suggesting that FBL may act as an oncogene and promote cancer cell proliferation. Moreover, there is definitive evidence that FBL overexpression contributes to tumorigenesis(Fig.R2-1) [Figures for referees not shown.] (Jin *et al*, 2021; Marcel *et al*, 2013). While our results showing knockdown of FBL significantly inhibiting tumor growth in mice (Fig 6A) support a potential role for FBL in promoting cancer, so we

also use the similar statement. We acknowledge that further research is needed to definitively prove this. We have adjusted our statement accordingly.

Comments:

The overall writing must be improved throughout the manuscript for clarity and some paragraphs in the introduction could be shortened (first one for example, since the Methyltransferase activity of FBL is not required for the new function described) so more important facts can be better articulated and expanded to allow some additional points to be mentioned in the discussion.

Response: Thank you for your valuable comments. We had a native speaker expert to revise the manuscript to improve its readability and clarity. We have shortened the first paragraph on the methyltransferase activity of FBL while also including key points and significant findings in the discussion section. We have addressed your specific concerns below.

1. *Figure 1: Statistics and the type of values (median, mean etc.) should be shown for panels A, B and C. We do not understand why colon and lung are chosen and why breast (and ovary, given the implication of BRCA1) or prostate are not shown. The cut off for low and High levels of FBL should also be defined.*
It would be great to show an immunoblot of FBL in the cell lines chosen for the reminder of the study compared to their normal, respective tissue.
Finally, the colony formation assays are good and convincing except for the lack of control immunoblot showing the FBL levels in the rescue settings. The shRNA chosen for these representative experiments should also be mentioned.

Response: Thank you for suggestions. In our revised manuscript, we have included statistical analysis and specify the type of values (median) for panels A, B, and C in the legend of Figure 1.

We chose colon and lung cancer as our study subjects due to their high incidence and mortality rates worldwide. In 2020, lung cancer was the leading cause of cancer death, while colon cancer ranks third in terms of incidence, but second in terms of mortality (Fig. R2-2) [Figures for referees not shown.]. These cancers are also the primary cause of cancer morbidity and mortality in China (Zheng *et al*, 2022). Therefore, there is an urgent need for better biomarkers for early detection and treatment of these

cancers, which motivated our interest in studying them. An additional crucial factor is that we are collaborating with clinical oncologists to conduct research on colon and lung cancer. Although we recognize the significance of breast and ovarian cancer, especially in the context of BRCA1 mutations, and the importance of prostate cancer as a significant health issue, we did not initially consider these cancers for our study. Furthermore, at the time of commencing our research on the role of FBL in these cancers, we were unaware of the potential role of FBL in regulating BRCA1. We hope this explanation clarifies our rationale for the choice of cancer types in our study. However, we now realize that studying breast and ovarian cancer would provide a more pertinent understanding of the role of FBL, given the involvement of BRCA1 in these cancers. Therefore, we plan to conduct further investigations to explore the role of FBL in breast and ovarian cancer in the future.

Regarding the cut-off values for low and high levels of FBL in Figure 1 D and E, we obtained the original figures from the LnCAR and cBioPortal databases, but were unable to locate the cut-off values for these figures. As such, in our revised manuscript, we utilized the online tool website (https://kmplot.com/analysis/) that includes GEO, EGA and TCGA databases. The tool enabled us to easily determine the cut-off values for obtaining Kaplan-Meier plots for lung and colon cancer patients with low and high levels of FBL. In the revised manuscript, the cut-off values have been defined based on the lower quartile of FBL levels, which indicates that the bottom 25% of the data is considered to be low expression, while the remaining 75% is considered to be high expression. The original KM plot figures have been replaced with the new ones.

In addition, we have included an immunoblot of FBL in colon and lung cancer cell lines, as well as their corresponding normal cell lines (Fig EV1A and B). We have also addressed an inadvertent omission from our previous submission by including a control immunoblot that shows the FBL levels in the rescue settings for the colony formation assays (Fig EV1E and F). We have also specified the shRNA used in our study in the methods section.

2. *Figure 2: My major comment in this figure is that the relevance of the sensitivity to each type of damaging agent is not discussed. These drugs do indeed create ultimately DSBs but they will be repaired by different means. So, explaining/suggesting why ICL*

damage is the only relevant DNA damage here is very important.
We also don't know which dose of MMC is used in the experiments shown in panels C-F. The authors should emphasize to which degree FBL KD sensitizes the cells to these drugs and correlate the colony formation assays with the EdU results. Additionally, to support the authors' claims regarding inability to repair the g-H2AX-labeled DNA breaks, IF data showing the recruitment of factors like BRCA1, RAD51, FANCD2 should be performed...The authors need to show results with and without MMC treatment upon FBL KD for the gH2AX staining as well as the HRR or NHEJ reporter assays. And finally, a positive control for each repair pathway (BRCA1or RAD51 and 53BP1 or Ku70, respectively) would be ideal so one can evaluate how critical, and novel is the role of FBL in the process.

Response: Thank you for your suggestion. In our study, we investigated the sensitivity of colon and lung cancer cell lines to several types of DNA-damaging agents, including MMC, cisplatin, etoposide, camptothecin, and H_2O_2. The rationale behind testing these agents is that they represent different types of DNA damage and are commonly used in cancer chemotherapy. MMC and cisplatin are both alkylating agents that form interstrand cross-links (ICLs). Etoposide and camptothecin are topoisomerase inhibitors that induce DNA damage by trapping the enzyme-DNA complex, leading to DNA strand breaks. H_2O_2 is a reactive oxygen species that can cause oxidative DNA damage. By testing the sensitivity of HCT116 or A549 cell lines to these different types of damaging agents, we aimed to investigate whether FBL expression is associated with a specific type of DNA damage response. Our findings indicate that FBL knockdown specifically sensitized cancer cells to DNA crosslinker agents, such as MMC and cisplatin, suggesting a potential role for FBL in interstrand crosslink (ICL) repair. Previous studies have shown that YBX1 is involved in ICL-induced damage repair, but not to camptothecin, etoposide, doxorubicin or vincristine (Ohga *et al*, 1996). In light of this, we hypothesized that the phenomenon observed in our study, where FBL knockdown specifically sensitized cancer cells to cisplatin and MMC, may be associated with YBX1. Therefore, we conducted further experiments to confirm our hypothesis in the manuscript. Our results on the relevance of sensitivity to DNA crosslinker agents may have potential implications in cancer therapy for patients with overexpressed FBL. A deeper understanding the molecular mechanisms underlying FBL/YBX1/BRCA1 axis in the response to DNA crosslinker agents-induced DNA damage could potentially inform the development of more personalized and effective cancer treatments.

We apologize for not specifying the dose of MMC used in panels C-F of Fig 2. To clarify, we used a concentration of 5 μM of MMC, which is a commonly used dose in other studies (Cheng *et al*, 2012). Our results from panels A and B of Fig 2 demonstrated that 5 μM of MMC was able to induce cell death in approximately 60% of control cells and 80% of shFBL-cells, respectively. Based on these preliminary findings and previous studies, we chose 5 μM of MMC for our EdU assays, which we have now detailed in the figure legend. Regarding the degree of sensitization of cancer cells to MMC and other DNA-damaging agents, our results demonstrated that FBL knockdown most significantly increased the sensitivity of cancer cells to MMC and cisplatin, compared to other agents such as etoposide, camptothecin and H_2O_2. We also performed EdU incorporation assay to assess the effect of FBL knockdown on cell proliferation and found that FBL knockdown affected cell proliferation and sensitize cells to MMC-induced growth inhibition. We appreciate the reviewer's suggestion to correlate the colony formation assays with the EdU results. In our study, both the colony formation assays and the EdU staining results yields similar results, indicating that FBL KD inhibited cell proliferation. However, it is worth noting that Wang et al recently discovered that EdU, which is widely used in the analysis of DNA replication, DNA repair, and cell proliferation, can be processed as “damage” in the human genome by the nucleotide excision repair system. This raises the possibility that such a reaction causes a futile cycle of excision and reincorporation into the repair patch, leading to eventual cell death (Wang *et al*, 2022). Therefore, in our revised manuscript, we have removed the Edu staining results and placed them in supplementary figures.

As suggested by the reviewer, we conducted additional experiments to investigate the recruitment of BRCA1 and RAD51 in response to DNA damage when FBL was knocked down using immunofluorescence staining. Our results showed that FBL knockdown significantly reduced the recruitment of BRCA1 to γH2AX foci in response to MMC treatment, indicating impaired DNA repair in the absence of FBL (Fig 2D-F). Intriguingly, we observed no significant effect on RAD51 foci (Fig EV2I), suggesting a distinct underlying mechanism. These additional experiments further support our claims regarding the critical role of FBL in DNA damage response and the inability of cancer cells to repair DNA damage when FBL is knocked down. We have incorporated these results into our revision and updated the manuscript

accordingly.

In our revised manuscript, we have presented the results of γH2AX staining in FBL KD or control cells with and without MMC treatment (Fig 2D and E). Furthermore, we utilized BRCA1 as a positive control for HR pathway and RIF1 as a positive control for NHEJ pathway (Fig 2G) in HR and NHEJ reporter assays. Our findings revealed that FBL knockdown significantly reduced HR but not NHEJ, indicating impaired HR repair in the absence of FBL. In the HR and NHEJ reporter systems, transfection of JS20 plasmid was used to induce DNA damage, and MMC treatment was not required. We have integrated these supplementary results into our revision and updated the manuscript accordingly. We appreciate the valuable suggestion from the reviewer.

3. *Figure 3: Since the GST pull was performed using purified GST-YBX1 added to cell lysates expressing SFB-FBL (and not purified FBL), the authors can't conclude that the interaction is direct.*
Otherwise, the experiments here are well done.

Response: We agree with the reviewer's comment and have made the necessary changes to the manuscript to reflect this. We have deleted the word “directly” and “direct” in the paragraph (Line 20, Page 8).

4. *Figure 4: There is one important information missing from this study which relates to what FBL/YBX1 do when FBL is in lower abundance and mostly in the nucleolus.*
This figure is claiming that FBL controls/modulates YBX1 translocation to the nucleus. The data shown only partially support it. Immunoblots of FBL should be shown in panels B so we can follow what happens to FBL.
Panel D with the co-IP is confusing as we don’t know in which compartment the IP is performed, and it is critical in order to support translocation of YBX1, as well as seeing the no MMC control.
Data showing a more detailed overview of what FBL and YBX1 do before and after MMC induction would benefit the study. I think a couple of simple experiments could be designed to address this concern: confocal imaging or reciprocal co-IP in the appropriate compartments. Indeed, FBL immunoblots are missing in panels B, E and F, so one can precisely follow what happens to FBL. A co-straining using the GFP-FBL and a reciprocal endogenous IP of YBX1 would clarify the message and strengthen the conclusions.

Response: Thank you for your suggestion. We apologize for any confusion. We agree that more detailed data on the dynamics of FBL and YBX1 before and after MMC induction would be informative. We have included immunoblots of FBL in Fig 4B, E and F to better follow the changes in FBL in response to MMC treatment in our revision. In this experiment of Fig 4D, the co-IP was performed in the nuclear fraction of HCT116 cells treated with MMC, which is where YBX1 translocates upon genotoxic stress. There is a panel with the no MMC control in Fig 4D. This experiment demonstrated that more YBX1 was translocated into nucleus upon MMC treatment, which lead to an increase interaction of FBL and YBX1. We have added this clarification to the figure legend. As suggested, we did additional reciprocal endogenous IP of YBX1 in nucleus (Fig EV4E).

Moreover, we also provided additional data in Fig EV4C to confirm that FBL overexpression as well as depletion impacts the nuclear translocation of YBX1 in response to MMC treatment. It further clarifies the role of FBL and YBX1 in response to MMC induction.

Regarding the concern about what FBL/YBX1 do when FBL is in lower abundance and mostly in the nucleolus, we acknowledge that this is an interesting question and an important area for further investigation. Our study focused on the role of FBL in regulating YBX1 translocation to the nucleus, but it is possible that additional factors may also play a role in this process.

5. *Figure 5: The data here are well introduced and we understand the focus on HRR. However, an important notion is that BRCA1 levels (both mRNA and proteins) are cell cycle regulated. Therefore, as shFBL induces a slowdown in proliferation, this aspect should be better controlled. And maybe if depletion of YBX1 is not inducing cell cycle arrest/slowdown, the experiments assessing the transcriptional activity on BRCA1 promoter in panels H and I, could have been done using sh/siYBX1 to reinforce the conclusions.*
Some supplemental data about RPA1/RPA2 (HRR at least) and BRIP1 (DSBR and ICL) or at least a comment (here or in the discussion) should be added.

Response: We appreciate the reviewer's suggestion to better control for cell cycle effects on BRCA1 levels in our study. As BRCA1 is a cell-cycle regulated protein, we

agree that the possibility of changed cell cycles upon FBL downregulation could impact BRCA1 expression. To address this concern, we conducted cell cycle analysis on shFBL-HCT116 and control cells and observed a slight but not significant reduction in the S phase in asynchronous shFBL-HCT116 cells compared to control (Fig EV5F). Additionally, we synchronized shFBL-HCT116 and control cells at the G1/S border using a thymidine double-block protocol. Consistent with previous findings, we observed that BRCA1 expression peaked during mid to late S-phase (4-6 h) and was lowest during G1(0 h or10 h) phases in both FBL knockdown and wild-type cells. Our results indicate that a decrease in the S phase in FBL knockdown cells compared with wild-type cells occurred at 2-4 hours release time periods. However, it is noteworthy that BRCA1 expression was significantly decreased even when there was no significant decrease in the S phase between FBL knockdown cells and wild-type cells at 6-8 hours release time periods. Therefore, our data suggest that the observed downregulation of BRCA1 is not solely due to the reduction of S-phase cells, although it is true that BRCA1 is predominantly expressed and functional in S-phase cells.

It was noteworthy that knockdown of either FBL or YBX1 leads to a similar phenotype, including increased cell sensitivity to DNA crosslinker agents (Fig 2A and B, Fig EV2A and B) and decreased MDR1 expression (Fig EV5B and C).

Moreover, since FBL directly interacts with YBX1, we propose that FBL may have a more direct role of in BRCA1 transcription via YBX1, which is another possible explanation for the observed phenotype in our study. Our proposed mechanism is supported by additional evidence, including results from immunofluorescence staining (Fig 4C and EV4C) and HR assay (Fig 4A), which indicate that FBL knockdown impairs DNA repair by HR, and induction of YBX1 could largely restore the HR defects induced by FBL knockdown, suggesting that YBX1 is a downstream target of FBL-mediated HR repair pathway. Therefore, we propose that FBL may be involved in repairing interstrand crosslinks (ICLs) by HR, and the FBL/YBX1/BRCA1 axis is one possible mechanism by which FBL downregulation impacts sensitivity to MMC. We have included our cell cycle analysis results in Fig EV5F to support this proposal.

Regarding the suggestion to use sh/siYBX1 to assess the transcriptional activity of the BRCA1 promoter, it is worth noting that previous studies have indicated that depletion of YBX1 affects cell cycle arrest (Kotake *et al*, 2017; Liu *et al*, 2020). Given that alterations in the cell cycle may affect BRCA1 expression, we have decided not to conduct this additional experiment.

We appreciate the reviewer's suggestion to include supplemental data on RPA1/RPA2 and BRIP1. We have performed additional experiments on RPA1/2 to address this concern. We found that FBL KD did not affect RPA1/2 expression and included these data in the revised manuscript (Fig EV5A).

6. *Figure 6: Nice results. Panel E needs to show magnification of the TUNEL results because it is hard to see. The experimental design explaining why the MMC treatment started at the same time as the edited tumor cells needs to be added. Given the conclusions the authors want to make, MMC treatment would have been best after the tumors had reached a similar size in all treatment conditions (+/- shRNA and +/- MMC).*

Response: Thank you for your suggestion. We have revised the Figure 6E to include a higher magnification image of the TUNEL staining. We apologized for any confusion on the figure legend of the experimental design. We did start MMC treatment after the tumors had reached a similar size (about 50 mm^3) in all treatment conditions. We have updated the legend of Figure 6 in our revision.

Minor comments:

1. *To strengthen the data, it would have been nice to see what happens to normal (colon/lung) cells upon FBL depletion.*

Response: Thank you for your suggestion. We have conducted additional experiments to investigate the impact of FBL depletion on normal colon (NCM460) and lung cells (BEAS-2B). Our results show that FBL depletion in normal cells affects cell viability (Fig EV1G and H). One possible explanation could be that FBL plays a crucial role in ribosome biogenesis and maturation, and therefore, its depletion could lead to defects

in protein synthesis, which may affect cell viability and cellular homeostasis.

2. *Table 4 should probably be Table 1.*

Response: Sorry for the error. In our revised manuscript, we have reorganized our tables and we thank you for your careful review.

3. *Figure 3: the nomenclature and color coding of panel E should be improved for an easier understanding.*

Response: As suggested, we have adjusted the colour and positioning of the labels to make them more visible in our revised manuscript.

4.Figure 4: IF on panel C should be shown in green for a better contrast.

Response: In our revised manuscript, we have redone the experiment, as shown in Figure 4C. We investigated the impact of FBL depletion and overexpression on YBX1 nuclear translocation with cisplatin or MMC treatment or not. Consistent with our previous result, DNA damage induced YBX1 nuclear translocation. Furthermore, FBL depletion decreased the nuclear translocation of YBX1, while FBL overexpression increased nuclear YBX1 levels (Fig 4C and EV4C). Thus, we also include FBL staining in Fig 4C and EV4C in our revised manuscript. We believe that this change can improve the clarity of the figure.

5. *Figure 5: the nomenclature and labeling of panel G needs to be simplified visually by maybe ordering the primer pairs tested from the farthest from the promoter to the closest. Also, it's mentioned and shown on the schematic that BRCA1 promoter has 4 Y-boxes but only 3 are tested (-2087 is missing). Why?*

Response: Thank you for your suggestion. We have reorganized the primer pairs in the order suggested by the reviewer, from the farthest from the promoter to the closest (Fig 5G). Regarding the number of Y-boxes tested, we apologize for any confusion.

In our experiments, we focused on testing the effects of the three most proximal Y-boxes to the BRCA1 promoter, as these have been previously reported to be critical for BRCA1 expression regulation (Thakur & Croce, 1999; Xu *et al*, 1997). We did not test the -2087 Y-box, as it is located further upstream and has not been previously implicated in BRCA1 regulation. Specifically, for the experiment depicted in Fig 5 H and I, we cloned the promoter sequence only 2000 bp upstream from the TSS, excluding the -2087 Y-box sequence

6. *Figure 6: Comment about BRCA1 induction after MMC.*

Response: Thank you for your suggestion. In our study, there is an induction of BRCA1 expression in MMC-treated xenograft tumors driven by HCT116 cells, as shown in Fig 6. Additionally, in Fig 2D and 2F, we also found that MMC treatment induced an increase in BRCA1 foci and BRCA1 expression. As is well known, MMC can induce severe DNA lesions and activate the DNA damage response to repair the damaged DNA. BRCA1 is a downstream factor of DNA damage response proteins and is recruited to chromatin to form damage-induced foci. The induction of BRCA1 after MMC treatment may be a protective mechanism that helps cells repair DNA damage and maintain genomic stability. Studies have reported that ionizing radiation also induces BRCA1 expression through a similar mechanism (Shabbeer *et al*, 2013; Susan A.Krum *et al*, 2010), which may explain the MMC-induced expression of BRCA1 proteins to promote cell survival.

7. *YBX1 is an DNA/RNA binding protein. We know BRCA1 is involved is HR, ICL-, R-loop- induced DNA damage repair. More recently, it was shown that HR requires an RNA molecule (forming an R-loop) to perform the repair appropriately. Therefore, a comment in the discussion about that notion should be added.*

Response: Thank you for your suggestion. Previous studies have showed that YBX1 is a non-canonical DNA-repair factor that participates in various DNA repair mechanisms, including homologous recombination repair (HRR), base-excision repair (BER), mismatch repair (MMR), and nucleotide excision repair (NER). Our study

focused on elucidating the regulatory role of YBX1 in BRCA1 expression. YBX1 is a DNA/RNA binding protein, and recent evidence suggests that RNA molecules play a crucial role in DNA damage repair through homologous recombination (HR) as pointed out by the reviewer (Francia *et al*, 2012; Michelini *et al*, 2018; Michelini *et al*, 2017; Santos-Pereira & Aguilera, 2015; Vohhodina *et al*, 2021; Zhang *et al*, 2020). Given the known involvement of BRCA1 in HR-mediated DNA damage repair, it is possible that YBX1 may also contribute to this process through its interactions with RNA. However, our proposed mechanism suggests that RNA molecule may not impact FBL-mediated HR repair, as we found that the interaction between FBL and YBX1 was not mediated by DNA or RNA (Fig EV3B). Nevertheless, the specific role of YBX1 in RNA-mediated DNA repair remains speculative, and further investigation is necessary to determine the exact mechanisms by which YBX1 binding RNA molecules facilitates BRCA1 mediated-HR repair. We have included a brief comment on this topic in the "Discussion" section in our revised manuscript(Page 14, Lines21-26), highlighting the current state of knowledge regarding the role of RNA molecules in HR repair and the potential implications for YBX1 function.

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Zheng R, Zhang S, Zeng H, Wang S, Sun K, Chen R, Li L, Wei W, He J (2022) Cancer incidence and mortality in China, 2016. *Journal of the National Cancer Center* 2: 1-9

Dear Prof. Wu,

Thank you for the submission of your revised manuscript. We have now received the enclosed reports from the referees, and I am happy to say that both support the publication of your study now. Referee 2 still has a few more minor suggestions that I would like you to address and incorporate before we can proceed with the official acceptance of your manuscript.

A few editorial requests will also need to be addressed:

- The conflict of interest subtitle needs to be corrected to "DISCLOSURE AND COMPETING INTERESTS STATEMENT"

- The authors credit section needs to be removed from the ms file. We now use CRediT to specify the contributions of each author in the journal submission system. CRediT replaces the author contribution section. Please use the free text box to provide more detailed descriptions, if you wish. See also guide to authors https://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines.

- None of the FUNDING INFO is listed in the ms file. Please add all funding info to the acknowledgement section in the ms file.

- Please upload all main and all EV figures as separate files. EV tables should also be uploaded individually.

- I am not sure whether all SOURCE DATA are provided now, as some files were missing but might be part of the deposited data. Can you please clarify? Submitted Source data files need to be organized into one file/folder per figure and ZIPed into 1 file for each main figure.

- The Data Availability Section (DAS) needs to be moved to the end of the materials and methods. Please make sure that all links are freely accessible upon the online publication of your ms.

- The legends for Tables EV1-EV4 should be removed from the ms file and added to each table file, for example in a separate tab.

- The order of the ms sections needs to be corrected to: abstract, introduction, results, discussion, materials & methods, data availability section, acknowledgments, disclosure statement and competing interests, references, main figure legends, EV figure legends.

- I attach to this email a related ms file with comments by our data editors. Please address all comments in the final ms.

I would like to suggest some minor changes to the title and abstract. Please let me know whether you agree with the following:

FBL promotes cancer cell resistance to DNA damage and BRCA1 transcription via YBX1

Fibrillarin (FBL) is a highly conserved nucleolar methyltransferase responsible for methylation of ribosomal RNA and proteins. Here we reveal a role for FBL in DNA damage response and its impact on cancer proliferation and sensitivity to DNA damaging agents. FBL is highly expressed in various cancers and correlates with poor survival in cancer patients. Knockdown of FBL sensitizes tumor cells and xenografts to DNA crosslinking agents, and leads to HR-mediated DNA repair defects. We identify Y-box binding protein-1 (YBX1) as a key interacting partner of FBL, and FBL increases the nuclear accumulation of YBX1 in response to DNA damage. We show that FBL promotes the expression of BRCA1 by increasing the binding of YBX1 to the BRCA1 promoter. Our study sheds light on the regulatory mechanism of FBL in tumorigenesis and DNA damage response, providing potential therapeutic targets to overcome chemoresistance in cancer.

I would also like to suggest that you modify the subheadings in the results section to be more specific about your data and findings.

EMBO press papers are accompanied online by A) a short (1-2 sentences) summary of the findings and their significance, B) 2-3 bullet points highlighting key results and C) a synopsis image that is exactly 550 pixels wide and 200-600 pixels high (the height is variable). You can either show a model or key data in the synopsis image. Please note that text needs to be readable at the final size. Please send us this information along with the final manuscript.

I look forward to seeing a final version of your manuscript as soon as possible. Please use this link to submit your revision: https://embor.msubmit.net/cgi-bin/main.plex

Best regards,
Esther

Esther Schnapp, PhD
Senior Editor
EMBO reports

Referee #1:

The authors have properly addressed all previous concerns particularly regarding the confounding role of cell cycle. I look forward to the publication of this important piece of work in EMBO reports.

Referee #2:

The authors have significantly improved their study by adding the additional experiments suggested by both reviewers to better supported the authors' claims. Their Point-by-Point answers were satisfying, and the addition of the technical details in the Methods and legends makes the study clearer. Therefore I believe that it is, with the few minor changes (mentioned below), ready for publication.

Important change: The authors have removed the word "direct" Line 20, Page 8 but they left it in other places. Since the demonstration is not complete, I believe that when it comes to describing the interaction of FBL and YBX1, they need to remove it.

Minor points:
• COAD or LUAD needs to be explained in the legend of Fig1.
• An hypothesis regarding RAD51 unchanged foci profile upon shFBL+ MMC should be added.
• YBX1 is always present in the nucleus (Fig4B), so I still don't fully understand how YBX1 is increasingly recruited to the nucleus, inducing the stronger binding to FBL, but the authors' hypothesis is valid and they should include it in the discussion.
• Other points to include in the discussion: p53 independent phenotype and the sensitivity to drugs like PARP inhibitors (Olaparib for example) since they have the data ready.

Dear Editor,

We are very grateful for the comments from editor and reviewers. Following the suggestions, we have modified the manuscript. As listed below, we have point-by-point addressed all the concerns raised by the editor and reviewers. Please note that page and line numbers that we mention here refer to our revised manuscript with changes tracked.

Response to Referee #2' comments:

General comments:
The authors have significantly improved their study by adding the additional experiments suggested by both reviewers to better supported the authors' claims. Their Point-by-Point answers were satisfying, and the addition of the technical details in the Methods and legends makes the study clearer. Therefore I believe that it is, with the few minor changes (mentioned below), ready for publication.

Response: Thank you for the positive comments! The specific questions have been addressed below.

Important change: The authors have removed the word "direct" Line 20, Page 8 but they left it in other places. Since the demonstration is not complete, I believe that when it comes to describing the interaction of FBL and YBX1, they need to remove it.

Response: Thank you for your suggestions. We appreciate the reviewer's attention to detail. We apologize for our carelessness. As suggested, we have removed the word "direct" or "directly" when describing the interaction between FBL and YBX1 throughout the manuscript (Line 2, Page 5; Line 2, Page 13; Line 18, Page 16; Line 4, Page 37).

Minor points:
1. *COAD or LUAD needs to be explained in the legend of Fig1.*

Response: As suggested, we have made the necessary revisions to the legend of Fig1 to include a brief explanation for COAD and LUAD.

2. *An hypothesis regarding RAD51 unchanged foci profile upon shFBL+ MMC should be added.*

Response: Thank you for your suggestion. It is widely recognized that BRCA1 can recruit the PALB2-BRCA2 complex to single-stranded DNA, thereby facilitating the recruitment of RAD51 to sites of DNA damage (Dali Zong *et al*, 2019; Rohit Prakash *et al*, 2015). However, when BRCA1 protein levels are reduced or in BRCA1-deficient cells, an alternative mechanism is activated in which PALB2 is recruited to single-stranded DNA in a manner dependent on RNF168. This recruitment of PALB2, in turn, promotes the recruitment of RAD51. Thus, the RNF168-mediated chromatin ubiquitylation pathway serves as a backup mechanism for RAD51 loading, as depicted in Figure R1 [Figures for referees not shown.] (Dali Zong *et al.*, 2019). This alternative pathway may explain the observed phenomenon in our study, where FBL knockdown led to reduced expression and foci of BRCA1 in response to MMC treatment, while having no significant impact on RAD51 foci. We have incorporated a brief discussion on this topic in our revised manuscript (Page 15, Lines 13-17).

3. *YBX1 is always present in the nucleus (Fig4B), so I still don't fully understand how YBX1 is increasingly recruited to the nucleus, inducing the stronger binding to FBL, but the authors' hypothesis is valid and they should include it in the discussion.*

Response: Thank you for your suggestion. The increased recruitment and stronger binding of YBX1 to FBL in the nucleus may be attributed to MMC-induced phosphorylation of YBX1, which facilitates its nuclear translocation. This, coupled with the relocalization of FBL from the nucleolus to the nucleus, likely promotes the

nuclear association of FBL and YBX1. Furthermore, the phosphorylation of YBX1 could potentially regulate its affinity for FBL. However, the precise mechanism underlying these observations requires further investigation. We have included a brief discussion on this possibility in our revised manuscript (Page 14, Lines 4-9).

4. *Other points to include in the discussion: p53 independent phenotype and the sensitivity to drugs like PARP inhibitors (Olaparib for example) since they have the data ready.*

Response: Apologies for the confusion. We appreciate the reviewer's suggestion to include the p53-independent phenotype and the sensitivity to PARP inhibitors in the discussion. While we recognize the importance of these aspects, we utilize the results to address specific concerns raised by the reviewers and unfortunately do not include the specific data related to these points in the revised manuscript. Hence, we have not included these points into the discussion.

However, we acknowledge the significance of investigating the p53-independent effects of FBL depletion and exploring the potential sensitivity to Olaparib. These are valuable areas of research that warrant further investigation, and we plan to pursue these directions in future studies.

Reference

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Response to editorial requests:

1. The conflict of interest subtitle needs to be corrected to "DISCLOSURE AND COMPETING INTERESTS STATEMENT"

Response: Thank you for your suggestion. We have corrected this in the revised manuscript accordingly.

2. The authors credit section needs to be removed from the ms file. We now use CRediT to specify the contributions of each author in the journal submission system. CRediT replaces the author contribution section. Please use the free text box to provide more detailed descriptions, if you wish. See also guide to authors https://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines.

Response: Thank you for providing the updated guidelines. We appreciate your clarification and have made the necessary adjustments to the manuscript. The authors' credit section has been removed, and we will now utilize the CRediT system to specify the contributions of each author during the journal submission process.

3. None of the FUNDING INFO is listed in the ms file. Please add all funding info to the acknowledgement section in the ms file.

Response: Apologies for the oversight. We have included all funding information in the acknowledgement section in the manuscript file.

4. Please upload all main and all EV figures as separate files. EV tables should also be uploaded individually.

Response: Thank you for your request. We have successfully uploaded all the required files in the appropriate format for this resubmission.

5. I am not sure whether all SOURCE DATA are provided now, as some files were missing but might be part of the deposited data. Can you please clarify? Submitted Source data files need to be organized into one file/folder per figure and ZIPed into 1 file for each main figure.

Response: We apologize for any confusion regarding the availability of the SOURCE DATA files. We have uploaded all the source data as indicated in the SourceData checklist provided by the Editor (please refer to the accompanying figure). However, due to the large file size, we were unable to upload the microscopy images' SOURCE DATA files to the author system. Therefore, these SOURCE DATA files have been uploaded to the BioImage Archive and can be accessed using the provided accession number S-BSST1095. (https://www.ebi.ac.uk/biostudies/studies/S-BSST1095?key=e043b993-3be3-4412-a328-a1d2090f43c7)

To clarify, we have organized the submitted Source data files into separate files for each figure. Additionally, we have compressed these files into a ZIP file for each main figure to facilitate easier access and download.

6. The Data Availability Section (DAS) needs to be moved to the end of the materials and methods. Please make sure that all links are freely accessible upon the online publication of your ms.

Response: Thank you for your request. The Data Availability Section (DAS) has been moved to the end of the materials and methods as per your instructions. Links to the data are properly accessible and functional for readers.

7. The legends for Tables EV1-EV4 should be removed from the ms file and added to each table file, for example in a separate tab.

Response: Thank you for your request. We have made the necessary changes to remove the legends for Tables EV1-EV4 from the manuscript file and added them to each table file individually.

8. The order of the ms sections needs to be corrected to: abstract, introduction, results, discussion, materials & methods, data availability section, acknowledgments, disclosure statement and competing interests, references, main figure legends, EV figure legends.

Response: Thank you for providing the revised order for the manuscript sections. We have made the adjustments to ensure that the sections are arranged in the correct order.

9. I attach to this email a related ms file with comments by our data editors. Please address all comments in the final ms.

Response: Thank you for providing the manuscript file with comments from your data editors. We have addressed all the comments in the final manuscript.

10. I would like to suggest some minor changes to the title and abstract. Please let me know whether you agree with the following:

FBL promotes cancer cell resistance to DNA damage and BRCA1 transcription via YBX1

Fibrillarin (FBL) is a highly conserved nucleolar methyltransferase responsible for methylation of ribosomal RNA and proteins. Here we reveal a role for FBL in DNA damage response and its impact on cancer proliferation and sensitivity to DNA damaging agents. FBL is highly expressed in various cancers and correlates with poor survival in cancer patients. Knockdown of FBL sensitizes tumor cells and xenografts to DNA crosslinking agents, and leads to HR-mediated DNA repair defects. We

identify Y-box binding protein-1 (YBX1) as a key interacting partner of FBL, and FBL increases the nuclear accumulation of YBX1 in response to DNA damage. We show that FBL promotes the expression of BRCA1 by increasing the binding of YBX1 to the BRCA1 promoter. Our study sheds light on the regulatory mechanism of FBL in tumorigenesis and DNA damage response, providing potential therapeutic targets to overcome chemoresistance in cancer.

Response: Thank you for your valuable suggestions. We greatly appreciate your input and have carefully considered your feedback. As suggested, we have made the necessary changes to the title and abstract in our final manuscript.

11. I would also like to suggest that you modify the subheadings in the results section to be more specific about your data and findings.

Response: Thank you for your suggestion to modify the subheadings in the results section. We agree that more specific subheadings can enhance the clarity of our data and findings. We have carefully reviewed the subheadings and made appropriate modifications to ensure that they accurately reflect the content of the section. The revised manuscript now incorporates these changes.

12. EMBO press papers are accompanied online by A) a short (1-2 sentences) summary of the findings and their significance, B) 2-3 bullet points highlighting key results and C) a synopsis image that is exactly 550 pixels wide and 200-600 pixels high (the height is variable). You can either show a model or key data in the synopsis image. Please note that text needs to be readable at the final size. Please send us this information along with the final manuscript.

Response: Thank you for providing us with the editing requirements for EMBO press papers. We have included the information in the final manuscript.

A) A concise summary of the findings and significance:

FBL is a nucleolar methyltransferase involved in ribosomal RNA and protein methylation, but its role in the DNA damage response has remained unclear. This study reveals FBL's crucial role in promoting cancer cell resistance to DNA damage and facilitating BRCA1 transcription via its interaction with YBX1, offering potential therapeutic targets for overcoming chemoresistance in cancer.

B) 2-3 bullet points highlighting the key results.

1. FBL is highly expressed in various cancers and promotes cancer cell resistance to DNA damaging drugs.
2. FBL interacts with YBX1, effectively promoting the expression of BRCA1 by increasing the binding of YBX1 to the BRCA1 promoter, which facilitates the DNA damage response.
3. The FBL/YBX1/BRCA1 axis plays a crucial role in HR-mediated DNA damage repair.

C) A synopsis image with dimensions of 550 pixels wide and a variable height between 200-600 pixels. The image should either showcase a model or present key data, with readable text at the final size.

In our manuscript, we have introduced a comprehensive working model (Figure 6H) that effectively elucidates the underlying mechanism of FBL in response to DNA damage. This figure serves as a concise synopsis. As suggested, we have made the image with the indicated pixels and submitted it in the final submission system.

Prof. Chen Wu
Hebei University
No.180 Wusidong Road
Hebei 071002
China

Dear Prof. Wu,

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.

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Abridged guidelines for 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.
- ideally, figure panels should include only measurements that are directly comparable to each other and obtained with the same assay.
- plots 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. Any statistical test employed should be justified.
- Source Data should be included to report the data underlying figures according to the guidelines set out in the authorship guidelines on Data Presentation.

2. Captions
Each figure caption should contain the following information, for each panel where they are relevant:
- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements.
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

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Select "Not Applicable" only when the requested information is not relevant for your study.

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New materials and reagents need to be available; do any restrictions apply?	Not Applicable	

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For **antibodies** provide the following information: - Commercial antibodies: RRID (if possible) or supplier name, catalogue number and or/clone number - Non-commercial: RRID or citation	Yes	Materials and Methods

DNA and RNA sequences	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Short novel DNA or RNA including primers, probes: provide the sequences.	Yes	Materials and Methods

Cell materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Cell lines: Provide species information, strain. Provide accession number in repository **OR** supplier name, catalog number, clone number, and/**OR** RRID.	Yes	Materials and Methods
Primary cultures: Provide species, strain, sex of origin, genetic modification status.	Not Applicable	
Report if the cell lines were recently **authenticated** (e.g., by STR profiling) and tested for mycoplasma contamination.	Yes	Materials and Methods

Experimental animals	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Laboratory animals or Model organisms: Provide species, strain, sex, age, genetic modification status. Provide accession number in repository **OR** supplier name, catalog number, clone number, **OR** RRID.	Yes	Materials and Methods
Animal observed in or captured from the field: Provide species, sex, and age where possible.	Not Applicable	
Please detail **housing and husbandry conditions**.	Not Applicable	

Plants and microbes	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Plants: provide species and strain, ecotype and cultivar where relevant, unique accession number if available, and source (including location for collected wild specimens).	Not Applicable	
Microbes: provide species and strain, unique accession number if available, and source.	Not Applicable	

Human research participants	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If collected and within the bounds of privacy constraints report on age, sex and gender or ethnicity for all study participants.	Not Applicable	

Core facilities	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If your work benefited from core facilities, was their service mentioned in the acknowledgments section?	Yes	Acknowledgements

Design

Study protocol	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If study protocol has been **pre-registered, provide DOI in the manuscript**. For clinical trials, provide the trial registration number **OR** cite DOI.	Yes	Materials and Methods
Report the **clinical trial registration number** (at ClinicalTrials.gov or equivalent), where applicable.	Yes	Materials and Methods

Laboratory protocol	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Provide DOI OR other citation details if **external detailed step-by-step protocols** are available.	Not Applicable	

Experimental study design and statistics	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Include a statement about **sample size** estimate even if no statistical methods were used.	Yes	Figure legends
Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. **randomization procedure**)? If yes, have they been described?	Yes	Materials and Methods
Include a statement about **blinding** even if no blinding was done.	Not Applicable	
Describe **inclusion/exclusion criteria** if samples or animals were excluded from the analysis. Were the criteria pre-established? If sample or data points were omitted from analysis, report if this was due to attrition or intentional exclusion and provide justification.	Not Applicable	
For every figure, are **statistical tests** justified as appropriate? Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. Is there an estimate of variation within each group of data? Is the variance similar between the groups that are being statistically compared?	Yes	Materials and Methods, Figure legends

Sample definition and in-laboratory replication	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
In the figure legends: state number of times the experiment was **replicated** in laboratory.	Yes	Figure legends
In the figure legends: define whether data describe **technical or biological replicates**.	Yes	Figure legends

Ethics

Ethics	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Studies involving **human participants**: State details of **authority granting ethics approval** (IRB or equivalent committee(s), provide reference number for approval.	Yes	Materials and Methods
Studies involving **human participants**: 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.	Yes	Materials and Methods
Studies involving **human participants:** For publication of **patient photos**, include a statement confirming that consent to publish was obtained.	Yes	Materials and Methods
Studies involving experimental **animals**: State details of **authority granting ethics approval** (IRB or equivalent committee(s), provide reference number for approval. Include a statement of compliance with ethical regulations.	Yes	Materials and Methods
Studies involving **specimen and field samples:** State if relevant **permits** obtained, provide details of authority approving study; if none were required, explain why.	Yes	Materials and Methods

Dual Use Research of Concern (DURC)	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Could your study fall under dual use research restrictions? Please check biosecurity documents and list of **select agents and toxins** (CDC): https://www.selectagents.gov/sat/list.htm	Not Applicable	
If you used a select agent, is the security level of the lab appropriate and reported in the manuscript?	Not Applicable	
If a study is subject to dual use research of concern regulations, is the name of the **authority granting approval and reference number** for the regulatory approval provided in the manuscript?	Not Applicable	

Reporting

The MDAR framework recommends adoption of discipline-specific guidelines, established and endorsed through community initiatives. Journals have their own policy about requiring specific guidelines and recommendations to complement MDAR.

Adherence to community standards	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
State if relevant guidelines or checklists (e.g., **ICMJE, MIBBI, ARRIVE, PRISMA**) have been followed or provided.	Yes	Date Availability
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.	Not Applicable	
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.	Not Applicable	

Data Availability

Data availability	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Have **primary datasets** been deposited according to the journal's guidelines (see 'Data Deposition' section) and the respective accession numbers provided in the Data Availability Section?	Yes	Date Availability
Were **human clinical and genomic datasets** deposited in a public access-controlled repository in accordance to ethical obligations to the patients and to the applicable consent agreement?	Yes	Materials and Methods
Are **computational models** that are central and integral to a study available without restrictions in a machine-readable form? Were the relevant accession numbers or links provided?	Not Applicable	
If publicly available data were reused, provide the respective **data citations in the reference list**.	Not Applicable	