molecular systems biology

Coordination of MAPK and p53 dynamics in the cellular responses to DNA damage and oxidative stress

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Transaction Report:

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

The reviewers' comments and authors' responses are not available with this article, as the initial review process took place with another journal.

Thank you once again for submitting your manuscript "The dynamics of MAPK activity and p53 levels are coordinated to generate specificity in the cellular responses to DNA damage and oxidative stress" to Molecular Systems Biology. I have discussed with the team the manuscript and the reviewers' comments from the other journal. We think that the topic fits well to the scope of MSB. In line with the reviewers' comments, we acknowledge that the findings seem relevant for the field. We would be happy to publish the study in Molecular Systems Biology, pending some minor modifications and editorial issues that would need to be fixed.

Overall, we think that the concerns of the reviewers from the initial submission at the other journal have been satisfactorily addressed. Indeed, it seems that the remaining concern of reviewer #2 regarding the conceptual issue related to the dependence (or lack thereof, as the reviewer is concerned) of cell fates/responses on p53 seems to be the result of a misunderstanding. As such, we do not think that this concern prevents the publication of the study. Reviewer #2 also has two remaining technical concerns. We think that the first one, referring to the need to complement the results showing the effect of the chemical inhibitors by additional perturbations of the target genes, does not seem essential to address at this point. Regarding the second one, i.e. requesting validations of the apoptosis findings with additional apoptosis assays, we think that this is a good suggestion that indeed would strengthen the related findings. However, if you do not have such data at hand, or if it is very difficult to obtain them in a reasonable time frame, it seems reasonable to omit these additional analyses. Overall, we think that both remaining technical concerns of reviewer #2 can be addressed by some rather minor text modifications.

On a more editorial level, we would ask you to address the following points:

Response to Reviewers' comments and identification of erratum to original data

Dear Dr. Polychronidou,

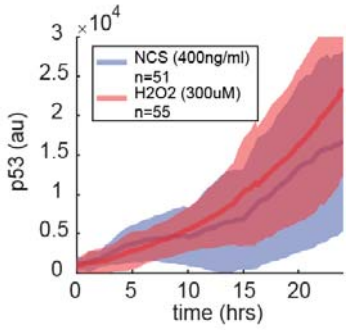
We have addressed the reviewer 2's remaining comment regarding the quantification of apoptosis following the guidelines you suggested through text changes.

Page 7, line 7: Replaced the phrase "activating apoptosis" with "inducing cell death"

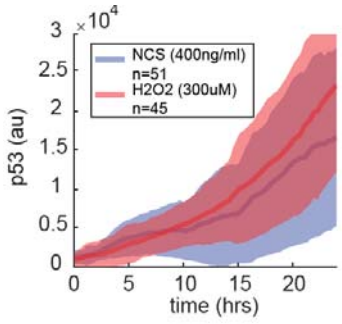
Page 8, line 4: In the Discussion, we included the statement: "While Annexin V and propidium iodide staining is typically indicative of apoptosis, other mechanisms of cell death are possible and further study is needed to elucidate the precise mechanism of cell death."

In the process of preparing the original data and analysis code for submission to GitHub, we noticed that some incorrect data had erroneously been included in some of the single cell data sets analyzed. Thankfully, correcting for the oversights did not alter any of the conclusions of the manuscript.

Specifically, in the third plot of Figure 1C, an additional 10 fluorescence traces had been mistakenly included in the H_2O_2 data set. We have corrected the number of samples in this figure from n = 55 (original plot) to n = 45 (corrected plot). This did not noticeably alter the associated third plot in Fig. 1C.

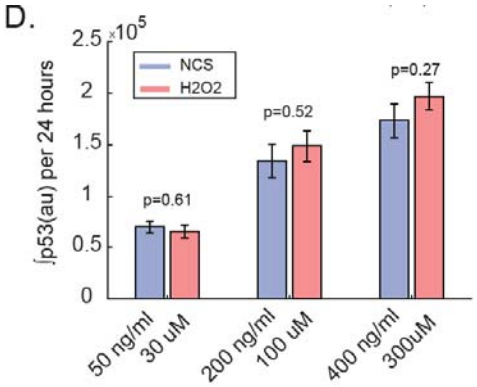


Original Fig. 1C

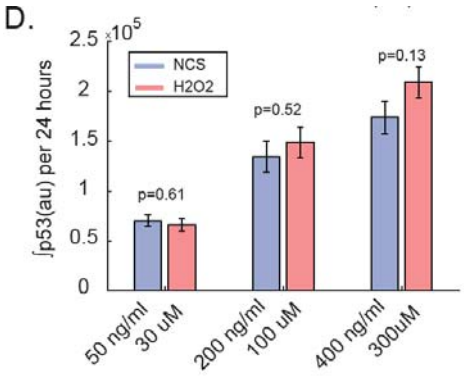


Corrected Fig. 1C

In Figure 1D, we provided statistical analysis of the data from Fig. 1C. Analysis of the corrected data set supports our original conclusion that there is not a statistically significant difference between the integrated p53 levels in the high DNA damage and the high H_2O_2 conditions, (p-values changing from p=0.27 to p=0.13 in the third set of bars, which is still not statistically significant).

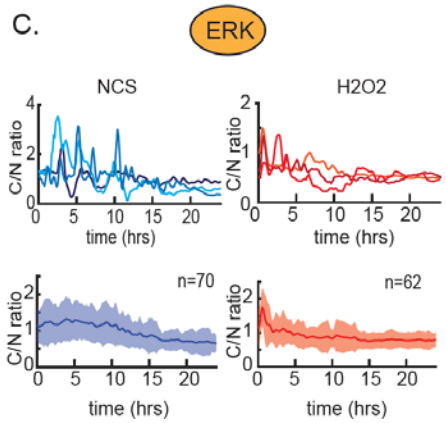


Original Fig. 1D

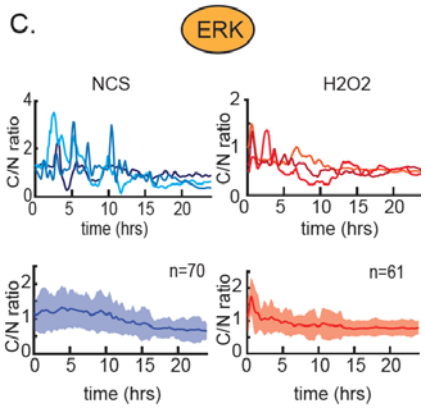


Corrected Fig. 1D

A similar error occurred in Fig. 2C, in which one erroneous trace was included in the H_2O_2 data set of ERK activity. We have removed the incorrectly included sample, and we updated the number of samples from n=62 to n=61. There was no appreciable effect on the figures, analysis, or conclusions drawn upon removal of the single sample.

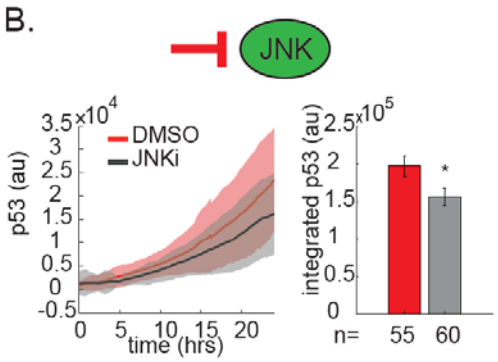


Original Fig. 2C

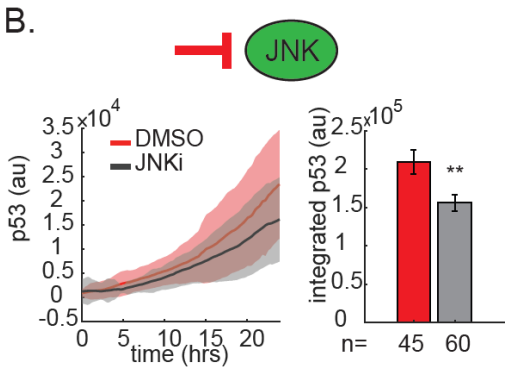


Corrected Fig. 2C

Data were also incorrectly included for the control condition in the experiment involving JNK inhibition, as presented in Fig. 6B. We have updated the figure to reflect the analysis of n=45 cells, accordingly. This resulted in the control bar having a slightly higher value (shown below). For the statistical analysis of these results, the corrected data actually provides greater statistical support for our conclusion that there is a significant difference between the control cells and the cells treated with the JNK inhibitor, with the p-value changing from 0.013 to 0.005. The correct p-value has now been reported in the caption for Figure 6B.



Original Fig. 6B



Corrected Fig. 6B

We apologize for the errors, which fortunately have been caught at this stage and which have not altered any of the conclusions of the manuscript.

Thank you again for sending us your revised manuscript. We are now satisfied with the modifications made and I am pleased to inform you that your paper has been accepted for publication.

EMBO Press Author Checklist

Corresponding Author Name: Eric Batchelor
Journal Submitted to: Molecular Systems Biology
Manuscript Number: MSB-2022-11401R

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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
- 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

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:
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  - are tests one-sided or two-sided?
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  - definition of error bars as s.d. or s.e.m.

Please complete ALL of the questions below.
Select "Not Applicable" only when the requested information is not relevant for your study.

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Report if the cell lines were recently **authenticated** (e.g., by STR profiling) and tested for mycoplasma contamination.	Yes	Materials and Methods

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Please detail **housing and husbandry conditions**.	Not Applicable	

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Include a statement about **sample size** estimate even if no statistical methods were used.	Yes	Figures
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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	Figures

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