EMBO reports

Interaction with the CCT chaperonin complex limits APOBEC3A cytidine deaminase cytotoxicity

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1st Editorial Decision 18th Dec 2020

Dear Dr. Green,

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

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

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

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

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

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

When submitting your revised manuscript, we will require:

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

2) individual production quality figure files as .eps, .tif, .jpg (one file per figure), of main figures and EV figures. Please upload these as separate, individual files upon re-submission.

The Expanded View format, which will be displayed in the main HTML of the paper in a collapsible format, has replaced the Supplementary information. You can submit up to 5 images as Expanded View. Please follow the nomenclature Figure EV1, Figure EV2 etc. The figure legend for these should be included in the main manuscript document file in a section called Expanded View Figure Legends after the main Figure Legends section. Additional Supplementary material should be supplied as a single pdf file labeled Appendix. The Appendix should have page numbers and needs

to include a table of content on the first page (with page numbers) and legends for all content. Please follow the nomenclature Appendix Figure Sx, Appendix Table Sx etc. throughout the text, and also label the figures and tables according to this nomenclature.

For more details please refer to our guide to authors:
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See also our guide for figure preparation:
http://wol-prod-cdn.literatumonline.com/pb-assets/embo-site/EMBOPress_Figure_Guidelines_061115-1561436025777.pdf

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

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

Please also follow our guidelines for the use of living organisms, and the respective reporting guidelines: http://www.embopress.org/page/journal/14693178/authorguide#livingorganisms

5) that primary datasets produced in this study (e.g. RNA-seq, ChIP-seq and array data) are deposited in an appropriate public database. If no primary datasets have been deposited, please also state this in the respective section (e.g. 'No primary datasets have been generated and deposited'), see below.

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

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

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

Data availability

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

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

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

Moreover, I have these editorial requests:

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

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

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

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

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

11) Supplementary Table 1 is a dataset. Please upload this as dataset file, named Dataset EV1 and change the callouts accordingly.

12) - Please order the sections like this: Title page - Abstract - Introduction - Results - Discussion - Materials and Methods - DAS (data availability section) - Acknowledgements - Author contributions - Conflict of interest - References - Figure legends - Expanded View Figure legends. Please add all the funding information to the acknowledgements.

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

I look forward to seeing a revised version of your manuscript when it is ready. Please let me know if

you have questions or comments regarding the revision.

Yours sincerely,

Achim Breiling
Editor
EMBO Reports

Referee #1:

"Interaction with the CCT chaperonin complex limits cytotoxicity from the APOBEC3A cytosine deaminase" is a well written and rigorous characterization of the interaction between the cancer mutator APOBEC3A and the CCT chaperonin complex. The authors identify through co-immunoprecipitation and mass spectrometry, that APOBEC3A co-purifies with multiple members of the CCT chaperonin complex. They confirm this interaction in multiple cell lines by reciprocal immunoprecipitation of over-expressed proteins. Importantly, they show that depletion of the CCT chaperonin complex by shRNA increases cell death induced by APOBEC3A over-expression, indicating that CCT likely regulates APOBEC3A and inhibits inappropriate activity of the enzyme. They also indicate that tumors with mutations within CCT chaperonin complex proteins have higher amounts of APOBEC-induced mutations. These results are important as APOBEC3A is a likely cause of the APOBEC mutation signature in cancers, which is the 2nd most abundant source of mutation during cancer development. Additionally, little is known about the potential APOBEC3A interactors or how the protein is normally regulated to limit the deleterious effects of the protein's activity on genomic DNA. I have the following criticisms that I believe will improve the manuscript if addressed.

Major:
1) For the analysis of effects of CCT chaperonin complex mutations on APOBEC-induced mutations, it is important to more clearly define what is meant by "deleterious mutations" in CCT chaperonin complex members. If these mutations are primarily base substitutions (as opposed to insertion/deletions or copy number changes), then there is a potential issue with the analysis. Tumors with higher base substitution burdens are more likely to contain a base substitution within a gene encoding a CCT chaperonin complex member. I have tried to conduct similar analyses with the result that tumors with a base substitution in almost any gene (regardless of its possible association with APOBEC-induced mutation) will have higher mutation burdens than tumors lacking a base substitution in the gene. Deletions or copy number changes may not be subject to the same effect if the number of these types of events do not correlate with the number of base substitutions across the tumors analyzed. I am unsure if using Signature 1 as a control will control for this effect, as Signature 1 is a minor source of mutation in the samples and skewed toward regions of the genome that are in CpG islands and not protein coding.
2) Comparison of CCT chaperonin complex expression by RNA-seq (which is publicly available for TCGA tumors) to Signature 2 and 13 mutations may provide a more reliable assessment of the effects of the CCT complex on APOBEC-induced mutation during cancer development. The authors already show that depletion of a single CCT complex member reduces the amount of the several complex members in cells, so this would seem to be a natural comparison.
3) The authors focus on effects of CCT complex mutation and APOBEC mutagenesis in breast, cervical, and bladder cancer, but only show the interaction of CCT complex with APOBEC3A in bone osteosarcoma or immune cell cancer lines. Support that the CCT complex regulates APOBEC3A in breast, cervical or bladder cancer cell lines would strengthen the reasoning behind

this analysis.
4) All experiments characterizing the association of the CCT complex with APOBEC3A and impacting APOBEC3A activity use over-expression of APOBEC3A. Can the authors provide evidence that the CCT complex is regulating endogenous APOBEC3A? This data may already exist as CCT4 knock-down appears to increase cell death without APOBEC3A over-expression. If this lethality were rescued by knock-down of APOBEC3A, then it would strongly suggest that the CCT complex is a major regulator of the activity of APOBEC3A at endogenous expression levels.

Minor:
1) APOBEC3A is a cytidine deaminase, not a cytosine deaminase. Cytosine deaminases deaminate the free cytosine base not cytidines in poly-nucleic acids.

Referee #2:

In this work, Green and coll. demonstrated that A3A enzyme could interact with the CCT complex. In addition, the depletion of the CCT complex induces a cellular cytotoxicity which was associated with A3A activity. The detected interaction is specific to A3A enzyme and other members of A3 family do not seem to interact (or weakly) with the CCT complex. Finally, the analysis of tumour genomes using bioinformatics data revealed specific signatures associated with A3A activity in presence of CCT mutated gene. These data suggest that the CCT complex interacts with A3A, and mutations that abolish the CCT gene imply specific A3A activity in the nucleus.

Although the work presented is interesting, several important points remain obscure, and probably the most important point is that the authors do not really demonstrate that the presence of A3A induced SB2 and SB13 signatures could be associated with mutations occurring in the CCT gene.

1) The authors used the HepaRG hepatic cell line. How do the authors justify the choice of this cells knowing that A3A is not physiologically expressed in hepatocytes?

2) The authors have demonstrated an interaction of A3A with CCT1, CCT4 CCT5 CCT7 in both U2OS and HeparRG cell lines. Why the experiments described in figure 4 were only performed with CCT4 and CCT7? What is the effect of CCT1 on cell viability?

3) The authors claim that mutations occurring in the CCT complex induce an A3A specific SB2 and SB13 signature. How could the CCT complex, which seems to be located in the cytosol influence the A3A DNA editing activity present in the nucleus? The authors do not show any confocal microscopy data to visualize the co-localization of the two proteins. Is the conformation of A3A different in presence of CCT? What about the half-life of A3A in presence of CCT?

4) The authors do not demonstrate that SBS2 and SBS13 signatures are associated with a loss of CCT activity. The authors should demonstrate the appearance of specific SB2 and SB13 signatures in presence of CCT siRNAs. This will prove that depletion of CCT is associated with nuclear A3A editing activity. Similarly, could mutated CCT gene be associated with other mutations in the genome that could be connected to A3A editing activity?

Referee #3:

In this study, the authors identified the CCT chaperon complex as a major interactor of APOBEC3A. Interestingly, depletion of CCT leads to reduced survival of APOBEC3A expressing cells. Furthermore, an increase of APOBEC signature mutations were observed in tumors with mutations in CCT genes. The authors propose that CCT limits the cytotoxicity of APOBEC3A. The experiments in this study are well designed and executed. All the main observations are clear. However, there are still a few issues that should be addressed before the manuscript is ready for publication.

1. All the interaction data on APOBEC3A and the CCT complex were obtained from cells expressing exogenous APOBEC3A. This leaves the possibility that the interaction could be an artifact of APOBEC3A overexpression. The conclusion of this study would be much strengthened if the authors can show that endogenous APOBEC3A (even when it is induced) interacts with CCT.

2. The authors suggest that the interaction of CCT with APOBEC3A limits its toxicity. However, there is no data to show that the interaction between CCT and APOBEC3A matters. Hypothetically, CCT could stabilize a protein X, which is an inhibitor of APOBEC3A. The effects of CCT on APOBEC3A may not be direct.

3. Along the same line, although depletion of CCT reduces the survival of APOBEC3A expressing cells, the protective effects of CCT may not be attributed to APOBEC3A itself. For example, some of the clients of CCT may be involved in DNA repair, which are required to suppress APOBEC3A induced DNA damage and keep cells alive.

4. In Fig. 4, can the authors provide some evidence that APOBEC3A induces more DNA damage in the absence of CCT? Furthermore, are the protective effects of CCT specific to APOBEC3A? Does it also protect against APOBEC3B?

5. The data in Fig. 5 are very interesting. Can the authors look into the mutation signatures more carefully to distinguish APOBEC3A and 3B signature mutations? The conclusion of this study would be stronger if only the APOBEC3A but not the 3B signature mutations are increased in tumors with CCT mutations.

Response to reviewers

We thank the reviewers for their insightful critiques, and have attempted to address each point raised either experimentally or in the manuscript text. Below we have outlined our additions and alterations to the manuscript, and our responses to reviewers' comments in blue text.

Referee #1:

"Interaction with the CCT chaperonin complex limits cytotoxicity from the APOBEC3A cytosine deaminase" is a well written and rigorous characterization of the interaction between the cancer mutator APOBEC3A and the CCT chaperonin complex. The authors identify through co-immunoprecipitation and mass spectrometry, that APOBEC3A co-purifies with multiple members of the CCT chaperonin complex. They confirm this interaction in multiple cell lines by reciprocal immunoprecipitation of over-expressed proteins. Importantly, they show that depletion of the CCT chaperonin complex by shRNA increases cell death induced by APOBEC3A over-expression, indicating that CCT likely regulates APOBEC3A and inhibits inappropriate activity of the enzyme. They also indicate that tumors with mutations within CCT chaperonin complex proteins have higher amounts of APOBEC-induced mutations. These results are important as APOBEC3A is a likely cause of the APOBEC mutation signature in cancers, which is the 2nd most abundant source of mutation during cancer development. Additionally, little is known about the potential APOBEC3A interactors or how the protein is normally regulated to limit the deleterious effects of the protein's activity on genomic DNA. I have the following criticisms that I believe will improve the manuscript if addressed.

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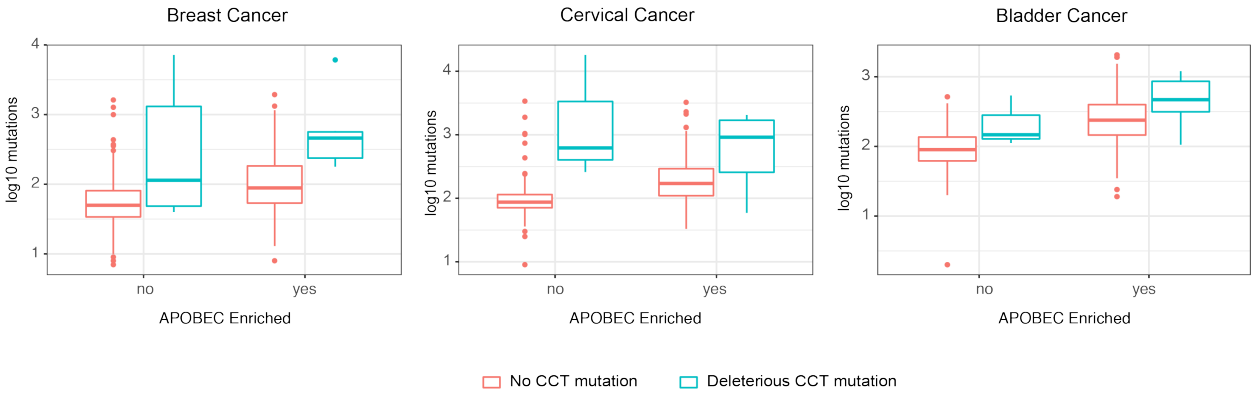
Reviewer 1 raises important points regarding our computational analysis and we have re-evaluated mutational signatures and CCT mutations in cancer genomes to improve the quality and clarity of data presented. The following points were specifically addressed:

1) Deleterious mutations are defined as those predicted to cause a negative effect on protein function including indels, substitutions, frameshifts, and non-sense mutations. We used established prediction models (refs: Choi Y et al, 2012. McLaren W et al, 2016.) to determine deleterious mutations in CCT subunit genes. This definition has been clarified in the manuscript text on pages 35 (figure legend), 11 (results), and 22 (methods). It is important to note that we excluded from all analyses tumors which have a mutation in the CCT gene that is predicted to be non-deleterious to avoid ambiguity in our analysis. This point has additionally been added to the manuscript text on page 22.

2) We investigated the association of CCT gene mutations with overall mutational burden in cancer genomes to determine whether mutations of the CCT genes were just a byproduct of heavy mutational burdens. We found that the frequency of CCT gene mutations was similar among samples with high A3A mutational burdens and low A3A mutational burdens (see table below). As a control, we evaluated the frequency of mutations in 10 randomly selected sets of 9 protein coding genes of similar size to CCT genes, the median of these results are shown below. Frequency of mutations is similar between CCT genes and other similarly-sized genes.

Cancer type	APOBEC mutational burden	Frequency of CCT gene mutations	Frequency of housekeeping gene mutations
Breast	Low	1.7%	1.1%
	High	2.4%	2%
Bladder	Low	4.5%	2.5%
	High	5.5%	4%
Cervical	Low	4%	3%
	High	4%	2.8%

Genomes with CCT gene mutations had a somewhat higher mutational burden overall (Fig 5A), which we have clarified in the manuscript text, however this was true regardless of the presence of APOBEC3 signature mutations. We analyzed genomes with and without APOBEC3 signature mutations (APOBEC enriched as defined in Roberts SA et al, 2013) and saw similar overall mutation burden in tumors with deleterious CCT mutations and those with wild type CCT genes. These analyses in breast, bladder, and cervical cancer are shown below. Thus we feel more confident that our data are not biased by overall tumor mutation burden.

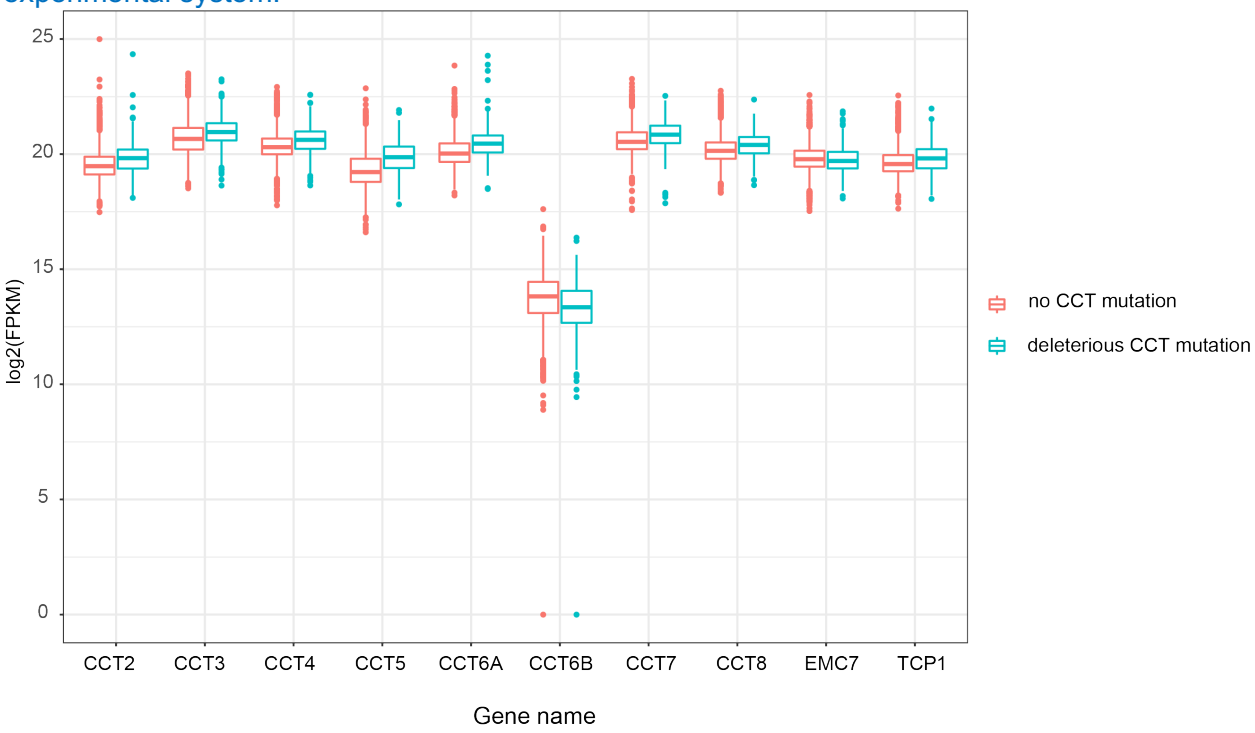


3) We agree with the reviewer's point about signature 1 being a suboptimal control and have removed this from Figure 5. In panel D, we show the contribution of all other SBS signatures compared to the two APOBEC-associated SBS signatures, which suggests that tumors with CCT gene mutations have an overall higher contribution of APOBEC-associated SBS signatures in comparison to all other SBS signatures. We have added to the revised Figure 5D the contribution of SBS2, SBS13, and all other SBS in all tumors within TCGA to further illustrate this point.

2) Comparison of CCT chaperonin complex expression by RNA-seq (which is publicly available for TCGA tumors) to Signature 2 and 13 mutations may provide a more reliable assessment of the effects of the CCT complex on APOBEC-induced mutation during cancer development. The authors already show that depletion of a single CCT complex member reduces the amount of the several complex members in cells, so this would seem to be a natural comparison.

We appreciate the reviewer's suggestion, and we evaluated RNA-seq from the tumor samples for which genomes were analyzed. Below is a depiction of transcript levels for each CCT gene in all of the TCGA tumors evaluated in Figure 5. Notably, CCT transcript levels are not globally decreased in the tumors with CCT gene mutations. This is not entirely unexpected however, because gene mutations, and even deleterious gene mutations, do not necessarily impact transcription. Of note, depletion of a CCT complex member at the protein level results in a stoichiometric imbalance of the complex, leading to degradation of additional CCT subunits (refs: Kasembeli et al, 2014 and Kunisawa et al, 2003) although this would not be expected to impact transcript levels. We have included a figure below (Reviewer 2, point number 2) to further illustrate this finding in our

experimental system.



3) The authors focus on effects of CCT complex mutation and APOBEC mutagenesis in breast, cervical, and bladder cancer, but only show the interaction of CCT complex with APOBEC3A in bone osteosarcoma or immune cell cancer lines. Support that the CCT complex regulates APOBEC3A in breast, cervical or bladder cancer cell lines would strengthen the reasoning behind this analysis.

We appreciate the reviewer's attention to the physiologic relevance of the A3A-CCT interaction. We have performed additional experiments in breast cancer cells to demonstrate the A3A-CCT interaction. The revised manuscript includes additional figures (Fig 2, panel D) which shows reciprocal co-IPs for A3A-HA and the CCT complex in MDA-MB-231 cell lines. Additionally to note, Fig 2C demonstrates the A3A-CCT interaction in hematopoietic cells which have also been shown to express A3A and harbor A3 signature mutations (refs: Koning et al, 2009. Green et al, 2017. Jalili et al, 2020.).

4) All experiments characterizing the association of the CCT complex with APOBEC3A and impacting APOBEC3A activity use over-expression of APOBEC3A. Can the authors provide evidence that the CCT complex is regulating endogenous APOBEC3A? This data may already exist as CCT4 knock-down appears to increase cell death without APOBEC3A over-expression. If this lethality were rescued by knock-down of APOBEC3A, then it would strongly suggest that the CCT complex is a major regulator of the activity of APOBEC3A at endogenous expression levels.

We performed additional experiments to confirm the association of CCT complex and endogenous APOBEC3A. In the revised manuscript, new data (Fig 3F) has been added to show that IP of endogenous CCT co-precipitates with endogenous A3A in BICR6 cells. There are very few cell lines that continuously express endogenous A3A, presumably because persistent expression of a potent deaminase is genotoxic. The BICR6 cell line (derived from a head and neck squamous epithelial cancer) demonstrates consistent A3A expression, although given how aberrant this finding is, we do not think that this cell line is an appropriate system in which to evaluate A3A-induced genotoxicity. In other words, we suspect that A3A expression/activity does not result in toxicity to BICR6 cells in the same way that it does in other cells. Thus, the reviewer's proposed experiment regarding cell viability after depletion of endogenous A3A is technically difficult to design in an appropriate cell context. We hope that the reviewers appreciate the feasibility issues we encounter with endogenous A3A experiments and are convinced by the viability data in ectopically expressed A3A as well as the new data showing an interaction between endogenous A3A and CCT as well as A3A-mediated DNA damage in the context of CCT depletion (Fig 4E).

Minor:
1) APOBEC3A is a cytidine deaminase, not a cytosine deaminase. Cytosine deaminases deaminate the free cytosine base not cytidines in poly-nucleic acids.

We appreciate the reviewer's attention to this detail and have revised the manuscript accordingly.

Referee #2:

In this work, Green and coll. demonstrated that A3A enzyme could interact with the CCT complex. In addition, the depletion of the CCT complex induces a cellular cytotoxicity which was associated with A3A activity. The detected interaction is specific to A3A enzyme and other members of A3 family do not seem to interact (or weakly) with the CCT complex. Finally, the analysis of tumour genomes using bioinformatics data revealed specific signatures associated with A3A activity in presence of CCT mutated gene. These data suggest that the CCT complex interacts with A3A, and mutations that abolish the CCT gene imply specific A3A activity in the nucleus.

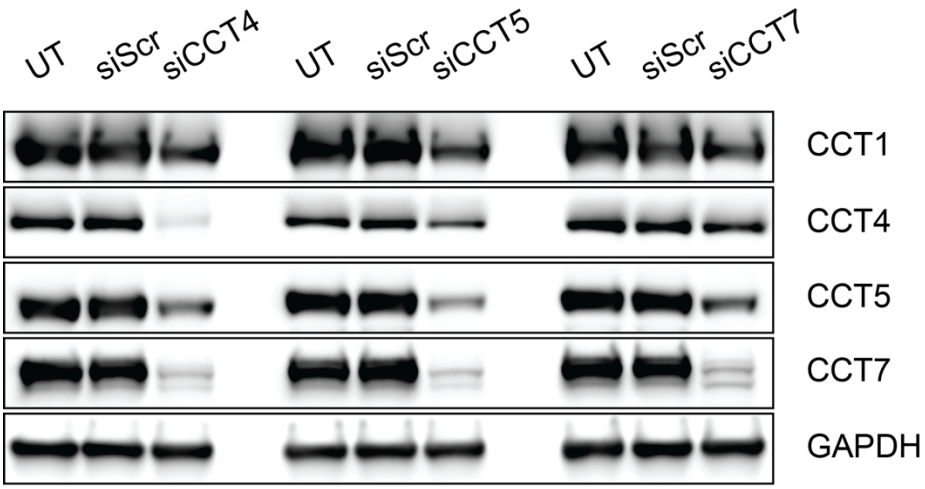
Although the work presented is interesting, several important points remain obscure, and probably the most important point is that the authors do not really demonstrate that the presence of A3A induced SB2 and SB13 signatures could be associated with mutations occurring in the CCT gene.

1) The authors used the HepaRG hepatic cell line. How do the authors justify the choice of this cells knowing that A3A is not physiologically expressed in hepatocytes?

The reviewer's point is well taken, we simply use HepaRG cells as a tool. We agree that physiologic relevance is important and thus included hematopoietic cell lines to reflect a tissue in which A3A is expressed in normal and malignant conditions (Fig 2C). To address the reviewer's query, we have done additional experiments to ensure that the A3A-CCT interaction is evaluated in physiologically relevant tissues. The first demonstration of A3 mutational activity in cancer was done in breast tumors (ref: Nik Zainal et al, 2012) and subsequent data have confirmed both expression and activity of A3A in breast cancers. Thus, we investigated the A3A-CCT interaction in a breast cancer cell line and show in the new Fig 2D reciprocal co-IPs of A3A and CCT in breast cancer cells.

2) The authors have demonstrated an interaction of A3A with CCT1, CCT4 CCT5 CCT7 in both U2OS and HepaRG cell lines. Why the experiments described in figure 4 were only performed with CCT4 and CCT7? What is the effect of CCT1 on cell viability?

The reviewer asks an excellent question. Consistent with prior reports in the literature (refs: Kasembeli et al, 2014 and Kunisawa et al, 2003), we show in figure 4A that knockdown of a single CCT complex member is associated with depletion of remaining CCT subunits. An expanded figure is shown below. This is based on the required ratio of each CCT subunit in complex formation, and subsequent depletion of excess CCT subunits in the absence of complex formation. We used RNAi targeting CCT4 and CCT7 based on the efficacy of siRNA for those targets rather than biology specific to CCT4 and CCT7.



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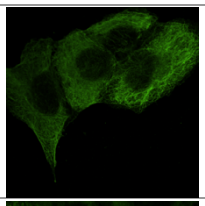
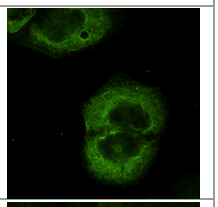
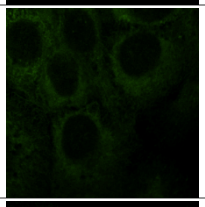
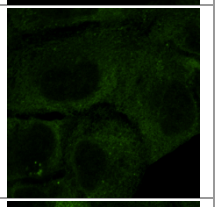
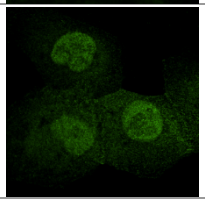
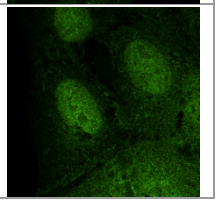
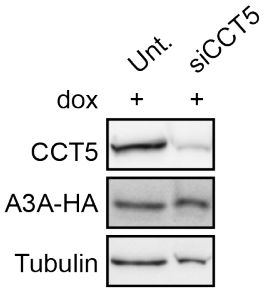
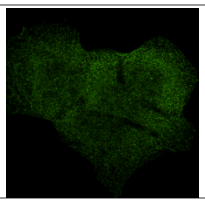
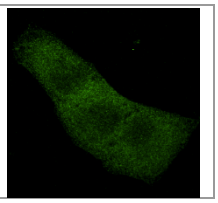
We agree with the reviewer that A3A subcellular localization in the presence and absence of the CCT complex would be informative regarding how altered localization during the interaction with CCT may prevent A3A access to the nucleus or to nuclear DNA, thereby mitigating genotoxic cell death. We have unsuccessfully tried to visualize the co-localization of CCT and A3A, and additionally the localization of A3A following CCT knockdown. Although we are able to image A3A by IF, we have tried several different antibodies to visualize CCT by confocal microscopy (listed in the table below), but none of the antibodies have been suitable for IF, thus we have not been able to identify cells in which CCT is depleted by RNAi nor to confirm specificity of the CCT antibodies for their targets. We are able to demonstrate efficacy of CCT5 knockdown by siRNA using immunoblotting, but not by IF in the same siRNA experiment.

Antibody	Brand	Species	Outcome	siCTRL	siCCT
Anti-CCT7	Abnova	Mouse	No detection of CCT	Not detected	Not detected
Anti-CCT5	Abnova	Mouse	Cross-reactivity		
Anti-CCT1	Abnova	Mouse	Cross-reactivity		
Anti-CCT2	Abcam	Rabbit	Cross-reactivity		



Anti-CCT7	Sigma	Rabbit	Cross-reactivity		

Since single-cell visualization was unsuccessful, we evaluated whether CCT depletion altered A3A localization in a population of cells. While we found a slight trend towards nuclear exclusion of A3A upon CCT depletion, the results are not significant and therefore not included in the revised manuscript.

The reviewer asks additional important questions regarding stability of A3A in the presence of CCT. We have indirectly evaluated this by immunoblot for ectopically expressed A3A with and without CCT depletion. We see no difference in A3A expression, at least in its denatured form, upon CCT depletion (see immunoblots in Fig 4).

4) The authors do not demonstrate that SBS2 and SBS13 signatures are associated with a loss of CCT activity. The authors should demonstrate the appearance of specific SB2 and SB13 signatures in presence of CCT siRNAs. This will prove that depletion of CCT is associated with nuclear A3A editing activity. Similarly, could mutated CCT gene be associated with other mutations in the genome that could be connected to A3A editing activity?

We appreciate the limitations in our experimental design pointed out by the reviewer and agree that we do not directly show alterations in mutational signatures correlated with CCT depletion. Instead, we used an indirect measure of CCT loss by evaluating tumors with deleterious mutations in CCT genes, which predict a nonfunctional gene product. Based on prior literature (refs: Kasembeli et al, 2014 and Kunisawa et al, 2003), depletion of a single CCT subunit will decrease/inhibit assembly and function of the entire CCT complex. Thus, a deleterious mutation in a single CCT gene should predict decreased function of the CCT complex. While showing directly that genome-wide mutational signatures change upon CCT depletion would be ideal, that would require significant experimental investment that would substantially delay reporting of the presented findings.

The reviewer raises an important additional point that mutations in CCT genes may be secondary to a mutagenic process, such as A3A-induced deamination itself, that would affect other mutations in the genome. It is certainly possible that A3A activity results in CCT gene mutations and subsequent CCT complex dysfunction. Regardless of the source of CCT gene mutation, we find that cancers with deleterious mutations in CCT genes are associated with an increased burden of APOBEC signature mutations genome-wide.

Referee #3:

In this study, the authors identified the CCT chaperon complex as a major interactor of APOBEC3A. Interestingly, depletion of CCT leads to reduced survival of APOBEC3A expressing cells. Furthermore, an increase of APOBEC signature mutations were observed in tumors with mutations in CCT genes. The authors propose that CCT limits the cytotoxicity of APOBEC3A. The experiments in this study are well designed and executed. All the main observations are clear. However, there are still a few issues that should be addressed before the manuscript is ready for publication.

1. All the interaction data on APOBEC3A and the CCT complex were obtained from cells expressing exogenous APOBEC3A. This leaves the possibility that the interaction could be an artifact of APOBEC3A overexpression. The conclusion of this study would be much strengthened if the authors can show that endogenous APOBEC3A (even when it is induced) interacts with CCT.

We appreciate the reviewer's critique and have performed additional experiments to address this concern. Figure 3 now includes an additional panel F which demonstrates co-precipitation of endogenous APOBEC3A and CCT complex subunits in BICR6 cells.

2. The authors suggest that the interaction of CCT with APOBEC3A limits its toxicity. However, there is no data to show that the interaction between CCT and APOBEC3A matters. Hypothetically, CCT could stabilize a protein X, which is an inhibitor of APOBEC3A. The effects of CCT on APOBEC3A may not be direct.

3. Along the same line, although depletion of CCT reduces the survival of APOBEC3A expressing cells, the protective effects of CCT may not be attributed to APOBEC3A itself. For example, some of the clients of CCT may be involved in DNA repair, which are required to suppress APOBEC3A induced DNA damage and keep cells alive.

Regarding points 2 and 3 above, the reviewer raises an interesting possibility regarding an indirect impact of CCT on A3A. This is possible on two levels: (1) the interaction between A3A and CCT may be mediated by a yet-unidentified protein, or (2) regardless of the A3A-CCT interaction, the impact of CCT on A3A-induced cytotoxicity may be mediated through CCT interaction with an alternate protein/complex. We appreciate the reviewer's input and have incorporated both possibilities into the manuscript discussion.

4. In Fig. 4, can the authors provide some evidence that APOBEC3A induces more DNA damage in the absence of CCT? Furthermore, are the protective effects of CCT specific to APOBEC3A? Does it also protect against APOBEC3B?

The reviewer raises an excellent point regarding A3A-induced DNA damage in the absence of CCT. To address this critique, we performed additional experiments in which we depleted CCT complex by RNAi and evaluated phosphorylation of histone variant H2AX (γH2AX), a marker of DNA breaks, in the presence and absence of A3A. We have added these data to Figure 4 (new panel E) which demonstrate an increase in γH2AX upon CCT depletion and A3A expression, consistent with increased DNA damage. We additionally address this finding in the manuscript text as the likely mediator of cytotoxity. We appreciate the reviewer's suggestion and feel that these added data have strengthened our findings.

Regarding CCT protection against APOBEC3B-induced cytotoxicity, the APOBEC3B enzyme is a less potent deaminase and induces far less DNA damage and minimal cytotoxicity in most model systems evaluated, including in ectopic expression in cell lines generated in our lab.

5. The data in Fig. 5 are very interesting. Can the authors look into the mutation signatures more carefully to distinguish APOBEC3A and 3B signature mutations? The conclusion of this study would be stronger if only the APOBEC3A but not the 3B signature mutations are increased in tumors with CCT mutations.

We appreciate the reviewer's point and agree that the distinction between A3A and A3B mutational signatures is an important tool. Our preliminary analysis suggests that tumors with CCT gene mutations are associated with an increase in A3A signature mutations, but not A3B signature mutations. Based on data included in our manuscript, the interaction between CCT and APOBEC proteins appears to be exclusive to A3A. Thus, we do not expect that dysfunction of the CCT complex would impact A3B mutagenesis.

1st Revision - Editorial Decision 20th Apr 2021

Dear Dr. Green,

Thank you for the submission of your revised manuscript to our editorial offices. We have now received the reports from the three referees that were asked to re-evaluate your study, you will find below. As you will see, referees #1 and #3 now support the publication of your study in EMBO reports. However, referee #1 has remaining concerns and some suggestions to improve the study, we ask you to address in a final revised manuscript. In contrast, referee #2 is not satisfied with the revised manuscript and requests whole genome sequencing data to experimentally determine APOBEC3 mutagenesis in cells with CCT depletion. However, considering your feedback regarding this point and feedback from the referees during cross-commenting, we decided to proceed without whole genome sequencing data. Nevertheless, as indicated above, the points and suggestions of referee #1 need to be addressed to provide a more convincing analysis of the tumour data. Please also provide a detailed point-by-point response addressing the remaining concerns of both referees.

Moreover, I have these editorial requests:

- I would suggest this slightly modified title:
Interaction with the CCT chaperonin complex limits APOBEC3A cytidine deaminase cytotoxicity

- Please provide the abstract written in present tense and with not more than 175 words.

- We would like to publish your manuscript (as also indicated by you) as Report. However, for a Scientific Report we require that results and discussion sections are combined in a single chapter called "Results & Discussion". Please do this for your manuscript. For more details please refer to our guide to authors:
http://www.embopress.org/page/journal/14693178/authorguide#researcharticleguide

- Regarding data quantification and statistics, please make sure that the number "n" for how many independent experiments were performed, their nature (biological versus technical replicates), the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values is indicated in the respective figure legends (also of the EV figures). Please provide statistical testing where applicable. Please consider this also for any new figure panels you will add to the manuscript.

- Please remove the granting information from the title page and put this into the acknowledgements. Please make sure that the funding information added in the online submission system is complete and similar to the one in the manuscript text.

- Please also remove the 'Disclosure statement' from the title page. The 'conflict of interest statement' later in the manuscript text is sufficient.

- Please add the legend of the dataset file on the first TAB of the excel file. Then please remove the legend from the manuscript text.

- As the Western blots shown are significantly cropped, could you provide the source data for all the blots (main and EV figures). The source data will be published in a separate source data file online along with the accepted manuscript and will be linked to the relevant figure. Please submit the source data (scans of entire blots) together with the revised manuscript. Please include size

markers for scans of entire blots, label the scans with figure and panel number and send one PDF file per figure.

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

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

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

Yours sincerely

Achim Breiling
Editor
EMBO Reports

Referee #1:

The authors have adequately addressed my minor points and major points 3 and 4. The authors also attempt to address major point 1. Figure 5A now shows that the CCT mutant tumors have higher mutation loads and figure 5D indicates that this is in part due to higher amounts of SBS2 and SBS13 mutations. However, it is still difficult to differentiate between CCT dysfunction causing increased mutation or CCT mutations being more likely in more mutated tumors, as both interpretations could be consistent with these results. In the rebuttal, the authors show that among APOBEC mutated tumors, those with CCT mutations have higher amounts of mutations than those without. But, among tumors that are not APOBEC mutated, tumors with CCT mutations also have higher levels of mutation than tumors without CCT mutations. This seems to suggest that the association of CCT complex mutations with higher overall tumor mutation loads is independent of APOBEC mutagenesis. There are possible mechanisms that could explain this that still support the authors' interpretation, such as mutations in the CCT complex increasing spontaneous mutation rates, however additional statistics would need to be done to determine if the effect in APOBEC mutated tumors is greater than in tumors that not APOBEC mutated. It also does not rule out that the occurrence of CCT complex mutations is more likely to occur in tumors with higher mutation rates when comparing tumors undergoing similar mutation processes. The frequency of CCT mutations in APOBEC high tumors versus APOBEC low tumors is the best attempt to address this issue. Additional description of the analysis is still needed to understand if this is a correct control. The total mutation loads are in each group are not described, which makes it difficult to determine if the similarity in the percent CCT gene mutations is due to similar overall mutation loads. As an alternative control, the authors could repeat their mutation load analyses in Figure 5 by grouping tumors by whether they have a mutation in one of the housekeeping genes described in their rebuttal and assess whether the tumors with mutations in the house keeping genes have higher amounts of APOBEC mutation. Additionally, major point 2 in my critique asked for a

comparison of CCT component expression to APOBEC-induced mutation abundance. Instead, the authors compared the expression level of different CCT components based on whether the tumors had a mutation in any of the CCT components. This does not address what was asked, which was to see if tumors with reduced CCT component expression have more APOBEC-induced mutations. If they do, it would provide further support that the analysis of APOBEC-induced mutation in tumors with CCT components mutations is not biased by higher tumor mutation loads. I believe the remaining portions of the manuscript to be well done, interesting, and sufficient for publication in EMBO Reports, regardless of whether the authors are able to establish a well-supported computational link between CCT complex dysfunction and the amount of APOBEC-induced mutation in cancer genomes. Although I recommend not over-stating conclusions based on an association of CCT gene mutation and APOBEC mutagenesis without further addressing either point 1 or point 2.

Referee #2:

The authors submitted this manuscript for the second time. They demonstrated in this work that A3A interacts with the CCT complex. New experiments have been performed in particular by showing that a depletion of the CCT complex induces an increase in double-stranded DNA breaks. Finally, the authors suggest that cancer patients having mutations in the CCT complex possess a higher number of mutations that are associated with a specific A3A signature (SB2 and SB13). The authors conclude that the CCT complex regulates A3A-induced mutations.

The authors showed an increase of double-stranded DNA breaks of ~15% (Figure 4E) in K562 cells. These experiments were performed in presence of siRNA directed against CCT7 in presence or absence of A3A expression. If the authors detect double-stranded DNA breaks, they should observe by deep sequencing, considerably more GC->AT mutations occurring specifically in the SB2 and SB13 signatures. These results, already requested in the first review, have not been accomplished and are important to validate the implication of the CCT complex in A3A-induced mutations.
The results in patients will only be convincing if the authors demonstrate the implication of A3A mutations occurring in SB2 and SB13 signatures.

Referee #3:

The authors have adequately addressed my comments. It is now suitable for publication in EMBO Rep.

Response to Referees

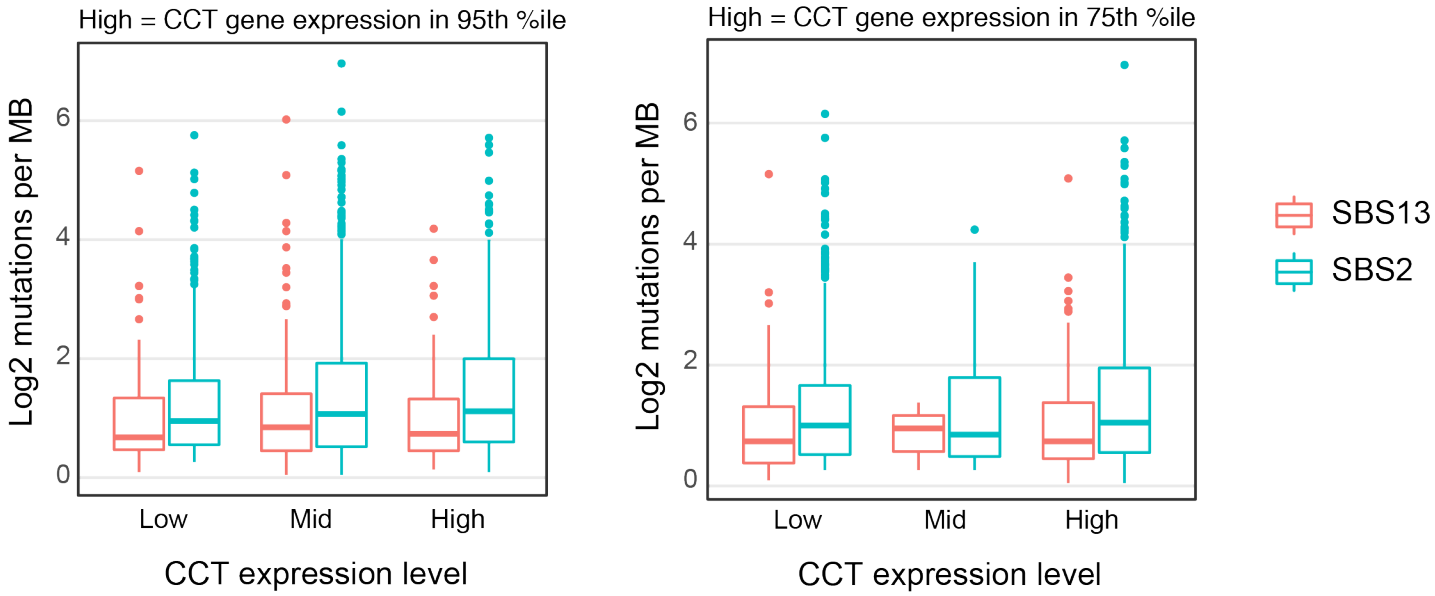
We thank the reviewers again for constructive feedback and have addressed the points made below in new analyses shown in the revised manuscript. Below are responses to the referees comments and explanations of additional data included in the manuscript, written in blue text.

Referee #1
The authors have adequately addressed my minor points and major points 3 and 4. The authors also attempt to address major point 1. Figure 5A now shows that the CCT mutant tumors have higher mutation loads and figure 5D indicates that this is in part due to higher amounts of SBS2 and SBS13 mutations. However, it is still difficult to differentiate between CCT dysfunction causing increased mutation or CCT mutations being more likely in more mutated tumors, as both interpretations could be consistent with these results. In the rebuttal, the authors show that among APOBEC mutated tumors, those with CCT mutations have higher amounts of mutations than those without. But, among tumors that are not APOBEC mutated, tumors with CCT mutations also have higher levels of mutation than tumors without CCT mutations. This seems to suggest that the association of CCT complex mutations with higher overall tumor mutation loads is independent of APOBEC mutagenesis. There are possible mechanisms that could explain this that still support the authors' interpretation, such as mutations in the CCT complex increasing spontaneous mutation rates, however additional statistics would need to be done to determine if the effect in APOBEC mutated tumors is greater than in tumors that not APOBEC mutated. It also does not rule out that the occurrence of CCT complex mutations is more likely to occur in tumors with higher mutation rates when comparing tumors undergoing similar mutation processes. The frequency of CCT mutations in APOBEC high tumors versus APOBEC low tumors is the best attempt to address this issue. Additional description of the analysis is still needed to understand if this is a correct control. The total mutation loads are in each group are not described, which makes it difficult to determine if the similarity in the percent CCT gene mutations is due to similar overall mutation loads. As an alternative control, the authors could repeat their mutation load analyses in Figure 5 by grouping tumors by whether they have a mutation in one of the housekeeping genes described in their rebuttal and assess whether the tumors with mutations in the house keeping genes have higher amounts of APOBEC mutation. Additionally, major point 2 in my critique asked for a comparison of CCT component expression to APOBEC-induced mutation abundance. Instead, the authors compared the expression level of different CCT components based on whether the tumors had a mutation in any of the CCT components. This does not address what was asked, which was to see if tumors with reduced CCT component expression have more APOBEC-induced mutations. If they do, it would provide further support that the analysis of APOBEC-induced mutation in tumors with CCT components mutations is not biased by higher tumor mutation loads. I believe the remaining portions of the manuscript to be well done, interesting, and sufficient for publication in EMBO Reports, regardless of whether the authors are able to establish a well-supported computational link between CCT complex dysfunction and the amount of APOBEC-induced mutation in cancer genomes. Although I recommend not over-stating conclusions based on an association of CCT gene mutation and APOBEC mutagenesis without further addressing either point 1 or point 2.

We appreciate that Reviewer 1 is positive overall about the revised manuscript and recognizes that drawing causal links using computational tools is inherently limited. We have modified the manuscript text in order to not overstate computational conclusions. We believe it is very difficult to carefully control the analyses done in Figure 5, and as such do not mean to make causal associations but rather draw a correlation between APOBEC3 mutagenesis and CCT complex dysfunction in human tumors. Further, it is very difficult to parse out the order of events: does hypermutation in a tumor lead to CCT gene mutation or does CCT gene mutation contribute to hypermutation? We anticipate that both processes occur to some extent simultaneously though cannot definitively determine from our analysis.

To improve controls as suggested by Reviewer 1, we have repeated the analyses shown in Figure 5 to include a third condition for all plots. This is displayed in the new Extended View Figure 2. This third condition utilizes a set of randomly selected genes that are similar in size and frequency of mutation to the CCT complex genes. These additional data show that the tumors with a mutated CCT complex gene have a similar overall mutation burden to those with a mutated gene in the random set. We analyzed tumors with deleterious mutations in the randomly selected gene set for whether there are different quantities of SBS2/13 mutations. This additional analysis demonstrates that the increase in SBS2/13 mutations is specific to tumors with CCT gene mutations. This was true when all tumors within TCGA were analyzed in aggregate and for most tumors when analyzed independently (breast, cervical). We found that in bladder cancers although an increase in SBS2/13 mutation burden occurs in tumors with CCT gene mutations, that was also true for tumors with mutations in the random gene set. Overall, we believe the analysis of a comparable, random gene set serves as a helpful control for our computational analyses and appreciate the Reviewer's advice towards this experiment.

Reviewer 1 additionally requests a comparison of CCT complex expression and APOBEC signature mutations. We have divided tumor genomes into those with low, middle, and high levels of CCT complex transcripts (by RNAseq). Shown below are two plots, one defining high transcript level as a sample with at least one CCT complex gene in the 95th percentile of gene expression, and one using the definition of high as at least one CCT complex gene in the 75th percentile of gene expression. Regardless of the definition used, there is no appreciable difference in SBS2/SBS13 signature contribution among genomes with low or high levels of CCT complex gene expression. While we cannot conclusively determine the reason for this result, we suspect that CCT transcript levels are not reflective of CCT protein expression and/or CCT complex function.



Referee #2

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The authors showed an increase of double-stranded DNA breaks of ~15% (Figure 4E) in K562 cells. These experiments were performed in presence of siRNA directed against CCT7 in presence or absence of A3A expression. If the authors detect double-stranded DNA breaks, they should observe by deep sequencing, considerably more GC->AT mutations occurring specifically in the SB2 and SB13 signatures. These results, already requested in the first review, have not been accomplished and are important to validate the implication of the CCT complex in A3A-induced mutations.
The results in patients will only be convincing if the authors demonstrate the implication of A3A mutations occurring in SB2 and SB13 signatures.

We appreciate Reviewer 2's attention to a genomic link between the CCT-A3A interaction and observed DNA damage/cytotoxicity in the absence of CCT. We are hesitant to undertake whole genome sequencing experiments because we do not think that they will add substantially to the scientific conclusions. We show that the combination of APOBEC3A activity and CCT depletion results in DNA damage and cell death. We additionally show that this is dependent on the deaminase (mutagenic) activity of APOBEC3A. Thus, sequencing data would add incrementally to these findings. Our lab is familiar with whole genome sequencing experiments for evaluation of mutagenesis and, while these experiments are possible, would take 4-6 months to accomplish including selection and growth of clones, library preparation, and time-consuming analysis. The proposed experiment is even more limited by the fact that A3A expression combined with CCT deletion results in cell death, thus analysis of clones with the combined aberrations may not be possible. We do not believe that the minimal benefit of including sequencing data warrants such a substantial delay.

Referee #3

The authors have adequately addressed my comments. It is now suitable for publication in EMBO Rep.

We thank Reviewer 3 for reading our revised manuscript, and for contributing to the overall improvement of our work.

2nd Revision - Editorial Decision 24th Jun 2021

Dear Dr. Green

Thank you for the submission of your revised manuscript to our editorial offices. I have now received the report from the referee that was asked to re-evaluate your study, you will find below. As you will see, the referee now supports the publication of your study in EMBO reports. The referee has 2 remaining points I ask you to address in a further revised version of the manuscript.

Moreover, I have these final editorial requests:

- Please mark in the source data the portion of the blots that show up in the final figure with a box. Please also label the panels throughout with figure number and panel letter (i.e. Fig. 3a - not just panel a).

- Please remove the referee access information from the Data Availability Section (DAS), but make sure that the dataset is public upon publication of the paper.

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

Kind regards,

Achim Breiling
Editor
EMBO Reports

Referee #1:

The authors have revised their manuscript a second time. They have now completed the requested analyses assessing whether CCT expression correlates with APOBEC-induced mutation load as well as comparing the number of APOBEC-induced mutations in CCT mutant tumors to that in tumors with a mutation in a set of randomly selected genes. They found that CCT expression does not correlate with the number of APOBEC-induced mutations in tumors. They also found that total mutation loads are elevated in both CCT mutant tumors and tumors with mutations in the randomly selected set of genes. However, CCT mutant tumors appear to have a higher number of APOBEC-induced mutations compared to tumors with mutations in the random gene set. This appears to be particularly true for breast and cervical cancers, but not true for bladder cancer. When analyzing across all cancer types, the difference between the number of APOBEC-induced mutations in CCT mutant tumors and tumors with a mutation in the random gene set seems very small. The authors recognize the difficulty in interpreting these analyses and have now controlled for influences of overall mutation load in the best way possible.

I recommend the following two minor changes prior to publication of the manuscript:
1) Include statistical analyses for expanded view figure 2 comparing the number of mutations in CCT mutant tumors to tumors with mutations in the random gene set to confirm that the observed differences are significant. This is currently absent and should be done for each panel of the figure.
2) Replace figure 5 with expanded view figure 2. These figures are identical except for the inclusion of the new control group, which is very helpful for allowing readers to accurately understand the

data.

Matthew D. Weitzman, Ph.D.
Professor of Pathology & Laboratory Medicine
University of Pennsylvania Perelman School of Medicine
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The Children's Hospital of Philadelphia

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weitzmanm@email.chop.edu

Achim Breiling, PhD
Editor | EMBO reports
a.breiling@emboreports.org

2nd July, 2021

Dear Dr. Breiling,

We are now submitting a revised version of our manuscript, entitled "**Interaction with the CCT chaperonin complex limits APOBEC3A cytidine deaminase cytotoxicity**" to *EMBO Reports*. We very much appreciate the critiques from reviewers and from yourself, which have guided improvement of the manuscript.

In the most recent round of review, Referee #1 requested two minor revisions:

1) "*Statistical analysis for expanded view figure 2*…" We have included statistical comparisons for all panels of EV2 in the new version of the manuscript.

2) "*Replace figure 5 with expanded view figure 2*…" While we have appreciated Referee #1's guidance regarding our computational analysis throughout the review process, and agree that controls requested and added throughout the review process have been important, we believe the comparison between tumors with and without mutated CCT complex genes is best displayed in the simpler format of Figure 5 for the main figure of the manuscript. Therefore, we have kept the original Figure 5 and included controls in the EV2. To address both of the Referee's points, we have additionally included statistical analysis in every panel of Figure 5.

We have additionally submitted the entire source files annotated as requested, and have made the dataset publicly available, as noted in the revised manuscript text. We trust that the manuscript is now in suitable condition for publication in EMBO Reports. Thank you for this opportunity to share our data with a broad scientific community. We believe that our findings will be impactful in the field.

Yours sincerely,

Abby M. Green, M.D. and Matthew D. Weitzman, Ph.D.

3rd Revision - Editorial Decision 5th Jul 2021

Abby Green
Washington University
Pediatrics
425 S. Euclid Ave.
St. Louis, MO 63105
United States

Dear Dr. Green,

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

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

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If you do NOT want this File to be published, please inform the editorial office within 2 days, if you have not done so already, otherwise the File will be published by default [contact: 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."

Should you be planning a Press Release on your article, please get in contact with emboreports@wiley.com as early as possible, in order to coordinate publication and release dates.

Thank you again for your contribution to EMBO reports and congratulations on a successful publication. Please consider us again in the future for your most exciting work.

Yours sincerely,

Achim Breiling
Editor
EMBO Reports

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PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

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

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

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

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

2. Captions

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

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

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

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

B- Statistics and general methods

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

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	Sample size consideration and power analyses were pre-determined for the TCGA data. See https://www.cancer.gov/tcga for details. Here we are using 9876 tumor samples with intact CCT genes and 326 tumors with deleterious CCT mutations (page 11).
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	NA
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	Samples excluded from our analysis of mutational signatures in cancer were from tumor types that are not frequently associated with APOBEC3 mutagenesis. This criteria was pre-established. Additionally we excluded samples that had mutations in any of the CCT complex members not classified as deleterious or having a high impact, this ensures the interpretability of the data (page 22).
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	Yes - We randomly selected sets of 9 protein coding genes that were similar in gene length to the CCT complex gene members (response to reviewers).
For animal studies, include a statement about randomization even if no randomization was used.	NA
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	No blinding of investigators was applied.
4.b. For animal studies, include a statement about blinding even if no blinding was done	NA
5. For every figure, are statistical tests justified as appropriate?	Yes, see methods section and figure legends.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Every MS-analysis was performed with three biological replicates to provide enough statistical power to apply parametric tests (either homoscedastic or heteroscedastic one-tailed t-test, depending on the statistical value of the F-test; heteroscedastic if F-test p value<0.05). The t-test was considered as a valuable statistical test because binary comparisons were performed, and the number of replicates was limited. No samples were excluded as outliers. Data analysis was not blinded. Proteins with a t-test p-value smaller than 0.05 were considered as significantly altered between the two tested conditions. Data distribution was assumed to be normal, but this was not formally tested. (Methods section pp 20-23). To assess if test assumptions are met, we log transformed data and applied the Shapiro-Wilk normality test where appropriate. When these assumptions were violated we applied the non parametric Wilcoxon rank sum test with continuity correction (pages 22-23).

USEFUL LINKS FOR COMPLETING THIS FORM

http://www.antibodypedia.com
http://1degreebio.org
http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-report

http://grants.nih.gov/grants/olaw/olaw.htm
http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm
http://ClinicalTrials.gov
http://www.consort-statement.org
http://www.consort-statement.org/checklists/view/32-consort/66-title

http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tumo

http://datadryad.org

http://figshare.com

http://www.ncbi.nlm.nih.gov/gap

http://www.ebi.ac.uk/ega

http://biomodels.net/

http://biomodels.net/miriam/
http://jij.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

Is there an estimate of variation within each group of data?	Coefficient of variation (CV) values were provided for each tested condition in the Expanded View Dataset 1. Methods section pp 20-23.
Is the variance similar between the groups that are being statistically compared?	Not in all cases the variance was similar between groups. In each case, adequate statistical methods have been used that consider whether variance is similar or different (F-test), in a case by case basis. Methods section pp 20-23. For mutational signatures analysis, the samples are tested for homoscedasticity. If this assumption is violated, we refrained from applying statiscal tests.

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	HA tag clone 16B12 (Biolegend #901502); HA tag clone C29F4 (Cell Signaling #3724S); CCT1 clone 2B2-D6 (Abnova #H00006950); CCT5 clone 4E5-4B1 (Abnova #H00022948); CCT7 clone 1D6 (Abnova #H00010574); CCT4 clone H-1 (Santa Cruz #SC137092); Ku86 clone B-1 (Santa Cruz #sc-5280), Tubulin clone 6A204 (Santa Cruz #sc-69969); GAPDH (Gene Tex #100118); APOBEC3B clone EPR 18138 (Abcam #184990); CCT1 clone EPR4082 (Abcam #109126); H2AX-p-S139-488 clone N1-431 (BD Biosciences #560445); CCT7 (Sigma #HPA008425); APOBEC3G/A (NIH AIDS Reagent Program #10082). Methods section, page 17.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	All cell lines were purchased from commercial sources such as ATCC or Millipore Sigma which includes authentication. All cell lines are tested routinely for mycoplasma in our laboratory and experiments are not conducted on cells found to be contaminated with mycoplasma. Methods section page 16.

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

D- Animal Models

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

E- Human Subjects

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

F- Data Accessibility

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

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	No