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Chromatin remodeler CHD1 promotes XPC-to-TFIIH handover of nucleosomal UV lesions in nucleotide excision repair

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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. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 27 October 2016

Thank you for submitting your manuscript on CHD1 roles in nucleotide excision repair to The EMBO Journal. We have now received comments from three expert referees, which I am copying below for your information. As you will see, these referees appreciate the interest of the topic as well as the potential importance of your current findings. However, there are also a considerable number of substantive, well-taken concerns (raised in particular by referees 1 and 2) that we feel would need to be fully addressed in order to make this work a strong candidate for EMBO Journal publication. These criticisms, which I prefer not to repeat in all detail here, include various major experimental issues (such as internal consistency and comparability, time course analyses, appropriateness of controls etc.), but also concerns of conceptual nature, such as conclusiveness of the data and discrepancies with other published findings, including the recent report on DDB2 roles in chromatin reorganization during repair.

Although the nature and extent of these concerns makes it unclear whether they may be satisfactorily addressable through a regular major revision, I would in light of the potential overall interest nevertheless be willing to to give you an opportunity to respond to the referees' criticisms by way of a revised version of the manuscript. It is clear that this may require substantial further time and efforts, but should you be able to validate and improve the present analyses along the lines suggested by the referees, we would remain interesting in considering this study further for publication. Since it is our policy to allow only a single round of major revision, I would be happy to discuss a possible extension of our normal three-months revision period - during which time the publication of any competing work elsewhere would have no negative impact on our final assessment of your own study. Also, should you have any specific questions/comments regarding

the referee reports or your revision work, please do not hesitate to get in touch with me already during the early stages of your revision work.

Thank you again for the opportunity to consider this work for The EMBO Journal! I look forward to hearing from you in due time.

REFEREE REPORTS

Referee #1:

Chromatin reorganization during DNA repair is an important and active area of research. This study focuses on nucleotide excision repair (NER) a general repair pathway involved in the removal of helix - distorting/destabilizing lesions like UV induced photoproducts. In this present study the authors present data supporting a role of the chromatin remodeler, CHD1 in helping XPC become displaced from sites of UV induced photoproducts facilitating repair. While other ATP-dependent remodelers have been associated with NER, CHD1 has not been shown previously to be involved in this process. In yeast Chd1 has been shown to be important for transcription elongation Arnt and coworkers (Simic R, et al. (2003) Chromatin remodeling protein Chd1 interacts with transcription elongation factors and localizes to transcribed genes. EMBO J 22(8):1846-56 PMID: 12682017) , and thus this study has the potential to make a novel contribution to the field. However, several important concerns diminish overall enthusiasm. For example, one major concern is that the authors try to claim that UVDBB is not involved in loading XPC at sites of UV damage, and since this idea goes against a large amount of data supporting a role for UVDDB enabling chromatin opening and access of photoproducts to XPC, the author need considerably more data to support this idea, as outlined below. Another concern is that while the authors provide data supporting a role of CHD1 in NER, since others have implicated other chromatin remolders in this process including, BRG1, ALC1, an INO80, experiments knocking down these remolders must be included in order to fully evaluate how large a contribution CHD1 makes in NER.

Specific concerns:

1. Literature citation is limited, work by Smerdon and Almouzni who independently have developed an access-repair-restore model of chromatin involvement should be better cited. Most recently Almouzni have further developed the model to include a prime step specifically when the lesion may be inwardly facing the nucleosome to provide access to the repair proteins.

2. Please justify why different experiments were performed in three different cell lines, HEK293 (Fig 1 only); U20S several figures, and HeLa (Figure 6). Thus comparing the different assays only performed on one cell line makes it difficult to formulate conclusions - it would be helpful if one cell line was analyzed the same in all the assays for a comparison. This is further complicated by the fact that no time course experiments are presented and in some experiments 1 hr or 3 hrs after UV damage are chosen for comparison. Lack of UVDDB involvement at later times might be expected as its binding to UV photoproducts occurs within seconds after damage (Vermeulen and coworkers).

3. The writing needs to be improved. For example on page 6, second line they mentioned UV-irradiation, but do not indicate how long after irradiation, it was not clear from what as written. At the bottom of this paragraph the authors make a confusing statement about co-immunoprecipation experiments in Figure 1. They use DDB2 to pull-down UV damaged chromatin, thus any co-immunoprecipation must be in the context of UVDDB, and not CHD1, thus there statement that "XPC, XPD, and DDB2 co-immunoprecipitate with CHD1 is not accurate. Also just because you can bring down multiple proteins with DDB2does not mean they are in fact part of a complex. Thus while CHD1 came down with DDB2 as a probe, it does not mean CHD1 is associated with any other of these proteins. They would have had to do a direct CHD1 pull down of the chromatin to make this claim.

4. Why did the authors use H3K4me3 and not H3 as a loading control in Figure 1C? Since H3K4me3 is highly enriched at active promoters near transcription start sites, are the authors implying the are looking at repair in active chromatin? This is not consistent with XPC's role in global genome repair.

5. Data in Figure 3 seems to have been generated 3 hrs after irradiation. No effect of knocking down UVDDB was seen on CHD1 association with chromatin. Without a better time course with earlier time points, these data are limited.

6. Why does XPC become more highly modified in the absence of CHD1? Two important control are to also KD DDB2 and directly probe with antibodies to poly-ubiquitin to show that this modification is poly-ubiquitin of XPC.

7. Figure 5 it would appear these experiments were done 3 hr after damage, again without a time course showing when exactly CHD1 is recruited to the damage sites and no DBB2 KD controls it is very difficult to accept no involvement of UVDDB in XPC recruitment. A better experiment would be a FRAP experiment with CHD1-eGFP to follow its recruitment.

8. The repair assay used in figure 6 would seem to have a very low dynamic range - especially for 6-4 photoproducts where background is already at 0.3 and the full signal is only 1.1. The slow repair of CPD after KD of XPC is odd. Since XPC is involved in global genome removal of CPD and is thus slower than transcriptional coupled repair, loss of XPC should not necessarily slow CPD removal kinetics. Perhaps CHD1 is playing a role in TCR, and thus a CSB KD in the presence and absence of a CHD1 KD, experiment would be warranted.

9. The range of cell survival in Figure 6G is limited, also a UVDDB KD should be added for comparison.

Referee #2:

In this manuscript, the authors study the involvement of the ATP-dependent chromatin remodeler Chromodomain helicase DNA-binding protein 1 (CDH1) in Global Genome Nucleotide Excision Repair (GG-NER). To verify if CHD1 is involved in GG-NER, the authors perform immunoprecipitation assays using the established GG-NER factor DDB2 as bait, before and after UV. They find that CHD1 co-purified in both conditions, but with increased amounts after UV. With chromatin fractionation assays, the authors demonstrate increased binding of CHD1 to chromatin after UV irradiation, which they report to be dependent on the GG-NER protein XPC. To validate CHD1 function in GG-NER, the authors perform immunofluorescence assays with local UVC damage and stain for several NER proteins following CHD1 knockdown. Results suggest that GG-NER protein XPC accumulates more at sites of damage in the absence of CHD1, while downstream proteins such as XPB and XPA, show reduced accumulation. Finally, the authors show that both UV survival and specifically CPD but not 6-4PPs repair is compromised after CHD1 knockdown.

The manuscript focuses on a very interesting topic that currently receives much attention, which is chromatin remodeling in relation to the DNA damage response, in this particular case NER. Most of the presented experiments are rather straight forward with a logical rationale, but some may need further considerations. The results presented by the authors indeed suggest that CHD1 functions in GG-NER. However, the authors conclude that this function is related to displacement of (XPC-bound) nucleosomes during NER, but they do not provide clear convincing evidence for this conclusion. Up to date, a clear mechanistic function is often lacking in the research field of chromatin remodelers and NER. Here the authors do provide some novel insight by showing the involvement of yet another chromatin remodeler (CHD1) in NER. However, they do not provide further insight on its exact function in GG-NER, thereby slightly lowering my enthusiasm and the potential impact of their findings. In my opinion, this manuscript is only suitable for publication if the following remarks are addressed.

<u>Specific comments:</u>

Page 5, 6 and Figure 1 (Results)
From Fig.1 A, the authors conclude that CHD1 co-purifies with DDB2-FLAG both with and without UV, but considerably more after UV. DDB2 staining (FLAG) in the input is clear, but not in the IP fraction. The authors justify this due to the presence of IgGs that interfere with the signal. However, a band for DDB2-FLAG is visible in the IP fraction only after UV, but not without UV. If indeed

more DDB2 was loaded in the gel after UV, this could be responsible for the stronger signal of both CHD1 and XPD. Therefore, the authors should improve DDB2-FLAG staining, or when this turned out not to be feasible include DDB1 staining (complex partner of DDB2) to demonstrate equal IP loading.

XPC would seem a more suitable control than XPD, as it directly interacts with DDB2 in GG-NER. It is also unclear why the authors chose to co-IP with DDB2 and not with XPC. They should also test if CHD1 interacts with XPC after UV, as later on in the manuscript the authors show that loss of DDB2 does not affect CHD1 binding to damaged chromatin, but loss of XPC does. Also, they find that loss of CHD1 affects XPC binding but they do not test DDB2. The authors state that DDB2-FLAG is used as a "molecular bait to isolate short chromatin fragments containing UV lesions and NER proteins". Therefore, a DNA binding protein such as Ku70 or Ku80 should also be taken as a negative control. Furthermore, no comments are made to the possibility of CHD1 interacting with DDB2, rather than being just present in the chromatin fragments pulled down with DDB2-FLAG. This should also be addressed.

In Fig1. C the authors use antibodies to a histone mark - H3K4me3 - to purify fragmented chromatin from U2OS cells. They should mention this in the main text of page 6, instead of suggesting that these antibodies are against H3 in general. They should also comment on why they chose this histone mark and address the fact that CHD1 is a specific reader for this histone modification. Additionally, this modification is associated with active transcription at promoter sites and with more open/relaxed chromatin structures. The authors should verify if overall levels of this modification change upon UV irradiation, which could explain stronger CHD1 binding after UV. This figure shows that the same amount of H3K4me3 is loaded in both IP and input, but in the input there are considerably larger amounts of CHD1 after UV, and also of DDB2, XPC and XPD (XPD and DDB2 are even almost absent in the input of the no UV condition). These huge differences in the input can very well explain why more protein is found in the IP after UV. The authors should resolve these issues or do other, more convincing IPs to show that CHD1 binds to histones after UV.

Page 7, Figure 2 (Results)
The authors study chromatin localization of CHD1 after UV by MNase fractionation. Histone H3 (not clear whether this is an antibody against general H3 or to a specific modified version, like in Fig.1C), which is used as control for nucleosomal DNA, is however also found in the soluble fraction (supposed to represent the linker DNA fraction). The authors state in the text of page 6 that this fraction also includes some dissociated nucleosome cores, but that the vast majority of nucleosome cores remain in the insoluble form. However, in Fig.2-B the amounts of H3 between soluble and insoluble chromatin seem approximately the same, thus not supporting this statement. This fractionation is also different from the one shown in Fig 3, where H3 is almost absent from the soluble fraction as one would also expect it to be. The authors should address these issues.
The authors state that GG-NER factors co-IP, without actually showing it. A GG-NER control in this panel would be appreciated, such as DDB2 and/or XPC, this was only shown in figure 3. The statement that XPC recruitment to UV-damaged nucleosomes follows a time course comparable to CHD1, seems a bit overstated since only two time points were investigated, and that the long time gap from 1 h to 6 h post-UV (in relation to repair kinetics) are surely not sufficient to speak about: 'with a time course comparable'. It should also be noted that the observed CHD1 dissociation, at 6 h post-UV does not match with the slow repair kinetics of CPDs. 6 h post-UV, CHD1 levels at damaged chromatin are back to base levels (similar or even lower than non-irradiated samples), whereas CPD repair only become more significant after this time point, as in the first few hours after UV, NER (particularly GG-NER) mainly removes 6-4 PP.

Pages 7, Figure 3 (Results)
In Fig3-A, the authors test the effect of knockdown of several NER factors on CHD1 recruitment to insoluble chromatin 3 h after UV. The authors do not explain why they test CHD1 recruitment 3 h after UV, which seems not appropriate considering that in Fig.2-B, they show that CHD1 binds to insoluble chromatin 1h after UV and leaves again within 6h post-irradiation. Secondly, this time-point is not appropriate to study the effect of GG-NER factors such as DDB2 and XPC, since these factors accumulate quickly after UV and 3h after 10 J/m2 may not be present at the damaged sites anymore. Therefore, the authors should perform this experiment at 1 h after UV, or show the presence of DDB2 and XPC at insoluble chromatin 3 h after 10 J/m2 of UV. Furthermore, DDB2 promotes XPC recruitment itself and CHD1 co-IPs with DDB2 after UV (Fig 1A), but the authors

mention that XPC can recruit independently of DDB2 and that this XPC fraction apparently recruits CHD1. This should be demonstrated, by e.g. knock down DDB2. The quantification in Fig 3C is not entirely convincing as the level of CHD1 chromatin binding after knockdown of XPC is similar to that in non-irradiated control conditions. In the western blot of Fig 3A, however, levels after XPC knockdown appear much higher than in the non-irradiated control. The authors should therefore either show another, more representative blot (with XPC levels similar to control). Additionally, it is not excluded that the absence of XPC influences the CHD1 binding under non-damaging conditions, as CHD1 also binds to non-damaged nucleosomes according to Figure 1.

Pages 8, Figure 5 (Results)
With immunofluorescence experiments, the authors study colocalization of NER factors with UV-DNA damage after CHD1 knockdown. DDB2 is used as damage marker in panel Fig 5C, but its co-localization with CPDs is not first tested in the absence of CHD1. Therefore, the authors should include DDB2 after CHD1 knockdown in these assays. XPC is shown to colocalize more, however, based on results from Fig.4, the authors should test whether this could be a consequence of higher XPC protein levels.
On page 8, based on the Fig.5 results the authors suggest that CHD1 stimulates the removal of XPC from lesions and thereby facilitate the transition or handover from XPC to later factors at UV lesions sites. However, the authors provide no convincing evidence that CHD1 acts on XPC itself, as alternative models could also easily explain increased XPC binding. For example, another possibility which should be investigated is whether perhaps CHD1 facilitates the recruitment of downstream factors rather than acting directly on XPC. Reduced recruitment of downstream factors would slow down repair and consequently there will be more unrepaired UV-lesions to which XPC will bind. The authors could test this by performing co-IP experiments with XPC and CHD1 (for instance also in the absence of downstream NER factors, to be sure that CHD1 interacts with XPC alone).

Page 9, Figure 6
In Fig.6-E, unscheduled DNA synthesis (by means of EdU incorporation) after UV is measured, to assess repair rates. Confusingly, the authors use XPA as a local damage marker, while in the previous Fig.5-E they show lower XPA accumulation in absence of CHD1. Immunofluorescence figures 5E and 6E contrast dramatically with each other as Fig.5E shows lower XPA accumulation, while Fig 6E XPA accumulation appears to be the same between control and siCHD1. The authors should address this apparent contradiction. Importantly, the authors show in Figure 6D that 6-4PP repair is not affected after depletion of CHD1. It is therefore even more remarkable that the UDS measurement, performed 1 h after filter irradiation is so strongly affected, as at the first hours after UV the NER-derived UDS is mainly dominated by 6-4PP repair.

Pages 9-12 (Discussion)
The authors provide a good reference coverage and interesting argumentation. However, there are slight discrepancies in the reasons - mentioned in introduction and discussion - why this study was initiated. In the introduction the authors highlight that the underlying mechanism by which several chromatin remodelers promote GG-NER still remains controversial and that they decided to test CHD1 in GG-NER due to the range of its genome-wide functions. Later in their conclusion, the authors comment on CHD1 role in transcription as a trigger to study its involvement in NER. With this in mind, it is unclear why the focus of this work was on GG-NER and why a potential involvement in transcription-coupled repair was not tested. A clear reason why the effect of CHD1 on TC-NER was not scrutinized should be provided.

A summary of their achievements is made in 4 main points, which nicely summarizes the main conclusions. However, some of these conclusions seem overstated and not fully supported by their data. Namely points (iii) and (iv): the authors did not unequivocally show with their experiments that CHD1 requires lesion recognition by XPC to be recruited to damaged chromatin nor that CHD1 displaces XPC from the nucleosomes. These ideas might hypothesized from their data, but not concluded (see remarks above), and they should provide (or be more open to) alternative models to explain their findings.

On page 11, the authors claim that their data is consistent with a model by "which CHD1 is required immediately after initial recognitions of CPDs", however this does not fit their experimental layout on Fig.3-A (3 after UV).

The authors further claim that the function of CHD1 immediately after UV is to expel the histone octamer, they do however provide no evidence whether indeed the histone octamer is expelled in CHD1-dependnent manner after UV.

Referee #3:

In mammalian cells, the UV photoproducts CPD and pyrimidine (6-4) pyrimidone photoproducts are repaired by the NER pathway, but CPD is repaired much more slowly than the (6-4) lesion. Efficient NER depends on the recognition of the lesions by the DNA damage-sensing factor XPC-RAD23B that binds to the (6-4) but not to the CPD photoproduct. A long-standing paradigm in the field of NER is that in mammalian cells, the DDB2 subunit of DDB (DDB1-DDB2) binds to the (6-4) photolesions, and less tightly to the CPD lesions. Subsequently, the trimeric Centrin2-XPC-RAD23B complex is recruited to the site of the lesion, followed by the recruitment of TFIIH and other downstream NER factors that foster the excision of short oligonucleotides containing the lesion. However, it is less clear how the assembly of these bulky NER factors and the sequential trafficking of these factors occurs in chromatin.

CHD1 is known as an ATP-dependent chromatin remodeling factor that plays a role in the regulation of transcription. In this work, the authors demonstrate that in UV-irradiated cells, CHD1 co-localizes with XPC and DDB2 at the sites of CPD lesions (and also 6-4 lesions) in chromatin environments. Based on detailed and thorough biochemical and in situ immunoprecipitation, immunofluorescence, and chromatin fractionation experiments, it is shown that XPC, but not DDB2 is responsible for the recruitment of CHD1. The function of CHD1 is to evict the histone octamer from the nucleosomes in order to allow downstream NER factors like TFIIH to access the site of the lesion, and also to displace XPC that is no longer needed in downstream NER events.

The new NER function reported for CHD1 is novel. This work is experimentally sound and convincing, and introduces a previously unknown step in the processing of UV photolesions, although it is not clear yet whether it is relevant to other kinds of NER substrates. A few minor issues should be addressed.

1. The title of the paper "The CHD1 remodeler displaces XPC-nucleosome intermediates during DNA nucleotide excision repair" may be misleading because it implies that CHD1 can function in the case of other NER substrates in general, and this has not been demonstrated. Suggested revised title: "The CHD1 remodeler displaces XPC-nucleosome intermediates during DNA nucleotide excision repair of UV-induced DNA lesions."

2. It is not clear whether CHD1 affects the recognition of CPD, or not, which is more slowly repaired than (6-4). Are the CPD/(6-4) repair ratios the same or not when the expression of CHD1 is suppressed?

3. Is there a role for centrin 2 in the recognition of CPD? Centrin is mentioned only once in this manuscript and then only in passing.

4. On p. 3, line 4 from the top, DDB2 is mentioned as a 'partner' of DCH1, but before that DDB (DDB1-DDB2) is mentioned. This nomenclature should be consistent from the beginning of the manuscript.

5. Bottom of P. 11: "...CHD1 promotes the simultaneous displacement of both the histone octamer and XPC bound to damaged DNA wrapped around these histones...." How can these events be simultaneous since eviction of the histone core is necessary to allow access to TFIIH so that this bulky NER factor can bind to the appropriate XPC domain? The XPC probably needs to be displaced after TFIIH binding.

1st Revision - authors' response 26 April 2017

Thank you very much for your letter of October 27th, 2016, stating that you would be willing to consider a revision of our manuscript addressing the referees' concerns. We are also grateful for

your follow-up correspondence granting us a three-month extension to perform additional key experiments.

We are now pleased to submit a thoroughly revised manuscript with several new experiments that have been carried out to handle the reviewers' criticisms. Indeed, the many useful reviewers' comments have contributed to substantially increase the quality of our report. The thoroughly revised manuscript includes the following 12 new figure items: Fig 2D & 2E (showing totally new experiments), Fig 3A & 3C (new experiments replacing the previous panels 3A & 3C), Fig 3D & 3E (totally new experiments), Fig 5C (new experiments replacing the previous Fig 5C), Fig 6E & 6F (new experiments replacing the previous panels 6E & 6F), Fig 6G (totally new experiments), Fig EV2E (totally new experiments) and Fig EV6 (totally new experiments).

These new data confirm our previous findings and further expand on the newly discovered role of the CHD1 chromatin remodeler in DNA excision repair of UV lesions. Briefly, our report shows that CHD1 stimulates a critical transition during nucleotide excision repair of specific UV lesions by driving the substrate handoff from the repair initiator XPC protein to downstream effectors. In our view, the major conceptual novelty of these findings is that this CHD1-mediated pathway transition takes place on the histone octamer of nucleosome core particles. This finding demonstrates that chromatin provides a structural scaffold to facilitate the UV lesion recognition rather than always posing a barrier to DNA repair processes.

The point-by-point response to the reviewers' comments is attached as a separate document.
We are confident that the thoroughly revised manuscript can now be accepted for publication and that the paper will appeal to a broad readership of the EMBO Journal.

POINT-BY-POINT RESPONSE

We wish to thank all three reviewers for their critical but constructive comments by which we were able to substantially increase the quality of our manuscript. The point-by-point response to the referees' comments is detailed below.

Referee No. 1

In his first paragraph, the referee raises several issues to which we respond as follows:

– The paper by Simic et al. (2003) is quoted in the manuscript (p. 8, L. 9 of the revised manuscript).
– We clearly point out in the abstract (p. 2, L. 13), in the introduction (p. 5, L. 3-4) and in the discussion (p. 12, L. 12-14) that CHD1 operates only on a subset of CPDs located on nucleosome cores. In the discussion, we also provide an alternative explanation for the apparently DDB2-independent recognition, i.e., that trace amounts of DDB2 remaining in the cells after siRNA-mediated depletion may be sufficient to load XPC complexes onto this subset of CPDs on nucleosomes (p. 12, L. 14-16).
– The effect of other chromatin remodelers, including BRG1, ALC1 and INO80, on CPD excision in HeLa cells is shown in the new Expanded View Figure 6, as requested by the reviewer. Please note that the inhibition achieved by CHD1 depletion is comparable to that seen after ALC1 depletion. These excision data are supported by careful monitoring of protein depletions by immunoblotting (new Expanded View Figure 2E).

Next, the referee lists specific concerns to which we respond as follows:

1. Additional papers from the Smerdon and Almouzni groups have been added to broaden the literature coverage (Czaja et al., 2012; Peterson & Almouzni, 2013). We also added the recent paper by Adam et al. (2016) on histone dynamics after UV irradiation.

2. The use of different cell lines, in addition to HeLa, is now justified. Briefly, HEK cells were employed because they are more permissive to transfections (p. 5, L. 14-16) and U2OS cells because they are more amenable to in situ fluorescence studies (p. 8, L. 2-3). The revised Figure 2 now shows a direct comparison between HeLa cells and U2OS cells, demonstrating the generality of the UV-dependent CHD1 recruitment to chromatin. The abnormal retention of XPC in the damaged chromatin of UV-irradiated cells is now demonstrated in both U2OS (Figure 5) and HeLa cells

(Expanded View Figure 5). Also, the revised Figure 2 displays a time course (0, 1, 3 and 6 h), as requested by the reviewer, revealing that the CHD1 recruitment to chromatin peaks at 1 h after irradiation.

3. We ensured that, in the revised manuscript, post-UV repair times are always clearly indicated. The confusing statement on p. 6 has been revised to "The subsequent immunoprecipitation of this chromatin fraction from UV-irradiated cells with anti-FLAG antibodies resulted in the enrichment of both CHD1 and XPD (a core NER subunit) relative to the control reactions without UV radiation or without DDB2-FLAG expression" (bottom of p. 5 and top of p. 6). We never claim that all these proteins are in the same complex, but argue that "this finding led to the hypothesis that CHD1 may contribute to GG-NER activity in UV-irradiated cells" (p. 6, L. 6-7), which is then proven in the follow-up figures.

4. The criticized Figure 1C has been removed and replaced by the new Figure 3D showing the co-immunoprecipitation of CHD1 with anti-XPC antibodies.

5. The experiment of Figure 3 has been repeated with 1-h incubations after UV exposure as requested by the reviewer. The time course of chromatin recruitment is shown in Figure 2.

6. We don't agree with the referee when he states that the ubiquitination of XPC is increased upon CHD1 depletion. The higher signal in Figure 4A arises from the overall increased expression of XPC protein. In the revised manuscript, we make clear that the higher level of ubiquitinated XPC reflects the increased expression of this factor (p. 8, L. 17-19). Taking into account this higher amount of XPC substrate, the DDB2-dependent ubiquitination in CHD1-depleted cells is indistinguishable from that in CHD1-proficient cells.

7. The experiments of Figure 5 are carried out 1 h after UV irradiation, not at 3 h as stated by the referee. The only exception is Figure 5A, where the recruitment of XPC had been tested both at 1 and 3 h after irradiation. However, a significant difference was observed only for the 1-h time point. The expression of CHD1-eGFP, as recommended by the referee, was not successful as all fluorescent fusion protein locates to the cytoplasm of transfected cells and there was not sufficient nuclear fluorescence to carry out FRAP experiments in chromatin.

8. The window of ELISA absorbance values (from around 0.3 to 1.4) is sufficiently broad to allow for quantitative determinations as demonstrated for example by Kobayashi et al. (2001) Pigment Cell Res. 14: 94-102. We don't understand the referee's comment regarding the low rate of CPD excision in XPC-deficient cells because it is generally known that about 85% of CPDs are processed by GG-NER, which is XPC-dependent, and only about 15% of CPDs are processed by TC-NER as demonstrated by Venema et al. (1990) Nucleic Acids Res. 18: 443-448 and many follow-up papers on this topic. In any case, the revised manuscript provides a new Figure 6G demonstrating that the CHD1 knockdown, unlike a CSB knockdown used as the control, does not impair TC-NER activity.

9. We also don't understand the referee's comment regarding the range of cell survival. The colony data are plotted in a logarithmic scale and we found that the reduction observed upon CHD1 depletion is statistically significant. We believe that the use of XPA and/or XPC knockdowns as controls is appropriate for this experiment aiming at demonstrating an increased UV cytotoxicity. In contrast, a DDB2 deficiency has been shown to diminish UV-induced cell death, as reported by Itoh et al. (2004) Proc. Natl. Acad. Sci. USA 101, 2052-2057, presumably because this factor is additionally involved in apoptosis.

Referee No. 2

This referee presented his concerns in the order of the figures followed by comments on the discussion section. Our point-by-point response is as follows.

Figure 1
– In the revised manuscript we clearly explain that the input chromatin fraction in Figure 1A contains more DDB2-FLAG after irradiation than without irradiation (p. 5, L. 30-32 of the revised manuscript). Importantly, in these input fractions the amount of CHD1 and XPD is not changed by

the UV treatment, demonstrating equal loading for these factors. However, the subsequent co-immunoprecipitation of CHD1 and XPD with antibodies against DDB2-FLAG indicates that these factors co-localize in UV-irradiated chromatin. We don't claim that they interact directly and actually we found in Figure 2 that XPC protein, rather than DDB2, is responsible for the UV-dependent recruitment of CHD1. We would prefer to stain XPC instead of XPD in the Western blot of Figure 1, but this was not possible due to antibody cross-reactivity.
– The immunoprecipitation with anti-XPC antibodies instead of anti-FLAG antibodies, as recommended by the reviewer, was carried out and presented in the new Figure 3D.
– The criticized Figure 1C has been removed and replaced in the revised manuscript by the new Figure 3D showing the co-immunoprecipitation of CHD1 with anti-XPC antibodies.

Figure 2
– In the revised manuscript, all blots are stained using antibodies directed against total histone H3.
– In the previous version of Figure 2B, we erroneously used an overexposed blot to show the low-level presence of histone H3 in the MNase supernatant. In the revised version of Figure 2B, all blots were equally exposed (please compare also with the blot of Figure 2D).
– We don't understand the referee's comment regarding an immunprecipitation in Figure 2 as this figure does not involve any immunoprecipitations.
– For the co-fractionation of NER factors we refer to our previous paper (Fei et al., 2011; see p. 7, L. 7-10 of the revised manuscript) but we do show in Figure 3B that XPC protein relocates to the nucleosome core fraction of chromatin at 1 h after UV irradiation.
– The criticized statement about the kinetics of CHD1 recruitment in comparison to XPC relocations has been deleted. We would like to point out that the time course of CPD repair in Figure 6C indicates that it is the initial excision that is mainly affected by a CHD1 deficiency. Starting from the 6-h time point, the kinetics of CPD excision in control and CHD1-depleted cells run in parallel, indicating similar repair rates during these later time periods.

Figure 3
– The referee is right when indicating a 1-h time point after UV radiation as being more appropriate. For the revised manuscript, we carried out new experiments with HeLa cells incubated for 1 h after UV exposure (see new Figures 3A and 3C).
– A DDB2 knockdown is included in the experiments of Figures 3A and 3C. The criticized sentence regarding a DDB2-independent recruitment of XPC has been deleted.
– Finally, the problem with quantifications has been solved by repeating all experiments of Figure 3.

Figure 5
– Figure 5C has been replaced by another image, where CPDs are used instead of DDB2 as a marker of damage spots. The outcome (reduced recruitment of XPB protein) is unchanged.
– In the revised manuscript, we clearly describe how the quantification of XPC spots reveals a higher retention of XPC molecules at the lesions rather than reflecting its overall higher level. The text is as follows: "to quantify protein redistributions, the fluorescence intensity at damaged spots was divided by the background fluorescence measured in each nucleus outside the UV lesion spots. This procedure ensures that the data demonstrate a truly increased accumulation of XPC protein at sites of damage rather than simply reflecting the higher overall level of this factor following CHD1 depletion" (bottom of p. 8 and top of p. 9).
– The new immunoprecipitation studies of Figures 3D and 3E, requested by the reviewer, indicate that CHD1 acts on XPC directly.

Figure 6
– Figure 6E has been replaced by the image from new experiments, where CPDs were used instead of XPA as a marker of damaged spots. The outcome (reduced EdU incorporation upon CHD1 depletion) is unchanged.
– In view of the criticism raised against the findings of the DNA repair synthesis assay, the experiments have been repeated using a different protocol. For the new Figures 6E and 6F, the cells were first allowed to recover for 2 h after UV irradiation before addition of EdU for another 1 h. The purpose of this protocol is that essentially all 6-4PPs are removed during the first 2 h (see the excision kinetics of 6-4PPs in Figure 6D) such that the measured EdU incorporation reflects DNA repair synthesis due to CPDs.

Discussion
– The discussion has been rephrased throughout to avoid discrepancies with the introductory section of the paper. See in particular the paragraph on the mechanistic role of CHD1 in transcription (p. 11, L. 19-24).
– In the revised manuscript, a role of CHD1 in TC-NER is excluded experimentally (new Figure 6G) and this finding is also highlighted in the discussion (p. 11, L. 16-18).
– We stronly believe that with the additional experimental evidence (new Figures 2D, 2E, 3A, 3C, 3D, 3E, 5B, 6E, 6F and 6G), the conclusions i) to v) on p. 11 (L. 5-12) are not anymore overstated.
– The new Figure 2 shows that the highest recruitment of CHD1 occurs at 1 h after UV irradiation. Therefore, we believe that the model whereby "…CHD1 is required after the initial recognition of CPDs…" (p. 12, L. 18-19) is correct.
– In the revised discussion, we make clear that the role of CHD1 in expelling the histone octamer is inferred from mechanistic studies on transcription initiation (p. 12, L. 19-21). Our finding that CHD1 is recruited in response to UV radiation specifically to nucleosome cores (Figure 2) is consistent with this proposed scenario.

Referee No. 3

To the five points raised by this referee we respond as follows.

1. The title has been changed as recommended by the referee (in a way that the maximum of 100 characters is not exceeded).

2. We point out in the description of the findings of Figure 6 (p. 9, L. 28-30) and in the discussion (p. 11. L. 12-14) that only the excision of CPDs is affected by a CHD1 deficiency. Instead, the excision of 6-4PPs remained unchanged (see Figure 6D). Therefore, the reviewer is right when noting that the CPD/6-4PP repair ratio changes upon CHD1 depletion.

3. A discussion of the possible function of RAD23B and centrin 2 (the interaction partners of XPC) is beyond the scope of this manuscript focused on the novel role of CHD1. However, we indicate in the introduction that both RAD23B and centrin 2 support the DNA-binding activity of the XPC subunit (p. 3, L. 23-24).

4. For consistency, we changed the nomenclature to UV-DDB (instead of DDB1-DDB2), throughout the manuscript, as suggested by the reviewer.

5. In the revised discussion, the term "simultaneous" has been replaced by "coordinated" to reflect the proposed mechanism by which the lesion is transferred from XPC to TFIIH (p. 12, L. 25).

2nd Editorial Decision 08 June 2017

Thank you for submitting your revised manuscript for our consideration. I apologize for the delay in its re-evaluation - we invited all three original reviewers to comment again on the revision, but to this point have still only heard back from referees 2 and 3, whose reports I am therefore now forwarding you. As you will see, while referee 3 considers your responses and modifications satisfactory, referee 2 still retains a several well-taken reservations. Although we generally allow only a single round of major/experimental revision, I would in this case like to give you an opportunity to address these outstanding issues in an additional round of revision, given the major overall improvements and the principally encouraging feedback from both reviewers. Please note however that it will be essential to address all points, except in cases where I particularly noted otherwise.

I am therefore returning the manuscript to you for an additional round of revision, hoping that you will be readily able to satisfactorily respond to the remaining points. Please do not hesitate to get back to me should you have any further questions.

REFEREE REPORTS

Referee #2:

My first concern regarding Figure 1 still stands and is not sufficiently addressed in this new version of the manuscript. More DDB2-FLAG is immunoprecipitated after UV and also more CHD1 and XPD is visible. The authors therefore cannot conclude that CHD1 and XPD are enriched in this UV fraction (page 5, line 34), as also more IP-ed DDB2 is present. The authors have not attempted to improve DDB2 staining, or include DDB1 staining or a negative control, as recommended, to demonstrate equal IP loading and specificity.

[EDITOR NOTE: please do improve the staining or add some of these recommended controls]

The authors have performed IP with XPC, as recommended, which indeed suggests an interaction between XPC and CHD1 (Figure 3D). However, also in this experiment, more XPC is immunoprecipitated after UV, making it unclear whether the increased band in CHD1 is UV-specific or not. This should be discussed in the text. The authors could also increase exposure times for CHD1 staining in Fig 3D to convincingly show that CHD1 is really not found in the XPC IP without UV.

[EDITOR NOTE: in addition to discussing in the text, please do follow the latter experimental suggestion]

My concerns regarding Fig 1C and Figure 2 have been addressed.

My concerns regarding Figure 3A have mostly been addressed. The fact that depleting XPC affects CHD1 recruitment but depleting DDB2 does not is still surprising given the fact that depleting DDB2 likely affects XPC recruitment. The possibility that a DDB2-independent fraction of XPC recruits CHD1 is addressed in the discussion, but it would have been more convincing if the authors would have experimentally confirmed this idea as was recommended. The authors could experimentally show that in this type of fractionation experiments, indeed a fraction of XPC is still recruited in the absence of DDB2 (siDDB2) and only depleting this XPC fraction (double siDDB2 and XPC), leads to diminished CHD1 recruitment.

[EDITOR NOTE: while I would encourage you to attempt such an experiment, it would probably not be essential at this stage]

My concerns regarding the damage marker in Fig 5C have been addressed. The new IPs of Fig 3D and 3E indeed suggest that CHD1 interacts with XPC only, if the lowered CHD1 levels after siDDB2 and siXPA are not statistically significant. I recommend that the authors also show a representative western blot image to accompany Fig 3E.

My concerns regarding Figure 6 have been addressed.

Concerns raised about the discussion have mostly been addressed. The authors have now experimentally tested for a role of CHD1 in TC-NER (Figure 6G). Unfortunately, this experiment was not performed correctly and does not inform about a potential role in TC-NER. TC-NER activity was measured by monitoring the incorporation of EU during 2 h immediately after UV irradiation (10 J/m2). This way, only transcription levels immediately after UV, which should reflect the UV-induced decrease in RNA synthesis, are measured. It is peculiar that a UV-induced transcription block is not observed in control and CHD1 depleted cells. Recovery of UV-blocked RNA synthesis takes much longer than 2 hrs and should therefore be measured at much later time points. The authors should perform this experiment the correct way, if they want to conclude anything regarding TC-NER activity.

The authors still do not provide any evidence that CHD1 expels nucleosomes during NER, although the title of the manuscript and the abstract suggest that CHD1 performs this function in NER. This concern was also raised in our initial report ('the authors conclude that this function is related to displacement of (XPC-bound) nucleosomes during NER, but they do not provide clear convincing evidence for this conclusion) but is hardly addressed in this new version. The title and abstract are therefore misleading and should more accurately reflect true results of this study. The authors only show a function for CHD1 in NER in either promoting the removal of XPC from damage or

promoting the recruitment of downstream factors (because of which, when CHD1 is depleted, XPC also recruits more and/or longer at sites of unrepaired damage). Both scenarios cannot be excluded. Also, the authors have not shown that catalytic activity of CHD1 itself is needed and that actual chromatin remodeling or nucleosome displacement takes place during NER, mediated by CHD1. In the abstract, the authors write that 'nucleosomes provide a scaffold facilitating the recognition of a subset of CPDs by XPC protein' and 'CHD1 is then needed to convey the resulting XPC-nucleosome intermediates to the GG-NER pathway'. As the results do not directly provide evidence for both conclusions, these sentences should be accurately rephrased (for instance by using phrases like: these results 'suggest' that...).

[EDITOR NOTE: unless you can provide concrete additional support, it will be essential to temper the claims in title and abstract to make sure they do not overreach the data]

Besides my concerns with the previous version of this manuscript, there are still some minor additional issues that need to be addressed. In the legend of Fig 5 (and Fig EV5) it is stated that 'the recruitment of NER subunits was quantified by measuring spot intensities followed by normalization to the nuclear background'. The same quantification and normalization method is indicated in the material and methods. However, based on this normalization, it is unclear what the Y-axis in the graphs represents. For instance in fig 5A, if XPC spot intensity at the damage is normalized by the nuclear background, how can it be around 1 in the siNC? Rather, I suspect that the authors have normalized recruitments to the recruitment in siNC (making this around 1). Please clarify this issue.

In the legend of Fig 2E, it is unclear what 'normalized to total level of CHD1' means. In the figure, without UV, the 'relative CHD1 recruitment' is around 1 but in the western blot only a minor fraction of the total level of CHD1 (=the free fraction + the insoluble fraction) is in the insoluble fraction. What does the 'relative CHD1 recruitment' then indicate? Please clarify this. The same also applies to the legend of Fig 2C. Rather, the authors should normalize to H3 levels, which represent the relative amount of protein lysate present in the insoluble fraction.

In the discussion, the authors hypothesize that CHD1 promotes the displacement of XPC from damaged sites, which stimulates the recruitment of downstream GG-NER factors after initial lesion recognition. In current generally accepted models of NER, however, XPC is not displaced to allow for the recruitment of downstream factors like TFIIH. Rather, TFIIH is recruited by and interacts physically with XPC. The authors should discuss their contradicting idea in light of current NER models and also discuss on which data or literature their idea that displacement of XPC promotes recruitment of downstream factors (including TFIIH) is based. It would also be helpful if the authors would discuss other potential scenario's, for instance the possibility that CHD1 promotes the recruitment of downstream factors like TFIIH and that the prolonged XPC binding after CHD1 depletion is merely an indirect effect of TFIIH not being recruited efficiently.

Referee #3:

This is a re-review of a previously sybmitted manuscript. I am satisfied with the revisions and recommend that this article be accepted for publication.

2nd Revision - authors' response 10 August 2017

We wish to thank the reviewer for further challenging our findings. Based on his comments and criticisms, we were able to further verify the findings of our study and improve the quality of the manuscript. The point-by-point response to the editor and reviewer is detailed below.

Reviewer's comments including editor's notes:

– Figure 1: We believe that the concerns with former Fig. 1A (now Fig. 1C) originated from the fact that we were trying to use this immunoprecipitation experiment to demonstrate two different features of CHD1 at once, i.e., its recruitment to chromatin upon UV irradiation and its co-localization in chromatin with DDB2 and other NER factors. Because of the relocation of these

NER factors to UV lesions in chromatin, it is not possible to obtain equal loadings of DDB2 or the associated DDB1 (as requested by the reviewer) when comparing UV-irradiated cells with mock-treated cells. Therefore, we introduced new experiments in Fig. 1A and 1B, where we first demonstrate that, on top of the constitutive association of CHD1 with chromatin, there is an additional UV-dependent recruitment of this remodeler to chromatin. The experimental approach is validated by the UV-dependent recruitment of DDB2 to chromatin shown in Fig. 1A. Also, the blot of Fig. 1A and the quantification of five independent experiments in Fig. 1B show that there is an about 40% increase of CHD1 levels in chromatin after UV exposure. This increase is very consistent across five completely independent experiments and is further demonstrated by the follow-up chromatin-binding assays of Fig. 2 and Fig. 3. In summary, the new experiment of Fig. 1A and Fig. 1B clearly demonstrate a recruitment or "enrichment" of CHD1 in chromatin upon UV irradiation and this finding is then followed by the aforementioned immunoprecipitation of short chromatin fragments demonstrating the co-localization of CHD1 with DDB2 (or XPD as an example of downstream NER factor) in chromatin. We believe that this two-step procedure now provides a sound experimental basis of the hypothesis that CHD1 is involved in the processing of UV lesions in the chromatin context. This initial hypothesis is then proven in the following 5 figures of the manuscript.

– Figure 3D: We included in Fig S3 a longer exposure of the blot of Fig. 3D, as recommended by the reviewer. Also, we point out in the legend to Fig S3 and in the main text of the revised manuscript that there is a low background association of CHD1 with XPC in unchallenged cells. This makes sense considering the constitutive association of XPC with chromatin even in the absence of UV irradiation. However, both the association of XPC with chromatin and the interaction of CHD1 with XPC in chromatin are increased upon UV irradiation (see p. 8, L. 4-7).

– Figure 3A: That XPC protein is able to bind to UV lesions in exactly the same fraction of nucleosome core particles even in the absence of DDB2 has been demonstrated in detail in a former publications (Fei et al., 2011, PLoS Biol. 9, e1001183, see list of references) and, therefore, we refer in the discussion to this earlier study (see p. 13, L. 5-8 of the revised manuscript).

– Figure 3E: A representative blot, as recommended by the reviewer, is included as new Fig. S4, and we confirm that none of the apparent differences are statistically significant when the CHD1 signal is normalized to the amount of immunoprecipitated XPC (see p. 8, L. 8-12, and legend to Fig S4 in the Appendix).

– Figure 6G: The TC-NER assay has been repeated with 16-h incubations, as requested by the reviewer. This extended incubation time did not change the outcome, i.e., that CHD1 is involved in GG-NER but not in TC-NER (see bottom of p. 10 and top of p. 11, and legend to Fig 6G, p. 29, L. 21-25).

– Title and abstract have been revised to reflect more faithfully the actual findings and avoid any mention of a possible nucleosome eviction. In the discussion, we clearly indicate that this hypothesis of a possible nucleosome eviction is inferred from analogous studies on the role of CHD1 in transcription (see p. 12, L. 7-19).

Minor additional issues:

– Figure 5A: In the figure legends, we clearly explain throughout the manuscript that control values were set to 1 (see for example p. 25, L. 11-12 and p. 28, L. 9-10). We confirm in particular that, in immunofluorescence studies, the signals at lesion spots were normalized to the corresponding background (see p. 17, L. 10-13). Again, the resulting fluorescence ratio of control cells was set to 1 for the graphical representation of data.

– Figures 2C and 2E: All quantifications of chromatin-bound proteins were carried out by normalization with histone H3. The text has been amended accordingly (see for example p. 26, L. 10-12 and L. 17-19).

– Discussion: The revised text now includes references to previous reports on the physical interaction between XPC and TFIIH (see p. 12, L. 7-9). We conclude that CHD1 stimulates these interactions in the chromatin context (see p. 12, L. 18-19). Finally, we provide additional

experimental evidence in the new Fig EV4 that the prolonged retention of XPC is not an indirect effect of downstream factors (TFIIH or XPA) not being recruited efficiently (see p. 9, L. 16-18 and L. 31-34).

3rd Editorial decision - acceptance 8 September 2017

Thank you for submitting your re-revised manuscript for our consideration. Referee 2 has now looked at it once more and I am happy to say that they have no more reservations against publication.

Referee #2

The authors have satisfactory addressed all our concerns and suggestions and recommend the manuscript for publication

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Hanspeter Naegeli
Journal Submitted to: The EMBO Journal
Manuscript Number: EMBOJ-2016-95742

Reporting Checklist For Life Sciences Articles (Rev. July 2015)

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 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.

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

Please ensure that the answers to the following questions are reported in the manuscript itself. We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

In the pink boxes below, provide the page number(s) of the manuscript draft or figure legend(s) where the information can be located. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable).

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B- Statistics and general methods

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2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No samples or animals were excluded from the analysis.
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.	In immunofluorescence experiments single cells have been chosen randomly all over two slides per condition. In other experiments no randomization was necessary as all technical replicates have been used for analysis.
For animal studies, include a statement about randomization even if no randomization was used.	non applicable
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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?	No
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C- Reagents

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7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Cell lines used in the study are regularly checked for mycoplasma contamination (Every 6 months) and they have been authenticated by STR profiling (Microsynth).

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D- Animal Models

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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.	non applicable

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	non applicable
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13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	non applicable

14. Report any restrictions on the availability (and/or on the use) of human data or samples.	Used cell lines are commerically available. No ethical restrictions.
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F- Data Accessibility

18. Provide accession codes for deposited data. See author guidelines, under '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	No data to be deposited.
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