EMBO reports

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UBL5 is essential for pre-mRNA splicing and sister chromatid cohesion in human cells

Yasuyoshi Oka, Hanne Varmark, Kristoffer Vitting-Seerup, Petra Beli, Johannes Waage, Anna Hakobyan, Martin Mistrik, Chunaram Choudhary, Mikkel Rohde, Simon Bekker-Jensen and Niels Mailand

Corresponding author: Niels Mailand, NNF Center for Protein Research, University of Copenhagen

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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.)

Editor: Nonia Pariente

1st Editorial Decision 20 March 2014

Thank you for your patience while your study has been under peer-review. 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, although the referees find the topic of interest, they raise some concerns that question the conclusiveness of the findings.

As the reports are below, I will not detail them here. However, the study would need to more conclusively show that defective Sororin mRNA splicing is crucial for the phenotypes observed

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through rescue experiments, as well as better control and characterize the cohesion and cell cycle defects, explore whether UBL5 interacts with other splicing factors and if defects in the splicing of other cell cycle/cohesion factors may have a role in the observed phenotypes. All referee concerns seem reasonable and would need to be addressed for a revision to be successful here. Please note that it is our policy to undergo one round of revision only and thus, acceptance of your study will depend on the outcome of the next, final round of peer-review.

Revised manuscripts must be submitted within three months of a request for revision unless previously discussed with the editor; they will otherwise be treated as new submissions. Revised manuscript length must be a maximum of 28,500 characters (including spaces). When submitting your revised manuscript, please also include editable TIFF or EPS-formatted figure files, a separate PDF file of any Supplementary information (in its final format) and a letter detailing your responses to the referees.

Do not hesitate to get in touch with me if I can be of any assistance during the revision process.

I look forward to receiving a revised version of your study.

REFEREE REPORTS:

Referee #1:

In this interesting paper, Oka and colleagues report a role for the ubiquitin-like protein UBL5 in pre-mRNA splicing, which indirectly affects sister chromatid cohesion.

First, Oka and co-authors showed that UBL5 is required for cell proliferation and sister chromatid cohesion. A quantitative Mass spectrometry analysis revealed that UBL5 associates with splicing factors. This was not totally unexpected, since the UBL5 homologue in yeast, Hub1, was previously shown to be required for pre-mRNA splicing. This was followed by RNAseq analysis upon UBL5 depletion and showed general effects in pre-mRNA splicing with general effects on intron retention.

The authors went on to show that the splicing defects observed include that of Sororin, a protein factor that is essential for sister chromatid cohesion (SSC).

Overall, this is an interesting and important study that describes a cellular role for UBL5 in higher eukaryotes. It clearly shows its effect on splicing affecting indirectly the production of Sororin.

This manuscript would benefit from the following revisions.

Specific comments/revisions

- Are there other splicing factors that have been reported elsewhere in the literature to have a role in cell cycle progression and/or sister chromatid cohesion. If so, do they interact with UBL5?

- The authors show that pre-mRNA splicing is required for the accumulation of Sororin and this has a central role in sister chromatid cohesion. On Figure 4, the authors should attempt to rescue the mitotic phenotype of cells depleted of UBL5 with ectopic expression of Sororin (a cDNA that should not be affected by general effects on pre-mRNA splicing).

- What remains yet unexplained is whether pre-mRNA splicing affects the expression of other players required for SSC.

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Referee #2:

Oka et al

UBL5 is essential for pre-mRNA spicing and sister chromatid cohesion in human cells.

In this work Oka et al present a series of experiments with which they investigate the role of UBL5 /Hub1 in splicing and chromosome cohesion. They first characterize the mitotic arrest phenotypes of cells with reduced UBL5 (siRNA) and then go on with proteomic analysis to show that UBL5 interacts physically with a number of other proteins that are known to play a role in splicing. They then show that splicing is globally affected by knock-down of UBL5 as well as other proteins in the same complex. Finally they show that Sororin levels are reduced in the absence of UBL5 function, and that cohesion can be rescued by wapl depletion.

Major concern:

My major concern with this paper is that the authors have not convincingly shown that Sororin is the target of the splicing machinery that is important for loss of cohesion. I list several reasons below.

1. If splicing of Sororin transcripts is limiting for Sororin protein levels under conditions of UBL5 (etc) knockdown, then it should be possible to rescue this defect by expression of a Sororin cDNA. Why was this experiment not done?

2. Similarly, Wapl is a negative regulator of cohesion, and RNAi of Wapl can suppress many defects that cause reduced cohesion establishment or maintenance (eg Esco, Sgo, SA2 dephosphorylation) so it is a stretch to say that "precocious sister chromatid separation arising from deregulation of the pre-mRNA machinery reflects to a large extent the downregulation of Sororin". There are other possible models.

3. If the argument is that Sororin is more affected than other cohesion factors by loss of splicing, then they should include all data to support this (including the "data not shown" on page 11, top). I also wonder why Sororin is not shown in the time course in Figure 4B, (and why Mcm6 appears to be reduced in this blot under siRNA conditions), and why the degree of Sororin knock-down is not exactly consistent with the phenotype observed (compare Figures 4A and 4D).

4. Have the authors ruled out the possibility that the anaphase delay they see in a majority of cells is due to cohesion fatigue and/or some a spindle or kinetochore defect? (See for example, Hofmann et al 2013,PLoS One 8, e74851)

In summary, while the effect of UBL5 (etc) knockdown on cell cycle progression is impressive, and the bioinformatics appear well done, it isn't clear that they have proven that Sororin is the relevant target in mitotic arrest. To do this they would need to show rescue with Sororin cDNA. In the absence of this control, and given the potential number of splicing targets (Figure 3) it is difficult to justify the authors' conclusions about Sororin.

Minor questions and concerns:

1. Statistical comparison bar in figure 3D is out of place?

2. Figure 1G. Please include scale bars.

3. Why did they choose FASN?

4. Some of the RNA bioinformatics are hard to evaluate because the tool is not published yet (spliceR)?

5. Critical flow data plots should be shown.

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Referee #3:

Timely establishment and termination of sister-chromatid cohesion are required for proper chromosome segregation and mitotic progression. Cohesion establishment occurs in S phase, and requires posttranslational modifications on the cohesin core and binding of sororin through the adaptor Pds5. Sororin antagonizes the cohesin releasing factor Wapl to stabilize cohesin on chromatin.
The Oka et al. study presents two novel findings. First, they show that the ubiquitin-like protein UBL5 binds non-covalently to and regulates spliceosome in human cells. This finding confirms and extends the findings in yeast, and establishes a conserved function for this UBL in splicing. Second, they show that sororin expression is highly sensitive to spliceosome dysfunction, although the mechanisms underlying this sensitivity are not explored. These findings will be of interest to scientists in the ubiquitin, splicing, and chromosome segregation fields, and are in principle appropriate for EMBO Reports. The following points need to be addressed before publication, however.
Major points:
(1) The RNA-Seq data are informative. Can the authors examine whether there are unique features of the retained introns, and if so, whether the retained sororin intron has these features? This may help answer why sororin expression is selectively affected.
(2) The authors need to confirm that the mature sororin mRNA is indeed down-regulated by UBL5 inactivation.
(3) The authors argue that sororin might be selectively down-regulated because it is a short-lived protein. The mechanism cannot be that simple, however, as another short-lived protein Sgo1 does not appear to be affected. It may be useful to examine whether sororin mRNA levels are regulated during the cell cycle and whether sororin mRNA is short-lived.

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Reply to the Reviewers

We would like to thank the Referees for their constructive and insightful comments on our manuscript. We were delighted to see that all Reviewers found our study interesting and worthy, in principle, of publication in *EMBO Reports*. In the revised version of the manuscript, we have included the results of a range of new experiments performed on the basis of the Reviewers' helpful suggestions, which address their concerns and further strengthen the conclusions made in our original manuscript. In particular, as requested by Referees #1 and #2, we now provide additional evidence that the downregulation of Sororin expression is a major cause of the sister chromatid cohesion defect seen in cells lacking UBL5. To this end, we show among other things that expression of an intron-less Sororin construct is able to rescue the cohesion defect of cells lacking UBL5 to an extent almost similar to that of expressing this construct in cells depleted of endogenous Sororin.

Below, we provide a detailed point-by-point response to the issues raised by the Reviewers.

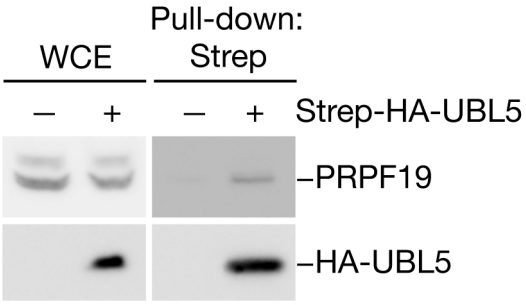
Point-by-point reply to the Reviewers' comments:

Referee #1:

Overall, this is an interesting and important study that describes a cellular role for UBL5 in higher eukaryotes. It clearly shows its effect on splicing affecting indirectly the production of Sororin.

Specific comments/revisions

- Are there other splicing factors that have been reported elsewhere in the literature to have a role in cell cycle progression and/or sister chromatid cohesion. If so, do they interact with UBL5?
Except for the MitoCheck screen (Hutchins et al., Science 2010; Neumann et al., Nature 2010), which uncovered a notable correlation between siRNA-mediated knockdown of splicing factors and mitotic defects (Fig E4B), the only spliceosome components that have been shown in more focused assays to affect mitotic progression are, at least to our knowledge, the factors in the PRPF19 complex (Hofmann et al., PLoS One 2013). In our analysis of UBL5-interacting proteins, we find potential interactions with these proteins, although only with a borderline significance (Table E1). This most likely reflects that UBL5 and the PRPF19 complex do not interact directly but are simply both present within the megadalton spliceosome complex. Consistent with this idea, we can detect a significant, but weak, interaction between UBL5 and PRPF19 in co-immunoprecipitation experiments (see data below). As judged from a range of similar UBL5 co-immunoprecipitation experiments, however, this interaction is much weaker than those observed between UBL5 and splicing factors such as SART1 and EFTUD2, which are likely to be direct binding partners of UBL5 in the spliceosome.



Interaction between UBL5 and PRPF19. HeLa cells were transfected or not with Strep-HA-UBL5 plasmid, and interaction between ectopic UBL5 and endogenous PRPF19 was assessed by immunoblotting StrepTactin pull-downs with PRPF19 antibody.

- The authors show that pre-mRNA splicing is required for the accumulation of Sororin and this has a central role in sister chromatid cohesion. On Figure 4, the authors should attempt to rescue the mitotic phenotype of cells depleted of UBL5 with ectopic expression of Sororin (a cDNA that should not be affected by general effects on pre-mRNA splicing).

We fully agree that such an experiment is of key importance for determining to which extent the downregulation of Sororin protein in UBL5-depleted cells is causative of the sister chromatid defect observed in these cells. As suggested by the Reviewer, we analyzed the ability of ectopically expressed, intron-less Sororin cDNA to rescue the sister chromatid cohesion defect arising from UBL5 knockdown. We found that relative to the effect of expressing an intron-less Sororin cDNA in cells lacking endogenous Sororin (where sister chromatid cohesion maintenance was restored in about 50-60% of cells), such ectopically expressed Sororin rescued the cohesion defect in UBL5-depleted cells almost as efficiently (new Fig 4G; Fig E5F). Together with the notion that the expression levels of no other known core chromosome cohesion factors were significantly affected by UBL5 knockdown (Fig E5C), this strongly suggests that the loss of Sororin expression is indeed a major underlying cause of the cohesion defect resulting from UBL5 depletion.

- What remains yet unexplained is whether pre-mRNA splicing affects the expression of other players required for SSC.

We analyzed the expression levels of all major known sister chromatid cohesion components, and found that with the exception of Sororin, the abundance of none of these are significantly affected by knockdown of UBL5 (Fig. E5C and data not shown). Together with our new findings that ectopic expression of intron-less Sororin largely corrects the sister chromatid cohesion defect in UBL5-depleted cells (new Fig 4G; Fig E5F), this strongly supports the notion that the precocious sister chromatid separation resulting from UBL5 knockdown reflects, to a large extent, the reduced expression of Sororin in these cells. Still, we cannot categorically rule out that there may be other factors whose expression is affected by UBL5 knockdown impact cohesion status more indirectly, or as-yet unknown cohesion factors, whose deregulation in UBL5-depleted cells also contributes to some extent to the observed sister chromatid cohesion defect. We clearly emphasize this possibility in the revised manuscript (p.9).

Referee #2:

Major concern:

My major concern with this paper is that the authors have not convincingly shown that Sororin is the target of the splicing machinery that is important for loss of cohesion. I list several reasons below.

1. If splicing of Sororin transcripts is limiting for Sororin protein levels under conditions of UBL5 (etc) knockdown, then it should be possible to rescue this defect by expression of a Sororin cDNA. Why was this experiment not done?
We agree that such rescue experiments using expression of intron-less Sororin cDNA, and which were lacking in the original manuscript, are indeed crucial for allowing robust conclusions as to whether the downregulation of Sororin is the main underlying cause of the sister chromatid cohesion defect arising from UBL5 knockdown. The results of such experiments have now been included in the revised manuscript. We find that relative to the effect of ectopic Sororin expressed in cells lacking endogenous Sororin (which in our hands is able to reverse precocious sister chromatid separation in approx. 60% of these cells), expression of such an intron-less Sororin cDNA almost as efficiently rescues the cohesion defect in cells depleted of UBL5 (new Fig 4G; Fig E5F). This provides additional, and more direct, evidence that the loss of Sororin expression is a primary cause of the cohesion defect resulting from UBL5 depletion.

2. Similarly, Wapl is a negative regulator of cohesion, and RNAi of Wapl can suppress many defects that cause reduced cohesion establishment or maintenance (eg Esco, Sgo, SA2 dephosphorylation) so it is a stretch to say that "precocious sister chromatid separation arising from deregulation of the pre-mRNA machinery reflects to a large extent the downregulation of Sororin". There are other possible models.
We concur with this notion, and have rephrased this sentence accordingly, so that it now reads: "*Consistent with a causal role of Sororin loss for the cohesion defect observed in UBL5- or SART1-depleted cells, we found that co-depletion of WAPL fully rescued this phenotype (Fig 4F)*" (p.8). Indeed, our new finding that introduction of intron-less Sororin rescues, to a large extent, the sister chromatid cohesion defect in UBL5-depleted cells (new Fig 4G; Fig E5F) provides more direct evidence that the loss of Sororin expression is a main underlying cause of this defect while the observed effects of WAPL RNAi merely support this notion.

3. If the argument is that Sororin is more affected than other cohesion factors by loss of splicing, then they should include all data to support this (including the "data not shown" on page 11, top). I also wonder why Sororin is not shown in the time course in Figure 4B, (and why Mcm6 appears to be reduced in this blot under siRNA conditions), and why the degree of Sororin knock-down is not exactly consistent with the phenotype observed (compare Figures 4A and 4D).
We have included a blot showing loading of Sororin onto chromatin in the time course (this data has been moved to the supplemental material (Fig E5A) in the revised manuscript). We find that significantly less Sororin is loaded onto chromatin in cells depleted of UBL5, and this may be at least partially responsible for the defective sister chromatid cohesion maintenance observed in these cells. The reason why less MCM6 is loaded onto chromatin upon mitotic exit in UBL5-depleted cells is unclear, but as knockdown of UBL5 affects the mRNA splicing pattern of multiple genes (Table E2), it is likely that changes in the expression levels/patterns of one or more such factors may, in one way or the other, contribute to lower levels of MCM6 being loaded onto chromatin in this time course.

Although the impact of knocking down splicing factors (UBL5, SART1, and EFTUD2) on the relative expression level of Sororin does show some degree of variability from experiment to experiment, we have consistently observed clear downregulation of Sororin but not other cohesion factors in multiple independent experiments, thus we are confident about the validity and reproducibility of this effect. If Sororin is rate-limiting for proper sister chromatid cohesion maintenance, which seems reasonable to assume, then it is possible that even a moderate reduction of its expression level is sufficient to evoke a serious cohesion defect.

4. Have the authors ruled out the possibility that the anaphase delay they see in a majority of cells is due to cohesion fatigue and/or some a spindle or kinetochore defect? (See for example, Hofmann et al 2013,PLoS One 8, e74851)
While we cannot formally rule out that such effects may contribute to some extent to the anaphase delay in cells lacking UBL5, the ability of ectopically expressed, intron-less Sororin to rescue the sister chromatid cohesion defect in the majority of these cells suggests that spindle/kinetochore defects and/or cohesion fatigue are unlikely to be major determinants of this delay.

In summary, while the effect of UBL5 (etc) knockdown on cell cycle progression is impressive, and the bioinformatics appear well done, it isn't clear that they have proven that Sororin is the relevant target in mitotic arrest. To do this they would need to show rescue with Sororin cDNA. In the absence of this control, and given the potential number of splicing targets (Figure 3) it is difficult to justify the authors' conclusions about Sororin.
Our new finding that expression of an intron-less Sororin cDNA rescues the cohesion defect in the majority of cells depleted of UBL5 (Fig 4G; Fig E5F) considerably strengthens the notion that the downregulation of Sororin is a primary cause of the sister chromatid cohesion defect arising from compromised pre-mRNA splicing. However, we cannot, nor do we wish to, rule out the possibility that additional mechanisms (e.g. the deregulation of one or more additional factors that are not part of the core cohesion machinery) may also contribute more indirectly to precocious sister chromatid separation triggered by the knockdown of splicing factors. In the revised manuscript, we now explicitly highlight this possibility (p.9).

Minor questions and concerns:

1. Statistical comparison bar in figure 3D is out of place?
We thank the reviewer for bringing this to our attention and have changed the visual representation of the statistical analysis accordingly, so that it now reflects the analysis more accurately.

2. Figure 1G. Please include scale bars.
A scale bar has now been added to this figure.

3. Why did they choose FASN?
The *FASN* gene was chosen simply because it provides a very illustrative, and fully representative, example of the the intron retention defect in cells depleted of UBL5 or SART1, and which we believe should be well visible even to non-experts. We have revised the legend for this panel, which now states clearly that *FASN* was chosen as a representative example of the splicing defect in UBL5- or SART1-depleted cells. Moreover, we swapped panels A and B of this figure, so that it now shows first the overall impact on pre-mRNA splicing by UBL5 or SART1 knockdown and then how this affects a representative gene (*FASN*).

4. Some of the RNA bioinformatics are hard to evaluate because the tool is not published yet (spliceR)?
We concur with this notion. Fortunately, the spliceR tool has now been published, and we now cite it appropriately in the revised manuscript.

5. Critical flow data plots should be shown.
We have included key flow data plots as suggested (new Fig. E1A,B).

Referee #3:

These findings will be of interest to scientists in the ubiquitin, splicing, and chromosome segregation fields, and are in principle appropriate for EMBO Reports. The following points need to be addressed before publication, however.

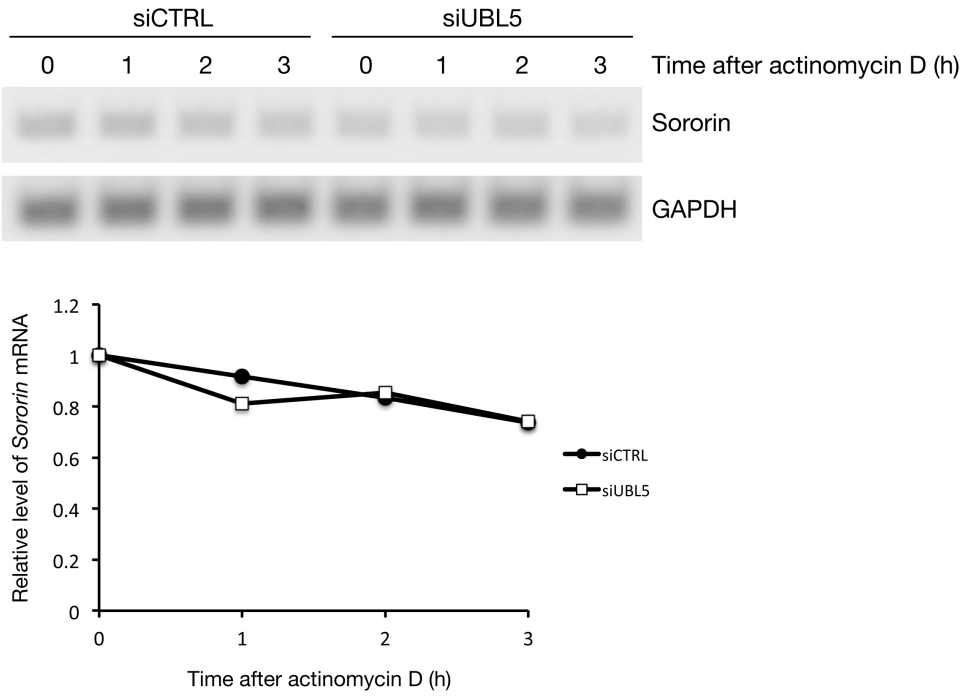
Major points:

(1) The RNA-Seq data are informative. Can the authors examine whether there are unique features of the retained introns, and if so, whether the retained sororin intron has these features? This may help answer why sororin expression is selectively affected.
This is an interesting and constructive suggestion, which we have investigated to the best of our ability. With our available tools to analyze RNA-Seq data (CLC Genomic Workbench and spliceR software) we have not been able to detect any clearly enriched or recognizable unique features among the large number of introns that are retained in transcripts from cells lacking UBL5 or SART1. This is now mentioned on p.7 of the revised manuscript. Thus, while we cannot formally rule out that some particular feature(s) of introns make them more prone to be retained in transcripts upon functional inactivation of UBL5 or associated splicing factors, searching for such patterns will require more extensive analyses, which we feel is beyond the scope of this study.

(2) The authors need to confirm that the mature sororin mRNA is indeed down-regulated by UBL5 inactivation.
We agree with this notion. We used PCR analysis of mature Sororin transcripts to confirm the RNA-Seq data predicting an intron retention defect in Sororin transcripts that selectively affects intron 1 (Fig. 4E in the revised manuscript). Indeed, as shown in the new Fig. E5D (left), transcripts in which intron 1 is retained are prominently upregulated in UBL5-depleted cells, consistent with our RNA-Seq data. Moreover, we observe a concomitant downregulation of the normal, mature transcript lacking this intron. Importantly, however, the total level of Sororin mRNA does not appear to be significantly downregulated following knockdown of UBL5 (Fig. E5D, right). Because the transcript in which intron 1 is retained contains internal stop codons and is thus predicted to be sensitive to nonsense-mediated decay, these data suggest that the reduced expression of Sororin on the protein level is largely a consequence of the increased proportion of transcripts containing intron 1, which does not give rise to a functional protein product. These data thus help to explain mechanistically why Sororin protein levels are decreased in the absence of UBL5.

(3) The authors argue that sororin might be selectively down-regulated because it is a short-lived protein. The mechanism cannot be that simple, however, as another short-lived protein Sgo1 does not appear to be affected. It may be useful to examine whether sororin mRNA levels are regulated during the cell cycle and whether sororin mRNA is short-lived.

We thank the Reviewer for bringing this to our attention. As suggested, we analyzed Sororin mRNA stability but found no evidence that it is short-lived, nor that its half-life is detectably altered by knockdown of UBL5 (please see figure below). On the other hand, as explained in the previous point, one major reason that Sororin is destabilized upon UBL5 knockdown is the increase in the relative level of transcripts that contain internal stop codons and are thus sensitized to nonsense-mediated decay (NMD). For Sgo1, our RNA-Seq data show that no NMD-sensitive transcripts become upregulated following knockdown of UBL5, which may plausibly explain why Sgo1 expression is not affected, despite it is a short-lived protein like Sororin. Because we cannot rigorously conclude that the short-lived nature of Sororin protein has any causal role in its decreased expression in UBL5-depleted cells, we have removed the statements about this from the revised manuscript.



Stability of Sororin mRNA and the impact of UBL5 knockdown. HeLa cells transfected with non-targeting control (CTRL) or UBL5 siRNA were treated with actinomycin D for the indicated times. Total RNA was then extracted and used to measure the levels of the Sororin and GAPDH mRNAs by reverse transcription followed by PCR.

2nd Editorial Decision 03 July 2014

Thank you for your patience while we have reviewed your revised manuscript. The study was seen by referees 1 and 3, as referee 2 was unavailable. Referee 3 thus assessed your responses to referee 2's concerns. As you will see from the reports below, the referees are now all positive about the publication of your study in EMBO reports. I am therefore writing with an 'accept in principle' decision, which means that I will be happy to accept your manuscript for publication once a few minor issues/corrections have been addressed, as follows.

- Include the discussion requested by referee 2

- Per journal policy, the RNA seq dataset needs to be deposited in an appropriate database, and its accession number provided in the manuscript

- Information regarding the error bars and the number of experiments performed seems to be missing from the legend to figures 3A and 4D.

- We now encourage the publication of original source data -particularly for electrophoretic gels and blots, but also for graphs- with the aim of making primary data more accessible and transparent to the reader. If you agree, you would need to provide one PDF file per figure that contains the original, uncropped and unprocessed scans of all or key gels used in the figures and an Excel sheet or similar with the data behind the graphs. The files should be labeled with the appropriate figure/panel number, and the gels should have molecular weight markers; further annotation could be useful but is not essential. The source files will be published online with the article as supplementary "Source Data" files and should be uploaded when you submit your final version. If you have any questions regarding this please contact me.

- As a standard procedure, we edit the title and abstract of manuscripts to make them more accessible to a general readership. I have slightly edited the abstract (not the title); please find the edited version below my signature and let me know if you do NOT agree with any of the changes.

- Every EMBO reports paper now includes a 'Synopsis' to further enhance its discoverability. Synopses are displayed on the html version and they are freely accessible to all readers. The synopsis includes a short standfirst text -I have added my proposal for this text below- as well as 2-4 one sentence bullet points that summarize the paper. These should be complementary to the abstract -i.e. not repeat the same text. This is a good place to be more informative and include, as appropriate, key acronyms and quantitative and organism (yeast, mammalian cells, etc) information. Could you supply a 211 pixels wide by 157 pixels high (or a 550 pixels wide by 400 pixels high) simple graphic outlining the main message of the study, and the bullet points to accompany the standfirst? Do let me know if you would like to modify the standfirst blurb:

"The ubiquitin-like protein UBL5 is shown to be required for pre-mRNA splicing. Its absence leads to aberrant Sororin mRNA intron retention, low Sororin protein levels, and thus defective sister chromatid cohesion.

2-4 bullet points"

After all remaining corrections have been attended to, you will receive an official decision letter from the journal accepting your manuscript for publication in the next available issue of EMBO reports. This letter will also include details of the further steps you need to take for the prompt inclusion of your manuscript in our next available issue.

Thank you for your contribution to EMBO reports.

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Edited abstract

UBL5 is an atypical ubiquitin-like protein, whose function in metazoans remains largely unexplored. We show that UBL5 is required for sister chromatid cohesion maintenance in human

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cells. UBL5 primarily associates with spliceosomal proteins, and UBL5 depletion decreases pre-mRNA splicing efficiency, leading to globally enhanced intron retention. Defective sister chromatid cohesion is a general consequence of dysfunctional pre-mRNA splicing, resulting from the selective downregulation of the cohesion protection factor Sororin. As the UBL5 yeast orthologue, Hub1, is also implicated in pre-mRNA splicing, our results show that UBL5 plays an evolutionary conserved role in pre-mRNA splicing, the integrity of which is essential for the fidelity of chromosome segregation.

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REFEREE REPORTS:

Referee #1:

Oka and colleagues have strengthened their manuscript with the results of new experiments which have addressed most of my previous concerns I therefore recommend publication in EMBO reports.

Referee #3:

The authors have addressed most of my concerns, and I am supportive of publication of this manuscript in EMBO Reports.

Their new results raise an interesting question, however. They showed RT-PCR data that were consistent with a splicing defect of intron 1 of sororin pre-mRNA caused by UBL5 depletion, and suggested that the aberrant Sororin mRNA with intron 1 included might be subjected to nonsense mediated decay (NMD). On the other hand, the levels and stability of the mature Sororin mRNA appear to be normal in UBL5-deficient cells. It is my understanding that NMD would reduce the stability of affected transcripts. The facts that the intron 1-containing transcript accumulates and that the mature Sororin mRNA levels do not decrease suggest that the (presumed) decreased Sororin translation is mediated by complicated mechanisms. The intron 1 containing transcript might not be subjected to NMD and may impede the translation of the normal transcript. This possibility needs to be discussed.

Reviewer 2 has suggested two important experiments to further strengthen the authors' claim that Sororin is a major, functional target of UBL5. The authors have performed these experiments and showed that co-depletion of the cohesin inhibitor Wapl or expression of an intronless Sororin cDNA effectively rescued the cohesion defects of UBL5-deficient cells. These results have greatly strengthened the paper. In my opinion, the authors have adequately addressed reviewer 2's major concerns.

2nd Revision - authors' response 14 July 2014

The following additions/corrections have been included in the final version of our manuscript, according to your instructions:

-Discussion suggested by Referee #3:
This is a valid point. We have included the following new sentence in the final manuscript (p.9): *'Unlike Sororin protein expression, however, the overall level of Sororin mRNA was normal in UBL5-depleted cells (Fig E5D), thus it is possible that the IR-containing Sororin transcripts are translated inefficiently or give rise to aberrant or unstable protein products'.*

-Deposition of RNA-Seq data:
The raw RNA-Seq data files reported in our study have been uploaded to Gene Expression Omnibus (GEO). Information on how to access these data has been provided in the materials and methods

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section (p.12). As mentioned during our recent correspondence, the accession number is currently indicated as 'XXXXXX'; I will forward you the actual accession number to you once I receive it from a GEO database curator.

-Information on error bars and number of experiments performed in Fig. 3A and Fig. 4D:
These missing details have now been added to the figure legends (p.17,18).

-Publication of original source data:
To ensure timely publication of the manuscript, we would like to opt out of including the original source data. With a number of co-authors on the manuscript currently being away, it will take us several weeks to collect all of the raw data, and I reckon this would delay the publication of our manuscript. I hope for your understanding. Please do let me know, however, if you do not agree with our decision.

-Edited abstract:
I essentially agree with your edits. However, I made a slight change to the last sentence in the modified abstract (p.2), so that it now reads: '*As the UBL5 yeast orthologue, Hub1, also promotes spliceosome functions, our results show that UBL5 plays an evolutionary conserved role in pre-mRNA splicing, the integrity of which is essential for the fidelity of chromosome segregation'* instead of: **'***As the UBL5 yeast orthologue, Hub1, is also implicated in pre-mRNA splicing, our results show that UBL5 plays an evolutionary conserved role in pre-mRNA splicing, the integrity of which is essential for the fidelity of chromosome segregation'*. This was in order to avoid using 'pre-mRNA splicing' twice in the same sentence. Please feel free to proceed with your own suggestion (or edit mine further) if you prefer.

-Synopsis:
I have added your suggestion for the standfirst text as well as 4 bullet points to the manuscript (p.3). In addition, I have uploaded a suggestion for a simple graphic outlining the major findings of our study. Please feel free to modify these new additions further as you see fit.

3rd Editorial Decision 14 July 2014

I am very pleased to accept your manuscript for publication in the next available issue of EMBO reports.

Thank you for your contribution to EMBO reports and congratulations on a successful publication.

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