EMBO
reports

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Ubiquitin specific protease like 1 (USPL1) is a SUMO isopeptidase with essential, non-catalytic functions

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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.)

Correspondence 14 March 2012

Thank you for your submission to EMBO reports. I have now had time to read it and discuss it with my colleagues, and have decided to further discuss the submission with you before making a final decision.

I appreciate the identification of a new class of SUMO protease, especially on the tails of our publication of DeSI as a second class of SUMO isopeptidase, which Mark Hochstrasser describes in a highlight in press as "the first unambiguous biochemical identification of a novel type of SUMO protease".

I agree that it would be fitting for EMBO reports to consider a study on this topic for publication. However, we place a strong emphasis on the functional significance of the findings we report and, in this respect, would require that an endogenous target of USPL1 be described. I am not sure where you are with this, I seem to remember from your presentation at the last CSH Ubiquitin meeting that you described USPL1 as is a low-abundance protein that specifically localises to Cajal bodies and the depletion of which impairs cell proliferation. Do you now have a clearer idea of a target? Alternatively, even in the absence of a target, if you included the information on its localization and function in a fifth main figure, this would indicate its functional relevance in the cell and we would

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be happy to send the study for peer-review.

I look forward to hearing from you.

Yours sincerely,

Editor
EMBO reports

Authors Response 15 March 2012

Thank you very much for your mail. Of course I can appreciate that you would prefer more physiological data - however, we have a number of reasons to send the biochemical identification of USPL1 out as is:

1) finding an endogenous target for the low abundant USPL1 is extremely difficult; we have been working on this for very long time, but not yet successfully.
As you well know this is a problem for all proteases including those working on Ubiquitin.
…
Of note, our key motivation was to search for novel isopeptidases - and we found one:
The DeSi paper authors stumbled coincidentally over a protease using their target in a yeast two hybrid.

2) The risk of being scooped with our key finding is very high - with me talking about USPL1 at meetings, even more so.

3) our biochemical data are of very high quality, and if you compare them side by side with your recent publication on DeSI, you will find that USPL1 is significantly more active as a SUMO isopeptidase both in vitro and in vivo.

The biochemical paper is in itself a round story.

4) The cell biological data that we have are obviously incomplete – cajal bodies disassemble and cells stop to grow, but the reason for this is unclear.
Integrating these data in any manuscript is unsatisfactory, and will obviously invite reviewers to ask for the underlying mechanism, which we can not address rapidly. This would delay publication dramatically, which I feel is took risky...

5) Our manuscript also includes, in contrast to the DeSI paper, mechanistic insights into the unique function of USPL1 as a SUMO rather than a ubiquitin protease ! Correct me if I am wrong, but your journal also publishes regularly strictly mechanistic papers on already known enzymesŠ...

Please note, there is already clear published evidence that USPL1 is highly relevant, this is mentioned in the manuscript:

The zebrafish USPL1 homolog, which we show to function as a SUMO isopeptidase, is essential for zebrafish development.

Our ongoing work on a knock-out mouse indicates that USPL1 is also essential in this organism - this is obviously beyond the scope for a first USPL1 manuscript, I just mention this to convince you that USPL1 will develop into an important player in the SUMO fieldŠ..

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In conclusion, if you are unwilling to send our manuscript out for review as is, I will have to submit the biochemical story elsewhere.
I believe this would not only be a pity for us, but also for your journal
-
this manuscript will certainly be highly cited.....

Correspondence 15 March 2012

I have now discussed the study and our correspondence with our Chief Editor and our Head of Publications. The study is clearly of potential interest and would be suitable for EMBO reports. Nevertheless, we feel the issue of functional relevance does need to be addressed, especially given that you have some data in that respect.

We all agree that the impact of the study would be much higher if there was some refinement of the biological function of USPL1 beyond the zebrafish data (which we had indeed noted). In our view, including the USPL1 localization and knock-down data would provide this evidence of physiological relevance. A study including this information would also be more compatible with the scope of EMBO reports (we do publish some mechanistic studies of known enzymes, but only if the physiological relevance and functional significance of the enzyme under consideration make such studies of general interest).

I want to assure you that we would NOT request the elucidation of the underlying mechanism for the growth impairment phenotype. EMBO reports emphasizes biological function over detailed mechanism, and we have a clear policy of asking referees to look at the 'data at hand', not its potential further development. We also have a 'one round of revision' policy, and I will explicitly ask the referees to judge this interesting dataset as far as it goes, because we agree this would be a realistic and exciting end-point. We have successfully done this in the past and do not see a problem.

I would also like to add that during peer-review of the DeSI study, the fact that an endogenous substrate had been identified was considered important by the referees, who also noted this took the work a step further from the Wss-1 yeast putative protease (which had been shown to act on artificial substrates).
Thus, the chances of your study of being successful during peer-review here would be much enhanced by the inclusion of the functional data.

We remain very interested in the study, and I hope that with my assurance that we will not require further elucidation of the growth impairment caused by depletion of USPL1, you will agree to include these data.

I would also request an expedited peer-review process, to ensure the timely publication of your study. If you agree, please let me know and we will reset the stage of your manuscript so you can edit the submitted files.

Yours sincerely

Editor
EMBO reports

Authors Response 16 March 2012

Thank you very much for your mail. With your assurance that our 'data at hand' are enough (of course pending critical review), and your willingness to request an expedited peer-review process, we are willing to include the USPL1 localization and knock-down data (as a 5th figure).

Would you please reset the stage of our manuscript so that we can edit the submitted files?

We should be ready to submit the extended version latest Monday evening.

1st Editorial Decision 10 April 2012

I have now heard back from the three referees that were asked to assess your study. Referee 1 took part in a structured referee report trial and so this report is in a different format. I am happy to say that, as you will see, all referees are supportive of the study and its suitability for EMBO reports. They nevertheless have a few minor concerns that need to be addressed in a revised version before we can make a final decision on your study.

For publication here, it would be important to provide quantitative data of USPL1 protease activity in comparison to SENP1; distinguish between C-terminal hydrolase and isopeptidase activity; strengthen figure 5D by showing knockdown, control and rescue in the same figure (using also catalytically dead USPL1 in the rescue); include costaining with a nucleolar marker in figure 5E; increase the size of the panels in figure 5G; and address points 1 and 2 of referee 2.

Determining whether there is an increase in cell death or lack of proliferation after USPL1 depletion, and more comprehensively analyzing the substrate specificity of C13orf221, would also be desirable as it would make the study more complete. However, it would not be strictly required for publication.

As we discussed, EMBO reports will not request the identification of a USPL1 substrate for publication, so you do not need to address questions two and three of referee 3.

If the referee concerns can be adequately addressed, we would be happy to accept your manuscript for publication. However, please note that it is EMBO reports 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.

I look forward to seeing a revised form of your manuscript when it is ready. In the meantime, do not hesitate to get in touch with me if I can be of any assistance.

Yours sincerely

Editor
EMBO Reports

REFEREE REPORTS:

Referee #1:

1. Do the contents of this manuscript report a single key finding? YES

2. Is the main message supported by compelling experimental evidence? YES

3. Have similar findings been reported elsewhere (e.g. on a closely related protein; in another organism or context)? NO

Please elaborate:
As far as I am aware this is the first indication in any species that USPL1, which is annotated as a ubiquitin specific protease, is in fact a SUMO-specific protease.

4. Is the main finding of general interest to molecular biologists? YES

Please justify:
For the last 10 years we have thought that there were only 6 SUMO specific proteases (SENPs). However the finding that one of the proteases annotated as a ubiquitin specific protease is in fact a SUMO specific protease opens the door for the expansion of the SUMO protease repertoire. This is also an important warning to those who take sequence alignments just a bit too seriously. It is also clear in the paper that USPL1 has an important role to play in a whole organism and I believe this should have wide interest.

5. After appropriate revision, would a resubmitted manuscript be most suited for publication:

[a] in EMBO reports

6. Please add any further comments you consider relevant:

In general this is a nice paper with a clear message and an important finding. However there are a number of points of detail that should be addressed prior to publication.
1. The authors indicate that USPL1 is a "potent" SUMO specific protease. However there is no quantitative data to back this up. Some indication of the turnover number (kcat) of the catalytic domain compared to that of SENP1 would be useful.
2. Fig 5D This should be a single set of graphs so that they are all on the same scale and the wild type rescue should be compared with rescue using the catalytically inactive form of USPL1.
3. Fig 5E It is stated that coilin redistributes to the nucleolus, but no nucleolar markers are used to back this up. Does it go to a specific compartment of the nucleolus or is it throughout the nucleolus.
4. Fig 5G I think this is a key, and very nice piece of data, but it is so small that it is difficult to visualize.
5. There was an issue about the substrates for USPL1, but as we don't have extensive knowledge of the substrates for the other SUMO proteases I don't think identification of substrates for USPL1 is within the scope of this manuscript.

Referee #2:

Schulz et al. identified USPL1 as a novel SUMO protease using an activity-based assay involving HA-tagged SUMO-vinylmethylester, a suicide substrate. The authors went on to show that the predicted catalytic domain of USPL1 cleaved precursor SUMO, albeit less efficiently than SENP1, and could cleave SUMO-AMC, SUMO3 chains, and SUMO2-RanGAP. USPL1 preferred SUMO2/3 over SUMO1 and did not hydrolyze ubiquitin-AMC in vitro, even at high concentrations. Overexpressing the catalytic domain of USPL1 in HeLa cells caused a general decrease in SUMO-2/3 conjugates, while a catalytically dead catalytic domain caused a slight increase in these conjugates. Knockdown had no effect on the general SUMO conjugate pattern, suggesting a more limited range of substrates than most SUMO proteases. Previously, all tested USP-class enzymes

were found to be ubiquitin proteases, but USPL1 is an extremely divergent family member. The authors mutated some of the residues that were conserved among USPL1 orthologs but not in other USPs and found that they, as well as the predicted catalytic cysteine, were indeed important for activity.

Among the orthologs of USPL1 in other organisms, a D. rerio protein, known as C13orf221, had already been identified as essential for early development. The authors demonstrated that C13orf221 binds and cleaves SUMO2/3 conjugates. Using immunofluorescence localization, USPL1 was found to concentrate in Cajal bodies (CBs) in both HeLa and zebrafish cells. USPL1 knockdown shifted the localization of the CB protein coilin to the nucleolus in 70-80% of HeLa cells and similar findings were seen in c13orf221hi3662Tg/hi3662Tg mutant fish.

This is a straightforward study that provides the first example of SUMO protease in the USP superfamily. A recent paper in EMBO Reports of a novel SUMO protease in another cysteine protease superfamily takes away a little of the novelty of the current work, but the analysis here is well done and provides the first link between the SUMO pathway and CB structure (although the mechanisms remain obscure). Unlike the earlier EMBO Reports study, this one does not identify a specific in vivo substrate for the SUMO protease. Nevertheless, because this and the earlier study uncover an expanded set of SUMO proteases beyond the long established ULP/SENP class, the two studies complement each other nicely. With attention to the relatively minor issues below, I would recommend acceptance.

Specific Comments:
1. The pulldown schematic and result should be moved to Figure 1 instead of Supplementary Data. If space is needed, Figure 1B and Figure 1C could be put in the Supplementary Data.

2. USPL1 belongs in the USP family, but it would be useful to have a more precise sense of percent identity and the range of species in which orthologs can be identified. A quick set of BLAST searches suggest that orthologs are found in a variety of unikonts, but I could not find anything beyond this supergroup. Also, are there likely USPL1 paralogs in any species? The bioinformaticist(s) on the paper should be able to clarify these issues.

3. In Figure 3D, the authors claim that the C13orf221 catalytic domain does not cleave preSUMO1 into mature SUMO1, but a band running with mature SUMO1 size can be seen in lane 3 (although faint). If real, then this result runs slightly counter to the proposal that C13orf221 prefers SUMO2/3. In general, the substrate specificity of C13orf221 is less well established than for USPL1, e.g., whether it has any activity toward ubiquitin.

Referee #3:

Schultz and colleagues reported the identification of a novel SUMO isopeptidase, USPL1, using a HA-SUMO-Vinylmethylester suicide substrate. The demonstrated that USPL1 binds to SUMO in vitro and prefers SUMO2/3 over SUMO1. C11orf221, a distant zebra fish homologue, also possessed SUMO isopeptidase activity. USPL1 co-localized with Coilin in Cajal bodies. And, depletion of USPl1 changes the pattern of nuclear localization of Coilin and reduced proliferation of Hela cells. Recently, EMBO report published a putative SUMO-specific protease, DeSI-1, which has a very limited substrate specificity. The current report would be a second example of a new SUMO-specific protease.
Major criticisms
The SUMO-AMC assay measures the C-terminal hydrolase activity. The authors need to specifically distinguish between the C-terminal hydrolase from isopeptidase activity in the description of their results.
Although the biochemistry of USPL1 is clear, the functional studies are very limited. The authors showed only co-localization with Coilin in the Cajal bodies. Is Coilin a SUMO substrate? Why was the localization of Coilin altered by USPL1 knockdown?
The effect of USPL1 on cell proliferation is not well-characterized. Fig. 5D needs to show knockdown, control, and rescue in the same figure. They also need to show whether this is due to cell death or lack of proliferation. A cell cycle profile should also be helpful.

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Revision - authors' response 02 July 2012

Step-by-step response to the reviewers:

Referee #1:

In general this is a nice paper with a clear message and an important finding.
However there are a number of points of detail that should be addressed prior to publication.

1. The authors indicate that USPL1 is a "potent" SUMO specific protease. However there is no quantitative data to back this up. Some indication of the turnover number (kcat) of the catalytic domain compared to that of SENP1 would be useful.

To give some indication of USPL1 "potency" we compared kcat/KM for the catalytic domains of USPL1 and Senp2 on our model isopeptidase substrate CFP-RanGAP1tail modified with YFP-SUMO2 (as described in Stankovic et al., 2009; quantitative analysis using FRET). Using substrate concentrations in the range of 50 to 600 nM, we determine the kcat/KM for Senp2 as 3 x 107 M-1 s-1 and the kcat/KM for USPL1 as 4 x 105 M-1 s-1 under our experimental conditions. These data show that the recombinant USPL1cat fragment is indeed an efficient enzyme (see, e.g., Bar-Even et al 2011, Biochemistry), yet 100fold less efficient than the Senp2 fragment. We mention the comparison with Senp2 in the manuscript and show an example experiment as a supplemental figure.

2. Fig 5D This should be a single set of graphs so that they are all on the same scale and the wild type rescue should be compared with rescue using the catalytically inactive form of USPL1.

As requested, we repeated these experiments (upon optimization of protocol to improve retransfection efficiency and reproducibility, and change to a colorimetric cell assay) to include the catalytic mutant USPL1 C236S. When this mutant rescued, we generated and tested a novel mutant, USPL1 C236A. Again it rescued cell proliferation. We then decided to also test for rescue of coilin localization, and indeed, wt and both mutants rescue coilin from nucleoli. This indicates that USPL1 has both catalytic and non-catalytic functions. Whether the non-catalytic functions involve non-covalent binding or are fully independent of sumoylation remains to be seen. These new data are presented in the new Figure 5.

3. Fig 5E It is stated that coilin redistributes to the nucleolus, but no nucleolar markers are used to back this up. Does it go to a specific compartment of the nucleolus or is it throughout the nucleolus.

Our interpretation that coilin is nucleolar upon USPL1 knockdown was based on comparison with phase contrast (not shown), and was consistent with literature findings: Coilin is known to accumulate in the nucleolus in response to diverse insults. For example, both cis- platin and γ-irradiation induce the colocalization of coilin with RPA-194 (the largest subunit of Pol I), and coilin can specifically interact with RPA-194 and the key regulator of Pol I activity, upstream binding factor (UBF) (Ref: Gilder et al., MBoC 2011). We now include a new suppl Figure to show co-localisation of coilin with UBF in the nucleolus upon USPL1 siRNA mediated knockdown.

4. Fig 5G I think this is a key, and very nice piece of data, but it is so small that it is difficult to visualize.

We enlarged part of the old Figure 5G in the new Figure 5 and moved the remainder of the figure to supplemental data.

5. There was an issue about the substrates for USPL1, but as we don't have extensive knowledge of the substrates for the other SUMO proteases I don't think identification of substrates for USPL1 is within the scope of this manuscript.

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We agree – thank you.

Referee #2:

Schulz et al. identified USPL1 as a novel SUMO protease using an activity-based assay involving HA-tagged SUMO-vinylmethylester, a suicide substrate. The authors went on to show that the predicted catalytic domain of USPL1 cleaved precursor SUMO, albeit less efficiently than SENP1, and could cleave SUMO-AMC, SUMO3 chains, and SUMO2-RanGAP. USPL1 preferred SUMO2/3 over SUMO1 and did not hydrolyze ubiquitin-AMC in vitro, even at high concentrations. Overexpressing the catalytic domain of USPL1 in HeLa cells caused a general decrease in SUMO-2/3 conjugates, while a catalytically dead catalytic domain caused a slight increase in these conjugates. Knockdown had no effect on the general SUMO conjugate pattern, suggesting a more limited range of substrates than most SUMO proteases. Previously, all tested USP-class enzymes were found to be ubiquitin proteases, but USPL1 is an extremely divergent family member. The authors mutated some of the residues that were conserved among USPL1orthologs but not in other USPs and found that they, as well as the predicted catalytic cysteine, were indeed important for activity.

Among the orthologs of USPL1 in other organisms, a D. rerio protein, known as C13orf221, had already been identified as essential for early development. The authors demonstrated that C13orf221 binds and cleaves SUMO2/3 conjugates. Using immunofluorescence localization, USPL1 was found to concentrate in Cajal bodies (CBs) in both HeLa and zebrafish cells. USPL1 knockdown shifted the localization of the CB protein coilin to the nucleolus in 70-80% of HeLa cells and similar findings were seen in c13orf221hi3662Tg/hi3662Tg mutant fish.

This is a straightforward study that provides the first example of SUMO protease in the USP superfamily. A recent paper in EMBO Reports of a novel SUMO protease in another cysteine protease superfamily takes away a little of the novelty of the current work, but the analysis here is well done and provides the first link between the SUMO pathway and CB structure (although the mechanisms remain obscure). Unlike the earlier EMBO Reports study, this one does not identify a specific in vivo substrate for the SUMO protease. Nevertheless, because this and the earlier study uncover an expanded set of SUMO proteases beyond the long established ULP/SENP class, the two studies complement each other nicely. ***With attention to the relatively minor issues below, I would recommend acceptance.***

Specific Comments:

1. The pulldown schematic and result should be moved to Figure 1 instead of Supplementary Data. If space is needed, Figure 1B and Figure 1C could be put in the Supplementary Data.

We changed Figure 1 and Suppl data along this good suggestion.

2.USPL1 belongs in the USP family, but it would be useful to have a more precise sense of percent identity and the range of species in which orthologs can be identified. A quick set of BLAST searches suggest that orthologs are found in a variety of unikonts, but I could not find anything beyond this supergroup. Also, are there likely USPL1 paralogs in any species? The bioinformaticist(s) on the paper should be able to clarify these issues.

We expanded information regarding USPL1 and homologs in the manuscript.
a) Sequence similarity of the USPL1 catalytic domain to that of conventional USPs is generally low, the highest similarity (19.5% residue identity) is observed for USP1.
b) Judging by the currently available genome sequences, the USPL1 family is restricted to the metazoan kingdom, where it is found in species with and without bilateral symmetry. Intact USPL1 sequences are generally present in vertebrates, chordates, and selected invertebrate phyla (see supplemental information). The catalytic domain of USPL1 has been lost in several lineages, including insects, where proteins with similarity to the non-catalytic USPL1 N-terminus exist. Other

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phyla, such as nematodes, are completely devoid of USPL1-like sequences.

3. In Figure 3D, the authors claim that the C13orf221 catalytic domain does not cleave preSUMO1 into mature SUMO1, but a band running with mature SUMO1 size can be seen in lane 3 (although faint).

Agreed… we soften our statement. Cleavage activity is very poor but detectable.

If real, then this result runs slightly counter to the proposal that C13orf221 prefers SUMO2/3.

Our statement refers to isopeptidase activity (and to C-terminal hydrolase activity on artificial substrates), not to C-terminal hydrolase activity on physiological substrates (preSUMO). We clarify this in the revised manuscript.

USPL1 and C13orf221 clearly prefer SUMO2/3 compared to SUMO1, as long as residues Cterminal of the cleavage site are identical (AMC assays, isopeptidase assays, reaction with SUMO-Vme). Consistent with this, USPL1 binds mature SUMO2 much better than mature SUMO1 in pulldown assays.

This is different in SUMO maturation assays, where sequence and lengths of the C-terminal extensions, which differ significantly between SUMO paralogs, can contribute to cleavage preferences. USPL1 matures both SUMO1 and SUMO2 with low (but comparable) efficiency and C13orf221 matures a bit of SUMO1 but not SUMO2.

In general, the substrate specificity of C13orf221 is less well established than for USPL1, e.g., whether it has any activity toward ubiquitin.

We characterized C13orf221 substrate specificity extensively, but left out data due to space constraints. In the revised version we include an additional supplemental Figure showing that C13orf221 cleaves SUMO2-AMC much better than SUMO1-AMC, and does not cleave Ubiquitin-AMC.
Referee #3:

Schultz and colleagues reported the identification of a novel SUMO isopeptidase, USPL1, using a HA-SUMO-Vinylmethylester suicide substrate. The demonstrated that USPL1 binds to SUMO in vitro and prefers SUMO2/3 over SUMO1. C11orf221, a distant zebra fish homologue, also possessed SUMO isopeptidase activity. USPL1 co-localized with Coilin in Cajal bodies. And, depletion of USPl1 changes the pattern of nuclear localization of Coilin and reduced proliferation of Hela cells. Recently, EMBO report published a putative SUMO-specific protease, DeSI-1, which has a very limited substrate specificity. ***The current report would be a second example of a new SUMO-specific protease.***

Major criticisms

The SUMO-AMC assay measures the C-terminal hydrolase activity. The authors need to specifically distinguish between the C-terminal hydrolase from isopeptidase activity in the description of their results.

We clarified this in the revised manuscript.

We measured C-terminal hydrolase activity both with the artificial SUMO-AMC substrates, and with physiologically relevant preSUMO substrates; We measured isopeptidase activity using SUMO chains and sumoylated model substrates.

Although the biochemistry of USPL1 is clear, the functional studies are very limited. The authors showed only co-localization with Coilin in the Cajal bodies. Is Coilin a SUMO substrate? Why was the localization of Coilin altered by USPL1 knockdown?

Of course we hope to learn more about the physiological role of USPL1 in the future, but this is certainly beyond the scope of this first manuscript in EMBO Reports. As described above, our novel data suggest a surprising non-catalytic function of USPL1 for cell proliferation and coilin localization.

Coilin is indeed a target for sumoylation (pers. Communication by Ron Hay, and confirmed in our hands), but we have no evidence for altered coilin levels or modification upon USPL1 knockdown (suppl. Figure 5). This does however not exclude a role for USPL1 in coilin desumoylation, because USPL1 knockdown may expose Coilin to SUMO isopeptidases in the nucleolus (Senp3/5) that it may normally not see.

The effect of USPL1 on cell proliferation is not well-characterized. Fig. 5D needs to show knockdown, control, and rescue in the same figure. They also need to show whether this is due to cell death or lack of proliferation. A cell cycle profile should also be helpful.

Visual inspection of cells shows no signs of increased apoptosis. We started initial characterization of the phenotype, and see a slight increase of G2/M cells in FACS analysis and a small increase in induction of apoptosis (based on PARP cleavage, see figure for reviewers attention below) after USPL1 knockdown. These subtle effects add little insights to our key findings, and in-depth analysis, such as measuring time needed to progress through individual cell cycle phases, is clearly beyond the scope of this manuscript.

We repeated rescue experiments (cell proliferation and coilin localization) to include two different USPL1 catalytic mutants in the analysis. Intriguingly, these experiment clearly show rescue both with wt USPL and with catalytically inactive variants. This indicates that USPL1 has both catalytic and non-catalytic functions. New data are included in the revised Figure 5.

2nd Editorial Decision 26 July 2012

I apologize for the time it has taken us to be able to contact you with a decision on your manuscript. The summer season unfortunately delayed this second round of peer-review! Nevertheless, as you will see below, all referees support publication of the study and I am thus very pleased to accept your manuscript for publication in the next available issue of EMBO reports. Thank you for your contribution to our journal.

Your study is therefore now officially in press and can be cited as such. I will ask for fast-track publication and will send you the doi as soon as possible, so that perhaps it can be formally cited as well.

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

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

Yours sincerely,

Editor
EMBO Reports

REFEREE REPORTS:

Referee 1:
The authors carried out all of the experiments that I requested and I'm happy that the paper is now suitable for publication. While USPL1 is clearly an active SUMO protease it's roles in proliferation and coilin localisation require the protease to be present but do not require it's catalytic activity. However I still think this is an interesting result that should be published. It is possible for instance that an inactive protease bound to and masking it's substrate might have the same effect as removing the SUMO.

Referee 2:
The authors have adequately addressed my relatively minor concerns regarding the original paper. The ability of inactive USPL1 enyzme to complement the siRNA knockdown is intriguing but has precedent among ubiquitin proteases. The data clearly link USPL1 with Cajal bodies and with an essential role in cell proliferation, although its exact molecular function remains unclear. The main point of the paper is that USPL1, despite belonging to the USP/UBP sequence family of DUBs, is in fact an active, substrate-specific SUMO protease. Understanding its physiological functions will likely take some time and need not be part of this initial report.

Referee 3:
The biochemical property of USPL1 is reasonably well established. However, it is clear from the new results that the catalytic activity of USPL1 is not responsible for the biological functions that the authors tried to establish. This has to be clearly annunciated in the title and abstract. Although USPL1 may be essential, it is not mediated through the catalytic activity of USPL1. Thus, both the title and the abstract have to be modified to reflect this new finding.

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