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Pin1 and WWP2 regulate GluR2 Q/R site RNA editing by ADAR2 with opposing effects

Roberto Marcucci , Brindle James, Simona Paro, Angela Casadio, Sophie Hempel, Nicholas Morrice, Andrea Bisso, Liam P. Keegan, Giannino Del Sal Mary A. O'Connell

Corresponding author: Mary A. O'Connell, MRC

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(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 15 April 2011

Thank you for submitting your manuscript for consideration by The EMBO Journal. It has been now been evaluated by three referees and I enclose their reports below. As you will see the referees find the post translational regulation of ADAR2 to be interesting, however, they do require further experimental analysis to make the study suitable for The EMBO Journal. THe referees require analysis of the effect of Pin1 on other RNA editing reactions and ADAR2 autoediting and that experiments are performed on the endogenous ADAR2 protein. As pointed out by the referees in some cases the current data also need to be strengthened. Given the interest in the study, should you be able to address the concerns we would be happy to consider a revised version of the manuscript for publication in The EMBO Journal.

I should remind you that it is EMBO Journal policy to allow a single round of revision only and that, therefore, acceptance or rejection of the manuscript will depend on the completeness of your responses included in the next, final version of the manuscript. When you submit a revised version to the EMBO Journal, please make sure you upload a letter of response to the referees' comments. Please note that when preparing your letter of response to the referees' comments that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process initiative, please visit our website: http://www.nature.com/emboj/about/process.html

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

Yours sincerely,

Editor
The EMBO Journal

Referee #1

Review of Marcucci et al,

Pin1 and Wp2 regulate GluR2 Q/R site editing by ADAR with opposing effects

In this manuscript the authors look for factors that may regulate the activity of ADAR2 via post-translational modifications. It has been known for a long time that editing levels vary, irrespective of ADAR levels thus suggesting a post translational regulation of ADARs.

Based on the presence of phosphorylation sites in the amino terminal end of ADAR2 the authors test for an interaction with Pin1, a prolyl isomerase known to interact with phosphorylated consensus sequences that are also found in the aminoterminus of ADAR2.

Based on mass spec analysis of protein co-purifying with ADAR2 the authors also identify the E3 ligase WWP2 as a potential interactor of ADAR2.

Based on these suggested interactions the authors build a model in which the interaction of phosphorylated ADAR2 with Pin1 would lead to its nuclear retention, while in the absence of Pin1 the protein would move to the cytoplasm. Once in the cytoplasm, the protein could get modified by WWP2 leading to degradation of ADAR2.

For the most part the paper is well written and the arguments can be followed. However, there are several major points that need further clarification:

1. While it is quite obvious that WWP2 destabilizes ADAR2, the effect of PIN1 is less obvious. The immunofluorescence images in figure 3 are hard to interpret and seem to be done with different exposures (as even the cell-free regions in the GFP channel of 3a show different levels of background pixels.) As a result, not only the cytoplasmic, but also the nuclear signals differ. Also, in figure 1c the Pin1 S67E mutant looks more like wt Pin1. Finally, even the western blots of nuclear and cytoplasmic fractions look not so convincing.

However, I am not so convinced that this part is most important. Pin1 might not only act by affecting the nucleo-cytoplasmic localization of ADAR2. If the authors wish to strengthen this point, they should try to get better IF images. Since they are working with transiently transfected cells (another point where quite some variation might emerge from) they should pick a region where a transfected cell can be cleary seen next to an untransfected one. A phase contrast or DIC image should be included to show the outline of the cytoplasm, and higher magnifications should be picked (same magnification in all images). In cases where both ADAR2 and Pin1 have been cotransfected, differences between cells that were only transfected with ADAR2 or with both constructs should be shown in the same microscopic field. I am sure that upon close inspection such areas with only one plasmid getting transfected can be found.

Finally, the nucleo-cytoplasmic western blot should be repeated a few times, quantified, and plotted into a graph with SD.

2. In figure 4b the immunofluorescence cannot be seen. (This is also true for the TIFF image). Also 4c needs quantification of several replicates. In its current version the figure does not support the statements on page 8.

3. A lot of the data emerges from transient transfections. Since these will give variable transfection efficiencies and expression levels, nuclear and cytoplasmic concentrations will also vary. It would

help if some of the experiments could be done with endogenous ADAR2. In the supplements the authors show the impact of Pin1 on GluRB editing only with endogenous ADAR2. There should be enough ADAR2 around to allow its visualization. Alternatively, a stable clone would help to get a clearer picture on ADAR's localization in response to Pin1 RNAi or overexpression.

4. On page 6 the authors state that only ADAR2 that has RNA bound to it can interact with Pin1. This statement is based on the fact that a mutant in both dsRBDs fails to interact with Pin1. This raises the question why ADAR2 can interact with Pin1 in vitro (Figure 1A). Is this interaction tested in the presence of dsRNA, or is the assay sensitive to RNAse treatment?

5. The in vitro interaction of Pin1 and ADAR2 has no clear negative control. Even pcDNA alone gives a signal in figure 1 A. This is confusing, especially as the input control shows that there is no ADAR protein present?

6. The abstract is confusing and partially misleading. In its current version one might think that GluRB is the only substrate for ADAR2. It is not clear what the authors mean by a "coordinately tightly regulation". It would suffice to say that Pin1 and WWP2 regulate ADAR2 levels.

Additional points:

In figures 1 D and E strong signals (from the IgG light chain I presume) are visible. These bands should be explained.

2. The editing signals derived from endogenous ADAR (supplementary Figures) should be included in figure 2. These sequencing lanes are more informative since transient transfection may lead to variable ADAR expression.

3. Figure 5D should be labeled so that it is clear that the triple mutant protein is monitored.

4. Figure 6A is lacking a negative control. A lane in which ADAR2-GFP is expressed but where FLAG WWP2 is missing should be included in the IP. In the current version there is no ADAR2 signal where there is no ADAR2 present-not too surprising.

5. In figure 6B mutants are being tested to identify the signal in ADAR required for an interaction with WWP2. Surprisingly, all constructs can interact with WWP2. A construct in which both interaction sites have been deleted should be included.

6. Page 5 The authors speak about loss of MPM-2 binding. If I understand the data correctly it is loss of binding of the MPM2 antibody?

7. Year is missing in the Aizawa reference (bottom of page 7)

8. The immunofluorescence image in S3 does not allow to see any of the important details it should show. Also exposure or contrast settings seem to differ in the immunostainings (as judged by the different levels of background in the "black" areas).

9. In figure 8 a boundary between nucleus and cytoplasm should be drawn. Also, structural studies do not support the notion that ADAR2 is a dimer. The authors might want to mention this.

10. Figure S4 shows cytoplasmic localization of the deletion construct. This is not surprising as the NLS had been deleted. This should be stated. In general, a drawing of the PIN 1 binding site and the nearby NLS would help to understand the data. Such a scheme could go into the supplements.

Referee #2

Keegan et al present highly novel results on the in vivo regulation of ADAR RNA editing activity by a prolyl isomerase activity that coordinately has effects on protein stability through the ubiquitin/proteasomal degradation system. THe experiments are through and support most of the

authors conclusions and bring a novel new insight into the regulation of RNA editing by other cellular physiological processes.

Minor points:

1. It would be of interest to know what effects Pin1 has on at least one other RNA editing site in a different target gene. There are several other targets that have high enough levels of editing to serve as interesting additional experimental data and either result (effects/no effects) could provide insightful.

2. What is the ADAR2 autoediting status in the Pin1 perturbation experiments and how does this affect the interpretation of the ADAR2 levels (protein/activity) results seen. This is important since autoediting is seen as a negative feedback regulatory system and there could be confounding effects here that accentuate or blunt the Pin1 effect.

Referee #3

Review on Marcucci et al, 2011 for EMBO J.

In the manuscript by Marcucci et al. the authors suggest a mechanism for how the A-to-I editing activity of the mammalian ADAR2 enzyme is regulated in vivo. They find that ADAR2 is interacting with the phosphorylation dependent Pin1 protein. The Pin1 interaction stabilize the editing enzyme and help localizing it to the nucleus, where most if not all of the site selectively edited substrates are located. Furthermore, they identify a motif in ADAR2 that is recognized by an E3 ubiquitin ligase, WWP2, making the protein susceptible for degradation by the proteasome when located in the cytoplasm. They propose that ADAR2 is tightly regulated by Pin1 and WWP2 with opposite effects on the editing activity. This is an excellent piece of work with a totally new view on ADAR regulation. It has previously been shown by several groups that editing is regulated during development. The low editing efficiency seen during embryogenesis increases gradually to adulthood. The present report suggests a mechanism for this regulation in a very elegant way. The manuscript is also well written and the experiments logic and well performed. Therefore, this reviewer only has a few minor points to address.

1. In the first paragraph of the results on page 5 the authors present two phosphorylated sites, S26 and S31, in ADAR2. In the next paragraph on the same page, a mutant with alanine (A) substitutions is described, S26A/S31A/T32A. Please explain why the T32 is mutated, this becomes clear later in the paper but at this stage it is not.

2. Page 7, last row: the year is missing in the reference Aizawa et al., both here and in the list of references.

3. Editing at the Q/R site of GluR2 is used as a control to detect a decrease in editing efficiency when the expression of Pin1 protein is reduced. The authors may consider analyzing also a less efficiently editing site like the R/G of GluR2 where they might see an even larger effect upon Pin1 reduction. This would also explain how editing at the R/G site is regulated during development.

4. Consider rephrasing the sentence "An increase expression of WWP2 in HeLa cells resulted in a reciprocal decrease in ADAR2 levels however the protein level of the ADAR2--PPxY mutant unable to bind WWP2 remained stable, demonstrating that WWP2 can cause a decrease in ADAR2 protein levels." on page 10, first paragraph.

5. If possible, it would be interesting to see if there is a difference in editing efficiency of a reporter cotransfected with wild type ADAR2 and ADAR2-PPxY. This would also better fit the proposed title of the paper.

Referee #1.

1. As requested by the reviewer we have repeated the immunofluorescence on Figure 3 and replace panel C and now there are transfected cells beside untranfected cells. Cells with both plasmids transfected can be clearly observed in the merge (iv).
The reviewer also requested that the nuclear cytoplasmic fractionation be repeated and quantified on a graph. The problem with this experiment is that there are two variables; 1) the export of ADAR2 into the cytoplasm and 2) ADAR2 is degraded in the cytoplasm by WWP2. At present we do not know the factors other than Pin1 that is required for ADAR2 localization to the cytoplasm nor do we know the rate of degradation of ADAR2 by WWP2 in the cytoplasm. Therefore we have repeated the nuclear cytoplasmic fractionation and graphed it in Figure 7 with the ADAR2 mutant that is that can no longer interact and therefore be degraded by WWP2. Thus we have removed one of the variables which is the rate of degradation by WWP2 and one can observe the accumulation of ADAR2 in the cytoplasm. We thank the reviewer for this comment as it illustrates the data better.

2. We apologise for the image in Figure 4B as we had made a mistake with the image settings. We have now corrected this. We have performed the nuclear cytoplasmic fractionation in Figure 7 and graphed it for the same reason as mentioned above as we do not know the rate of degradation of these ADAR2 mutants with WWP2 in the cytoplasm and want to perform the experiment with more stable protein.

3. I absolutely agree with the reviewer and it would be wonderful if all experiment could be performed with endogenous ADAR2. In Figure 2 we show editing in HeLa by endogenous ADAR2 and endogenous editing of *GluR2* Q/R in SH-SY5Y cells. In Figure 5 we show protein stability with endogenous ADAR2 In addition some experiments were performed with endogenous Pin1 (Figure1) and endogenous WWP2 (Figure 6). Unfortunately the one set of experiments we cannot perform is immunofluorescence of endogenous ADAR2. The commercial ADAR2 antibodies we have detect ADAR2 on immunoblots but unfortunately not with immunofluorescence. Having purified ADAR2, I can assure the reviewer that there is very little ADAR2 present so very good antibodies are required to visualise it. We no longer have our own original ADAR2 polyclonal antibodies.
Unfortunately I do not think that stable ADAR2 clones will not address the problems of transfection efficiencies. We would still have to transfect *GluR2 B13* minigene in addition to siRNAs to Pin1 into an ADAR2 stable cell line. We would probably also have to transfected siRNA to WWP2 as it will degrade ADAR2. I am reluctant to make an ADAR2 mutant that no longer interacts with Pin1 and WWP2 as it would introduce many mutations that may make the protein unstable. I think that once the import and export factors that regulate ADAR2 are identified then it will be easier to visualize ADAR2 in the cytoplasm. We have endeavoured throughout the manuscript to draw comparisons between Figure 3 and Figure 7 and stress that when ADAR2 can no longer interact

with WWP2 in *Pin1*$^{-/-}$ MEF cells and is therefore more stable, its cytoplasmic localization is very apparent.

4. We would like to thank the reviewer for this comment as we should have mentioned this in the manuscript. We have now rectified this with the comment '. In the *in vitro* binding assays with GST-Pin1 and recombinant ADAR2 purified from *P. pastoris.* (Fig.1B) ADAR2 appears to interact with GST-Pin1 in the absence of dsRNA. However in our experience is difficult to eliminate all the dsRNA present in the purified protein fraction from yeast (Gallo et al. 2003) so therefore we presume that this *in vitro* reaction is also mediated by dsRNA.' When performing experiments for a previous publication (Gallo *et al,* 2003 EMBO J.) we found that when we overexpressed and purified ADAR from *Pichia*, it was difficult to get rid of dsRNA that was present in the yeast even if you treated with RNases as the bound protein protects the RNA from digestion. It is only when you have a mutant that can no longer bind to RNA can you be certain that there is no RNA present. For that reason the ADAR2 mutant we use in our study is that characterized by K. Nishikura who has published extensively on it.

5. We apologise that in Figure 1B (right panel) we cropped the lane with the negative control by mistake. We have now rectified this and replaced it with the correct panel which shows ADAR2 that does not interact with GST (Left panel lane 5). We apologise for the background band in the negative control pcD3 in Figure 1A it is contamination from another lane and have included a figure for the reviewer showing that normally this lane is empty. We have included a statement in figure legend 1 to clarify this and to avoid any confusion. 'The minor band in the lane with pcD3 is contamination from the neighboring lane.'

6. We apologise if the abstract is misleading as this is not our intention. We have modified it to state that: 'ADAR2 catalyses the deamination of adenosine to inosine at the *GluR2* Q/R site in the pre-mRNA encoding the critical subunit of AMPA receptors and this is its most important substrate.' We have also modified the statement and now state that activities are 'coordinately regulated' and removed the word 'tightly'. We believe that this is more accurate than the statement 'Pin1 and WWP2 regulate ADAR2 levels' as it does not convey the understanding that the activities of these two proteins are linked. For example in Figure 7, ADAR2 that can no longer bind WWP2 appears almost like wild type in MEF wildtype cells. It is in only MEF cells from *Pin1* $^{-/-}$ mice can you see it localized to the cytoplasm.

Minor comments

1. The strong bands in Figure 1D and E are indeed are IgG light chain and we have added this comment to the Figure legend 1; 'Asterisks represent IgG light chain.'

2. Figure 2 has been modified as requested.

3. We apologise for not correctly labelling figure 5D and have rectified this.

4. We have replaced Panel A in Figure 6 with a new one that has a negative control. Even though it was not requested we have also replaced Panel A in Figure 7.

5. We have replace Panel C in Figure 6 with one where both we include an ADAR2 mutant with both WWP2 sites mutated as requested.

6. We have rectified the statement and now is 'loss of binding of the MPM-2 antibody was particularly evident with the triple mutant'

7. We have added the year to the Aiwa reference.

8. We have replaced Figure S3 with another in which the cytoplasmic localization of ADAR2 the triple mutant is clearly seen.

9. We have added the boundary line again between the nucleus and cytoplasm that somehow disappeared. The reviewer is correct in that structural studies were performed with high concentrations of dsRBDs alone and deaminase domains alone of ADAR2 and these do not dimerize. However five different groups have reported that ADAR2 is active as a dimer. Therefore to reconcile the data we have redrawn Figure 8 and indicate that if ADAR2 dimerizes it is probably with the amino terminal and dsRBD. We have also added the statement to the figure legend 'The mechanism of dimer formation is still unclear.'

10. We have a previous publication (Desterro *et al.* 2003, J. Cell Science116) where we endeavored to identify the NLS in ADAR2. We delete some putative NLS and found that in ADAR2 there is a non canonical NLS within the first 64 amino acids. To my knowledge no other group has been able to pinpoint it further. Therefore it is not possible to draw a figure depicting the Pin1 sites and the NLS in ADAR2 as we really do not know where it is. We have added a statement to clarify this. 'Interestingly a deletion of the amino terminal residues 4-72 renders ADAR2 cytoplasmic (Wong et al., 2003) and it has also been demonstrated that this region is required for nuclear localization as it contains a non canonical NLS within the first 64 amino acids ((Desterro et al., 2003).'

Referee #2.

Minor points

1. In SH-SY5Y cells we tried to investigate endogenous editing by ADAR2 at the R/G site in *GluR2* transcript but it was too low. Therefore we transfected ADAR2 into these cells in the presence of either a siRNA *Pin1* or a siRNA control. We sequenced over 90 clones for each construct and found that in the absence of siRNA editing at the R/G site was 73%, in the presence siRNA control, editing was 69%, and in the presence of an siRNA *Pin1* editing decreased to 45%. Therefore editing at the R/G site in the *GluR2* transcript is also influenced by Pin1. We now state this in the manuscript in the middle of page 7: 'We also analyzed editing at the R/G site in the *GluR2* transcript and found it was 69% but dropped to 45% when siRNA specific for *Pin1* was co-transfected whereas editing was 73% when a control siRNA was co-transfected.' We have also included a supplementary Figure 2 to show the levels of Pin1 when the cells were transfected with an siRNA to Pin1. We also tried to look at the level of the endogenous serotonin transcript in SH-SY5Y cells however we could not detect it.

2. We analysed ADAR2 autoediting in total RNA from the brain of the *Pin1*$^{-/-}$ mice and have included a new Figure 2 for the reviewers. We do not observe any difference between 2 wildtype mice and 2 knockout mice. It is likely that there is some other protein compensating for Pin1 in these mice as the phenotype in the knockout mice is not as severe as expected as its homologue in yeast is essential. Also the level of +47 in RNA from wildype mice is very similar to that published (Reuter *et al.* 1999 Nature, Figure 3). In this paper the authors also show that most cell lines do not have ADAR2 autoediting so unfortunately we could not analyse autoediting in any other cell line. In the future we plan to culture primary neurons and hopefully we will be able to address this question.

Referee #3

1. From our Mass Spectrometry data on ADAR2 expressed in *Pichia*, we identified Ser26 and Ser31 as being phosphorylated, therefore we mutated them. However the binding site for Pin1 is Ser/Pro or Thr/Pro so we presumed that Thr32 would also be phosphorylated so we made the triple mutant. In Figure 1B there is a decrease in interaction with Pin1 when the triple mutant is made versus Ser 26. We also show that Pro33 is a key residue for Pin1 activity (Figure 4). Therefore we predict that Thr 32 is phosphorylated even though we have no evidence for it. We have added a statement to page 5 'We mutated T32 as this is the residue that precedes the proline so it may be important for Pin1 binding'. In the Discussion to clarify this we state 'Also we would predict from our results both with the ADAR2 triple mutant (Fig. 1) and with the ADAR2^{P33A} (Fig. 4) that Thr32 is phosphorylated and that this is a key phosphorylation event.' Also at the bottom of page 8 we state: 'This implies that the second proline is the critical one, thus the phosphorylation of T32 may be the critical site for Pin1 binding and P33 for isomerization. Notably this is also the most conserved Pin1 site in vertebrate ADAR2 sequences (Supplementary Fig. 1).'

2. We have added the year to the Aizawa reference

3. As stated above in response to referee 2 we do observe a decrease in editing at the R/G site in the presence of a siRNA specific for Pin1.

4. We have rephrased the sentence as requested. 'An increase in the level of ADAR2 was observed however there was not a reciprocal increase in the levels of the ADAR2^{-PPxY} mutant. This mutant could no longer bind WWP2 so its protein level could no longer be regulated by the proteasome therefore the proteosome inhibitor had no effect on its stability.'

5. We have performed the experiment as requested in HeLa cells transiently transfected with increasing concentration of *GluR2 B13* minigene however we do not see any difference between ADAR2 and the ADAR2^{-PPxY} mutant (Figure 3 for reviewer). I am concerned that transient transfections is not the best way to perform this very interesting experiment as there is transiently high concentration of ADAR2 in the nucleus but it may be labile and on the other hand there is more cytoplasmic localization with ADAR2^{-PPxY} mutant but it is stable. Therefore both effects cancel each other so the editing level is the same. Stable cell lines with the wildtype and mutant ADAR2 may be a better system to address this question but I am not sure.

Reviewer Figure 1

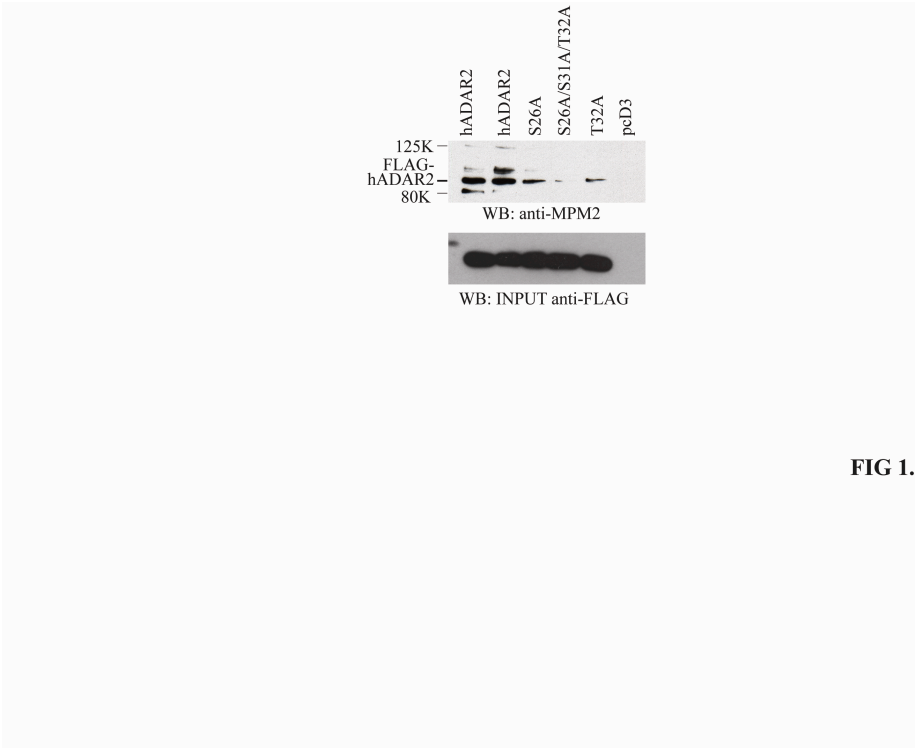
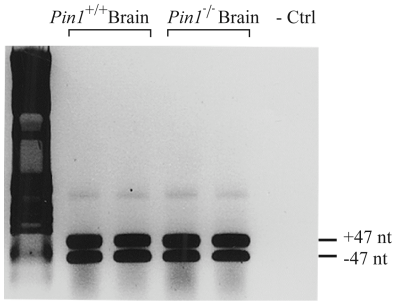


FIG 1.

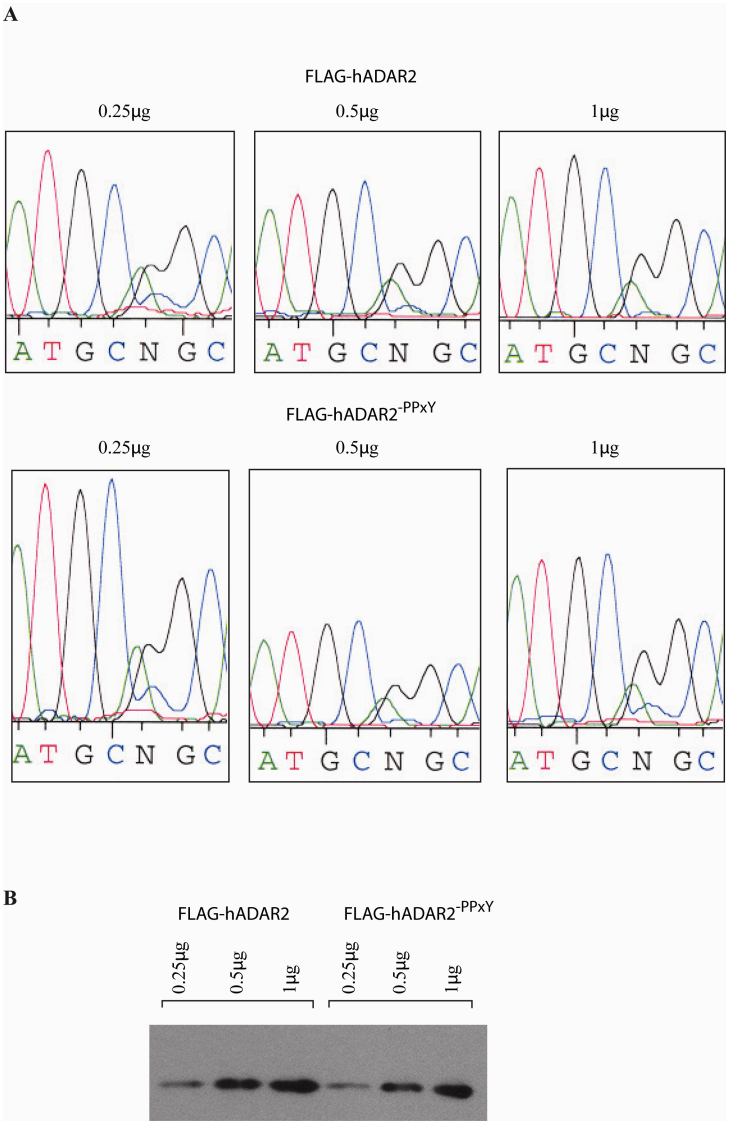
Immunoblot analysis with anti-MPM-2 antibody of anti-FLAG immunoprecipitates from lysates of HEK 293T cells transfected with FLAG-tagged hADAR2 (150mM NaCl as washing step), FLAG-tagged hADAR2 (200mM NaCl as washing step), ADAR2^{S26A}, ADAR2$^{S26A/S31A/T32A}$, ADAR2 ^{T32A} or pcD3.

Reviewer Figure 2



RT-PCR from brain total RNA isolated from two Pin1 wildtype (Lane 1, 2) and two Pin1 knockout (Lane 3, 4) mice. Autoediting of endogenous *Adar2* transcript was analysed. No difference was observed.

Reviewer Figure 3



A. Upper panel. Sequencing of RT-PCR product of *GluR2 B13* minigene transiently transfected in HeLa cells with increasing amount of FLAG-ADAR2 (0.25µg, 0.5 µg, 1.0µg). Lower panel. Sequencing of RT-PCR product of *GluR2 B13* minigene transiently transfected in HeLa cells with increasing amount of FLAG-ADAR2^{-PPxY} (0.25µg, 0.5 µg, 1.0µg). B. Immunoblot of FLAG-ADAR2 and FLAG-ADAR2^{-PPxY} mutant with anti-FLAG antibody to visualize the protein expression level in HeLa cells.

2nd Editorial Decision 12 July 2011

Thank you for submitting your manuscript for consideration by the EMBO Journal. It has now been seen by two of the original referees, they find that the study has been significantly strengthened and

are positive regarding publication once some additional data has been added directly demonstrating the effect of Pin1 and the mutant on ADAR2 localization.

Yours sincerely,

Editor
The EMBO Journal

Referee #1

In this version of the manuscript the authors have nicely addressed the majority of points raised before. Particularly the quantification of nuclear and cytoplasmic ADAR2 levels in figure 7 is an important piece of data.

Only figure 3 still needs some improvement: A direct comparison of the nucleo-cytoplasmic distribution of ADAR2 in cells ectopically expressing either Pin1 S67E or -more importantly- wild type Pin1 is still missing. The three ADAR2 positive cells in panel C are also expressing the Pin1 mutant and there is no ADAR2 positive cell that does not express Pin1. More importantly, the same is true for panel B.

It would be most convincing if the authors could show in one microscopic field that ADAR2-GFP localizes to the cytoplasm in the Pin1-/- cells but that a cell that expresses HA-Pin1 at the same time has ADAR2-GFP localized to the nucleus. The authors should have these data in their already prepared microscopic slides.

Referee #2

In my opinion, the author's have now quite adequately addressed the referees comments.

Additional correspondence 19 July 2011

One of the reviewers wants us to show in Figure 3 cells expressing one plasmid versus the other in the same microscopic field. The reviewer is under the assumption that we have this data but I can assure we do not.

I would like to clarify what we are show in Figure 3. This is a co-transfection in MEF cells to demonstrate the localization of either wt and mutant ADAR2 and Pin1. When co-transfections are performed the DNA of the different plasmids are mixed together with Lipofectamine//to maximise transfection efficiency. Therefore both plasmids enter the cells and it is very unlikely to have cells with one plasmid, in particular MEFs as they are notorious for their low transfection efficiency. This is why many researchers transfect GFP into their cells with thier plasmid of interest to estimate the transfection efficiency.

Therefore to support our immunofluorescence data in Figure 3 we have also performed nuclear and cytoplasmic fractionation to show that in the absence of Pin1 ADAR2 is present in the cytoplasm. In addition we have performed immunofluorescence of various ADAR2 mutants to show that by mutating the binding site of Pin1 in ADAR2, ADAR2 mislocalizes to the cytoplasm. However the most compelling data is Figure 7 where we demonstrate that when a mutant of ADAR2 is made so that it can no longer interact with WWP2, a ubiquitin E3 ligase, the protein accumulates in

the cytoplasm in the absence of Pin1. The reason one does not observe strong accumulation in the cytoplasm in Figure 3 is because the protein is being degraded.

We have endeavoured to emphasise this point throughout the manuscript.

2nd Editorial decision 22 July 2011

Thank you for your correspondence. I am pleased to inform you that your paper can now be accepted for publication. You will receive the formal acceptance letter shortly.

Yours sincerely,

Editor
The EMBO Journal

