

Manuscript EMBO-2010-73815

## Overlapping functions of Hdac1 and Hdac2 in cell cycle regulation and hematopoiesis

Roel H. Wilting, Eva Yanover, Marinus R. Heideman, Heinz Jacobs, James Horner, Jaco van der Torre, Ronald A. DePinho and Jan-Hermen Dannenberg

*Corresponding author: Jan-Hermen Dannenberg, Netherlands Cancer Institute*

---

### Review timeline:

Submission date:	29 January 2010
Additional Correspondence:	26 February 2010
Additional Editorial Correspondence	26 February 2010
Editorial Decision:	02 March 2010
Additional Correspondence :	23 April 2010
Additional Editorial Correspondence:	23 April 2010
Revision received:	03 May 2010
Editorial Decision:	17 May 2010
Accepted:	20 May 2010

---

### 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.)

Additional Correspondence

26 February 2010

---

My name is Jan-Hermen Dannenberg, junior group leader at the Netherlands Cancer Institute in Amsterdam and I submitted a manuscript (EMBOJ-2010-73815) to EMBO Journal on 29th of January 2010.

Since submitting the ms I have learned that a ms with similar finding to ours is in press in Genes and Development and will be published on March 1th. Given this situation it would be of great help if you could let us know if there has been a decision on our manuscript. In case our manuscript is rejected I would like to know asap since that will give us a chance to submit our ms somewhere else. If a different decision has been made it also would be of great help to know.

Additional Editorial Correspondence

26 February 2010

---

Many thanks for your message and for alerting me to this competing manuscript. We have received two of the referees' reports on your manuscript, but unfortunately we are still awaiting the third; the referee has promised to get this to us within the next few days, and I'm sure you understand that (assuming the report does come in soon) I would prefer to wait until I have all three reports before making a final decision. I would, however, like to reassure you that – as a matter of editorial policy - the EMBO Journal does not consider manuscripts published since the date you submitted your

study to negatively impact upon our assessment of the work. Therefore, we will not take this competing manuscript into account when making our decision.

I am sorry that I can't give you a formal decision right now, but I hope you understand our position, and I will be in touch early next week with a decision. Many thanks for your patience until then!

Yours sincerely,

Editor  
The EMBO Journal

1st Editorial Decision

02 March 2010

Many thanks for submitting your manuscript EMBOJ-2010-73815 to the EMBO Journal. As I told you on Friday, I have received the comments of two of the three referees, which are enclosed below. Unfortunately, referee 3 has still not returned his/her report, and given the just-published Genes and Development paper, I do not want to delay things further before making a decision. As you will see from the reports, both referees express interest in your study and are broadly in favour of publication. We would therefore like to invite a revision of your manuscript. However, while referee 1 has only relatively minor comments, referee 2 raises more serious concerns. Addressing these should primarily involve significant re-writing, but he/she does also highlight some issues with the presented data that will likely require repeating a number of experiments to address concerns as to consistency and interpretation. One other point I would like to highlight: given the Yamaguchi et al publication, it will be essential to cite and discuss this study appropriately in your manuscript. For example, I note that they find that p21 and p57 knockdown can partially rescue the cell cycle arrest induced by HDAC1/2 depletion, whereas you show that loss of p21 can not rescue the phenotype; do you attribute this distinction to p57 or might there be other reasons for the apparent discrepancy?

Given the situation, we would obviously like to speed things up as much as possible. However, it will be important to satisfactorily address the concerns of referee 2, as well as those of referee 1. Moreover, should the third report come back, we will of course forward it on to you and may ask you to respond to any criticisms therein. Ideally, it would be great if you could submit your revision within the next few weeks; please can you let me know whether you think this is a realistic target or whether you feel you will need more time. Should you wish to do so, we can certainly also discuss in greater detail what data we would deem necessary for the revision.

When preparing your letter of response to the referees' comments, please bear in mind 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 reading the revised manuscript.

Yours sincerely,

Editor  
The EMBO Journal

## REFEREE REPORTS

Referee #1 (Remarks to the Author):

In the manuscript by Wilting et al., the authors describe the functions of HDAC1 and HDAC2 in cell cycle progression and hematopoietic differentiation. The experimental approach, based on the

generation of different combinations of HDAC1/HDAC2 null alleles in primary or transformed fibroblasts, is well designed and the results clearly show that HDAC1 and 2 have redundant and requisite roles in cell cycle progression. The authors demonstrate that genetic deletion or ectopic expression of inactive HDAC1/2 in mouse embryonic fibroblasts (MEFs), as well as in transformed cells causes a senescence-like G1 cell cycle arrest. The authors provide genetic proof that this contribution of HDAC1/2 in cell survival is p53 and p21 independent. In addition, the inactivation of different combinations of HDAC1 and HDAC2 allowed the authors to conclude that while both these histone deacetylases are required for survival of hematopoietic cells, only HDAC1 is essential for erythrocyte-megakaryocyte differentiation. These results could, at least in part, provide a mechanistic explanation of the hematological side effects observed in patients treated with HDAC inhibitors.

Specific comments:

1. In Figure 1B, add the quantification of the SA- $\beta$ -Gal staining (as was done in Fig. 5D).
2. Add the genotypes associated with the curves in all the cell survival graphs. This would clarify the graphs.
3. In Figure 3A, p27Kip seems to be up-regulated but in the text (page 7) it is stated that the level of p27Kip was not altered. Correct the discrepancy.
4. On page 6, the authors write: "...we generated Hdac1 and Hdac2 catalytic inactive mutants... The authors must show data confirming that the HDAC1D99A, HDAC1Y303F, HDAC2D100A and HDAC2Y304F are inactive. For example, perform an in vitro assay to determine the activity of mutant HDACs on substrate proteins.
5. On page 12, the last sentence: the authors state that the hematological side effects of HDACi relate to "on-target actions" of HDAC1. However, the authors have not ruled out "off-target" effects and should incorporate this to the text.
6. On page 13, the last sentence of the first paragraph is incomplete. Please fix.  
There are many typographical errors:  
On page 13,  
line 8, "provide a rational for"  
line 25, "HDAC1 and a contain"  
last line, change up regulation to up-regulation
7. There is no callout in the text to Supplemental Figure 2.
8. In the figure legend of Figure 1 there are typographical errors.
9. In the figure legend of Figure 2 define control and vector.
10. In the figure legend of Figure 5, panels B and C are inverted.

Referee #2 (Remarks to the Author):

In general, I feel that the findings of this paper are sound and significant. However, this manuscript should have been proofread before it was submitted for peer-review as there are a number of obvious, unacceptable and distracting errors. Some of the figure references in the text are incorrect as are some of the figure labels/legends themselves (e.g. Cdk4 vs. tubulin as loading control in Fig. 1). Considering that the western blot samples are from cultured MEFs, the loading controls are too variable for clear interpretation and certainly for publication (e.g. in Figure 3A, and esp. Figure 5A). There are numerous obvious spelling and grammar errors, confusing phrases, and even unfinished sentences (e.g. the last sentence of the first Discussion paragraph). All of these things make it very difficult and time-consuming to evaluate the scientific quality of the experiments and their interpretation, and serious corrections of the writing itself -- too numerous for me to list one-by-one -- are required before eventual publication.

Nevertheless, I generally like the experimental design, results, and their implications, and feel these are of good quality overall. I would, however, like to offer several specific suggestions for improvement.

Figure 1A: In the text describing Figure 1A, the authors state that levels of Hdac3 remain unchanged upon deletion of Hdac2, but this really cannot be determined based on the Hdac3 blot the authors present -- the signals are too variable. Also, please match the labels of the figure with the figure legend (tubulin vs. Cdk4).

Figure 2A) In both the legend for this figure and the figure itself the authors should be much more explicit that these lysates are from a RCM2+;Hdac1L/L;Hdac2-/- background and treated with 4-OHT. These are mentioned 2/3 of the way through the figure legend, but it would be much clearer to not only state this at the beginning and also label it in the figure.. Furthermore, in both A and B of this figure, nowhere is it mentioned what the "control" sample is. As a general comment for all the figures in this paper, it would be easier for most readers to understand if the genetic backgrounds were explicitly noted in the figure itself.

Figure 3A) In addition to my loading control criticism above (the uneven loading really does make this figure difficult to interpret), I have several further points. In my opinion, this set of blots would be easier for a reader to compare if they were presented as an allelic series rather than in their current jumbled order. Either one of the following orders is preferred: Hdac1(+/+);Hdac2(+/-) then Hdac1(L/L);Hdac2(+/-) then Hdac1(L/+);Hdac2(-/-) and finally the double null, or instead Hdac1(+/+);Hdac2(+/-) then Hdac1(L/+);Hdac2(-/-) then Hdac1(L/L);Hdac2(+/-) and finally the double null.

Next, I'm troubled by the inconsistency between the blots in Figure 3A and Suppl. Fig. 3A. Notice, for example, that in Suppl Fig 3A, the levels of p27 and p16 remain basically constant regardless of Hdac2 copy number in the control lines or Hdac1 levels. In Figure 3A, however, there is clearly more p27 in the Hdac2(-/-) lines expressing the control than Hdac2(+/-) lines, and the level of p16 is also quite variable (with no obvious trend). Furthermore, expression of shRNAs against either p21 or p53 appears to have substantial effects on p27 and p16 levels in different Hdac backgrounds, especially as compared to controls. Considering the other phenotypes of Hdac-deficient cells discussed in the paper, the authors certainly need to address this in the context of G1 regulation.

Also, in the "Hdac1 and Hdac2 cooperative regulate p21" section of the main text, the authors state that MEFs lacking Hdac1/2 do not stably express p53, but do not show any data to support this. Considering that they later spend a good deal of time examining the regulatory role of p53 in these genetic backgrounds, it's not clear why they have chosen to omit this data. As an additional further comment, near the bottom of this section, the authors abbreviate the genotypes of the MEFs. I feel it would be much easier to refer to the figures if the genotypes in the text and the genotypes in the figures were the same (i.e. there is no sample in Figure 3A labelled "Hdac1(-/-)" -- what one has to deduce is that they actually mean RCM2+;Hdac1(L/L);Hdac2(+/-) or Hdac2(-/-) MEFs treated with 4-OHT.

In the main text paragraph starting with, "Although we obtained hardly detectable levels of p21..." there are several points of concern. First, the authors claim that p19 and p27 levels are unaffected by expression of Hdac1 shRNAs, citing Suppl Fig 3. Furthering my comments above, in Suppl Fig 3A, p19 levels in Hdac1 KD Hdac2(+/-) lines are obviously much lower than in the control (if we are to believe these lanes were equivalently loaded). This either needs to be explained or redone to be believable. Furthermore, the authors say that p21 is increased in Hdac2(-/-)p21(+/-) as compared to the control, but the bands are so faint in these samples that this claim is not well supported. Next in this paragraph, the authors refer to "cycling Hdac1 shRNAs expressing Hdac Hdac2(+/-)p21(+/-) MEF cultures," but I truly have no idea what they mean by this. Please clarify.

Figure 4C,D) It would be useful to label matching isolates in Figure 4C and D so that the reader can tell which is which.

Figure 5) I assume the cells in A were treated with 4-OHT, but this is not stated either in the figure or the legend. The authors should do so. Also, I feel the variation in the amount of protein loaded in each well is unacceptable for a publication figure.

In the main text discussing oncogenic Ras, the authors should describe what effect this has on p53 and Rb, for the benefit of readers outside the cancer field. Similarly, further along in the paper, they should describe the purpose and effect of injecting p(I);p(C) for people outside the field.

Finally, I generally found the discussion somewhat incoherent, and it was difficult to follow the logic throughout. Upon revision, the authors should carefully rewrite the discussion to be more acute and coherent, because in its current form it simply will not do.

In summary, my main concerns with this manuscript have to do with the presentation of the science, not the science itself. Therefore, I feel with substantial revision, this paper could be acceptable for publication in EMBO.

---

Additional Correspondence

23 April 2010

This week we discovered that Figure 6 of our manuscript contains an error which has consequences for the message of our research. Can we have a phone conversation about this today? The problem we encountered is as follows:

In Figure 6 we show that deletion of Hdac1 or Hdac1 and Hdac2 using the MxCre transgene results in severe anemia and thrombocytopenia (Fig6A,B) and is caused by a drop in megakaryocytes/thrombocytes and erythrocytes (Fig6C,D,E). Our conclusion based on these data is that Hdac1 has a specific function in the erythrocyte-megakaryocyte lineage.

For reasons I will explain below we found out this week (yesterday) that the mice that are indicated as MxCre<sup>+</sup>;Hdac1L/L are actually MxCre<sup>+</sup>;Hdac1L/L;Hdac2L/+ mice. Although the fact that Hdac2 is still expressed in the liver and bone-marrow (as indicated in Suppl Fig4 and 5) we found actually this week that MxCre<sup>+</sup>;Hdac1L/L;Hdac2<sup>+/+</sup> mice do not have a phenotype. The phenotypes we attributed to the loss of Hdac1 only are actually due to the loss of Hdac1 and a reduction in Hdac2 levels. The conclusions with respect to wild-type and MxCre<sup>+</sup>;Hdac1L/L;Hdac2L/L mice do not change and are still valid.

In conclusion the data we have now indicates that Hdac1 and Hdac2 are functionally redundant in the differentiation of the haemopoietic system. Loss of Hdac1 or Hdac2 does not result in a phenotype, while combined loss It also suggests that it is the total level of Hdac1 and Hdac2 are crucial for the development of the hematopoietic system since Hdac1 loss in the presence of reduced Hdac2 results in a severe phenotype. Complete loss of Hdac1 and Hdac2 enhances these phenotypes and results in apoptosis.

In the past we have performed experiments with MxCre<sup>+</sup>;Hdac1L/L;Hdac2<sup>+/-</sup> mice. In these instances the absence of a phenotype was attributed to problems with pIpC activity (our former supplier of pIpC (Invivogen) had difficulties with the production of this substance. Therefore we switched to Sigma pIpC which doesn't work basically (confirmed by others). Currently we have Amersham pIpC which works great). Combined with an administrative error in which MxCre<sup>+</sup>;Hdac1L/L;Hdac2L/+ mice were indicated as MxCre<sup>+</sup>;Hdac1L/L;Hdac2<sup>+/+</sup> and an immunostaining in which we can not distinguish between Hdac2<sup>+/+</sup> and Hdac2<sup>+/-</sup> (after Cre deletion of the L allele) we concluded that Hdac1 loss results in the observed phenotypes.

Two weeks ago we started new experiments in which we injected MxCre<sup>+</sup>;Hdac1L/L;Hdac2<sup>+/+</sup> (100% confirmed) with pIpC that we tested for activity. At the beginning of this week it became clear that these mice did not develop the phenotypes that we had observed before. This triggered us to review and re-genotype the mice we had used in the experiments described in the manuscript which resulted in the conclusions described above. We have analyzed these mice this week and confirmed the absence of a phenotype in these mice.

I hope this explains the situation. I apologize for the inconvenience caused by the mistakes we made.

Just a quick message to follow up on our phone conversation. Thanks again for alerting us to this problem with your data in Figure 6, and good to hear that you do now have the data with the correct genotypes. Since this does change one of the main messages of the paper, we will have to have your revision stringently re-reviewed by both referees, and there can be no guarantee as to the outcome. However, we will ask the referees to bear in mind that they should not dismiss your paper on the grounds of the Yamaguchi et al study in *Genes and Development*.

I look forward to receiving your revision.

Yours sincerely,

Editor  
The EMBO Journal

We appreciate the comments by the reviewers and thank them for suggestions to improve the manuscript. Below we have addressed the comments point-by-point and indicated the changes made to text and figures. We are confident that these changes in response to the reviewers' comments have improved the readability and quality of the manuscript.

We would like to mention that Figure 6 has changed significantly. The reason for this is that during the time of the revision of this manuscript we became aware that the "MxCre+;Hdac1L/L" mouse data presented in Figure 6 was actually obtained from analysis of MxCre+;Hdac1L/L;Hdac2L/+ mice. The mislabeling of the data occurred through an administrative error in documenting "MxCre+;Hdac1L/L;Hdac2L/+" as "MxCre+;Hdac1L/L". Since our immunohistochemistry for Hdac2 (as shown in Suppl. Fig 4 and 5) does not distinguish between Hdac2WT and Hdac2HET we interpreted the data as a result of Hdac1 deletion only.

While this manuscript was under revision we performed additional experiments with correctly genotyped and correctly documented MxCre+;Hdac1L/L;Hdac2+/+ mice. These experiments revealed that MxCre+;Hdac1L/L;Hdac2+/+ mice, upon efficient ablation of Hdac1 did not show the phenotypes (anemia and thrombocytopenia) that we attributed to deletion of Hdac1. Upon careful evaluation of all the data and re-genotyping of all mice used in the experiments presented in the manuscript we have to conclude that Hdac1 does not have a unique function in the hematopoietic development. Instead, Hdac1 and Hdac2 have, similar to our observations in fibroblasts, redundant functions in hematopoiesis, in particular in the development of the erythrocyte megakaryocyte differentiation. In the revised version of the manuscript we have added the analysis of the correct MxCre+;Hdac1L/L;Hdac2+/+ mice (in Figure 6, Suppl. Figures 4, 5 and 6). Additionally, the data previously labelled "MxCre+;Hdac1L/L" is retained in figure 6 and correctly labelled "MxCre+;Hdac1L/L;Hdac2L/+". Although the current data no longer supports our previous conclusion for a specific function of Hdac1 in hematopoiesis, it shows that total levels of Hdac1 and Hdac2 are critical for the development of the erythrocyte-megakaryocyte lineage. A reduction of Hdac2 levels in the absence of Hdac1 results in severe anemia and thrombocytopenia (similar to dual inactivation of Hdac1 and Hdac2) but still supports the survival of (dysfunctional) megakaryocytes. Accordingly we have changed the title of the manuscript and the last part of the abstract. We sincerely apologize for the mistakes we made by not correctly documenting the genotypes. We would like to emphasize that the data presented in revised manuscript has been (re)checked carefully and faithfully represents the analysis of indicated genotypes.

Reviewer #1

We thank the reviewer for his/her comments. We have addressed below the comments by the reviewer point-by-point and accordingly changed the figures and text.

1. We have quantified the senescence in Hdac1/2 deficient MEFs and control MEFs (Hdac1 or Hdac2-deficient MEF cultures) by counting SA- $\beta$ gal+ cells in three independent MEF cultures.

These data are added to figure 1 in panel D.

2. We added the genotypes of all the survival curves. In general, we have changed the annotation of genotypes to wild-type, Hdac1KO and DKO to enhance the readability of the manuscript.
3. We have corrected our conclusion regarding p27Kip levels in Hdac1/Hdac2 deficient MEFs. We would like to emphasize that the up-regulation of p27Kip in DKO MEFs, in contrast to up-regulation of p21Cip, did not correlate with this genotype. As shown in figure 3B, p27Kip was also upregulated in the absence of Hdac2.
4. We have over-expressed, immuno-precipitated and subsequently measured the Hdac catalytic activity of wild-type and mutant forms of Hdac1 and Hdac2 using a fluorogenic-based Hdac activity assay. As shown in Supplemental figure 2, mutant forms of Hdac1 and Hdac2 displayed a dramatic (up to 60%) reduction in Hdac activity compared to wild-type Hdac1 and Hdac2. These data clearly suggest that the Hdac1 and Hdac2-mutants used in our studies are impaired in their capacity to remove acetyl groups from target sequences.
5. We have changed our conclusion regarding on-target effects by stating that our results "at least partly relate to on-target effects on HDAC1 and HDAC2"
6. We have carefully read through the manuscript and changed the typographical errors throughout the manuscript.
7. We have included a callout in the text to Supplemental Figure 2.
8. We have corrected the typographical errors in the figure legend of figure
9. We have defined the control (wild-type cells) and vector (DKO MEFs) in the legend of figure 2.
10. The text referring to panels B and C of figure 5 has been corrected.

Reviewer #2.

We thank the reviewer for his/her critical remarks and suggestions to improve the presentation of the science presented in this manuscript. We have implemented the suggestions in the revised manuscript and indicate below point-by-point the changes we have made to the text and figures.

1. Concerning figure 1, we have repeated the western blot analysis for Hdac3 and loading control and inserted this into Figure 1A. Since the protein loading of the last "Hdac2-/-" sample was slightly elevated (with respect to the tubulin loading control) we omitted the last "Hdac2-/-" sample. We corrected the labels for the used loading control.
2. In order to improve the readability for the readers we have inserted the genetic backgrounds of the MEF cultures in the figures. Since most MEF cultures contain a combination of genetic alleles which makes the readability difficult due the size of the letter we have to use to fit the figures and amount of information, we have decided to change the genetic backgrounds to WT (wild-type) Hdac1KO, Hdac2KO or DKO (Hdac1/2 double knockout). In the beginning of the results section we have indicated how these genotypes were obtained (through addition of tamoxifen to RCM2+;Hdac1;Hdac2 MEFs).
3. We agree with the reviewer that the presentation of the western blot analysis in figure 3A requires improvement. Following up on his suggestions we have changed the order of the lysates in a more logical way (WT, Hdac1KO, Hdac2KO and DKO). The reviewers' criticism concerning the loading of the lysates in figure 3A is addressed by analyzing the same lysates for tubulin. In contrast to what we observed for Cdk4, tubulin signals suggest a more equal loading between all samples. Since we quantify protein concentrations in all our lysates using BCA methods (Biorad), we are confident that tubulin is a better loading control for our experiments than Cdk4. Although for many experiments Cdk4 seems to work well as a loading control, for other experiments (like in Figure 5A) we have observed that tubulin staining compared to Cdk4 gives a different result concerning equal loading. A possible explanation for this may relate to the fact that Cdk4 is nuclear protein that is less efficient released during lysis compared to the cytoplasmic tubulin. For future studies we will use tubulin as a loading control
4. Figure 3B and Supplemental Figure 3A raises questions for the reviewer concerning p16Ink4a and p27 levels. Before addressing these questions we would like to emphasize that the increase in p21Cip levels is the only consistent change in levels of tested cell cycle inhibitors (p16Ink4a, p19Arf, p21Cip, p27Kip) in Hdac1/2 deficient MEFs that we have seen in many independent experiments. The data in Figure 3 emphasize this and show that p21Cip (and its positive regulator p53) are not required for such a cell cycle arrest. Comparing the p16Ink4a levels in control infected MEFs (figure 3A) indicates that wild-type and Hdac2KO MEFs have slightly elevated levels of p16Ink4a compared to DKO MEFs and Hdac1KO MEFs. In Supplemental Figure

3 p16Ink4a levels in Hdac2<sup>+/+</sup> and Hdac2<sup>+/-</sup> are also slightly elevated compared to Hdac2<sup>-/-</sup> MEFs. In conclusion these data suggest that the levels of p16Ink4a, in contrast to p21Cip levels, do not correlate with the cell cycle arrest observed in DKO MEFs. In agreement with this deletion of p16Ink4a and p19Arf do not allow proliferation in the absence of Hdac1 and Hdac2 (Fig 4E). As with p16Ink4a and correctly noted by the reviewer p27Kip levels change in the different genotypes but do not correlate with the cell cycle phenotype. This suggests that p27Kip levels are not relevant for the observed cell cycle arrest in the absence of Hdac1 and Hdac2. While p27Kip levels are somewhat elevated in DKO MEFs compared to wild-type and Hdac1KO MEFs they are equally elevated in Hdac2KO MEFs (Fig3B). Since the latter one does not undergo a cell cycle arrest we conclude that these levels are not relevant for the observed cell cycle arrest.

With respect to the situation in which p21 or p53 are absent (Fig 3B) we agree with the reviewer that p27Kip are significantly elevated. Again, as indicated by the reviewer, this increase occurs regardless the status of Hdac1 and Hdac2. Since only DKO MEFs undergo a cell cycle arrest, we conclude that p27Kip is not relevant for the observed phenotype. We cannot exclude that DKO MEFs are more sensitive to the observed levels of p27Kip as compared to Hdac2KO MEFs. We have included these considerations in the main text of the manuscript.

5. Upon request of the reviewer we have included data that supports our conclusion that p53 is not activated in the absence of Hdac1 and Hdac2 (Fig 3A).

6. In Figure 4 we show that in Hdac2KO;p21<sup>+/+</sup> MEFs, Hdac1 KD results in upregulation of p21Cip. p21Cip is also induced in Hdac2KO;p21<sup>+/-</sup> levels upon downregulation of Hdac1, albeit that the levels of p21 are lower due to the presence of one functional p21Cip allele. We have included a longer exposure of the p21Cip westernblot analysis to support the statement that p21Cip is induced upon dual inactivation of Hdac1 and Hdac2

7. Figure 4C and D have been adjusted to present the data more clearly. We also included labels to indicate the origin of the analyzed clones in Figure 4D.

8. In the legend of figure 5A we included that the MEFs, from which the lysates were isolated, were treated with 4-OHT. Additionally, we have repeated the westernblot analysis shown in Figure 5A and used tubulin as a loading control.

9. We have included an explanation of the effect of oncogenic Ras on p53 and pRB pathway regarding the experiment mentioned in Figure 5.

10. We described the effect and purpose of injecting pIpC.

11. The discussion part is carefully rewritten.

2nd Editorial Decision

17 May 2010

Many thanks for submitting the revised version of your manuscript. It has now been seen again by both referees, and I am pleased to be able to tell you that, despite the changes to the conclusions of your study, they are both still supportive of publication in the EMBO Journal. Their comments are enclosed below.

While neither referee asks for any further modification, we do need higher resolution figure files from you before we can accept the manuscript. I would therefore ask you to submit a revised version including better quality files, as well as combining the supplementary files into a single PDF. Once we have these, we should then be able to accept the manuscript without further delay.

Yours sincerely,

Editor  
The EMBO Journal

## REFEREE REPORTS

Referee #1 (Remarks to the Author):

The authors addressed the comments made during the first review. The fact that the authors revised their conclusions regarding the redundancy of HDAC1 and HDAC2 in hematopoiesis does not compromise the study even if it takes away part of the novelty in their investigation of these two class I HDACs.



As pointed out during the first review, in fact, HDAC1 and HDAC2 has been previously described to play redundant roles in many different cellular contexts. However, the authors properly cited the previous literature, including the work from the Matthias laboratory. The manuscript can be published in the current revised form.

Referee #2 (Remarks to the Author):

I thank the authors for addressing my concerns and those of the other reviewer. This revised manuscript is much improved, and I feel this paper is now ready for publication in the EMBO Journal.