

## **Corrigendum: Selective class II HDAC inhibitors impair myogenesis by modulating the stability and activity of HDAC-MEF2 complexes.**

Nebbioso A, Manzo F, Miceli M, Conte M, Manente L, Baldi A, De Luca A, Rotili D, Valente S, Mai A, Usiello A, Gronemeyer H, Altucci L.

Corrigendum on EMBO Rep. 2009 Jul;10(7):776-82. doi: 10.1038/embor.2009.88. Epub 2009 Jun 5

### **Appendix**

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## People involved

Lucia Altucci, University of Campania "L. Vanvitelli", Corresponding Author

Achim Breiling, EMBO reports, Editor

Antimo Migliaccio, Head of the Department of Biochemistry, Biophysics and General Pathology, University of Campania "L. Vanvitelli"

Guisepe Paolisso, University of Campania "L. Vanvitelli"

Bernd Pulverer, EMBO Press, Head of Scientific Publications

Erica Wilfong Boxheimer, EMBO Press, Data Integrity Analyst

## Initial communication

January 2017

**Date: 09.01.2017: 13:41**

From: Achim Breiling

To: Lucia Altucci

Dear Dr. Altucci,

I am contacting you as scientific editor for EMBO reports regarding your scientific report published with us in 2009 ("Selective class II HDAC inhibitors impair myogenesis by modulating stability and activity of HDAC-MEF2 complexes", Nebbioso et al.). As you might be aware of, a comment regarding this paper has recently been published on PubPeer:

<https://pubpeer.com/publications/19498465>

In such cases we routinely re-analyse the figures of the papers in question and we indeed noted inconsistencies in some panels of the Western blot data of your paper. We would therefore ask you to provide the original source data for all figures in your manuscript (EMBOR-2008-32509) showing Western blots, in order to re-assess the consistency of the data shown.

Please let me know a.s.a.p. when you would be able to send me the required files.

Kind regards,

Achim Breiling

Editor

EMBO Reports

**Date: 09.01.2017: 13:52**

From: Lucia Altucci

To: Achim Breiling

Dear Dr. Achim Breiling,

I was not aware of it, but of course I will recover the files and come back to you asap.

Best,

Lucia Altucci

**Date: 10.01.2017: 16:13**

From: Lucia Altucci

To: Achim Breiling

Dear Dr Breiling,

I start sending you some files related to the paper in question.

The experiments have been carried out between 2005 and 2009 and thus it is not easy to find files and we are looking also into the original films.

I will send a wetransfer with additional data related to figure 2.

Best regards,

Lucia Altucci

**Date: 10.01.2017, 18:08**

From: Achim Breiling

To: Lucia Altucci

Dear Dr. Altucci,

Thanks for sending the files. However, it is unclear to me to which figure panels these blots belong and which region was used for the panel. Please, could you prepare a pdf for each figure panel that contains the sourcedata (the un-cropped blots) for the cropped panels shown, including size markers, best also highlighting the area that was used for the final figure. There is no hurry. It would be fine to have these files during next week.

Kind regards,

Achim Breiling

**Date: 10.01.2017, 18:22**

From: Lucia Altucci

To: Achim Breiling

Dear Dr Breiling,

since I was very surprised of the situation and in view of the time passed (some experiments are of 2005-6) I tried my best to send immediately something.

I will prepare a file as you say with the files or rescanned files if I do not find the insets.

Best Lucia

**Date: 12.01.2017: 18:08**

From: Lucia Altucci

To: Achim Breiling

Dear Achim,

I send you the pdf containing files that have been used for the western blots.

The image that has been highlighted on the site is present in two different expositions, one of which shows the remaining signal after reprobing the filter for normalization with Tubulin.

I decided also to add this last since to me is useful for the evaluation.

All experiments have been performed between 2005-2009 so between 6 and 11 years ago. Thus, some have been reacquired from films (when films were used), for some other images we started form a digital file.

In addition, we added the images referring to additional items mainly of Figure 2 and 3. The items are in the same order of the paper.

In these 3 days, we did not yet find all files/scan, although we hope this already enough.

Please let me know whether this is ok to close the issue.

Best,

Lucia

**Date: 17.01.2017: 15:05**

From: Achim Breiling

To: Lucia Altucci

Dear Lucia,

Thank you for sending us a pdf-file with Western blot source data for EMBOR-2008-32509. However, the file you sent is not sufficient for a thorough analysis of the data. We require

the unprocessed and uncut original data of high resolution (best primary scans of the exposed films) in order to compare these to the panels shown in the paper. You provided in many cases already cropped pictures that are identical to the final panels (e.g. for Fig. 2A). Further, the quality is often so low, that an evaluation of the data is not possible (e.g. for Figure 2B – MEF2D).

I attach two examples. In Figure 2B (MEF2D) the first and last band look very similar (marked with an asterisk). In order to rule this out, high-resolution scans of the original blot would be necessary. In Figure 2C the IgG lane for MEF2D seems to be missing (or is cut half), which seems to be the case also in the source data you provided. Further, in the source data for this Figure also the IgG lane for the AcMEF2D co-IP is not present, which casts some doubt on the origin of the empty lane in the final figure panel (marked with an arrow).

Therefore, please provide us with unprocessed and uncut original source data of high resolution for the entire Western blot panels of your manuscript. We ask you to send us these files till end of next week. Thanks for your collaboration.

Sincerely,

Achim

**Date: 26.01.2017: 17:45**

From: Lucia Altucci

To: Achim Breiling

Dear Achim,

I am sending you the data that we have been able to recover back.

In some cases you will see the originals rescanned, in other cases you will see the files as we have found them (but very few are cropped). Some experiments have been acquired digitally at that time, so between 2004-2009, nearly 13-8 years ago.

In addition, you will find the scan of a letter from Dr De Luca in whose lab the experiment of Fig 2A was done. He cannot find the originals. They have moved (as us), changing Department and locations.

Since some of the points that you have been indicating are in the files, we do really hope that the data are sufficient this time to solve the criticism.

I send you the files as pdf with the image of reference near. 2 Files, one main, one supplementary.

We are really trying our best.

Best,

Lucia



Dear Lucia Altucci,

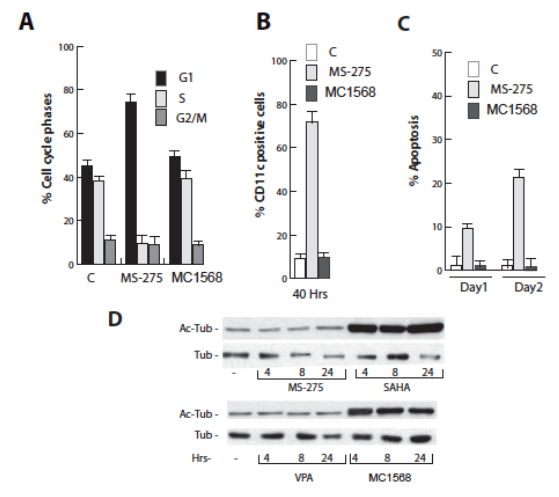
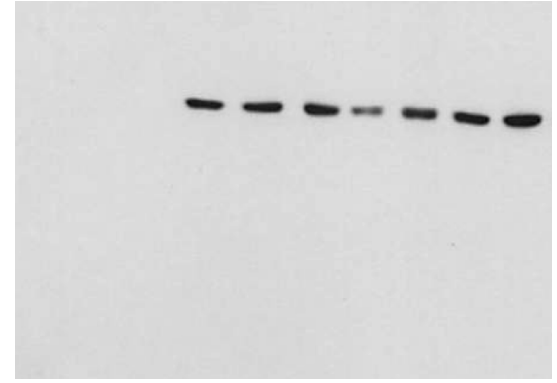
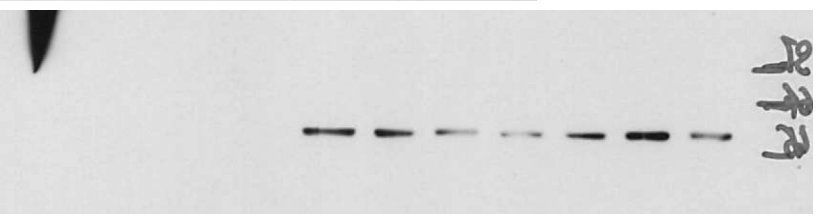
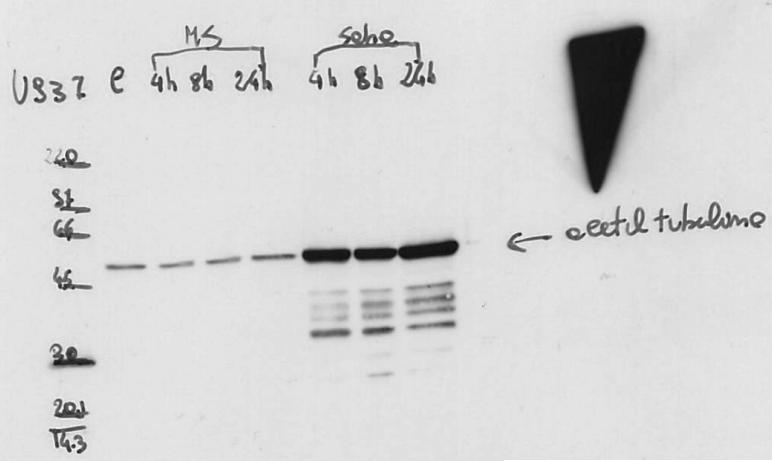
I am really sorry to communicate that we have not found the original film of the figure 2a of your manuscript: "Selective class II HDAC inhibitors impair myogenesis by modulating the stability and activity of HDAC-MEF2 complexes".

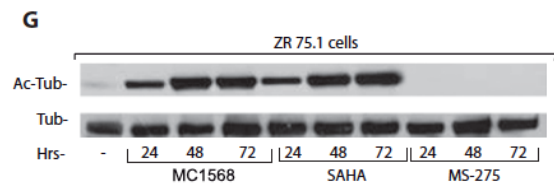
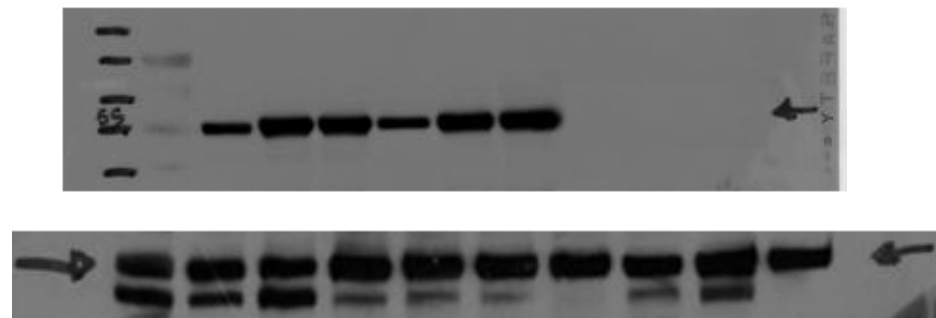
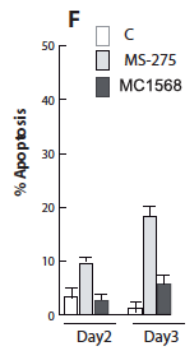
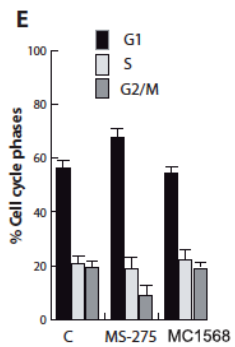
Regarding this project I remember very well the collaboration and the support to prepare for your group of research reagents and treatment of C2C12. In particular you are requesting a figure that was made in April 2005. Since that time my lab moved from the original area for infiltration of water followed by collapse of the roof. In that occasion it was not possible to recover some of the material, while some other was discarded because we moved in a smaller lab. Moreover, as you can imagine, pc has changed several times and the PhD that was working on the project left more than 7 years ago.

I will continue to search in old backup of our pc and in several old boxes but at today I am not able to find the original film. I am really sorry for this inconvenience.

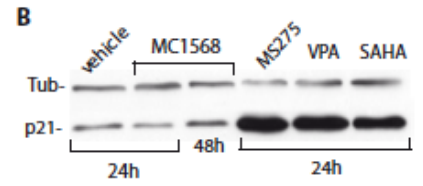
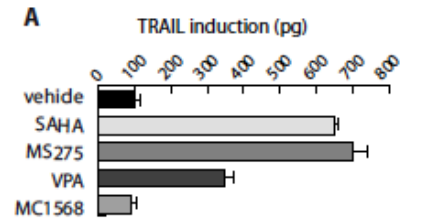
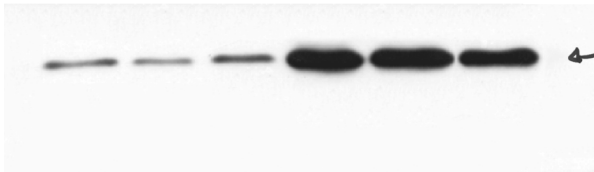
Sincerely

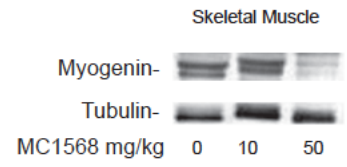
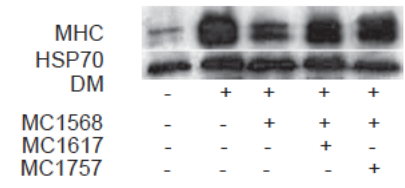
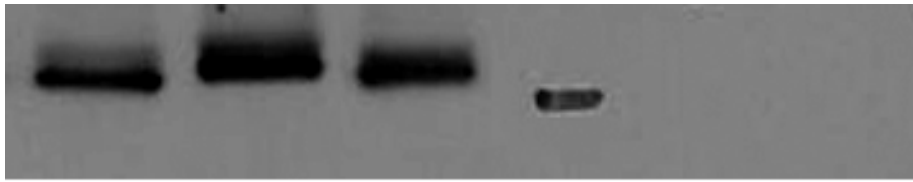
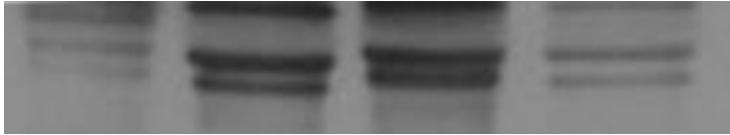
*Antonio De Luca*

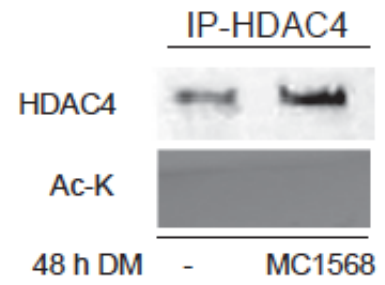
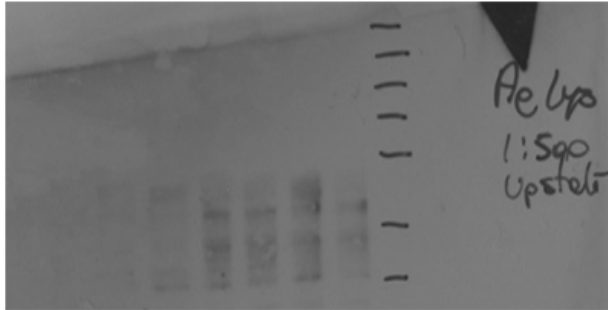
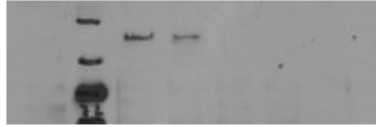


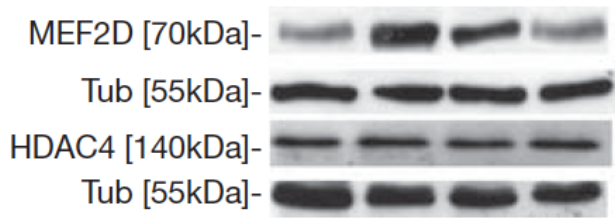






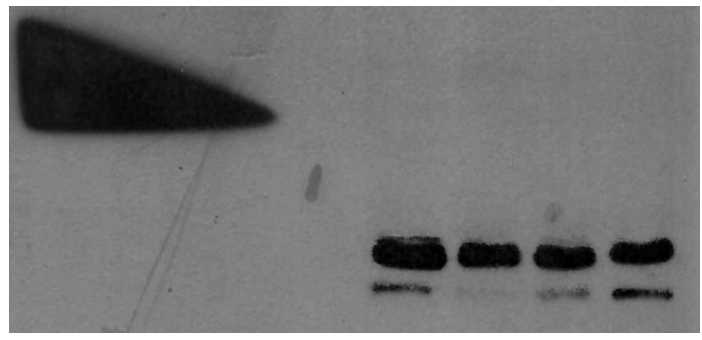
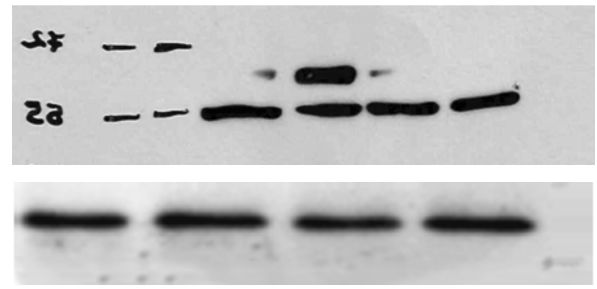
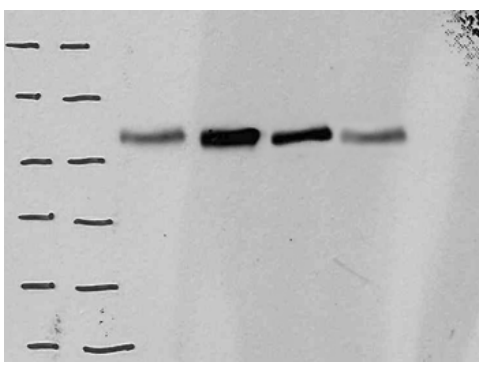




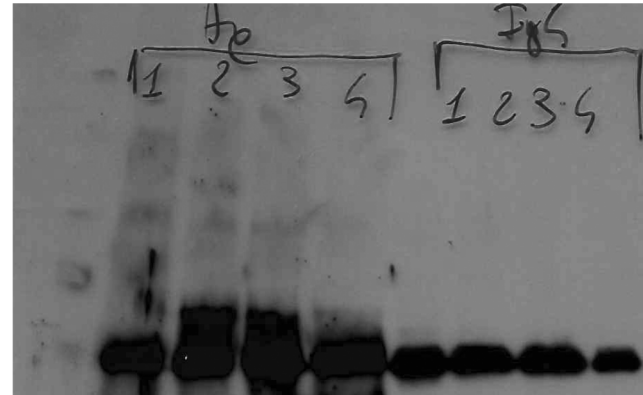
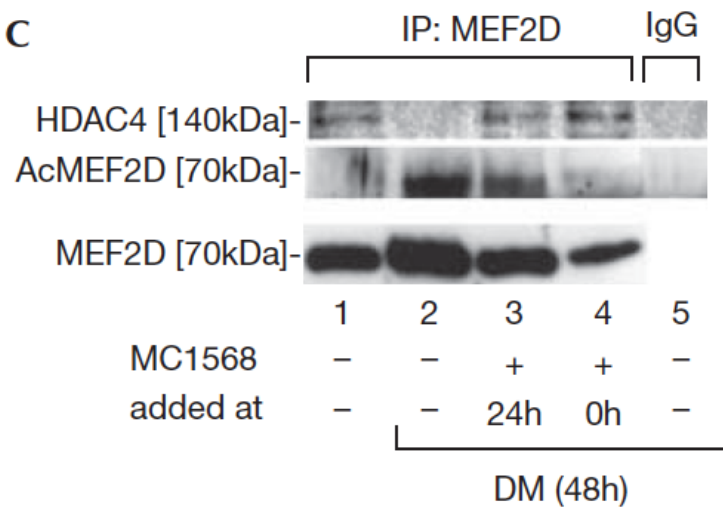


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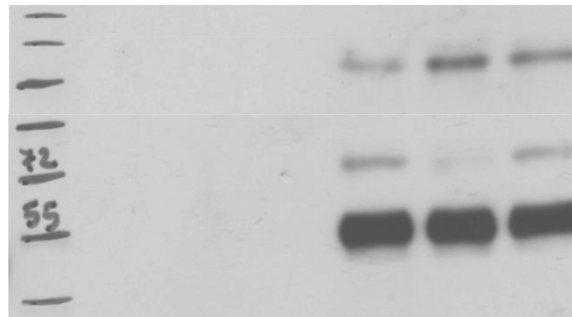
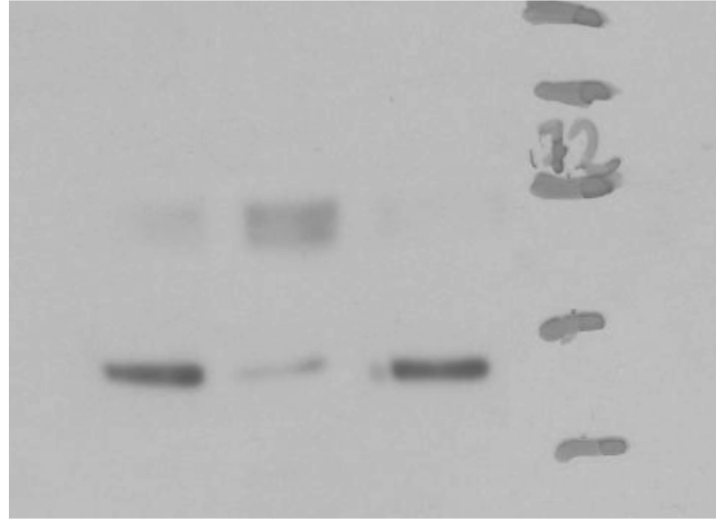
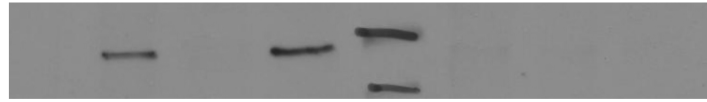
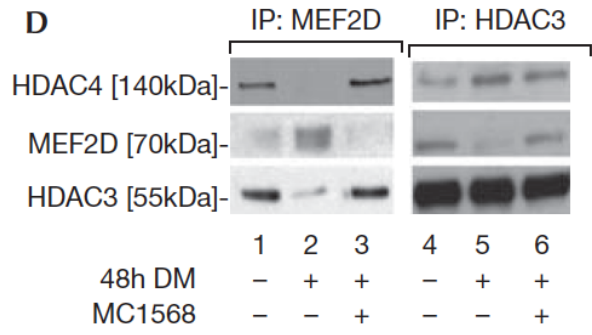
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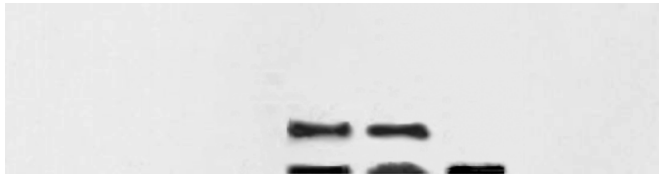
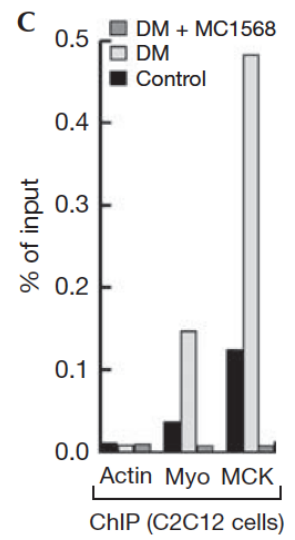
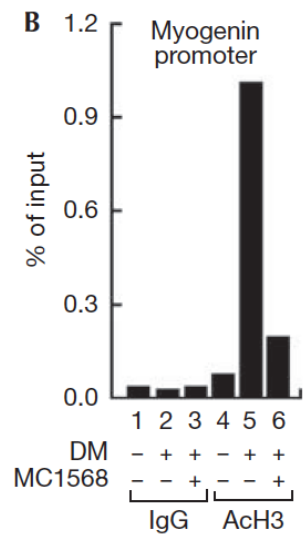
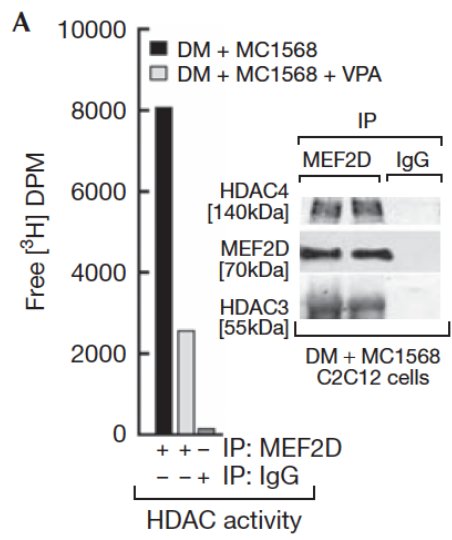


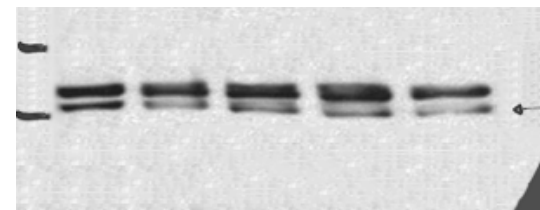
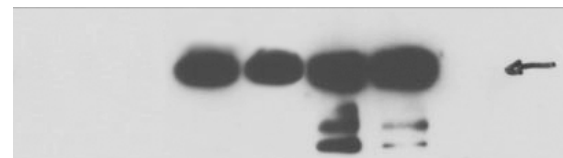
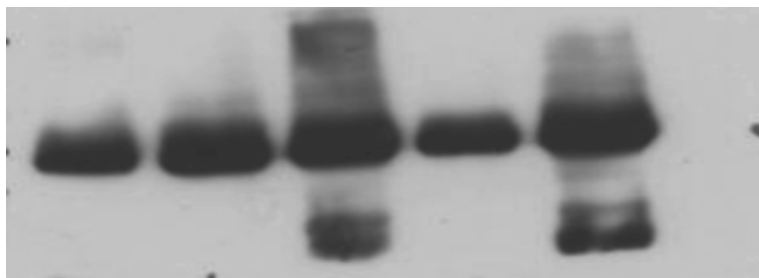
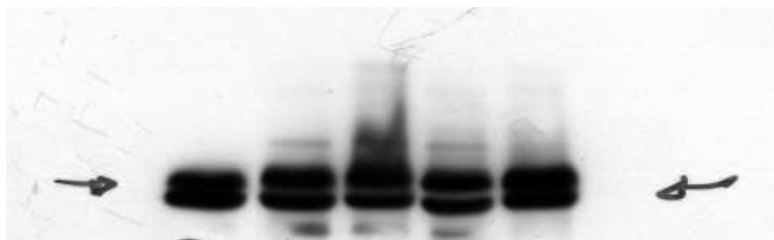
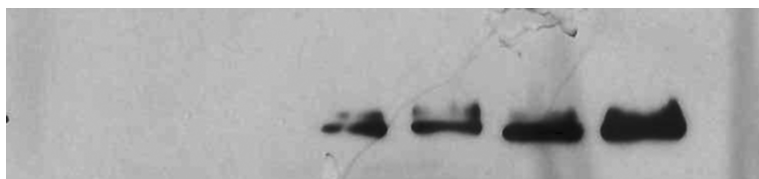
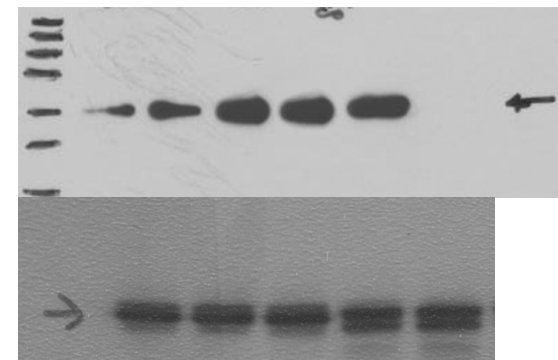
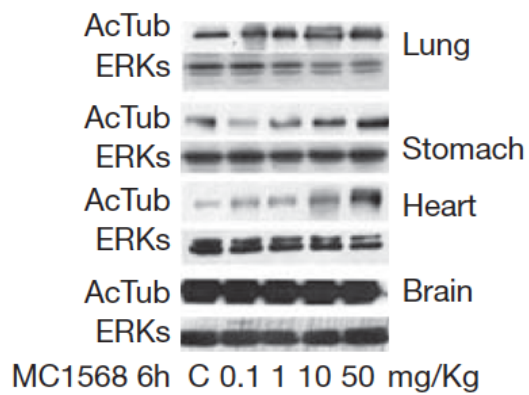
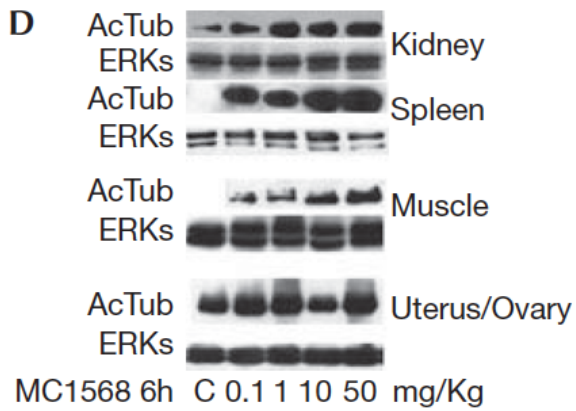
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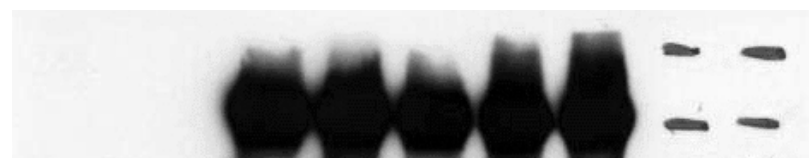
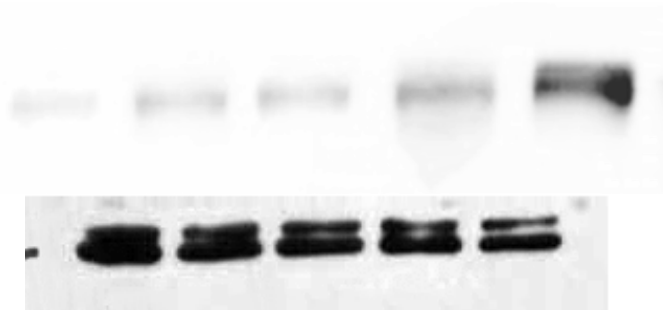
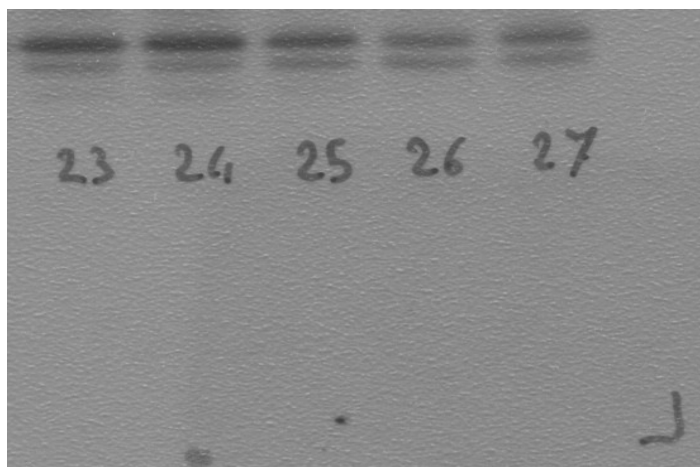
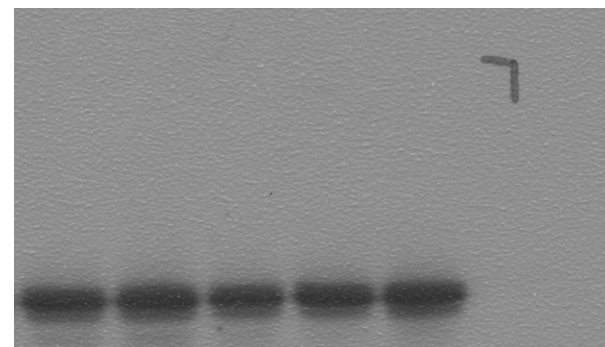
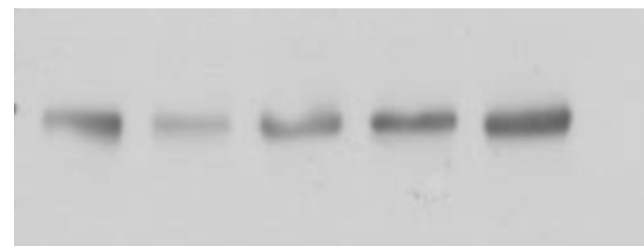
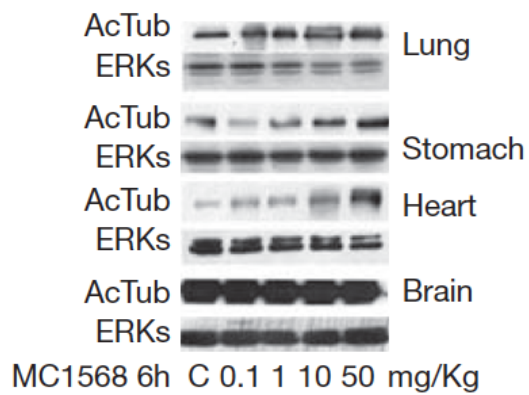
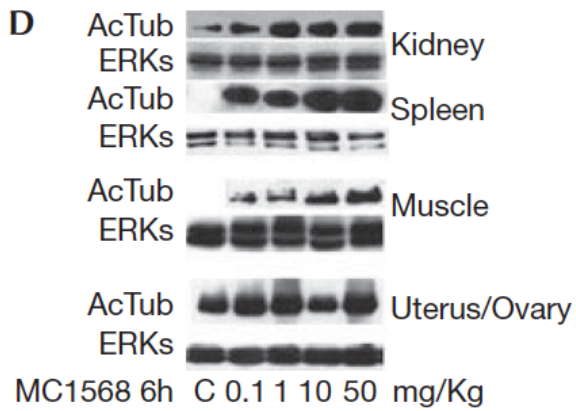
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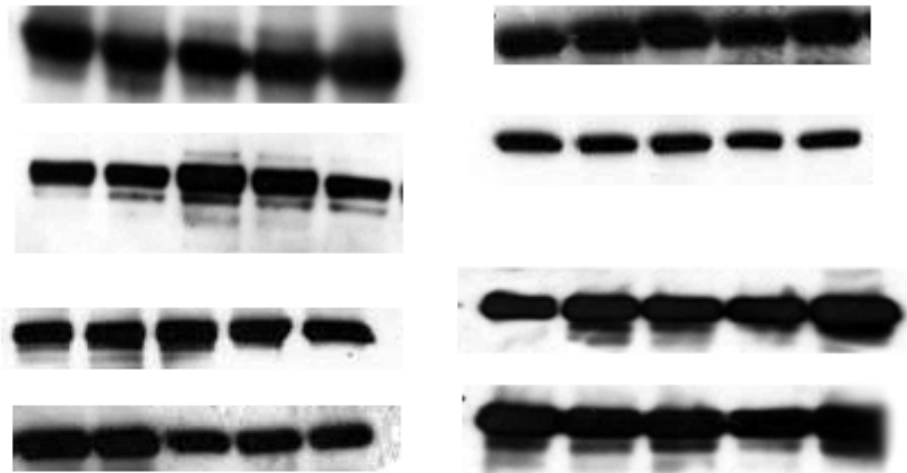
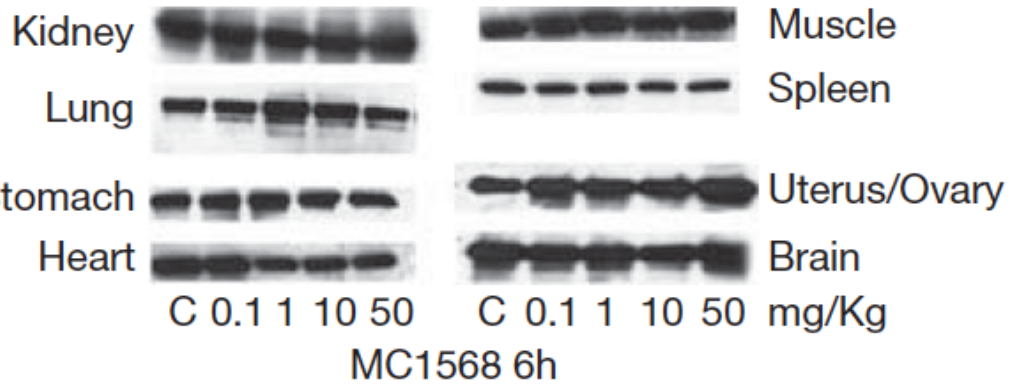


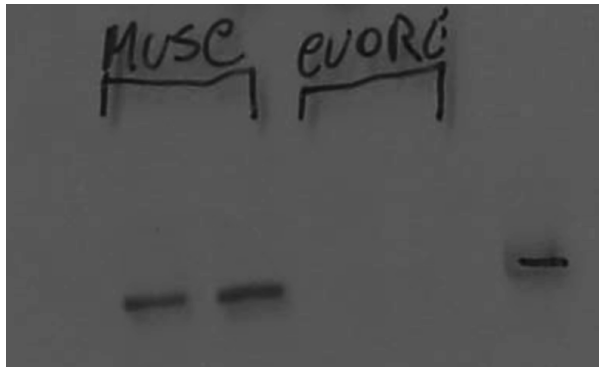
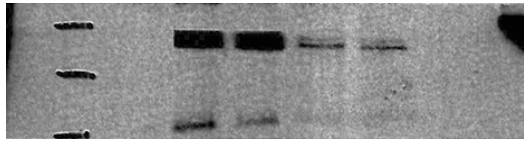
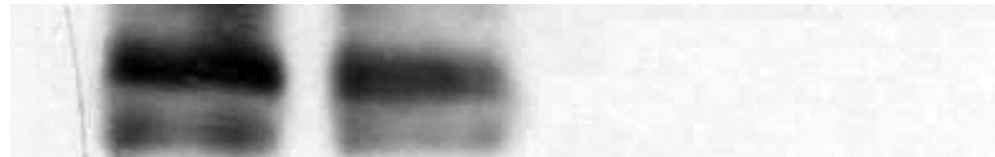
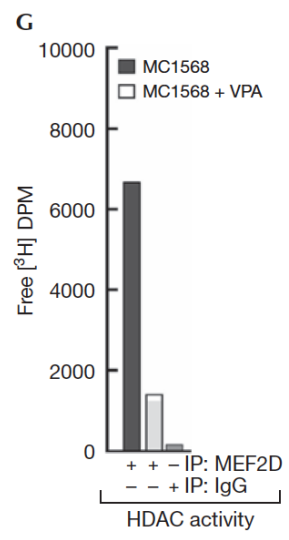
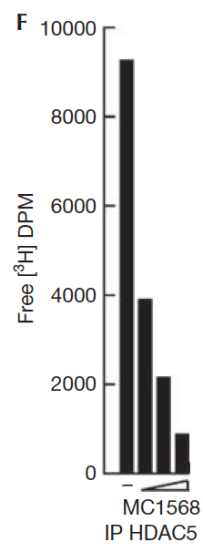
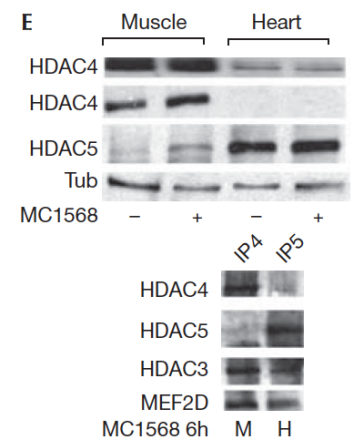






Unmodified tub





## Follow-up after EMBO Press internal investigation

April 2017

**Date: 13.04.2017: 15:33**

From: Erica Boxheimer

To: Lucia Altucci

Dear Dr. Altucci,

Thank you for your patience while we have been looking into your case. Achim and I have been in close communication with Bernd Pulverer, our chief editor (cc'd here). We agree that the splice marks noted in the figure (MEF2D) do not appear in the source data. However, this figure is extremely important to the conclusions of your paper, and we are concerned with the similarity of the bands in lanes 1 and 4. Therefore, we would request high-resolution source data that would help to resolve this issue. I've attached a document to help illustrate the issue. We are looking forward to your response.

Have a nice Easter!

Kind regards,

Erica

**Date: 13.04.2017: 17:13**

From: Lucia Altucci

To: Erica Boxheimer

Dear Erika,

we will try to solve this issue and provide the data.

I understand the concerns, although similarity of bands can very often occur.

Note also, that, along these lines, we have provided you with the original data of the normalization (tubulin) for the MEF2D blot where the previous MEF2D signal is clearly visible.

Despite this data is not in the manuscript, it supports in full the conclusions and also suggest the consistency of the MEF2D blot.

Nevertheless It is also in our interest the clarification of this issue (as we always have shown immediately responding and collaborating) and we will make our best to provide the data.

Since Easter is coming, this will be after the Easter holidays.

Best,

Lucia Altucci

**Date: 13.04.2017: 18:00**

From: Bernd Pulverer

To: Lucia Altucci

Dear Lucia,

Let me just respond briefly on behalf of my colleagues: I agree that bands can look similar and we look very carefully at these issues. Often people are indeed misled and make black and white judgments based on non-definitive data.

Since we look at many image processing aberration every day, we have a good sense of when problem are worth following up. In this case, we agree that the lines noted on PubPeer are not a significant issue based on the source data that you provided. However, in both the source data and the published figure the two bands are very similar indeed, including noise/background around the bands. There is a finite probability this is entirely by chance, but to make a clear call on this, we will need to assess higher resolution primary data.

Please also not that this is an important piece of data in the paper, hence our concern.

It is of course fine to wait until after Easter.

best wishes,

Bernd Pulverer

**Date: 13.04.2017: 18:09**

From: Lucia Altucci

To: Bernd Pulverer

Dear Bernd,

sure, I understand and, as previously stated, we will send the data.

I do know this is an important part and that is why we had also included the full image of the normalization in our reply, where the bands of MEF2D were also visible and were supporting the conclusions.

But, I understand your point and we will send the data.

Let me state: the reason why I do not do it right now is that I am not in the office and do not have the original files with me.

Best,  
Lucia

**Date: 18.04.2017: 17:58**

From: Lucia Altucci

To: Bernd Pulverer

Dear Bernd,

following our exchange of mails I send you the file of MEF2D bands at a higher resolution (300). These experiments have been performed in April 2006 and this is the resolution that I have.

While for this specific image, only digital acquisition was performed/found now, for the file of the tubulin (where you can still see the signal of MEF2D, on the same blot) we have also found the original film which we are ready to send you materially in support of the conclusions.

I appreciate your suggestions and inputs.

Best,

Lucia

**Date: 18.04.2017: 22:02**

From: Bernd Pulverer

To: Lucia Altucci

Dear Lucia,

thanks you for digging up this data. We will assess and get back shortly.

best wishes,

Bernd

EMBO Press communication with the institute

August 2017

**Date: 17.08.2017: 16:26**

From: Bernd Pulverer

To: Antimo Migliaccio

Dear Dr. Migliaccio,

We have consulted your institutional website and gather that any issues related to research integrity or quality assurance should be directly reported to you

<https://www.unina2.it/index.php/ateneo/organi/organismi/presidio-della-qualita-di-ateneo/assicurazione-della-qualita-della-ricerca>).

We are writing because we have detected significant potential aberrations in a paper from the laboratory of one of your colleagues, Lucia Altucci, who listed your institution as primary affiliation in the paper:

[Selective class II HDAC inhibitors impair myogenesis by modulating the stability and activity of HDAC-MEF2 complexes.](#)

**Nebbio** A, Manzo F, Miceli M, Conte M, Manente L, Baldi A, De Luca A, Rotili D, Valente S, Mai A, Usiello A, Gronemeyer H, **Altucci** L.

EMBO Rep. 2009 Jul;10(7):776-82. doi: 10.1038/embor.2009.88. Epub 2009 Jun 5.

It is our policy to report such matters to the relevant institution to ensure that response on this matter can be coordinated where possible. In the first instance, we would like to understand if your institute would plan an investigation of the matter.

We will send details once we can ascertain that you are the right contact for further discussion and I would therefore be grateful for your guidance in this matter.

Best wishes,

Bernd Pulverer

**Date: 17.08.2017: 17:43**

From: Antimo Miglaiccio

To: Bernd Pulverer

Dear Dr. Pulverer,

Thank you for your e-mail. I can confirm of being, as Department Director, the person in charge for the issues regarding the scientific integrity and quality assurance of our Department. Of course, I am ready to ensure any possible collaboration about this matter. I have also to say that I have been informed some months ago by Dr. Altucci about this controversial, raised by an anonymous report appeared in the PubPeer site. However, our Department is prepared to set up a deeper investigation on this question.

with my best regards,

Antimo Miglaiccio

**Date: 19.08.2017: 09:37**

From: Bernd Pulverer

To: Antimo Miglaiccio

Thank you very much for the rapid response. We attach a report from our image forensic analysis. I would like to note that since the aberrations we may have detected were rather surprising to us, we commissioned an independent expert in such analyses for a second opinion. This expert reported the same issues that were noted in our analysis.

We would be grateful, if you were able to share your opinion on the matter from the institutional vantage point. If you could let us know how long this will take and what level of analysis you intend to undertake, we can plan our response at the journal level. It is our duty to correct the scientific record in the most appropriate manner possible - this can range for a full retraction of a paper, to the addition of reliable source data or independent experimental results in form of a corrigendum or addendum. Where possible, we take into account the results of an institutional investigation if that is made available to us.

Best wishes,

Bernd Pulverer

EMBO Press; image forensic analysis of Nebbioso et al.  
(EMBO Rep. 2009 Jul;10(7):776-82)

In January 2017 we were alerted in a comment on PubPeer about potential splice lines in Figure 2B of a paper published in EMBO reports in 2009 by Lucia Altucci (corresponding author) and colleagues entitled "Selective class II HDAC inhibitors impair myogenesis by modulating the stability and activity of HDAC-MEF2 complexes", DOI 10.1038/embo.2009.88). We detected discontinuities in the background that are atypical of such data, visible between lanes 3 and 4 of the respective panel of Fig. 2 (MEF2D blot). We therefore asked the authors to provide source data for all the Western blots shown in the paper, which the authors provided without delay.

After a thorough analysis of the source data and a comparison with the published images, we came to the conclusion that intentional manipulations may have taken place in the generation of two of the figures of the paper (Figs. 2B and 2C) and possibly the source data related to these figure panels.

In detail, we observed significant differences between the provided source data and the published figure panels in Fig. 2B and 2C. Please refer to the attached file. Most strikingly, one band (lane 3 in Figure 2B, MEF2D blot) is likely to have been turned by 180° comparing the source data and the published figure. Further, the first MEF2D band and fourth MEF2D band in Fig. 2B are similar to a degree that is unlikely to be explained by chance (both in the source data, as well as in the published panel).

In the AcMEF2D blot of Fig. 2C, the IgG control lane in the published images looks significantly different to the source data provided by the authors (see the attached blot), suggesting that a part of a different image was spliced in.

Taken together, it seems likely that both images referred to above were put together using parts of images from different sources. These observations were also confirmed by a second expert analysis conducted independently of EMBO Press.

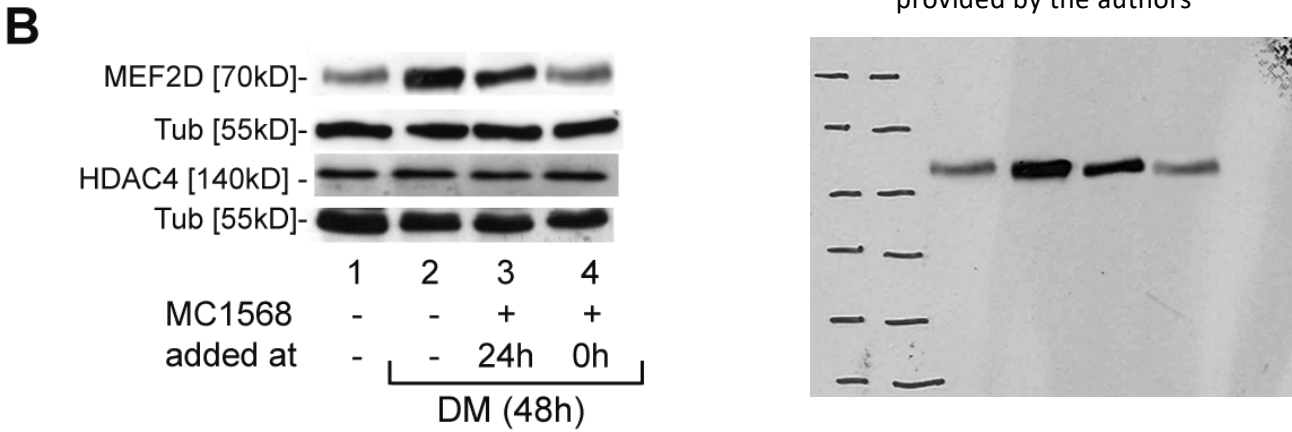


**Selective class II HDAC inhibitors impair myogenesis by modulating the stability and activity of HDAC-MEF2 complexes**

Angela Nebbioso, Fabio Manzo, Marco Miceli, Mariarosaria Conte, Lucrezia Manente, Alfonso Baldi, Antonio De Luca, Dante Rotili, Sergio Valente, Antonello Mai, Alessandro Usiello, Hinrich Gronemeyer, Lucia Altucci

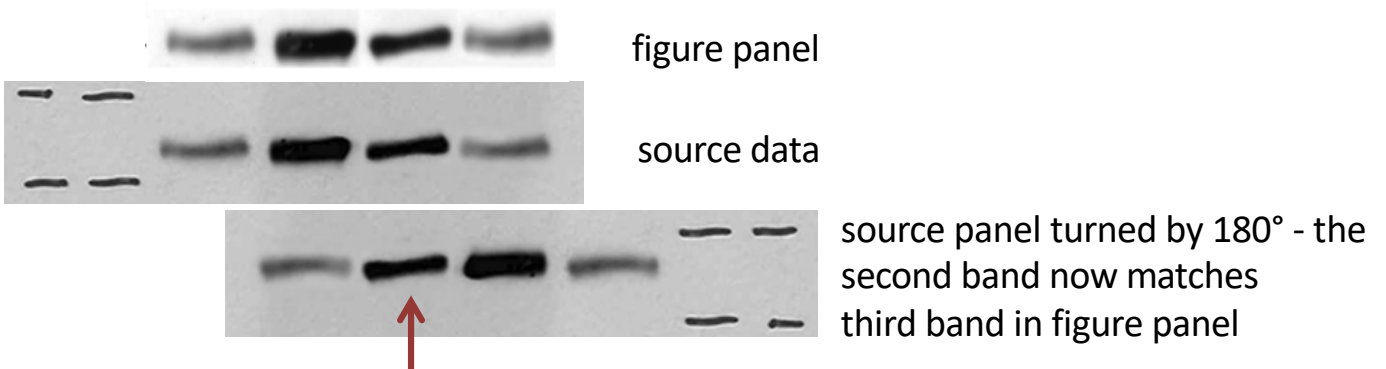
Fig. 2B, figure panel

Fig. 2B, MEF2D, source data provided by the authors

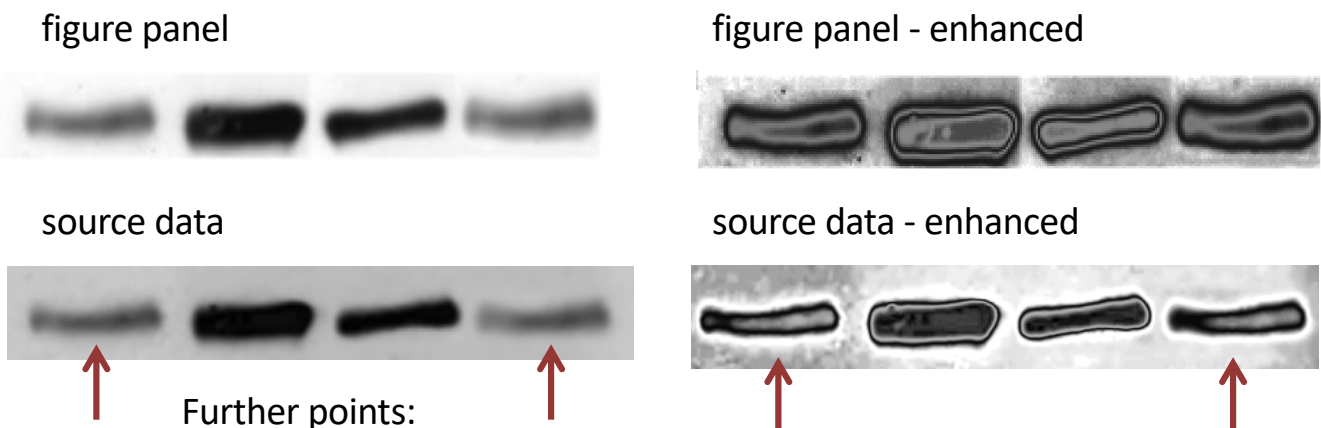


Issues with Fig, 2B:

1. Third MEF2D band in source data and figure panel is rotated (180°).



2. First MEF2D band and fourth MEF2D band in both the source data and the figure panel are too similar to be explained by chance. Further, potential splice lines are visible in the figure panel.



Further points:

- background in panel and source data next to bands is different
- space between bands is not even (also in the tubulin lane)

Fig. 2C, figure panel

enhanced contrasts

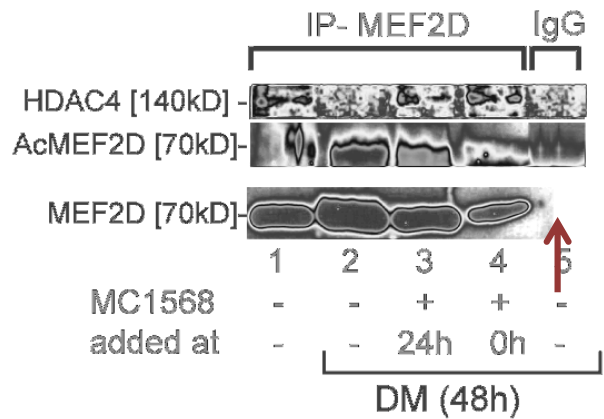
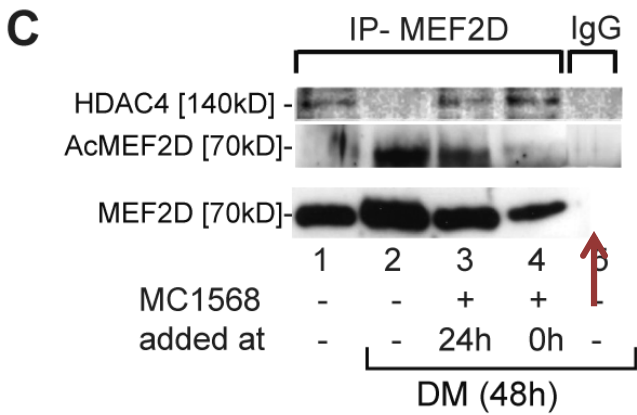


Fig. 2C, source data  
AcMEF2D

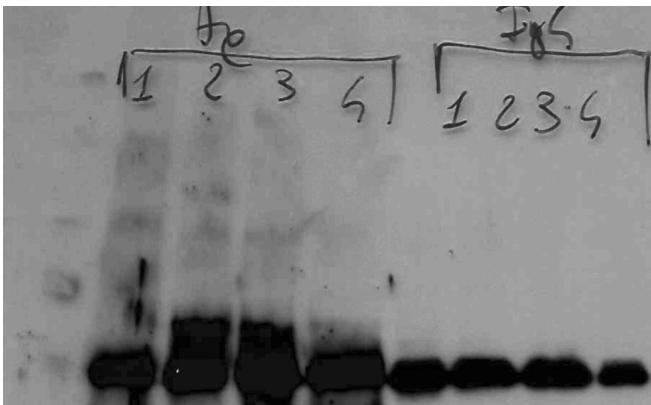


Fig. 2C, published AcMEF2D  
panel on top, source data below

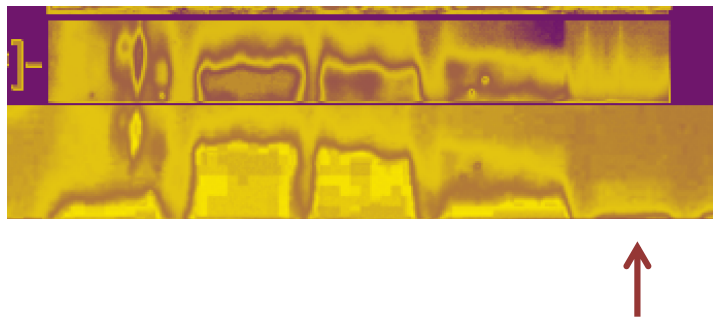


Fig. 2C, source data  
MEF2D



Issue with Fig. 2C:

→ IgG lane for the AcMEF2D panel differs from source data, splice line visible

minor issue:

→ IgG lane for the MEF2D panel cut off or never loaded

**Date: 21.08.2017: 17:06**

From: Antimo Miglaiccio

To: Bernd Pulverer

Dear Dr. Pulverer,

thank you very much for your prompt and exhaustive reply. I have already contacted Lucia Altucci. As I told in my previous mail, we discussed this issue a few months ago when the figure under debate appeared in the PubPeer site. I asked her to provide us with all raw data, the electronic images and, when possible, the auto-radiographies regarding the paper by Nebbioso et al, published in EMBO Report 2009. However, most of the experiments shown in the paper published in 2009 were performed between 2006 and 2007 and Fabio Manzo, the co-first author who cared for the assembly of several final images, left the Department more than five years ago. This makes understandably more difficult to collect the raw experimental results.

I plan to get all data Dr. Altucci will be able to provide. The images under discussion will be scanned at highest resolution possible and compared to published figure by some colleagues of ours with a good expertise in image processing. Shouldn't they reach unequivocal conclusions we will seek the advice by an outside professional. Of course, we will share with you all results. Anyway, Dr. Altucci has offered to repeat the experiments related to the data shown in these figures or allow replying these experiments by an independent investigator. I hope that this will help to dispel all doubts about the truthfulness of the findings reported in the paper.

To do this I need not less than twenty days, also in consideration that August is considered in Italy as a holyday time.

I thank you again and remain sincerely yours

Antimo

September 2017

**Date: 07.09.2017: 13:04**

From: Antimo Miglaiccio

To: Bernd Pulverer

Dear Dr. Pulverer,

we have thoroughly analyzed the images in the paper by Nebbioso, et al. EMBO Rep. 2009 Jul;10(7):776-82, and in particular those indicated for potential aberrations (substantially, the Fig. 2). To avoid any potential bias in evaluating these images we decided to submit them to an external professional expert, which has provided the attached report (in Italian with english translation) where he concludes that *"the supplied digital images and the original autoradiograph support material are compatible with the published figures for the above-mentioned images. With these elements it cannot be asserted that any image*

*manipulations have been performed*". However, to corroborate and strengthen the scientific reliability of the data and avoid any possible doubt, the experiment described in the figure 2B, which is object of the main concerns raised, has been reproduced "ex novo" under the same experimental conditions described in the paper. You will find attached the scan (I have personally made) of the autoradiograph showing the results of this experiment, which are substantially identical to those shown in the Fig. 2B of the EMBO Rep. paper. Of course, I can mail the autoradiograph if you need it. Further work is in progress to confirm the MEF2D immunoprecipitation experiment shown in Fig. 2C, but it will need still some time due to much larger cell amount required. We will send the results of these experiments as soon as possible. I sincerely hope that these data will address all your concerns about this article. I look forward to receive your reply and remain

sincerely yours,

Antimo Migliaccio

P.S. I am afraid that in my previous message have omitted one of attachments. Please accept my apologies

29 08 2017

Oggetto: Consulenza esterna per analisi di immagini Figura 2, lavoro scientifico: Selective class II HDAC inhibitors impair myogenesis by modulating the stability and activity of HDAC-MEF2 complexes. EMBO Rep. 2009 Jul,10(7):776-82. doi: 10.1038/embor.2009.88.

Riceviamo la precisa richiesta di analizzare le immagini di lavoro scientifico come determinato in oggetto. Nel dettaglio, la nostra ditta ha *expertise* in campo grafico, di design e di analisi d'immagine sia nell'assemblaggio che nella generazione di immagini e figure scientifiche con l'utilizzo di programmi di generazione ed analisi di immagine informatica. Dunque, ci si richiede di verificare la presenza di eventuali anomalie nella Figura 2 (pannelli B e C) del lavoro menzionato in oggetto.

Tale valutazione viene richiesta dal Dipartimento di Biochimica Biofisica e Patologia generale dell'Università della Campania 'Luigi Vanvitelli per corroborare, confermare o smentire i dati d'immagine presenti nella summenzionata figura 2 pannelli B e C.

I dati da paragonare ai dati del lavoro sono inseriti nel presente rapporto:

Figura 2B lavoro/Immagine da comparare

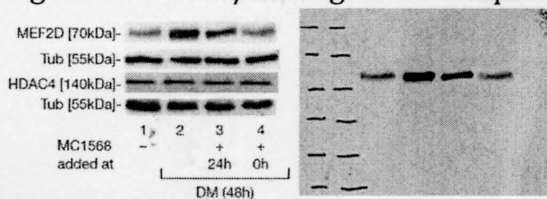
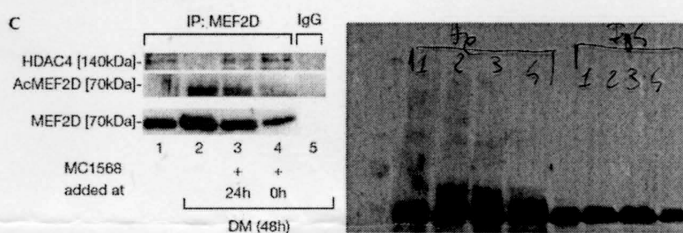


Figura 2C lavoro/ Immagine da comparare



In relazione alla figura 2 del lavoro in oggetto, ed in particolare alle immagini 2C e 2B i dati vengono analizzati per corrispondenza o duplicazione di pixels con software di analisi di immagini. I dati presi in esame sono i dati presenti in alcuni pannelli della figura 2B e 2C del lavoro pubblicato e citato in oggetto e confrontate alle immagini fornite dal committente per le bande MEF2D della Figura 2B e acMEF2D e MEF2D della Figura 2C.

Il committente indica che tali immagini si riferiscono ad esperimenti svolti nell'anno 2006 ed acquisite con supporto materiale o digitale all'epoca. Il committente indica altresì, di essere in possesso solo di alcune delle esposizioni prodotte all'epoca, essendo passati circa 11 anni.

Sul primo punto immagine Fig. 2B: MEF2D: non sono presenti duplicazioni di pixels. Viene fornita come fonte originaria un'immagine digitale. Si nota una somiglianza fra la prima (indicata come 1) e l'ultima banda (indicata come 4), ma non v'è corrispondenza di pixels o duplicazione o altre distorsioni (inversione, duplicazione).

In assenza del supporto di lastra autoradiografica, si accerta che l'immagine fornita dal committente è coerente con quella pubblicata. Nell'immagine pubblicata è presente un diverso contrasto, compatibile con l'uso di un'esposizione diversa dell'immagine o con un contrasto d'immagine digitale.

Per l'immagine 2C (bande per MEF2D-ac e MEF2D), mediante analisi di pixels, si reperta la perfetta corrispondenza delle prime 4 bande (da 1-4 acMEF2D, banda più alta). Lo spazio successivo alle bande, indicato come banda 5 mostra delle diversità, ma è compatibile con l'uso di una lastra diversa o anche con l'uso di un'esposizione digitale diversa. Infatti, l'immagine a paragone risulta più esposta di quella in Figura.

Per l'immagine della figura 2C relativa a MEF2D viene materialmente fornito il supporto autoradiografico originale che è, con assoluta certezza, quello utilizzato per assemblare la figura. Si ritiene pertanto che la mancanza della parte finale dell'immagine nel lavoro sia un mero errore di assemblaggio della figura e non una manipolazione.

In conclusione, le immagini digitali fornite ed il supporto autoradiografico originale materialmente fornito, sono compatibili con la figura pubblicata per ciò che concerne le immagini come sopra indicato.

Con i presenti elementi non è possibile affermare con assoluta certezza che siano state eseguite manipolazioni d'immagine.

Al contrario, è verosimile che le piccole differenze laddove repertate siano dovute a diverse esposizioni ed aloni presenti in esse.

We receive the precise request to analyze the images of a scientific work as determined in the object. In detail, given our expertise in graphics, design and image analysis both in assembly and in the generation of images and scientific figures with the use of computer image generation and analysis programs, you are asked to verify the presence of any possible anomalies in Figure 2 (panels B and C) of the mentioned article.

This evaluation is requested by the Dipartimento di Biochimica Biofisica e Patologia generale dell'Università della Campania 'Luigi Vanvitelli' to corroborate, confirm or deny the image data in the aforementioned Figure 2 (B, C).

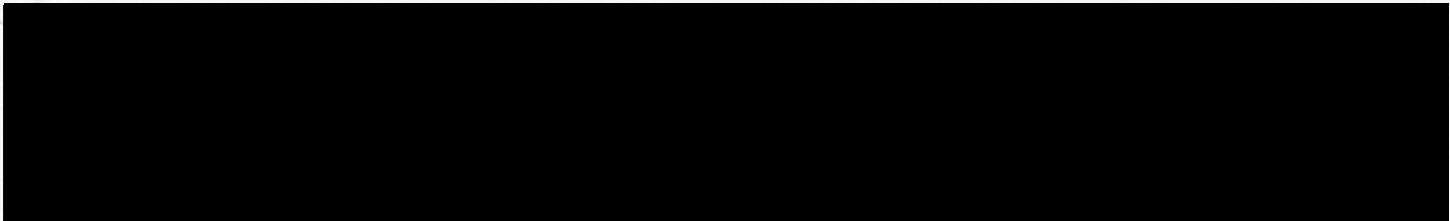
Data to be compared to figure 2 are included in this report (Italian part).

With reference to Figure 2 of the work in question, and in particular Figures 2C and 2B, the data are analyzed by matching or duplicating pixels with image analysis softwares.

In details, we are requested to analyse and look for potential aberrations.

The data examined are present in some of the panels shown in Figs. 2B and 2C of the published work. The data are compared to the images provided by the comittent for the MEF2D bands of FIG. 2B and acMEF2D and MEF2D of FIG. 2C.

The client indicates that these images refer to experiments carried out in 2006 and acquired with material or digital support at that time. The client also indicates to still have only some of the files produced at that time since it was about 11 years old.



On the first image point Fig. 2B. MEF2D: There are no duplications of pixels. A digital image is provided as the original source. A similarity between the first (indicated as 1) and the last band (indicated as 4) is identified, but there is no match of pixels or duplication/inversions or other distortions that can be further detected.

In the absence of autoradiography material support, we can state that the image provided by the customer is consistent with the published one. There is a different contrast in the image in the publication that seems compatible with the use of a different image exposure or with application of digital image contrast.

For image 2C (bands for MEF2D-ac and MEF2D), pixel analysis shows a perfect match for the first 4 bands (1-4 acMEF2D, upper bands). The band indicated as 5 shows some diversity between the published figure and the image given for reference, but looks potentially compatible with the use of a different material source or with the use of a different digital display (different exposition). Indeed the image provided looks more exposed than the one used in the Figure.

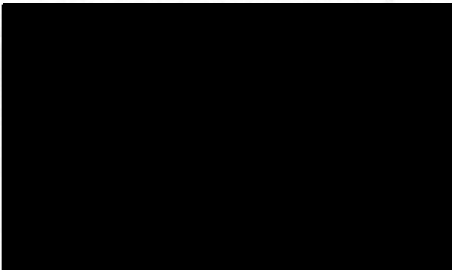
For the picture of Figure 2C for MEF2D, the original autoradiograph support is materially provided, which is, with absolute certainty, the one used to assemble the figure.

It is therefore considered that the lack of the final part of the image in the published work is a mere mistake of assembling the figure and not a manipulation.

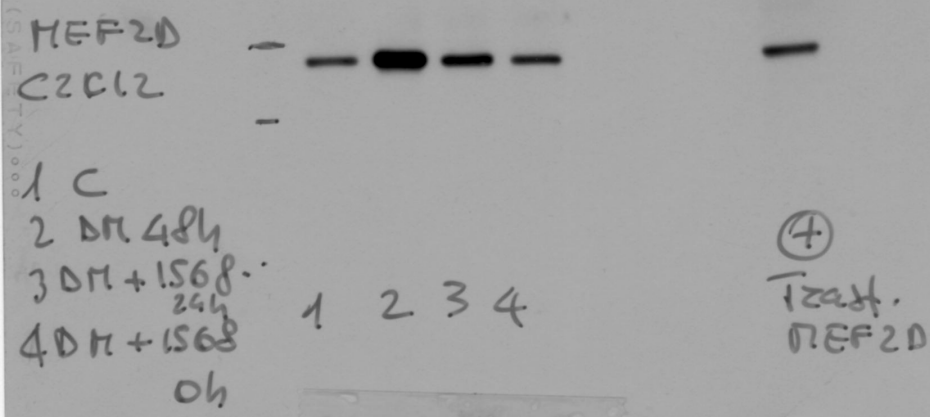
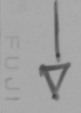
In conclusion, the supplied digital images and the original autoradiograph support material are compatible with the published figures for the above-mentioned images.

With these elements it cannot be asserted that any image manipulations have been performed.

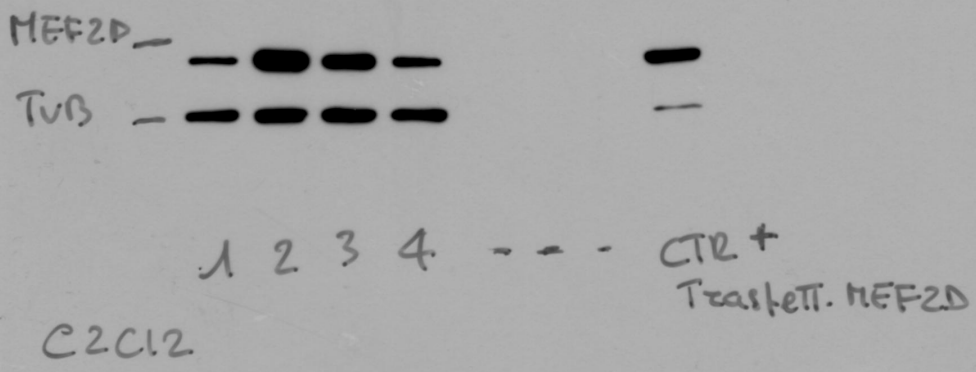
On the contrary, it is possible that the small differences that have been found are due to different exposures and different spots present in them.



31/8/17



01/09/2017



- 1 CTR.
- 2 DM 48h
- 3 DM + 1568 e 24h
- 4 DM + 1568 a 0h



**Date: 22.09.2017: 14:44**

From: Antimo Miglaiccio

To: Bernd Pulverer

Dear Dr. Pulverer,

just a few lines to inform you that immunoprecipitation experiments repeating those shown in Fig. 2C are ongoing and I hope to be able to show you them in the next week.

all the best,

Antimo Migliaccio

**Date: 26.09.2017: 10:32**

From: Antimo Miglaiccio

To: Bernd Pulverer

Dear Dr. Pulverer,

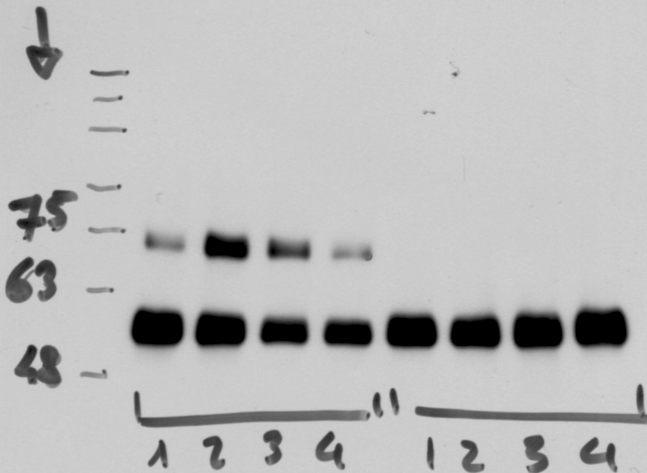
as I anticipated the last week, here is the radiographic sheet scan showing the proteins immuno-precipitated by MEF2DAb and probed with antiAc-Lys Abs. This was the experiment described in the Fig. 2C of the paper published in EMBO Rep. 2009 Jul;10(7):776-82. The results of the experiments carried out now are, in my opinion, completely superimposable to those shown in the aforementioned paper. These experiments have been repeated twice and, if you want, I can send you the original radiographic plates from the different experiments.

At this point, I have to say that, as far as I'm concerned, all the potential issues about the scientific integrity and reproducibility of these data have been addressed. It's now of paramount importance for me knowing your conclusions about this matter. If you prefer, we can also discuss the question by phone. However, I look forward to receive your reply

with my best wishes

Antimo Migliaccio

MARKER XL-OPN  
G-266



WB pan Ac  
lys

- 1 c
- 2 DM 4F
- 3 HL1568 at 20h DM
- 4 HL1568 at 0h DM

1 2 3 4    1 2 3 4  
IP NEF2D    IP IgG

C2C12 cells

22/9/17

**Date: 26.09.2017: 11:56**

From: Bernd Pulverer

To: Antimo Migliaccio

Dear Dr. Migliaccio,

Thank you for your updates dated 7th and 22nd September.

We have evaluated the formal image forensic analysis that you commissioned from an independent graphical design company. I am afraid that we cannot fully agree with the outcome of this analysis, which largely considered direct pixel duplications. This is of course not the only aspect to consider and in fact not the issue at hand in this case. The report apparently did not consider the arguments we made and I assume they did not in fact see our report as the major issues we raised remain unexplained, in particular that in two cases the provided source data does not fit to the published figure panels, suggesting that manipulations have been taken place during the preparation of the original manuscript.

We have noted your efforts to reproduce the data independently. This is certainly an important and meaningful exercise in order to understand the effect of these issues on scientific knowledge. However, the impact on the corrective measures that we will have to undertake to ensure the veracity of the published literature is only affected peripherally by whatever the outcome is: even if all the conclusions stand in a paper, but the reported experiments were manipulated in serious ways that undermine the confidence in the data published, we would need to conclude that the paper as published cannot stand. To put this more simply: if the reported results are presented in a flawed manner and no source data is available that support the veracity of the published data, we still need to act to correct that published data and we cannot simply replace flawed data with new data generated years later. Such data can of course still in principle be published separately.

Yours sincerely,

Bernd Pulverer

**Date: 26.09.2017: 12:23**

From: Antimo Migliaccio

To: Bernd Pulverer

Dear Dr. Pulverer,

thank you for your prompt and exhaustive reply. I assume that you have already checked my today mail with new data. I understand that this is not the point, as you are not satisfied with the conclusions of the forensic analysis by the independent graphical company I asked for. Actually I didn't submit your report to this company just to avoid any possible bias in evaluation and only requested an analysis for pixel duplication and any image manipulation. You have already read the report.

Now I can submit your report to this company and ask them their opinion and a reply of this analysis finalized to clarify specifically your issues. I would try to call you by phone to discuss with you this matter and find a coordinated solution if possible,  
I thank you again and remain,

sincerely yours

Antimo Migliaccio

**Date: 26.09.2017: 18:36**

From: Bernd Pulverer

To: Antimo Migliaccio

Dear Dr. Migliaccio,

Thank you for the rapid response. We will take a more detailed look at your findings. The journal will take its autonomous decision how to correct the paper, taking fully into account your institutional findings.

To summarize them, would it be accurate to state that you have independently repeated the experiments in question and conclude the results are reproducible. Furthermore you have commissioned independent advice that leads you to conclude that there is no evidence for any digital image manipulation.

Given this, you have not pursued an internal investigation to assess the cause of any manipulations.

We are certainly happy to follow up with a call, as it may clarify how we both view the issue. I am just returning from a trip and would be mostly available for the rest of the week (timeplan permitting)

best wishes,

Bernd Pulverer

**Date: 28.09.2017: 12:27**

From: Antimo Migliaccio

To: Bernd Pulverer

Dear Dr. Pulverer,

Thanks, once again, for your mail. Of course, I will wait with the greatest confidence for the journal autonomous decisions about the paper published in EMBO Rep. 2009 Jul;10(7):776-82. I am also sure that the Department findings and the external report will be taken in the proper consideration. In this regard I can officially confirm that:

- The new experiments shown were performed by an independent group under my supervision and the images were scanned by myself or one of my co-workers. Of course, all original materials (radiographic plates, filters, etc...) are available for mailing, upon your request. The experiments carried out so far fully corroborated the findings reported in Fig. 2B and 2C of the paper, supporting reproducibility of the data and scientific confidence.
- The questioned images have been analyzed by an external professional company with a recognized expertise in image processing. As an unusual similarity between some protein bands was highlighted and pointed out in the report that you sent to me, I specially asked this company to look for pixel duplications and any other potential image manipulations in addition to the correspondence between source data provided by Dr. Altucci and the published figures. In that occasion I didn't provide the company with your forensic analysis to ensure a completely independent and unbiased evaluation. The report by the analysts, I have sent to you, concluded that there is not any evidence for image manipulations. Therefore, I didn't find matter to pursue further investigation. Nevertheless, I am fully willing to submit again the images under discussion to the external experts together with your forensic image analysis, asking to specifically address the issues you raised, if you think that this is necessary.
- We got most of the raw data but we have to be aware that something could be missing, as, in some instances, we are dealing with experiments carried out in the mid 2006! Of note, the dates of the original experiments can be stated by the fact that, together with raw data, the original, hand-written lab protocols of that time have been also provided to me.

I will be more than happy if we can follow up with a call when you will find it appropriate. Please let me know at your earliest convenience, a couple of dates/times that would be fine for you.

All the best

Antimo Migliaccio

October 2017

**Date: 04.10.2017: 13:50**

From: Antimo Migliaccio

To: Bernd Pulverer

Dear Dr. Pulverer,

I am writing just to know whether you did receive my mail of 28 September that, at any rate, I copied below.

*"Dear Dr. Pulverer,*

*Thanks, once again, for your mail. Of course, I will wait with the greatest confidence for the journal autonomous decisions about the paper published in EMBO Rep. 2009 Jul;10(7):776-82. I am also sure that the Department findings and the external report will be taken in the proper consideration. In this regard I can officially confirm that:*

- *The new experiments shown were performed by an independent group under my supervision and the images were scanned by myself or one of my co-workers. Of course, all original materials (radiographic plates, filters, etc...) are available for mailing, upon your request. The experiments carried out so far fully corroborated the findings reported in Fig. 2B and 2C of the paper, supporting reproducibility of the data and scientific confidence.*
- *The questioned images have been analyzed by an external professional company with a recognized expertise in image processing. As an unusual similarity between some protein bands was highlighted and pointed out in the report that you sent to me, I specially asked this company to look for pixel duplications and any other potential image manipulations in addition to the correspondence between source data provided by Dr. Altucci and the published figures. In that occasion I didn't provide the company with your forensic analysis to ensure a completely independent and unbiased evaluation. The report by the analysts, I have sent to you, concluded that there is not any evidence for image manipulations. Therefore, I didn't find matter to pursue further investigation. Nevertheless, I am fully willing to submit again the images under discussion to the external experts together with your forensic image analysis, asking to specifically address the issues you raised, if you think that this is necessary.*
- *We got most of the raw data but we have to be aware that something could be missing, as, in some instances, we are dealing with experiments carried out in the mid 2006! Of note, the dates of the original experiments can be stated by the fact that, together with raw data, the original, hand-written lab protocols of that time have been also provided to me.*

*I will be more than happy if we can follow up with a call when you will find it appropriate. Please let me know at your earliest convenience, a couple of dates/times that would be fine for you.*

*All the best"*

I would also ask you whether you still think that a call could be useful and when I can contact you.

In the meanwhile I remain

Sincerely yours

Antimo Migliaccio

**Date: 05.10.2017: 22:20**

From: Bernd Pulverer

To: Antimo Migliaccio

Dear Dr. Migliaccio,

thank you for your note and apologies for the delayed response.

We would indeed find a discussion useful at this point. Would Monday at 3 or 4pm work for you?

I would aim to include my colleague Achim Breiling in this discussion.

Thank you for offering to share the lab-book pages relevant to the two experiments under discussion. If we could obtain scans, we would be grateful. We ask exceptionally for this as it is clear to us that either the figure 2c and / or the source data for 2C were manipulated. If it is both, this is in our view a much more serious problem.

Best wishes,

Bernd Pulverer

**Date: 06.10.2017: 19:00**

From: Antimo Migliaccio

To: Bernd Pulverer

Dear Dr. Pulverer,

it's me that I have to apologize for insistence. Maybe I am anxious, but our Department would clarify as soon as possible this situation. I am happy to discuss the matter with Dr. Breiling too. If you agree, Monday 9 October at 4 p. m. could be fine for me.

As I told in my previous mail, we got from Dr. Altucci all the raw data related to this paper and I am sending you the scans of the lab book pages concerning the experiments of Fig. 2C. These pages are written in Italian and also contain other data, which aren't related to this Figure. These experiments have been carried out in the early April of 2006. Let me know if you need a brief description in English. Concerning the Fig 2C, I would stress that I got the original digital scan of blot with Pan-Ac Ab of Anti-MEF2D IP from Dr. Altucci, which she already sent to you, This image was also uploaded in the digital archive of the Department. According the lab-book, the images from this blot were both captured from radiographic sheets and using a digital imager. In this regard Dr. Altucci previously told me of being not sure that the digital image sent to you was the same exposition used to prepare the figure of the paper. It is possible that differences between the figure and the original scan is due to different exposures or acquisition method. This possibility was also indicated in the report of the blind analysis performed by the external company I asked for the forensic evaluation.

I thank again and look forward to discuss with you on Monday.

Best Regards

Antimo Migliaccio



31/3/06

Germinati WB di ieri

Esempio 3 esposizioni + acquisizione digitale

Centate US37 -  $0.6,8 \times 10^5$  e patati e  $2 \times 10^5$  m3

fosfemi - 180 ul / fosfo

DNA modificato con bisolfite e pette PER x

RFR B2

DNA 32

buffa (10x) 2.52

DNTP 100ul 12

primer A 20ulM 2.52

primer B (11) 2.2

Taq 0.32

H<sub>2</sub>O 13.2  
252

84°C x 5'	84°C x 5'
84°C x 11'	84°C x 11'
57.5°C x 1'	52°C x 1'
72°C x 1'	72°C x 1'
72°C x 5'	72°C x 5'

x 35 aditi

primer M

primer U

03/04/06

2000g -> (1/2 x coupling e 1/2 x normale)

1) adanti 250 ul + 250 ul (v per 122 del vecchio protocollo)

2) Diff 48h 370ul + 130 ul

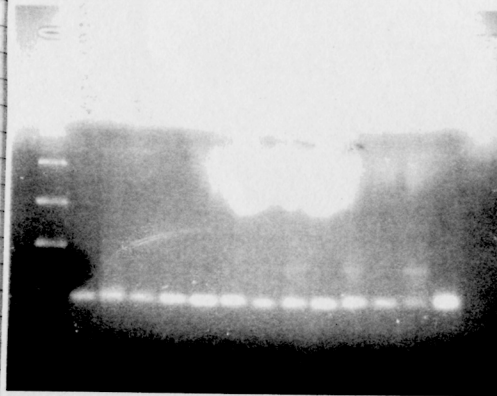
3) Diff 24h + 1568 o 24h 303 + 197 ul

4) Diff + 1568 o 48h 351 + 143 ul

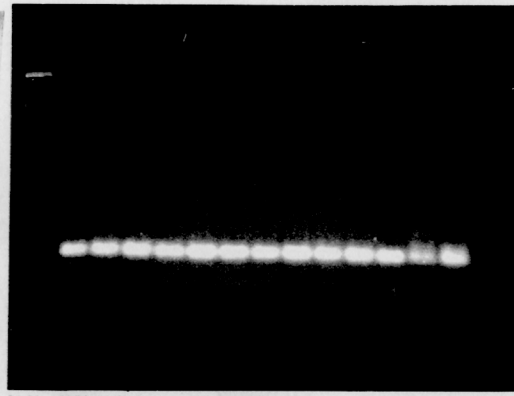
Mix TAP buffa -> 550 ul aux / sample  
note 4°C

U

M



31-Mar-2006 14:15:11 Low=0 High=4095 Gamma=1.0 Exposure = 0.467 se



31-Mar-2006 14:20:31 Low=0 High=4095 Gamma=1.0 Exposure = 0.339 secs

C sele Aze S 5000 5008

-> + MeJ 2D } 38/pto  
 IgG (mouse)  
 ↓  
 0.N 4°C su water

↳ protocollo normale  
 ↳ protocollo coupling

- Analisi FAES US37 Ch e 24h Sehe (campione tenuti in frigo x tutto we: Attenzione!!!)

04/04/06

- Fatti stops in Cep e SKBR3 e Consumati e -80°C

- Ip MeJ 2D e IgG

2h con A/G plus a 4°C (x met normale)

↳  
 lavaggi con TAP\* (5' each) (x i 2 met)

→ + sample buffer 2x

↓  
100°C x 3'

↓  
easy-eat gel

1) M  $\frac{1234}{Mef2D}$   $\frac{1235}{I94}$

2) M  $\frac{1234}{Mef2D}$   $\frac{1235}{I94}$   
coupling

Embete e 50V, course e 100V  
transblot 1h e 1/2 a 70V e 4°C  
inente con Mef2D e Aek 0,1N e 4°C

- Procediti US37 e eseguito protocollo esty  
MSPRARβ2. juno e proteina di 0,1N e 56°C

05/09/06

Esaminati WB di veri.

↓  
EEL

↓

diverse espressioni (lip di sub. pro vecchi!)

Eseguita anche acquisizione digitale

N.B. Cadute lastre in vasca dei liquidi  
Stressati! X seam pelle e che ne sporente

- MSPRARβ2 150 ul / pto dwell a  $3 \times 10^5$  / ml  
escheare juno - clorofurmo (in tubi con te  
e scelto) 5 ul + 5 ul di eshebo

Preparazione con Auk e 10M e Et-OH

↓

β 13000 rpm x 5'

↓  
pellet lavato con Et-OH 70%

↓

Resospension in H<sub>2</sub>O mediante e celle OD

Campione	260nm	280nm	260/280	OD 1/2
Ch	0.371	0.218	1.7	3.4
2	0.230	0.144	1.6	2
3	0.367	0.224	1.6	3.3
4	0.286	0.181	1.6	2.6
5	0.255	0.152	1.6	2.7

06/09/06

- Incubati filtri di veri β<sub>2</sub>β<sub>2</sub> con HDAC3 (106)  
(55 kDa)

↓

0,1N e 4°C

- Incubi 2 gels

1) M  $\frac{1234}{Mef2D}$   $\frac{1235}{I94}$

2) M  $\frac{1234}{Mef2D}$   $\frac{1235}{I94}$   
coupling

Embete e 50V, course e 100V  
transblot 1h e 1/2 a 70V e 4°C  
Inenteti con HDAC<sub>3</sub> (Sigma) 140 kDa

**Date: 09.10.2017: 17:33**

From: Antimo Migliaccio

To: Lucia Altucci

CC: Bernd Pulverer, Achim Breiling

Dear Lucia,

Today I have thoroughly discussed with Drs. Pulverer and Breiling the issues concerning some images of the paper by Nebbioso, et al. published in EMBO Rep. 2009 Jul;10(7):776-82. They would like discuss with you for further clarifications. So I am kindly asking you to contact them at your early convenience.

Thanks a lot

Antimo

November 2017

**Date: 02.11.2017: 13:48**

From: Antimo Migliaccio

To: Bernd Pulverer, Achim Breiling

Dear Drs Pulverer and Breiling,

I received some days ago the second analytic report about the well-known paper by Nebbioso et al. (EMBO Rep. 10, 776-82). In this report the professional company I commissioned for the image analysis, specifically addresses the questions you raised as I had this time shown to them your original forensic report. I would like share it with you, hoping that it could be useful. Anyway, please don't hesitate to contact me if you think that I can be still of help.

All the Best

Antimo Migliaccio

22 October 2017

Object: Second external consulence for analysis of images in Figure 2  
EMBO Rep. 2009 Jul;10(7):776-82. doi: 10.1038/embor.2009.88.

As a second consulence, we are asked by the Dipartimento di Biochimica Biofisica e Patologia generale to specifically comment on the points raised by a different source whose identity we are not aware of.

In details, we are requested to re-analyse these specific points:

1. Fig 2B. Potential rotation of the third band of 180°

We have deeply re-analysed this point. We have applied both pixel identity analyses as well as background comparison. The differences are compatible with a different exposition or contrast used. We have particularly analysed the third band comparing the figure data with the source got from the Department. In this case we have applied a deep pixel-matching analyses to better define a potential rotation/background. There is a different contrast in the image that is compatible with the use of a different image exposure or with a digital image contrast. This also applies for the similarity of band 1 and 4 as previously stated by us. It is still our conclusion that the image provided by the customer is consistent with the published one.

2. Fig 2C. The band 5 shows diversity, but is compatible with the use of a different source or with the use of a different digital display as stated previously

Since we also got the original lab-book (scanned) we could definitely read that different images were taken in 2006 and that problems occurred in some acquisition since this is reported in the lab-book.

In conclusion, the images are compatible with the published figures for the above-mentioned images. With these elements it cannot be asserted that image manipulations have been intentionally performed. It is, however, possible that the small differences that have been detected are due to different exposures and different spots present in them.

**Date: 05.11.2017: 22:57**

From: Bernd Pulverer

To: Antimo Migliaccio

Dear Dr. Migliaccio,

Thank you for sending this follow-up analysis by the graphics company that you commissioned to analyze the images.

We struggle to follow the logic in this report, as three of the 4 bands are apparently identical between the figure panel and the source data, while the third band is not. It is simply very hard to understand how this could happen - especially also taking into account the similarity between bands 1 and 4 as well the similarity between a 180o rotation of band 3.

The report also reminds me to emphasize that the interview with Dr. Manzo is also essential to try to uncover the reasons for the entry in his labbook at the time that there were 'some problems' with the image acquisitions in the 2006 images. Maybe this will help understand the reason for these apparent aberrations.

best wishes,

Bernd Pulverer

**Date: 05.11.2017: 22:48**

From: Bernd Pulverer

To: Antimo Migliaccio

Dear Dr. Migliaccio,

Last week we had a constructive Skype-conversation with Dr. Altucci. We agreed that the best way to proceed would be that we (EMBO press) directly analyse the original source data that is still available. Dr. Altucci stated that for the MEF2D panel shown in Fig. 2B in the paper published in EMBO reports no original primary data (i.e. exposed autoradiographs) is available, despite her best efforts to locate it. However, she mentioned that such data would be available for a replicate experiment performed around the same time in 2006). She also indicated that a blot probed for tubulin (the loading control) would be available that shows residual signals of MEF2D (after stripping). Dr. Altucci noted that you were in possession of all these files at this time. We would therefore kindly ask you to send us these films by registered post.

Regarding Figure 2C, it seems that the autoradiographs that had been used for the assembly of the published figure are available for AcMEF2D and MEF2D. We therefore would ask you to send us also these two originals films.

Should you have additional original exposures related to both figure panels, also of replicates done at the time of the original experiments, we would be grateful to be able to view these also.

We will of course reimburse you for the sending costs and return the files as soon as possible. He hope this will be a useful exercise as we cannot follow the arguments made based on the low resolution scans provided.

Finally, we advised Dr. Altucci to invite the co-author who apparently performed these experiments (Fabio Manzo) to comment on these experiments in light of the apparent image aberrations. We do not intend to influence institute internal process, but it may be advisable for all concerned if this interview is carried out in your presence or by yourself alone. A written statement by Dr. Manzo about these experiments, or a report on the conversation by you, could be very useful in our deliberations.

It looks like this is all the information we will be able to obtain and we will make a final decision with all this in hand. As discussed previously, this can in principle be an editorial note of concern, a correction, a partial retraction of the figure panels in question, or indeed in principle a full retraction of the whole paper.

best wishes,

Bernd Pulverer

**Date: 10.11.2017: 17:45**

From: Antimo Migliaccio

To: Bernd Pulverer

Dear Dr. Pulverer,

I will send the original films monday morning. Have a good WE.

Antimo



Bernd Pulverer, Ph.D.  
Chief Editor | The EMBO Journal  
Head | Scientific Publications  
EMBO  
Meyerhofstrasse 1  
D-69117 Heidelberg

Naples, 10 November 2017

Dear Dr. Pulverer

as you kindly asked, I am sending the films related to Figs. 2B and 2C of the paper by Nebbioso, et al. published in EMBO Rep. 2009 Jul; 10(7):776-82. Therefore, you will find enclosed 5 numbered autoradiography films. More in detail:

1. (05/04/2006) corresponds to exp. shown in Fig 2C Ip-MEF2D probed for MEF2D.
2. (06/04/2006) WB using anti-tubulin Abs, used for normalization of Fig 2B. The signals of the previous blotted MEF2D are still detectable after stripping and correspond to the MEF2D shown in Fig 2B.
3. (08/03/2006) experiment of IP MEF2D on C2C12 cells probed with ac-lysines to detect MEF2D-ac. The experiment is basically a duplicate of the experiment shown in Fig 2C, not used for any figure.
4. (08/06/06) WB of MEF2D on C2C12 cells as in Fig2B. This experiment substantially replicates the exp. shown in Fig. 2B. This kind of experiments was repeated different times to control the efficacy of the various lots of MC 1568, which was not commercially available at that time. In fact, it is reported that the blot was performed as a control of the activity of a new lot of MC1568. The first 4 are basically a duplicate of Fig2B MEF2D signal. From band 5 to 8 only MC1568 (no differentiation protocol) was used as indicated.
5. (09/06/2006) western blot for tubulin as normalization for the MEF2D signal shown in film (4).

In addition, I would inform you that two days ago I met Dr Fabio Manzo, which is one of the main co-Authors of the paper, and interviewed him about the experiments under discussion. Unfortunately, after almost 10 year from paper publication and more than 11 after these experiments were carried out he was unable even to remember who performed them, and only answered verbatim: *"It's been 10 years and at the moment I have difficulty remembering who did the experiment or editing the image. Anyway, I assure you that in no*



*case has any mystification or alteration of experiments or images been made, since it is not in my person or in the people of the group I worked with in doing so".*

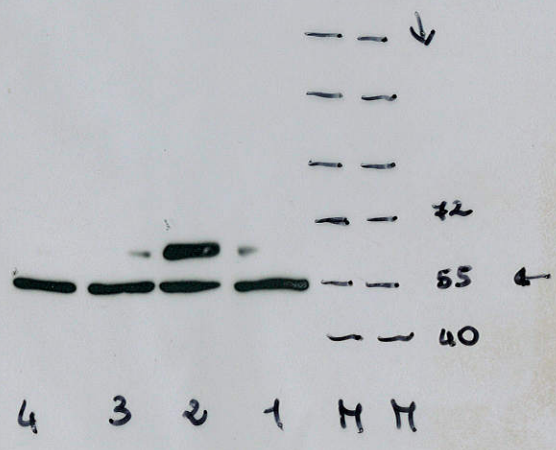
I hope that your analysis will dispel all the doubts about this question. I only would kindly ask you to inform me about the autonomous editorial decisions before you take any eventual action.

My best wishes

Artimo Migliaccio, M. D.  
Director of the Department



6/04/06  
2-Tubuline  
(Met 2D)



1

coupling

5/04/06

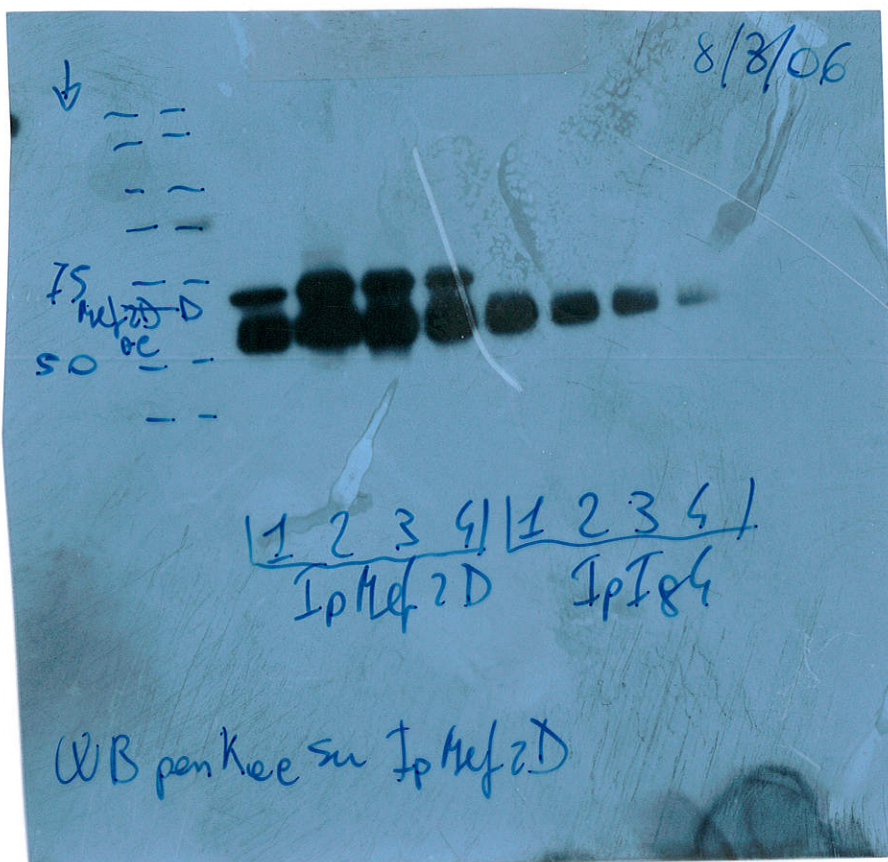
IP e<sub>2</sub>-e<sub>12</sub>

2D				I <sub>8</sub> C			
1	2	3	4	1	2	3	4



↳ coupling

3



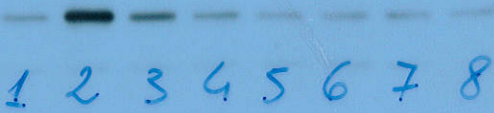
↗

4

- 1 = C
- 2 = DM
- 3 = DM + 1568 @ 24h
- 4 = DM + 1568
- 5 = 1568
- 6 = 1568 48
- 7 = DM + 1568 48
- 8 = )

Navolato 1568  
de testare

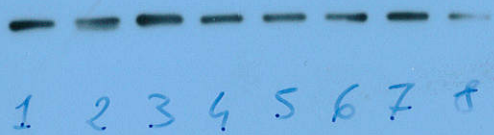
MeFeD



8/6/06

5

Tub



8/6/06

**Date: 11.11.2017: 01:42**

From: Bernd Pulverer

To: Antimo Migliaccio

Dear Antimo,

thank you for the update. We hope this will help bring all of us to a conclusion. Has there been any progress with contacting the first author for his statement?

best wishes,

Bernd

**Date: 11.11.2017: 18:31**

From: Antimo Migliaccio

To: Bernd Pulverer

Dear Bernd,

thank you so much for your mail. Indeed, I talked twice in this week with Fabio Manzo. He said that after so much time (he left from our Department in 2011) can not even remember what experiments he did personally. He has only strongly asserted that he and people working with him at that time never did manipulations of data or images and the experiments were carried out exactly as described in the lab book (of which I sent to you a few pages). In my letter you will find the literary text (translated in english) of his declaration. I sincerely hope that image analysis you can perform will allow an exhaustive conclusion, satisfactory for all of us.

All the best

Antimo

[January 2018](#)

**Date: 03.01.2019: 18:02**

From: Bernd Pulverer, Achim Breiling

To: Antimo Migliaccio

Dear Antimo,

We have reviewed all the information provided and will return these files by post. We decided that a 'partial retraction' is the most effective way to correct the literature at this point.

We share the attached draft text first with you in order to clarify your suggestions for the reference to your institutional investigation and the replicated experiments, which we

understand were carried out under your own supervision. We will contact the authors directly as soon as we hear back from you. We would prefer if this communication with the authors is run directly through the editorial office and hope you agree to this process.

Since additional papers by these authors published elsewhere also show potential aberrations, we would also be interested to learn more about the status of these papers, within the bounds of confidentiality.

Best wishes for 2018.

Bernd and Achim

**Date: 09.01.2018: 16:45**

From: Antimo Migliaccio

To: Bernd Pulverer, Achim Breiling

Dear Bernd and Achim,

First of all, let me wish you all the best for the New Year. I am honoured that you wanted share with me the draft text of “corrigendum” that you are going to publish. Although I fully understand your point of view, I have to confess that it is rather hard for me accepting, in this case, the concept of retraction, as I know, having repeated the experiments, that the findings reported in the paper are highly reproducible and reliable. Most important, these results, as far as I know, have been confirmed in several laboratories worldwide. The trustworthiness of the scientific data coming out from our Department is for me a key point, which I would be made clear. Needless to say, I cannot nor will pretend to interfere in any way with the Journal autonomous determinations. I am returning attached your draft with a few comments that, hopefully, will help to summarize my thoughts.

As concerns the other papers by these authors published elsewhere indicated for potential aberrations, if you are referring to the PubPeer report, I am generally disgusted by this kind of anonymous delations. In my opinion, anyone who raises suspicions of scientific misconduct should take responsibility for his allegations, which badly affect the reputation not only of the Authors but also of the Reviewers and Journals that allowed the publication. Therefore, we will act in this regard only if and when concerned Journal Editors will advance doubts about the published papers. So far we haven't received any warning by any of them.

Regarding our institutional position, as I told in my previous mail, we have decided to not proceed further with this case, as we were unable to find any evidence of aberrant behaviors by Dr. Altucci and her group. I also feel the duty of specifying that my group and Altucci group started collaboration a few months ago. This, obviously, did not and never will affect my institutional duties.

I have been more than gratified to collaborate with you and hope of having met your expectations.

Your faithfully,

Antimo

**Date: 09.01.2017: 11:44**

From: Bernd Pulverer

To: Antimo Migliaccio

Dear Antimo,

We will contact Dr. Altucci and co-authors with the appended text now so that we can address this in a timely manner.

Best wishes,

Bernd

**Date: 14.01.2018: 14:52**

From: Bernd Pulverer, Achim Breiling

To: Antimo Migliaccio

Dear Antimo,

Thank you for your letter. We sympathise with your comments, but please consider the arguments we shared previously: the issue at hand is the data within the paper we published - we are doing what we consider necessary to correct the scientific record, which is centred on the data in the paper, not merely the conclusions made.

On a general note, not related to this paper, figures in research papers cannot become caricatures of rigorous experimental evidence, nor do they serve merely an illustrative purpose to provide some visual support to an author's conclusions: the data are the core of the research paper. I am putting this in slightly glaring language, as we see so very many examples, where proper data documentation is not taken seriously - through lack of training, sloppiness, rushed publication, or worse.

In this case, we simply do not have sufficient confidence that the published panels are legitimate - we have shared all the arguments before. We are NOT questioning the conclusions. This is important and was certainly taken into account when we decided not to retract the whole paper in this case.

Regarding the issue of retracted vs. withdrawn: thank you for raising this. We have a clear functional definition of both terms: retracted means in our terminology the decision of the journal to remove a piece of data or a whole paper from the scientific record due to an infringement of research ethics or scientific integrity. If an author requests deletion due to a mistake or oversight on their part, we want to show this by using the term withdrawn (please see <http://emboj.embopress.org/content/34/22/2721> for a more detailed

discussion). Since we are requesting this selective removal of data based on likely image integrity issues, we would formally label it 'retracted'.

(NB: The figure I added was from another paper where we had a 'withdrawal' - I am sorry that I missed the inconsistency).

Regarding your 'comment 1' on 'discrepancy': the image looks identical, apart from a single band. We think the word 'discrepancy' is correct here. This is unlikely to be an image based on another experiment done in parallel at the same time.

Regarding comment 2: This is the stripped and re-probed (for the loading control tubulin) blot, that the authors also sent us, claiming to show the remaining signals of MEF2D, which match to the signals seen in the aberrant blot (panel 2B - MEF2D). Is your point that the residual signals fit to those in the panel shown in the paper (in terms of intensity), or just that signals in lanes 3 and 4 are clearly reduced compared to lane 2 (which is the major message of panel 2B)? Either way, this hints that the conclusions may be true (as confirmed also by the replicates you provided), but it does not explain what happened to the panel shown in the paper, and also does not explain why panel 2B does not match to the source data provided (and why one band may have been rotated 180 degrees). It is also surprising that of the nearly identical bands 1 and 4, nothing is left after stripping of band 4, but we have signal left of band 1. We cannot in our view be certain that this stripped blot fits to the panel shown.

Best wishes,

Bernd and Achim

**Date: 16.01.2018: 17:35**

From: Antimo Migliaccio

To: Bernd Pulverer, Achim Breiling

Dear Bernd and Achim,

thanks for your kind letter. As I previously told, I cannot but comply, in all respects, with the independent Journal decision. However, there are some perplexities left on my side that, obviously, will remain between us. In the "corrigendum" you clearly indicate that, in the panel 2B, the band 1 and 4 are the "nearly identical" and another band (band 3) has been spliced and rotated by 180 degrees. This suggests that in the first case (bands 1 and 4) we would deal with an example of "band cloning", in the second one (band 3) with a "cut and paste" operation. Both these image manipulations are, in general, easily recognizable. As you know, I commissioned an external analysis performed twice by a private company. I would stress that I haven't known this company before and it was recommended it to me as a renowned team with expertise in using and detecting image manipulations. They usually deal with artistic reproduction of drawings and paintings. The analysts concluded that it couldn't be asserted that image manipulations were performed and even when I have shown the results of your forensic analysis they reiterated that no manipulations were detectable. Should I think that they did lie to me?

Another question that remain unclear to me is the motive of manipulating images to represent “true” results, but I wouldn’t bore you with issues we have previously and repeatedly discussed. Rather, the collaboration Authors provided throughout all the steps of this investigation, as you too acknowledged in our correspondence, makes me trust in their good faith. These were the reasons why it seemed to me reasonable to ask the Authors to withdraw the figures in question rather than subject them to the “humiliation” of a retraction. I would be happy to get your reply in this regard. Needless to say, I would be extremely grateful, also on behalf of my Institution, if you could consider such a possibility.

Anyway, I am happy that this question has come to an end and am very grateful for your smooth collaboration.

All the best

Antimo

June 2018

**Date: 21.06.2018: 15:44**

From: Achim Breiling

To: Antimo Migliaccio

Dear Antimo,

Please accept my sincere apologies for coming back to you regarding this issue belatedly. We have now discussed the issue again, and we have decided that a partial retraction is the only way to proceed (as already explained by Bernd in a previous message).

Please find attached the revised version of the corrigendum. We need to clarify suggestions for the reference to your institutional investigation (which we feel is mandatory), and the replicated experiments.

Please let me know if you can provide a link to an official documentation of the institutional investigation, and/or to a figure showing the replicate experiments. The latter we can also discuss directly with Dr. Altucci. I will contact her directly as soon as I hear back from you.

Yours sincerely,

Achim



**Date: 22.06.2018: 17:48**

From: Antimo Migliaccio

To: Achim Breiling

Dear Achim,

thank you very much for your e-mail. No need for apologies. In Naples we say that late is always much better than never...However, I acknowledge your decision, which is essentially the same as Bernd had outlined. Of course, also my considerations remain the same, needless to reply. I would only be sure of having correctly understood your requests. If I am right, you are asking to post the figures derived from replicate experiments performed at the same time as the published data for both panels, with related description and methods section, on Figshare or Dryad. Furthermore, you request that the images from the experiments corresponding to the ones shown in figures 2B and 2C of the original publication performed recently by independent institutional investigators under my supervision will be placed in a permanent repository. In this regard I am wondering whether I could also use Figshare or Dryad. Alternatively we could create a link to the Department website. If this completely satisfy your needs I would be ready to proceed in this way.

Yet, on my side, I have to inform you that, having discussed this morning about your decision with the Chancellor (Rector) of our University, who was already informed by Dr. Altucci from the beginning of this history, he has decided to take directly the responsibility of dealing with this issue. Therefore, I kindly ask you to contact him at the address [rettorato@unicampania.it](mailto:rettorato@unicampania.it). However, I remain at your disposal for any further question.

All the best

Antimo

**Date: 25.06.2018: 11:30**

From: Achim Breiling

To: Antimo Migliaccio

Dear Antimo,

Thanks for the quick reply. Most importantly, we request that the recent replicate experiments directed by you are posted/deposited by you in a permanent repository. We would prefer a deposition at Figshare or Dryad, not at the department website. This should contain all necessary information (authors, methods, description of results) to qualify for a stand alone piece of data.

We will offer to Dr. Altucci to do the same for her replicates performed at the same time as the published data. However, this would be up to her, and not requested by us.

If you are positive about the deposition of the recent data as indicated above, we will proceed contacting Dr. Altucci and the Chancellor.

Kind regards,

Achim

**Date: 25.06.2018: 18:36**

From: Antimo Migliaccio

To: Achim Breiling

Dear Achim,

thanks for your e-mail. I can confirm I'm ready to archive data on Dryad or Figshare. However, since the Rector has decided to take charge of this matter directly, I cannot do anything without his authorization. Therefore I must ask you to contact him ([rettorato@unicampania.it](mailto:rettorato@unicampania.it)) before anything else. I thank you sincerely for your cooperation.

My best wishes,

Antimo

## Escalation to the Rector of University of Campania "L. Vanvitelli"

July 2018

**Date: 02.07.2018: 13:53**

From: Achim Breiling

To: Guisepe Paolisso

CC: Bernd Pulverer

Dear Prof. Paolisso,

I am contacting you regarding a case of potential image aberrations in a paper from the laboratory of one of your colleagues, Lucia Altucci. We have discussed the case in detail with Prof. Antimo Migliaccio, head of the department, who told us that you already have been informed about the case. Prof. Migliaccio indicated that he would require your authorization in order to proceed.

Please find attached our letter detailing the case and indicating our decision, and a draft text of the corrigendum we plan to publish.

Yours sincerely,

Achim Breiling

Editor

EMBO reports

PS: This note is in CC to the head of scientific publications and acting chief editor of EMBO Reports, Dr. Bernd Pulverer.

*Dear Prof. Paolisso,*

*I am writing to you because we have detected significant potential aberrations in a paper from the laboratory of one of your colleagues, Lucia Altucci (affiliated to the University of Campania "L. Vanvitelli"), in the paper:*

*Selective class II HDAC inhibitors impair myogenesis by modulating the stability and activity of HDAC-MEF2 complexes.*

*Nebbioso A, Manzo F, Miceli M, Conte M, Manente L, Baldi A, De Luca A, Rotili D, Valente S, Mai A, Usiello A, Gronemeyer H, Altucci L.*

*EMBO Rep. 2009 Jul;10(7):776-82. doi: 10.1038/embor.2009.88.*

*It is our policy to report such matters to the relevant institution to ensure that response on this matter can be coordinated where possible. We had therefore contacted Prof. Antimo Migliaccio, as head of the department, who then undertook an institutional investigation. Prof. Migliaccio told us, that he and Dr. Altucci have informed you about the case already.*

*We have now decided that a partial retraction is the appropriate way to proceed in this case, and have communicated this to Prof. Migliaccio (as an alternative, we would agree to a full retraction, if the University decided that was more appropriate in this specific case. It is our policy to coordinate with institutional investigations wherever possible). Please find attached the text of the corrigendum we plan to publish.*

*Most importantly, we request that the recent replicate experiments directed by Prof. Migliaccio are posted/deposited in a permanent repository (Figshare or Dryad), which is then linked to the published corrigendum. This should contain all necessary information (authors, methods, description of results) to qualify for a stand-alone published unit of fully documented, reproducible research. Prof. Migliaccio has agreed to do this. We note that Prof. Migliaccio indicated that he would require your authorization in order to proceed.*

*We will offer to Dr. Altucci to do the same for her replicate experiments performed at the same time as the published data. However, this would be up to her, and not a formal requirement from us. The reason is that we have no means to formally ensure that data are authentic and unmodified. I will discuss this directly with Dr. Altucci as soon as I hear back from you.*

*Yours sincerely,*

*Achim Breiling*

**Date: 18.07.2018: 11:58**

From: Guiseppe Paolisso

To: Achim Breiling

Letter from the rector

Dr. Achim Breiling, PhD,  
Editor  
EMBO reports  
Meyerhofstrasse 1  
Heidelberg 69117  
Germany

18 July 2018

Dear Dr. Breiling,

I recently got your message regarding the paper by Nebbioso et al (Embo Rep 2009) and I spent a noteworthy part of the time since your message to analyze the disappointing story about the paper by Nebbioso *et al.* describing experiments mostly performed between 2006 and 2008.

It is more than 10 years since these experiments were carried out that somebody in anonymous way raised some concerns. Please let me know why “anonymous” source could raise such concerns after so huge time. In other words, I am wondering how long one should await before to be out of such problem, and if you (the Journal) take into account that concerns moved after such long time from data publication might also and mainly hide aims different than the scientific ones, especially if not demonstrated as in this case. Notwithstanding, I understand that we are dealing with the scientific need of the journal to make clear a “gray zone” of a previous published scientific paper. Please also note that our policy requires that original data of research must be stored for five years from publication date, otherwise we should use whole buildings to keep all original results (especially those that are not – or not yet – digitalized) of our researchers for decades. Nevertheless, I got the original images still available and all the materials concerning the case from Prof. Migliaccio, Head of Department, who initially dealt with this question. Of course, I have in my hands your “forensic analysis” of images together with your comments that, I feel, show a deep discrepancy between the preliminary statements and the conclusions.

But let just analyze the scientific facts:

More than a year ago (December 2016) an anonymous source (PubPeer?!) raised some concerns about some images from panel B of the figure 2.

Concerning the Fig. 2B, at eye examination, as far I can evaluate even enlarging the image at max, the panel concerning the MEF2D blot looks absolutely consistent with the source data provided by Altucci. Your analysis claim that *“the first MEF2D band and fourth MEF2D band in Fig. 2B are similar to a degree that is unlikely to be explained by chance (both in the source data, as well as in the published panel).* On the other hand, I got a different analysis, provided by Migliaccio, who commissioned a new investigation to an external independent company concluding: *“it cannot be asserted that any image manipulations have been performed”.* Which one of two conclusions is reliable and why? Does an officially certified method exist allowing to definitely establish whether these images have been manipulated or not? The similarity degree between lane 1 and lane 4 is *“unlikely”* or *“impossible”* to be explained by chance?

I would rather make the point on the veracity of these images. Prof. Migliaccio gave to me the scans of some unpublished experiments, actually in your hands, performed at the time of those represented in Fig. 2. From the image scans I can judge that the authors have reproduced these data completely and repeatedly at the time of those investigations. Furthermore, an x-ray film of the experiment shown in Fig. 2B, should be physically in your hands: this experiment corresponds to western-blot with anti-tubulin Abs for band normalization, where is present the “residual MEF2D signal” after stripping of the previous probe. I have carefully examined the scan of this film, which is, to my view, absolutely consistent with the data published and all other data.

Concerning the Fig. 2C, it seems to me that images represented in the middle and lower panels of this Figure are perfectly corresponding to source data. I am not able to discuss about the background discontinuities you mention but I can refer to image analysis commissioned by Prof. Migliaccio, which states “band 5 shows diversity but is compatible with a different source (a different exposure, I presume) or use of a different digital display”. Once again, in my opinion, only the reliability of the data can drive the final conclusion. In this regard, I see the scan of an X-ray film (that should also be in your hands) showing a WB with pan anti-K-Ac Abs carried out in 2006 (the same exp. of Fig. 2C) with a pattern superimposable to that shown in the paper.

In addition, Prof. Migliaccio has reported that both experiments shown in Fig. 2B and 2C have been reproduced the last summer from independent researchers under his control and supervision. He showed the results of these experiments fully confirming the validity of all the data.

Finally, before drawing conclusions and due to the fact that almost 10 years have been elapsed since Nebbioso et al published their paper, analyses of literature should help to understand the problem dimension. So far, the work by Nebbioso *et al* has been cited by 66 times (48 articles; 14 reviews discussing mainly the association between class II HDACs and Mef2 proteins leading to the repression of Mef2-based transcriptional activity and the use of MC1568 to modulate the stability of the HDAC-Mef2 complexes; 2 book chapters; 1 conference paper; 1 short survey) out of self-citations. Notably, all papers support the findings that Class II HDAC-specific inhibitors have a detrimental effect, inhibiting rather than promoting skeletal myogenesis. More interestingly, 4 different independent papers (see the enclosed table 1) have repeated the experiments and confirmed them. I strongly believe that if any “retraction” of any data of paper by Nebbioso et al. would be made, I should be obliged to move a warning to all authors about such data, also displaying your choice and the potential consequences on their manuscripts (please note that despite any potential technical problems the DATA HAS BEEN LARGELY CONFIRMED). Indeed, I am sure your final intent is not a “witch hunt” but the research of the true.

#### Conclusions:

On the basis of these considerations I sincerely believe that a retraction, although partial and in form of “*corrigendum*”, of this paper reporting reliable and influential data (as EMBO Rep., usually does) would be basically unfair, as it suggests that the data produced have been manipulated, whereas the reliability and the scientific message of this paper is out of any doubt. Furthermore, it looks afflictive towards the Authors from our Institution and discredits the Institution itself I represent. It is my own knowledge that prof. Altucci has been always fully collaborative, immediately replying to your concerns and tried to clarify the situation at best. So far, I feel obliged to defend the reputation of this University with every legitimate means as well as the Prof Nebbioso and Altucci’s international reputation, which has never been grazed, if not by such “anonymous” concern (after 10 years for publication date).

Nevertheless, I understand that seeking clarity the journal would prefer to correct or implement the images under discussion. I would in this respect, as well as of the undiscussed scientific quality of the journal, to propose on the basis of the data provided, a potential solution that might find a consensus of both parties, *such as a “corrigendum” including the link to the images corroborating the conclusions already present in the manuscript without the use of the word “retraction” in any part of the comments*. I strongly believe that the “new results” combined with the analyses of the “original data” does not support, allow and justify in any part of the comments the word “retraction” which has to be considered injurious for the University and, much more, for the quality of my researchers’ work and the integrity of their international reputation. The images might be chosen among those provided by the authors from similar experiments performed at same time. As I know,

these experiments are physically at your offices. On our side, we can take charge of depositing all figures and all significant data in a public repository such as Dryad or Figshare.

I am sure that a you will understand my position which I feel is strengthened by the scientific and objective results provided and I hope that you will be able to actively support my conclusions.

Sincerely yours

Giuseppe Paolisso  
Rector University of Campania – Luigi Vanvitelli



Table 1: Manuscripts confirming the experiments by Nebbioso *et al* (Fig 2b):

1. In the paper “Promiscuous Actions of Small Molecule Inhibitors of the Protein Kinase D-Class IIa HDAC Axis in Striated Muscle” (<https://febs.onlinelibrary.wiley.com/doi/epdf/10.1016/j.febslet.2015.03.017>) Lemon and colleagues confirm that the class IIa HDAC inhibitor MC1568 (purchased from both Sigma-Aldrich and Selleck chemicals) inhibits differentiation of C2C12 myoblasts into multinucleate myotubes repressing a MEF2-dependent reporter gene (Fig.1F);
2. In the manuscript “AMP-activated protein kinase mediates myogenin expression and myogenesis via histone deacetylase 5” (<https://www.physiology.org/doi/10.1152/ajpcell.00124.2013>) Fu and colleagues confirmed that the treatment of C2C12 cells with MC1568 (5µM) stabilizes the repressor structure of HDAC5. They showed MC1568 treatment decreased myogenin mRNA level (Fig. 2A) and MHC protein levels (Fig. 2B). As expected, MC1568 also decreased the number of mature myotubes compared with the control group (Fig. 2C).
3. In the paper “The MEF2-HDAC axis controls proliferation of mammary epithelial cells and acini formation in vitro” (<http://jcs.biologists.org/content/128/21/3961.long>) Clocchiatti and colleagues in showing the MEF2D mRNA levels in normal breast and cancer counterparts (Figure 2B) said that were similar to that described previously during muscle differentiation (Nebbioso *et al.*, 2009; Sebastian *et al.*, 2013);
4. In the “Increases in intracellular sodium activate transcription and gene expression via the salt-inducible kinase 1 network in an atrial myocyte cell line” (<https://www.physiology.org/doi/10.1152/ajpheart.00512.2011>) Papov and colleagues examined the effect of monensin on MEF2 activation in cells treated previously with MC1568 and showed that pretreatment of HL-1 cells with MC1568 prevented the increase in MEF2 activity induced by increases in [Na]<sup>+</sup><sub>i</sub> (Fig. 6A).





February 2020

**Date: 06.02.2020: 09:31**

From: Achim Breiling

To: Lucia Altucci

Dear Dr. Altucci,

Please accept our apologies for proceeding slowly with this issue; we only now implemented a new and more granular list of correction types (summarized in EMBO J in 2019, Volume 38 Issue 18, DOI 10.15252/emj.201970001) and given your concerns about labelling this a partial retraction, we are now able to offer a type of correction that suits the necessary changes optimally.

We have decided that revoking the two figure panels in question is the appropriate way to proceed in this case, and we believe this reflects fully our previous discussion on this matter. Please find attached the text we will publish to explain this course of action.

Most importantly, we would request that the recent replicate experiments directed by Prof. Migliaccio are posted/deposited in a permanent repository (BioStudies, Figshare or Dryad), which is then linked to the published expression of concern. This should contain all necessary information (authors, methods, description of results) to qualify for a stand-alone published unit of fully documented, reproducible research. Dr. Migliaccio had agreed to this course of action in his last correspondence with us.

We can offer that you can post the replicate data you provided (as indicated above) as a separate post on one of the repositories mentioned above. However, this would be up to you, and not a formal requirement from us. The reason we cannot use this data to replace the problematic figure panels in the published article is that we have no means to formally ensure that these data originated as part of the experimentation included in the original paper.

As indicated in the attached text, we require specific e-mails from each author affirming this change. We will thus contact all co-authors directly in this matter with the attached text. If an author is unwilling to comment, disagrees with the statement, or if an author cannot be reached, that will be indicated. Our records show the following e-mail addresses; please provide the most recent e-mail contacts, in case you have this.

Nebbioso Angela – [REDACTED]

Fabio Manzo – [REDACTED]

Marco Miceli – [REDACTED]

Mariarosaria Conte – [REDACTED]

Lucrezia Manente – [REDACTED]

Alfonso Baldi – [REDACTED]

Antonio de Luca – [REDACTED]

Dante Rotili – [REDACTED]

Sergio Valente – [REDACTED]

Antonello Mai – [REDACTED]

Alessandro Usiello – [REDACTED]

Hinrich Gronemeyer – [REDACTED]

Please confirm receipt of this letter, and confirm if you agree with this course of action and the draft statement attributed to you in the expression of concern statement.

Yours sincerely,

Achim Breiling, Bernd Pulverer

**Date: 06.02.2020: 10:12**

From: Lucia Altucci

To: Achim Breiling and Bernd Pulverer

CC: Antimo Migliaccio

Dear Achim & Bernd

I acknowledge the reception of this email.

I will need some days to read the content and reply to you with comments suggestion and my own position. I am currently out of office but this issue will be top of the list once back.

In the mean time I will also inform the authorities of my University (included Prof. Migliaccio which is in cc of this email)

Best,

Lucia

**Date: 27.02.2020: 08:24**

From: Lucia Altucci

To: Achim Breiling and Bernd Pulverer

Dear Achim & Bernd

I would like to give you an update.

I am discussing with the Director on how to submit the data on the replication studies so that they become available to all authors.

I have contacted Dr Gronemeyer with whom I am discussing the points contained in the attachments of your email.

We are currently drafting some potential suggestions and would kindly ask you to see not only the text, but also the connected figures as it would appear to better work on these points.

I believe that we will still need a bit of time due to the fact that we are also involved in additional activities as mentioned in the acknowledgment letter.

Best,

Lucia

March 2020

**Date: 03.03.2020: 13:43**

From: Achim Breiling

To: Lucia Altucci

Dear Lucia,

Thanks for the update. In order to move forward with this issue in a timely manner, we would prefer to get your agreement on the existing previously agreed text of the 'editorial expression of concern' as soon as possible. This text has been discussed in great detail (also with our publisher and legal experts), and in our view it describes the matter in a transparent, sufficiently detailed and fair manner. As we need to contact all co-authors directly, too, we would like to proceed within the next 2 weeks (that is by March 19th).

Kind regards,

Achim

**Date: 18.03.2020: 14:54**

From: Lucia Altucci

To: Achim Breiling and Bernd Pulverer

Dear Achim and Bernd,

Thank you for your constructive proposition. You will certainly understand that it is rather difficult to agree on a text without seeing the figures. Therefore, I would kindly ask you to see the final version once it is ready.

You will definitely understand that for me and my colleagues it is of highest importance that the following messages are clearly conveyed in the "Editorial Expression of Concern":

1. This is a concern of the editors, not of the authors – This seems obvious from the text but I would like to point this out.
2. I appreciate the last paragraph "All the authors ...." and the corresponding explanatory text before, specifying that the data have been reproduced by an independent team of scientists and that, in addition, we have provided replicate data.

3. I have to insist, however, that the “Editorial Expression of Concern” is published only, when these reproduced data are publicly available and can be referred to in the “Editorial Expression of Concern”. Only this way, the scientific community can compare all available data in the specific issue that gave rise to your concern. I will not accept that the “Editorial Expression of Concern” is published when the replicated data cannot be checked by the scientific community. I think it is a question of fairness that anyone who was anonymously ‘accused’, resulting in a public action, can at the same provide the facts to defend the accuracy of the scientific conclusions originally reported.
4. Concerning the editorial statement:’ "A digital image provided by the authors as representative of the source data for lane MEF2D of Fig2B (see Image A below) does not match to the published image in one of the four lanes (lane 3). The authors did not provide an explanation for this discrepancy", I would like to point out -as previously stated- that we found this image on a repository of 2006 with no additional info; thus, we have indicated differences might be related to a different exposure used in the publication. Note that an independent forensic analysis supported these points. Thus you might consider changing the statement into: "A digital image provided by the authors as representative of the source data for lane MEF2D of Fig2B (see Image A below) does not match to the published image in one of the four lanes (lane 3). The authors do not agree with this analysis, also based on an independent forensic image analysis."
5. You use the term ‘revoked’ panels. I understand (and seek) the need of clarity, but cannot a different term be used (Concerned)? I would like to stress that as far as I was made aware by the Institution, data related on figure 2B (exposure of tubulin after previous probing with MEF2D: MEF2D signal shows still up together with tubulin in the same film) are physically in your hands; these data (clearly of the time of the publication, given the tubulin signals, which are those published) support the scientific message suggesting that MEF2D is induced during differentiation (lane 2 - 48h differentiation medium), compared to lane 1 (control). This differentiation-specific MEF2D induction is reduced by inhibitor treatment (lane 3 and lane 4). While this point does not shed light on the raised technical issue on lane 3, the trend of the MEF2D modulation is clear and support the scientific message in full, and can be dated at the time of the publication (and not later). Given that this can be proven, may this be linked to the manuscript since you have the film and you can date it independently of lab books or authors/external scientists replicas?

I would be very pleased, if we can reach an agreement and I would like to thank you for your fairness to provide the means of defense to anonymously accused scientists.

A for the requested emails update, those are the emails that I have in my latest contacts:

Nebbioso Angela – [REDACTED]  
Fabio Manzo – [REDACTED]  
Marco Miceli – [REDACTED]  
Mariasosaria Conte – [REDACTED]  
Lucrezia Manente – [REDACTED]  
Alfonso Baldi – [REDACTED]

Antonio de Luca – [REDACTED]  
Dante Rotili – [REDACTED]  
Sergio Valente – [REDACTED]  
Antonello Mai – [REDACTED]  
Alessandro Usiello – [REDACTED]  
Hinrich Gronemeyer – [REDACTED]

At the time of my latest contact with you both, for a transparent process, I had advised all authors of the editorial concerns after anonymous accuses. This was around 2017 in which is also my last direct contact with you as far as I can recall now.

I am very much looking forward to hearing from you.

Bets regards,  
Lucia Altucci

May 2020

**Date: 18.05.2020: 15:58**

From: Achim Breiling

To: Lucia Altucci

Dear Lucia,

Thank you for your detailed response. We have now prepared the figure, which I have attached.

Let me first point out that we have no concern regarding the scientific message of the study, but we have an obligation to the research community to assure the integrity of the published data to the highest level possible, to ensure that confidence is retained in the reliability of high level literature. The data in question are the figure panels in the attached file that we discussed previously. We acknowledge that the data have been reproduced, but this does not explain what has happened to the published images or the corresponding source data. As the originally published images have issues that have not been resolved by the source data, we have to revoke these images, accompanied by an editorial expression of concern to explain why this was done.

We certainly agree that your replicate data can be published in parallel (or even earlier) on a suitable publicly accessible archival repository (e.g. Figshare, Dryad or BioStudies). This will be indicated in the editorial note, and a link will be included. Please let us know about the content of this submission, and which data will be shown.

We state in the expression of concern that 'we have been informed by Antimo Migliaccio, head of the department which corresponding author Lucia Altucci is presently affiliated to (...), that an institutional investigation formally concluded that no illicit image manipulation is apparent in the manuscript.' I think this clearly indicates our and your point of view, and is transparent. We have discussed this text extensively now over a long period of time, and

feel that it perfectly describes the case. Thus, we cannot offer further major changes to the text in the interest of bringing closure to this.

Kind regards,

Achim

**Date: 25.05.2020: 17:24**

From: Antimo Migliaccio

To: Achim Breiling

Dear Achim,

I hope that mail will find you in an excellent shape. This wish is mandatory as all we are concerned about the pandemic present condition. As you will imagine, I am writing about the querelle concerning the EMBO REp. paper by Nebbioso et al. . I am happy that this question is finally coming to the end. I had the opportunity of discussing the issue with Lucia Altucci, who is still rather disappointed. I have to say that I substantially agree with her as, although I did look for it thoroughly (you can trust me), I didn't find evidence of image manipulation and, on the other hand, I have directly verified the reliability of that findings. I am deeply convinced that EMBO done a good deal in publishing them. I have loaded the original films of the experiments described in Fig. 2 and their replicate in Figshare, doi: [10.6084/m9.figshare.12349841](https://doi.org/10.6084/m9.figshare.12349841). You can include the link in the editorial note. Now, I am writing to ask you whether you can send me back the films I mailed you on November 10, 2017 so that I can save them for the Department files and possible further queries. This would be very important for us.

I am also wondering whether you have taken steps to inform the Rector about your final decision since I remember that he was also involved.

I thank you once again for your smooth collaboration in this story and wish you,

All the best

Antimo

**Date: 27.05.2020: 16:44**

From: Achim Breiling

To: Antimo Migliaccio

Dear Antimo,

Thanks for sending the figshare link. This is fine, and we will include it in the expression of concern.

I will certainly send back the films. Presently this is rather difficult, though, as the blots are on my desk at EMBL and we are still on lockdown; i.e. I am working from home, and am not allowed to enter the office. I will send them of course as soon the situation gets different. If this is urgent, though, I have to find a way to get them, and have them shipped.

Regarding the Rector, we did inform him, as you know. His major point to come to an agreement was that we do not use the word retraction, which we do not. Moreover, the Rector confirmed/agreed that the additional data will be put on a public repository from your side, which now has happened. Thus, we do not see why to involve him again.

Kind regards,

Achim

**Date: 27.05.2020: 18:15**

From: Antimo Migliaccio

To: Achim Breiling

Dear Achim,

Thank you very much for the kind and prompt reply. Absolutely don't worry for the films. After almost three years, a few weeks (let's hope) more or less will not change anything. Of course, you can send them, at your early convenience, when you will get access to your office again. I just need to keep them in our Department files.

Concerning the Rector, I know that he expected to be informed once the Editorial Office will have taken the definitive decision. As he dealt this issue directly with Bernd and you, I thought you would have contacted him. Of course, I can do it, if it seems more appropriate.

I wish a quick end of lockdown for you and all the Germany. The experience of COVID-19 pandemia has been horrific in Italy. In Southern Italy we were relatively lucky but in the North there was a real catastrophe.

My best wishes

Antimo

June 2020

**Date: 08.06.2020: 8:57**

From: Achim Breiling

To: Antimo Migliaccio

Dear Antimo,

I had a bit of vacation (at home) for pentecost.

It would be nice if you could talk to the Rector.

Kind regards,

Achim

**Date: 10.06.2020: 13:39**

From: Erica Boxheimer

To: Lucia Altucci

Dear Dr. Altucci,

This is a formal notification to let you know that we are moving forward with the expression of concern that has been in progress for the past three years. I have attached the final text and the figure that will be published.

I will email each of your co-authors separately and ask for each of them to indicate whether or not they are in agreement with the expression of concern. Authors who disagree will be noted as such.

Best regards,

Erica Boxheimer

**Date: 11.06.2020: 12:36**

From: Lucia Altucci

To: All co-authors, Antimo Migliaccio, EMBO Press

Dear all,

some of you yesterday have received the email related to the Note of concern from Embo Reports. I would like to clarify some points raised by the authors to me in the afternoon of yesterday.

Let me first recapitulate.

At the beginning of 2017, following an anonymous indication, I was contacted by the Journal and I immediately answered. At the same time, I advised all authors.

In the following months the head of the Department (Prof. Migliaccio, in cc) has been contacted by the Journal (without any notice to me...); following this, he asked me to materially provide the data and protocols etc. I did it as far as I could (experiments in question are dated 2006...)

At that time I also informed the authorities of my University (Rector).



As you can all read at the bottom of this mail (in the exchange of message with Editors), the value of the scientific message is out of question in consideration not only of the high number of citations, but also of the fact that data have been reproduced not only by my group, but also by independent, external researchers. In addition, I heard that, in order to clarify the situation, an image analysis has been commissioned to a professional third party without proving problems with the 'questioned' images.

I also was informed that the Rector (in cc) was contacted and replied to the Journal without getting feedback.

Only very recently (Feb-March 2020, 3 years later) I got a text for the note of concern. I do not know what exactly has been discussed (and with whom) within this time, I was not part of it.

As all authors know, in March 2020, I informed them about this development via email.

I want to underline that the text of the email from Ms. Boxheimer that I got (see below, in green), is different from the text that the authors apparently got and forward to me to get info (in pink).

She is kindly writing to the authors, that I would have 'agreed' on the text. For me, the fact that I had to accept the text (if you read the email of Dr Breiling in bold blu, major changes were not allowed), does not mean that I fully share this view and all sentences.

Finally, I would like to point out that I gave the emails of all of you to the Editors upon request, (see in the text of the email to Dr Breiling, blu).

I do not know why the authors are contacted separately and why some have not been contacted (yet?).

I go on the assumption, that this is the transparent policy of the Journal to deal with those type of issues.

I thank all of you for the time spent in reading the email.  
I wish you are all in good health.

Best, Lucia

**Date: 11.06.2020: 16:31**

From: Antimo Migliaccio

To: Erica Boxheimer

Dear Ms. Boxheimer,

I've just read the e-mail by Lucia Altucci, concerning the "editorial expression of concern" of EMBO Rep on the paper published in 2009 by Nebbioso et al. I was also asked yesterday

evening by some of the Authors, reached by an e-mail of yours, about my opinion on the attached text of the “note of concern”, as it is said that this text was agreed upon by the journal, the publisher, Dr. Altucci and the head of her department (me). For sake of clarity I have to say that this is not completely correct. As I previously told to Drs. Achim Breiling and Bernd Pulverer, in my opinion, there is no matter of concern on the Figures 2B and 2C, as no evidence of image manipulation was found and, on the other hand, I have directly verified the reliability of that findings. Nonetheless, I am aware that the final decision on this issue lies solely with the Editorial Office and I can do nothing but respect it, but it doesn't mean that I endorse it. I would be very grateful if you can make clear this position in the correspondence with third parties

With my best wishes

Antimo

**Date: 15.06.2020: 6:30**

From: Guiseppe Paolisso

To: Achim Breiling

Dear Dr Breiling,

I have been astonished on becoming aware from Prof Migliaccio that you have already discussed with me about the presumed issue on the manuscript by Nebbioso et al EMBO Reports 2009 and fulfilled my only request not to use the word "retracted". In my function of Rector, I wrote you on almost 2 years ago and you had never the institutional courtesy to reply (for my role of Rector of an Institution involved in a revision process). We have documentation that you received and read my message.

I would like to underline that in all other procedures for similar (or other cases) that I have been involved in, I always got a reply. This is not only considered a form of politeness, but also the evidence of a balanced discussion among the parties. Anyway for your convenience I just report the conclusions of my letter on July 18, 2018 :

Conclusions:

On the basis of these considerations I sincerely believe that a retraction, although partial and in form of “corrigendum”, of this paper reporting reliable and influential data (as EMBO Rep., usually does) would be basically unfair, as it suggests that the data produced have been manipulated, whereas the reliability and the scientific message of this paper is out of any doubt. Furthermore, it looks afflictive towards the Authors from our Institution and discredits the Institution itself I represent. It is my own knowledge that prof. Altucci has been always fully collaborative, immediately replying to your concerns and tried to clarify the situation at best. So far, I feel obliged to defend the reputation of this University with every legitimate means as well as the Prof Nebbioso and Altucci's international reputation, which has never been grazed, if not by such “anonymous” concern (after 10 years for publication date).

Nevertheless, I understand that seeking clarity the journal would prefer to correct or implement the images under discussion. I would in this respect, as well as of the undiscussed scientific quality of the journal, to propose on the basis of the data provided, a potential solution that might find a consensus of both parties, such as a “corrigendum” including the link to the images corroborating the conclusions already present in the manuscript without the use of the word “retraction “ in any part of the comments. I strongly believe that the “new results” combined with the analyses of the “original data” does not support, allow and justify in any part of the comments the word “retraction” which has to be considered injurious for the University and, much more, for the quality of my researchers’ work and the integrity of their international reputation. The images might be chosen among those provided by the authors from similar experiments performed at same time. As I know, these experiments are physically at your offices. On our side, we can take charge of depositing all figures and all significant data in a public repository such as Dryad or Figshare.

I am sure that a you will understand my position which I feel is strengthened by the scientific and objective results provided and I hope that you will be able to actively support my conclusions.

Thus, I am still wondering along these two years why you did not have the time to give/discuss a feedback. Did you forget, or did you perhaps believe that was not useful? Coming to the substance of the matter, from your last communication to Prof Altucci (which again I only had the chance to see because I was included in c/c!), I understand that you push for agreement on the use of word “revoked“ rather than “retracted”. However, it is clear to me that you use the two words as synonyms, despite different rephrasing. That is why it seems also unbecoming to brand the figures with the word "revoked". This would deny all value to two figures you agree to be central for the message conveyed to readership. Therefore, a more agreeable solution could be publishing your note as a CORRIGENDUM, as said two years ago, and introducing its link in the first page of the original article and in the legends of “questioned” figures. Needless to say, you are free to do not take in account my previous and present mail , but it should be also clear that, without any further communication and formal justification of your decision directly to me, as Rector of University of Campania, I will feel also free to undertake any appropriate action to defend our University reputation and the colleagues involved. I have now formally asked to Profs Altucci and Migliaccio to DO NOT PROVIDE any additional consensus to this very "unusual" procedure that you have been one-sidedly put in place and of course you will be obliged to publish your note without any consensus by authors. It should be clear that in absence of any agreement between them (Altucci and Migliaccio) and you, your note will be treated as a violation of the reputation of our University. For such reasons I formally ask you that any your further communication to Profs Altucci and Migliaccio should be forward even to me.

Sincerely yours  
Giuseppe Paolisso

July 2020

**Date: 14.07.2020: 14:26**

From: Bernd Pulverer

To: Guiseppe Paolisso

Dear Dr. Paolisso,

Please find enclosed a letter in response to your e-mail dated June 15th.

I am afraid the letter is lengthy, as I feel that I need to explain in as much detail as possible why we are undertaking the corrective process we proposed and to respond specifically to a number of statements in your two letters. I remain hopeful that you and your colleagues can follow the issues at hand and understand our motivations.

I also hope that we can come to an understanding on a corrective route to take in order to settle this incredibly protected case. We offer three options.

Yours sincerely,

Bernd Pulverer

Dr. Bernd Pulverer | EMBO Press | Meyerhofstr.1 | 69117 Heidelberg | Germany

Dr. Giuseppe Paolisso  
Rector University of Campania  
Viale Abramo Lincoln, 5  
81100 Caserta CE  
Italy

**Dr. Bernd Pulverer**

Head of Scientific Publications, EMBO  
Chief Editor, The EMBO Journal

phone +49 6221 8891 501  
fax +49 6221 8891 200

bernd.pulverer@embo.org

14-7-2020

Re. editorial note on image aberrations in EMBO Rep. 2009 Jul; 10(7):776-82

Dear Dr. Paolisso,

I write in response to your note to my colleague Achim Breiling, date June 15th 2020. I am sorry for the lack of communication to your previous letter from 18 July 2018. We have been in protracted communication separately with you, the authors and Dr. Migliaccio. Parallel communication with three different entities in one institution is something we have not typically seen, and I am sorry that it remained unclear to us who was making decisions at the institutional level in this case. I also admit that this case is rather awkward, as there seems to be a limited willingness to engage with the reasoning we provided to explain our recommendations. Instead, a number of strong statements were made that we take issue with. It may be useful if I respond to a number of points made in your original letter specifically in the hope that we can come to an understanding. We have a long history in cooperating constructively with authors and research institutions in resolving matters around image aberrations constructively, and it remains my hope that this is possible also in this case.

Please note that we believe it is our responsibility as editors of a leading scientific journal to ensure the scientific record is both reliable and reproducible. Most research is publically funded, and both the public and the scientific community depend on an authoritative scientific record. There are innumerable cases one could mention to illustrate the importance of this gatekeeper role of scientific journals. I will just mention two recent retractions in the Lancet and NEJM on the application of Hydroxychloroquine as one example.

You may already know that EMBO Press has been among the leading publishers to institute *pre-publication* image integrity screening. The rate of issues uncovered by us, and in fact anyone else who cares to look, is striking (Pulverer B. The EMBO Journal Vol 34 | N o 20 | 2015). These problems can often be rectified before publication as they can be traced to mistakes in data presentation, data acquisition or indeed data processing. Unfortunately, the paper in question predates these pre-publication screenings, and therefore we have to embrace a more publically visible corrective step. Post-publication, issues cannot be 'silently' corrected, given that the scholarly record is archived. It has to be formally corrected, and I assure you that we follow standard best practice procedures.

I do hope it is appreciated by professionals such as yourself that our motivations are not, as alleged in your letter, to pander to unreasonable individual whistle blowers – why would we? Our reputation as a journal is just as much at stake as the reputation of authors and their institutions. Our sole aim is to protect the scientific record. The large number of serious issues uncovered in the scientific record should, in my view, convince anyone of the importance of this matter. We will only retain the public trust in our research endeavours if we are seen to self-correct transparently.

Regarding specific points raised in your 2018 letter:

- 1) *'it suggests that the data produced have been manipulated, whereas the reliability and the scientific message of this paper is out of any doubt'*: I respectfully note that the editorial expression of concern explains in some detail why the panels are in question. There is no statement anywhere that data has been manipulated. In fact, the proposed author statement states 'the conclusions drawn from figure 2B and 2C, and from the paper as a whole remain unchanged'. The reliability of the 'message' (i.e. the conclusions) of the paper is not directly relevant in considering a correction, as I believe we explained

previously in our communication: a research paper is based on conclusions built up of data presented within the same paper and the previous literature. If the data presented in the paper is in doubt, it has to be corrected or removed. If the main conclusions are impacted by the data in question, they are therefore also in doubt, and in such cases the whole paper is usually retracted. In this particular case, we have not suggested to retract the paper, but instead to remove a very limited, but nonetheless important subset of data images that are in doubt. Please note that subsequently executed data is certainly useful to support the conclusions of a research paper, and this is why we link to it (something few journals in fact currently do), but this data is not relevant to the data published in the paper in 2009. We can only consider replacing data in a figure panel with compelling data that we are convinced was generated at the time of the original experimentation. If we did not follow this (standard) policy, any author could publish placeholder data figures to rush a paper out and later to thorough experiments and replace the figures. This would not be a scholarly scientific process.

- 2) *'it looks afflictive towards the Authors from our Institution and discredits the Institution itself I represent.'* With all due respect, we do not share this interpretation. Please point to specific text that you regard as unreasonable and explain why. Please note that self-correction is in fact a core part of the scientific process and can therefore be regarded as reputation enhancing, as pointed out elsewhere by experts on research integrity.
- 3) *'if not by such "anonymous" concern (after 10 years for publication date).'* It is irrelevant if the note is anonymous or not, what matters is the substance of the issues that were brought to the editor's attention. As I noted, we are in no way beholden to whistle blowers. In fact, we don't act on many such notes where we cannot see any definitive case for concern. We will always act cautiously and we always give the authors the benefit of the doubt in the many cases we see with ambiguous claims. If you follow the whole correspondence we had with your colleagues, this is precisely what happened in this case – we pointed out issues and requested source data to support the figure panels in question in an entirely neutral, non-judgemental manner. There is no formal rule that issues are dropped after 10 years. The paper remains in the literature and scientists will read it and rely on the data presented. We are well aware that data retention policies have changed and not all data is available after 10 years. I assure you that we have taken this into account in our deliberations of this case.
- 4) *'a potential solution that might find a consensus of both parties, such as a "corrigendum" including the link to the images corroborating the conclusions already present in the manuscript without the use of the word "retraction".'* We have chosen a format called 'Editorial expression of concern' as this is a generally recognized corrective mechanism that articulates best the situation here: the editors are concerned about specific data and have decided that it is essential that the reader is alerted to the issues in a maximally transparent manner, so that they can judge themselves. The authors and research institution appears not to share out concern despite repeated discussion, and therefore we articulate this very explicitly and clearly also. The interested reader is thus optimally informed about who has issue with what.

*'The images might be chosen among those provided by the authors from similar experiments performed at same time.'*: Such data is indeed useful to support the conclusions drawn, as is repeat data carried out later. Note, however, that the data supplied by the authors was generated in a similar time frame in 2006 as the published study, but not formally replicate data from the same experimental context. Altucci and colleague describe the supplied data related to *fig 2B* in the figshare post as *'the blot was performed as a control of the activity of a new lot of MC1568'*, thus implying that a different lot of the MC1568 inhibitor was used, i.e. different reagents. This is in line with the marking on the autoradiographs we viewed, which state *'nuovo lotto 1568 da testare'*. We can conclude that this data is *consistent* with the published figure but not formally a *replicate*. Irrespective of that, note that the originally published image is in question, and given that the data is in doubt, it has to be corrected in a visible manner. The source data provided by the authors for *fig 2B* and *2C* is at odds with the published images. In fact, the partial, but not complete overlap of figure and source data in *2B* is what continues to cast doubt on the integrity of the published images. Please also note that in *fig 2C* there are three apparent modifications in the figure panel that would be highlighted: the apparent duplication of background between lanes 2 (IP MEF2D) and lane 5 (IP IgG) in the HDAC4 panel, the likely added lane 5 in panel AcMEF2, The truncation of the panel MEF2D after the 4th lane.

- 5) We ensured that the wording of the editorial expression of concern remains rather circumspect on the details, again given the concerns that you raised. We are not stating a reason for the discrepancy, let alone do we suggest any illicit motivations—we show the evidence so that the interested reader is informed.
- 6) *‘this very “unusual” procedure that you have been one-sidedly put in place and of course you will be obliged to publish your note without any consensus by authors’*: A number of the author (3) have actually agreed with the proposed statement. Regrettably, journals do not always manage to obtain consensus from all of the authors – in this case despite our best efforts. This divergence in opinion is therefore noted in concrete terms in form of an *editorial note* and a clear partition into a *journal statement* and an *author statement*. The journal has a mandate to correct the scientific record to its best ability.

I believe that your line of argument indicates that you simply don’t believe in the validity of the issues we raised about the data and the source data that was supplied later by the authors. It is regrettable that your independent expert advice (we believe this was carried out by [REDACTED]) has - in our view - not provided an authoritative digital forensic assessment. We would very much like to convince you that our own assessment is in fact valid. Since we were surprised by the partial inconsistencies between the published figure panel and the source data supplied later, we in fact obtained an independent assessment from another image forensic expert, who immediately came to the same conclusion without having been made aware of the nature of our concerns. It may thus be helpful for you to contact independent world experts on this topic at this stage to get an authoritative assessment. In case this is useful, we would recommend one of the following. This case has not been discussed with either of them previously:

- 1) Elizabeth Bik (Nature **581**, 132-136 (2020), doi: 10.1038/d41586-020-01363-z; <https://www.the-scientist.com/news-opinion/eye-for-manipulation--a-profile-of-elisabeth-bik-65839>)
- 2) Mike Rossner (<http://www.imagedataintegrity.com/about.html>)

I close by quoting a statement you made in your most recent letter: *‘your note will be treated as a violation of the reputation of our University’*. It is my sincere belief that you protect the reputation of your institution and your researchers best by demonstrating a willingness to self-correct the literature according to international standards of best practice. I state this especially in light of other apparent aberrations noted on other papers by a subset of the authors.

If we fail to reach an understanding on the editorial expression of concern text we proposed, I can propose two alternatives that follow our general ethos of transparency, which we apply to all steps of the publication process in the hope that we can reach a level of constructive understanding and a way forward:

- 1) We would be willing to change the visualization of the *revoked* figures from a very visible red wordmark across the image to our new *retract and replace* format. While the ‘similar’ data supplied by the authors is not formally a replicate (see #5 above), we believe that it was carried out in a similar time frame and may support the conclusions. The published figure would display the new data instead of the original data, with a footnote in the figure legend stating the image was *‘retracted and replaced as outlined in editorial note of concern [hyperlink]’*. The editorial note would show both the old and the new figure side by side to archive the original figure. We would also show the source data provided with an annotation of the third band and the image aberrations noted, to explain why this is an issue at all. The revised text would not use the word ‘revoked’, but rather refer to the formal corrective modality *‘retract and replace’*. Note that the data would therefore not be linked to via the figshare platform, but be republished within the actual paper. We would retain the link to the more recently generated, confirmative data supplied independently.

*alternatively*

- 2) We would be willing to publically release all our written exchanges as supplementary information alongside a modified text as a *corrigendum*. Further, we would add our full image forensic analysis with

annotation to the corrigendum text, including the figure panels and the source data provided. In this way, the interested reader can make up their own mind.

Both of these suggestions for alternative modalities to correct the paper suggested in good faith to provide more options to you and your colleagues. Please advise which of the three alternative corrective modalities you wish to pursue so that we can close this case.

However, if you continue to believe that our proposed course of action, despite the alternatives provided above, unfairly undermines the reputation of your institution, we would appreciate if you would state in concrete terms what action you plan to take. Your statement, 'I will feel also free to undertake any appropriate action to defend our University reputation and the colleagues involved' may be interpreted as a plan to start legal proceedings. We will take the necessary action to prepare for the consequences once you inform us of the concrete meaning of this statement.

Yours sincerely,

A handwritten signature in blue ink that reads "Bernd Pulverer". The signature is fluid and cursive, with a long horizontal stroke at the end.

Bernd Pulverer

Head of Scientific Publications



**Date: 20.07.2020: 9:35**

From: Guiseppe Paolisso

To: Bernd Pulverer

Dear Dr. Pulverer,

First and foremost, let me thank you for the prompt and detailed reply to my note dated, 15th June, 2020.

I realize that it will be truly difficult reach a fully shared agreement about the EMBO Rep. paper issue. It seems that we look at same things in a different way.

Far be it from me debating, point by point, about all the statements in your letter, but just to give some examples:

You wrote: "Parallel communication with three different entities in one institution is something we have not typically seen, and I am sorry that it remained unclear to us who was making decisions at the institutional level in this case. It quite strange what you said and thus I will try to make clear such point. Each University has a Rector responsible for the governance and chief of the hierarchic decision/s encompassing questions related to public image of University itself. After the Rector, there is the chief of the Department and finally, the single researcher. So, all decisions regarding University image or controversies have to be also supported by the Rector. Just to make you an easy work, as you have already made, you just need to copy to the all subjects interested in.

You wrote "it is our responsibility as editors of a leading scientific journal to ensure the scientific record is both reliable and reproducible". This is true, and I am absolutely convinced about it, but I am also convinced that the data reported (and that we are discussing) are both "reliable and reproducible", as they have been verified and confirmed beyond all reasonable doubt in the last ten years. Indeed, you assert: "There is no statement anywhere that data has been manipulated." But then, what does it mean talking of "integrity" of published images?

Again, I agree with you and also believe that "I protect reputation of our institution and researchers best by demonstrating a willingness to self-correct the literature according to international standards of best practice". That's what I've been aiming to do for over 30 years and that's why I also feel the duty to prevent anyone from casting doubts where these doubts do not exist.

Moreover, it is quite "worrying" the statement "in light of other apparent aberrations noted on other papers by a subset of the authors". Who and what are you referring to? (Please clarify). How is this statement applied to the EMBO Rep. or how does it influence it?

All in all, it seems to me that you are convinced that some aberrations have been made, whereas I have reached certainty that this is not the case. These two positions are obviously irreconcilable.

Nonetheless, the goal is now to end this seemingly endless story. Therefore, I would be inclined to accept the proposal numbered 2, it's to say a public release of all our written exchanges (do you mean the correspondence between you, Dr. Breiling, Dr. Migliaccio and me? Please clarify) as supplementary information with a modified text as a corrigendum.

I understand that this may include the image analysis with annotation to the corrigendum text, and the figure panels and the source data provided. Of course, I expect that you also add the link to FigShare to show the experiments performed by independent investigators, supporting the presented data.

I agree that in this way, the interested readers can judge from their mind. However, I would be grateful if you can kindly provide an example or (best) a preview of how this corrigendum will appear since it is very difficult to take this decision without seeing the data, as you will certainly recognize.

Finally, in your last period you wrote: However, if you continue to believe that our proposed course of action, despite the alternatives provided above, unfairly undermines the reputation of your institution, we would appreciate if you would state in concrete terms what action you plan to take. Your statement, 'I will feel also free to undertake any appropriate action to defend our University reputation and the colleagues involved' may be interpreted as a plan to start legal proceedings. We will take the necessary action to prepare for the consequences once you inform us of the concrete meaning of this statement. Please note that I have no intention of revealing to you which action this University will put in place where the controversy is not resolved with everyone's approval, since different possibilities can be taken into account. Obviously, you can feel free to think of any hypothesis. However, I am sure that the solution will be found.

Finally I would appreciate very much if you release back a message for receiving and READING such mail.

I thank you in advance and remain,

Yours sincerely,

Giuseppe Paolisso

**Date: 20.07.2020\_ 18:40**

From: Bernd Pulverer

To: Giuseppe Paolisso

Dear Dr. Paolisso,  
Please find comments inserted below.

Sincerely,

B. Pulverer

*Dear Dr. Pulverer,*

*First and foremost, let me thank you for the prompt and detailed reply to my note dated, 15th June, 2020.*

*I realize that it will be truly difficult reach a fully shared agreement about the EMBO Rep. paper issue. It seems that we look at same things in a different way.*

*Far be it from me debating, point by point, about all the statements in your letter, but just to give some examples:*

*You wrote: "Parallel communication with three different entities in one institution is something we have not typically seen, and I am sorry that it remained unclear to us who was making decisions at the institutional level in this case. It quite strange what you said and thus I will try to make clear such point. Each University has a Rector responsible for the governance and chief of the hierarchic decision/s encompassing questions related to public image of University itself. After the Rector, there is the chief of the Department and finally, the single researcher. So, all decisions regarding University image or controversies have to be also supported by the Rector. Just to make you an easy work, as you have already made, you just need to copy to the all subjects interested in.*

*You wrote "it is our responsibility as editors of a leading scientific journal to ensure the scientific record is both reliable and reproducible". This is true, and I am absolutely convinced about it, but I am also convinced that the data reported (and that we are discussing) are both "reliable and reproducible", as they have been verified and confirmed beyond all reasonable doubt in the last ten years. Indeed, you assert: "There is no statement anywhere that data has been manipulated." But then, what does it mean talking of "integrity" of published images?*

**BP:** Manipulation and image integrity are different terms. The former implies active manipulation of the image to mislead the reader. The latter can be caused by mistakes or ill-informed use of image processing tools and a lack of understanding of the scientific process. You will find more information is available in the expert literature. I can only repeat again that the reproducibility of the data is not the primary issue here, it is the reliability of the images in the published paper.

*Again, I agree with you and also believe that "I protect reputation of our institution and researchers best by demonstrating a willingness to self-correct the literature according to international standards of best practice". That's what I've been aiming to do for over 30 years and that's why I also feel the duty to prevent anyone from casting doubts where these doubts do not exist.*

**BP:** May I suggest a more accurate wording would be that these doubts do not exist for you?

*Moreover, it is quite “worrying” the statement “in light of other apparent aberrations noted on other papers by a subset of the authors”. Who and what are you referring to? (Please clarify). How is this statement applied to the EMBO Rep. or how does it influence it?*

**BP:** As you will undoubtedly be aware, other papers by the corresponding author have been subject to retraction (<https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0014229>; we appreciate that LA did not agree with this retraction) or correction. At EMBO Press, we are considering the paper published in EMBO Reports and the issues in that specific paper.

*All in all, it seems to me that you are convinced that some aberrations have been made, whereas I have reached certainty that this is not the case. These two positions are obviously irreconcilable.*

**BP:** We had suggested that it might be helpful for you to involve another internationally recognized expert for an independent view. As we noted, we had already involved such an expert for a ‘second opinion’ (without informing them either of the details of the paper or the issues we noted).

*Nonetheless, the goal is now to end this seemingly endless story. Therefore, I would be inclined to accept the proposal numbered 2, it’s to say a public release of all our written exchanges (do you mean the correspondence between you, Dr. Breiling, Dr. Migliaccio and me? Please clarify) as supplementary information with a modified text as a corrigendum.*

*I understand that this may include the image analysis with annotation to the corrigendum text, and the figure panels and the source data provided. Of course, I expect that you also add the link to Fig share to show the experiments performed by independent investigators, supporting the presented data.*

**BP:** Of course, just as we had already done. This information is relevant, as we have stated from the start.

*I agree that in this way, the interested readers can judge from their mind. However, I would be grateful if you can kindly provide an example or (best) a preview of how this corrigendum will appear since it is very difficult to take this decision without seeing the data, as you will certainly recognize.*

**BP:** We have assembled the document and it is under review. You will receive it in due course.

*Finally, in your last period you wrote: However, if you continue to believe that our proposed course of action, despite the alternatives provided above, unfairly undermines the reputation of your institution, we would appreciate if you would state in concrete terms what action you plan to take. Your statement, 'I will feel also free to undertake any appropriate action to defend our University reputation and the colleagues involved' may be interpreted as a plan to start legal proceedings. We will take the necessary action to prepare for the consequences once you inform us of the concrete meaning of this statement. Please note that I have no intention of revealing to you which action this University will put in place where the controversy is not resolved with everyone's approval, since different possibilities can be taken into account. Obviously, you can feel free to think of any hypothesis. However, I am sure that the solution will be found.*

*Finally I would appreciate very much if you release back a message for receiving and READING such mail.*

**BP:** As you can see, we have READ and taken note of your statement.

*I thank you in advance and remain,  
Yours sincerely,  
Giuseppe Paolisso  
Rector of University of Campania "Luigi Vanvitelli".*