Laboratory statement

Pubpeer requested explanations about some of our previous publications because of splicing of Western blot-gel images or duplications. We would like to state that splicing western blot image was a normal practice 10 years ago and never represented an issue for our group. This practice has been done in many of our studies without indicating this in the figure legends based on the fact that the rearrangement of the images were done properly using the (i) same film exposure and (ii) the same western blot.

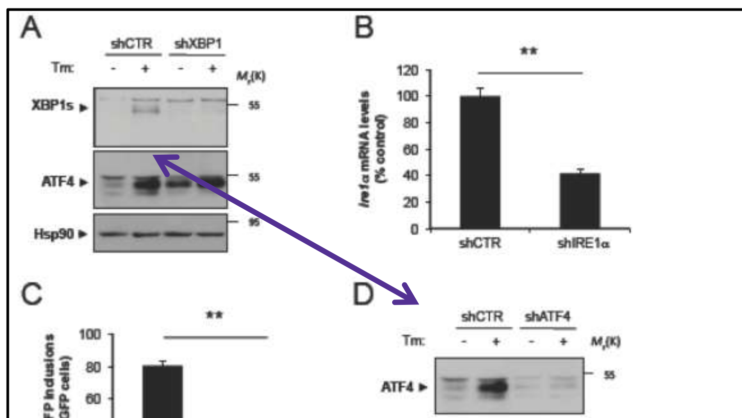
However, because of changes in editorial policies in the last years in most Journals, in all our recent publications we now state in the legend the images that were spliced and also provide full scan of gels. We have also generated a data repository in our website to give open access to full scans of all our publications.

We thank Pubpeer for identifying these unintentional errors. When they were relevant to the message of the study we informed the journals, who decided to do corrections or not depending on the case, or just replaced data without any further action. Here we provide raw data, explanations of experimental setup and also copies of the communications with editorial offices when we addressed these issues.

For full transparence we also requested an ethic investigation to the Faculty of Medicine. An inter-institutional commission was generated with no-conflict of interest, and concluded that errors when existed where unintentional.

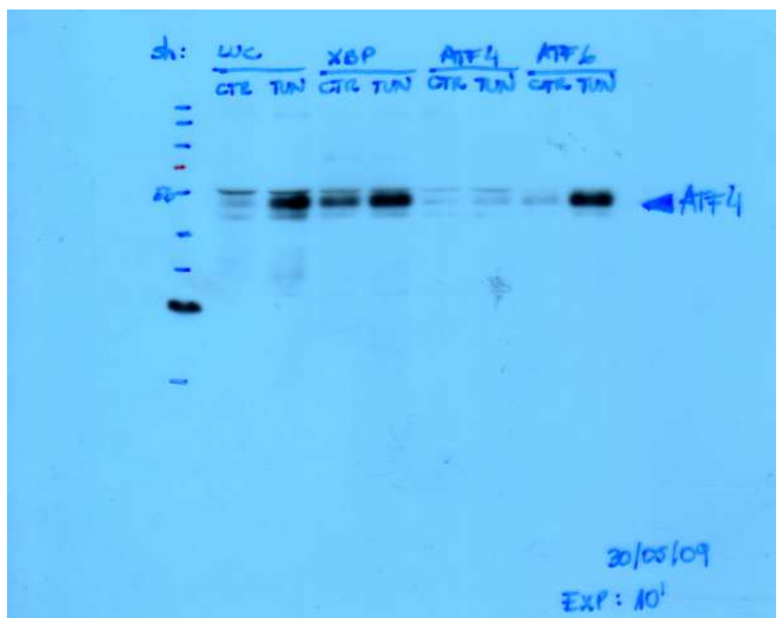
Targeting the UPR transcription factor XBP1 protects against Huntington's disease through the regulation of FoxO1 and autophagy. Vidal RL, Figueroa A, Court FA, Thielen P, Molina C, Wirth C, Caballero B, Kiffin R, Segura-Aguilar J, Cuervo AM, Glimcher LH, **Hetz C.** Hum Mol Genet. 2012 May 15;21(10):2245-62.

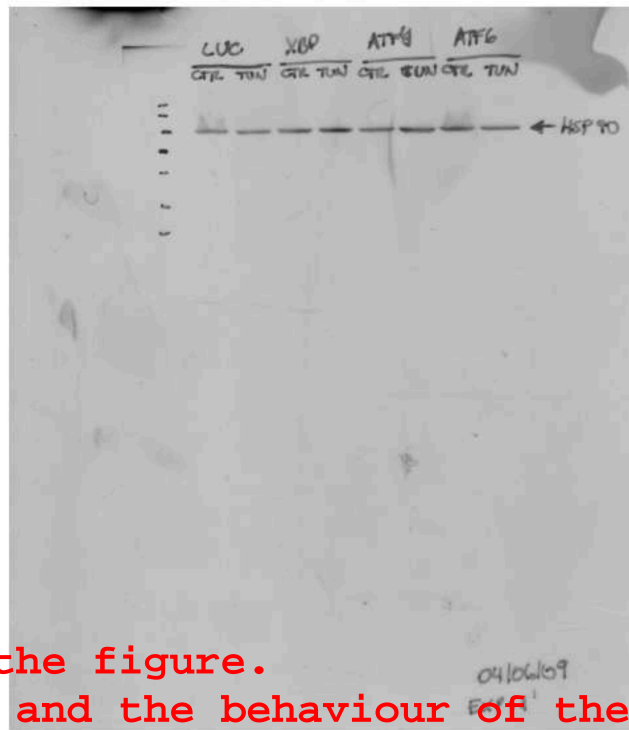
It was noticed that there was a control (shCTR) repeated in supplementary figure 4A and D.



Answer:
In panels A and D we tested the efficiency of shRNA knockdown in stable cell lines for the genes XBP1, ATF4, and control and then monitored ATF4 and XBP1 expression by Western blot. These 3 cell lines were generated at the same time and thus used the same control shRNA in most experiments.
This is why in both panels A and D the control image (shCTR) are the same (from the same blot), repeated twice in both panels figure. This was not mention in the figure legend.

Action:
As soon as we noticed this error we contacted to the editor and send the original data. They decided to replace the supplementary material without any correction since there was no error (see interchange of emails 2012 in the next pages).





Dr Hetz admits to have fabricated the figure.
His rationalization is irrelevant, and the behaviour of the journal is appalling. Also, the loading control seems to be on a different gel

Claudio Hetz <claudio.hetz@gmail.com>

replacement supplementary figure HGM Manuscript

OUP Team (Pam) <oupteam@techset.co> Fri, Nov 2, 2012 at 7:35 AM
To: HMGeditorial <hmg.editorialoffice@oup.com>, claudio.hetz@gmail.com
Cc: oupteam <oupteam@techset.co>, HMG <hmg@oup.com>

Dear Stephanie/Claudio

The supplementary data has been replaced as requested. Please let me know if there are any further queries or problems?

Regards

HMG team

Claudio Hetz <claudio.hetz@gmail.com>

Clarification/Erratum on figure legend HGM Manuscript?

Claudio Hetz <claudio.hetz@gmail.com> Mon, Oct 29, 2012 at 2:47 PM
To: hmg.editorialoffice@oup.com
Cc: Rene Vidal <renevidalg@gmail.com>
Bcc: chetz@hsph.harvard.edu

Dear Editor
since I didn´t receive an answer from this important email, I am sending it again.

Many thanks in advance.

Sincerely yours,

Claudio Hetz

Dear all
I am contacting you because we just realized we omitted some minor, but relevant, information in the legend of supplementary Figure 4 of our manuscript "Hum Mol Genet. 2012 May 15;21(10):2245-62"

In panels A and D we tested the efficiency of shRNA knockdown for the genes XBP1 and ATF4, and then monitored ATF4 expression by Western blot. Both cell lines were generated in parallel and used the same control shRNA line in most experiments.

This is why in both panels A and D the control image (shCTR) is the same (from the same blot), repeated twice in both panels figure. This was not mention in the figure legend.

To avoid misinterpretations, I wanted to discuss with you if it will be necessary to add an errata or clarification to the figure legend.

As you could see here in the attached file (full scan of the films), all the runs are from the same blot where we tested actually 3 shRNAs for different genes and one control. Since these are general reagents of the lab, these type of controls are done all the time, and we have dozens of independent repeats. There was an unintentional mistake when the loading of the image was added but as you could see from this full scan all correspond to the same experiment.

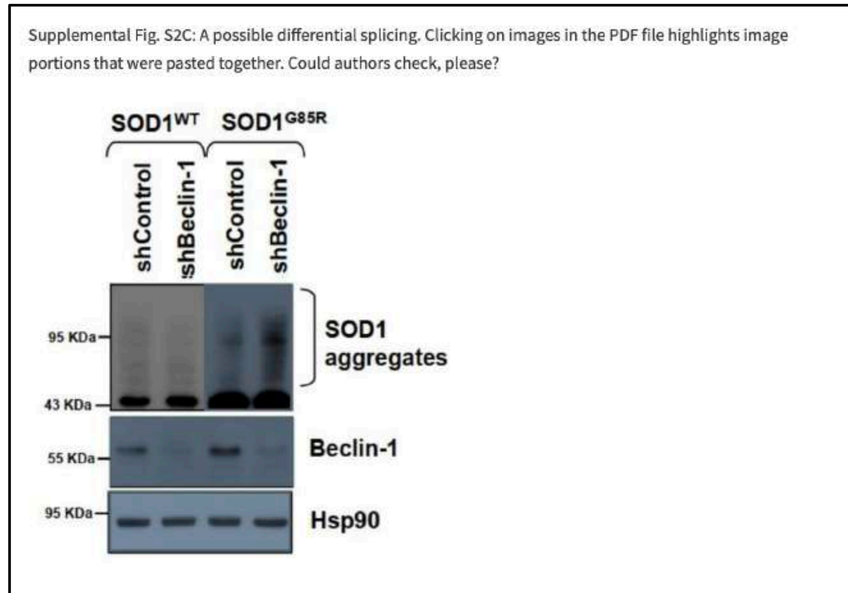
Normally when we repeat a control on a paper we state it in the legend and here we missed it. If you consider we need to add a clarification for the legend, the following text could be used:

"In the original legend of Supplementary Figure 4A and D authors have omitted the fact that the control shCTR in both panels correspond to the same Western blot image because all runs were done in the same gel. The loading control was cut in the wrong position to assemble the image. Full scans were provided to the editorial office of these experiments".

We apologize to the journal for this unintentional mistake.

Sincerely yours,

XBP-1 deficiency in the nervous system protects against amyotrophic lateral sclerosis by increasing autophagy. Hetz C, Thielen P, Matus S, Nassif M, Court F, Kiffin R, Martinez G, Cuervo AM, Brown RH, Glimcher LH.Genes Dev. 2009 Oct 1;23(19):2294-306.

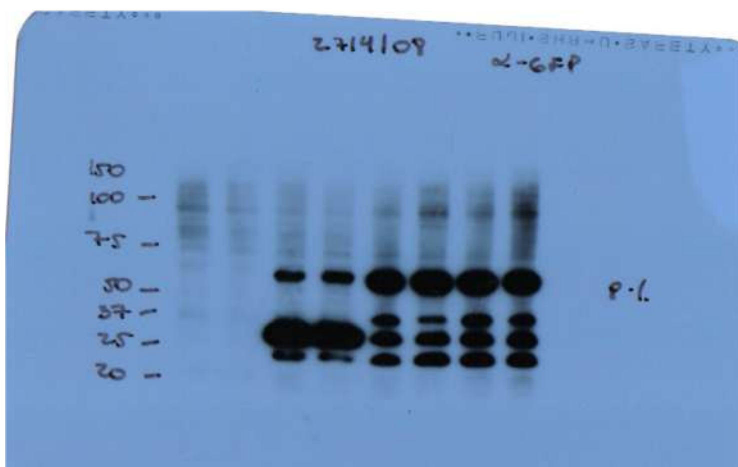


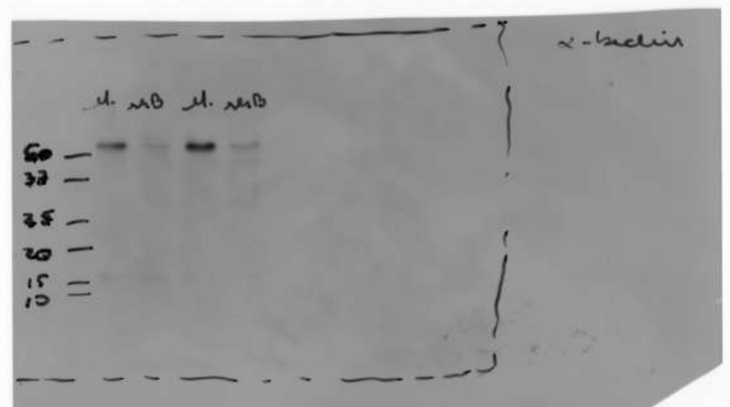
Answer:

Western blots to measure at SOD1 aggregation are always run without DTT on a separate gel to see basal aggregation. This experiment included other samples with another SOD1 mutant (see film). The pairs include its own control for comparison but importantly the lanes come from the same western blot from the same film exposure.

The confirmation of the knockdown was performed on a different gel. These were stable cell lines with the pLKO.1 lentiviral vectors (selection with puromycin), so the knockdown levels of Beclin 1 are maintained. We checked the knockdown on a routine basis and re run the samples to construct the panel here.

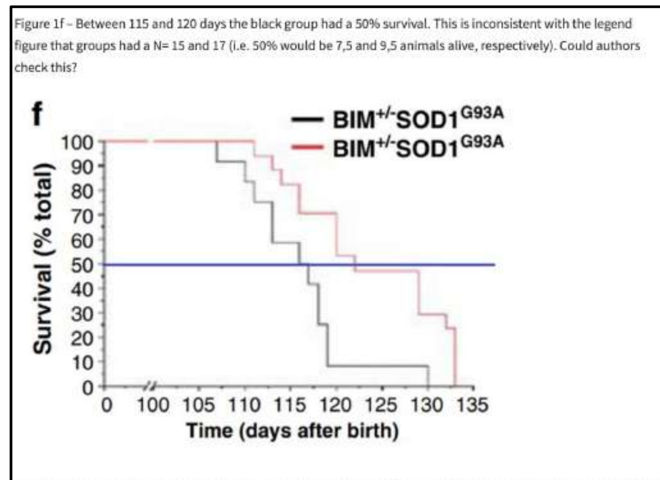
I notice Dr Hetz failed to provide the original HSP-90 gel (he has others, so where is HSP90?)It's very important to check if Beclin-1 and HSP-90 were done on the same gel.





The proapoptotic BCL-2 family member BIM mediates motoneuron loss in a model of amyotrophic lateral sclerosis C Hetz, P Thielen, J Fisher, P Pasinelli, R H Brown, S Korsmeyer, L Glimcher

Cell Death Differ. 2007 Jul;14(7):1386-9.

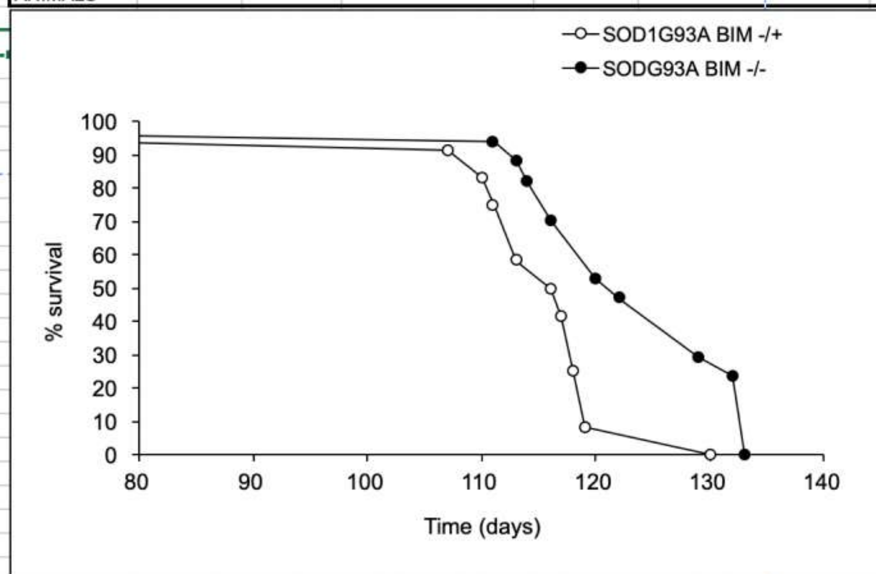


Answer:

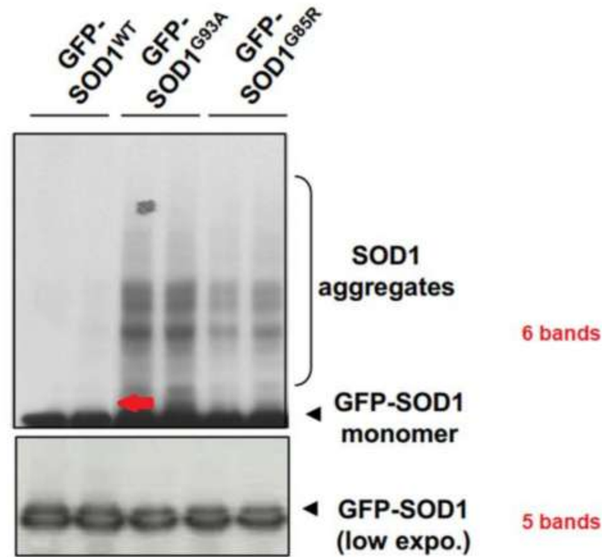
Yes, we made a typo error in staying the N of animals in the survival curve. N is 12 and 17. The curve and statistics do not change since both were automatically generated by the software to built the Kaplan Meyer curves. The message of the article do not change. We apologize for this unintentional mistake.

Survival	number animals		
HET	AND WT	number	%
0			100,0
107	1	1	91,7
110	1	2	83,3
111	1	3	75,0
113	2	5	58,3
116	1	6	50,0
117	1	7	41,7
118	2	9	25,0
119	2	11	8,3
130	1	12	0,0
TOTAL ANIMALS	12		

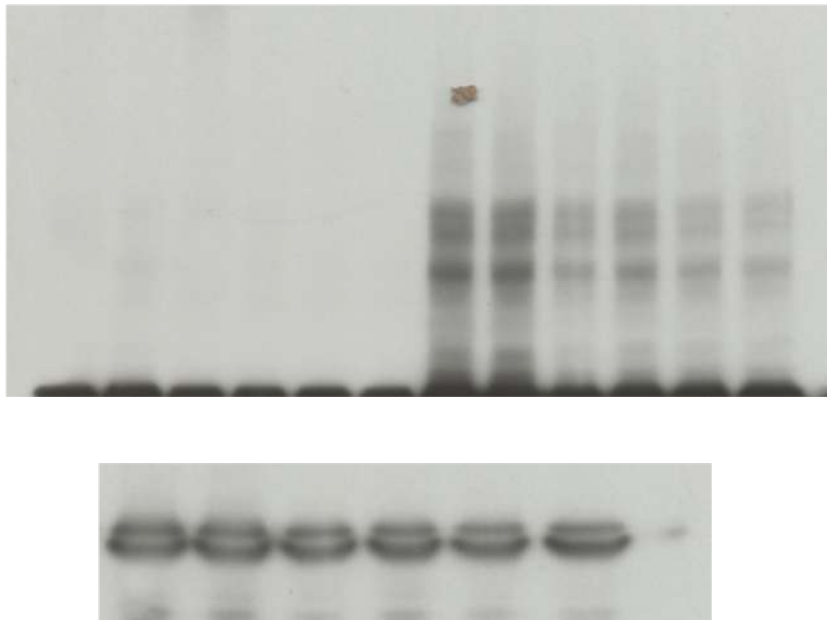
Survival	number animals		
BIM KO			%
0			100
111	1	1	94,1
113	1	2	88,2
114	1	3	82,4
116	2	5	70,6
120	3	8	52,9
122	1	9	47,1
129	3	12	29,4
132	1	13	23,5
133	4	17	0
total	17		



Supplemental Fig. 2C: Different number of bands. Undeclared splicing in GFP-SOD1 monomer



Answer:
Yes, we made a mistake when preparing the figure and one band was missing from the original gel. Full scan of upper gel is provided and low exposure of lower panel.



There is a mismatched gel again, 6 vs 5 lanes. It's like a signature joke.
Also: both images are supposed to show the same gel, with different exposure times.
But the top gel has 13 lanes, the lower one just 7. This makes no sense, unless the lower gel is something unrelated, an utterly different gel.

Endoplasmic reticulum stress leads to accumulation of wild-type SOD1 aggregates associated with sporadic amyotrophic lateral sclerosis.Medinas DB, Rozas P, Martínez Traub F, Woehlbier U, Brown RH, Bosco DA, **Hetz C.**Proc Natl Acad Sci U S A. 2018 Aug 7;115(32):8209-8214

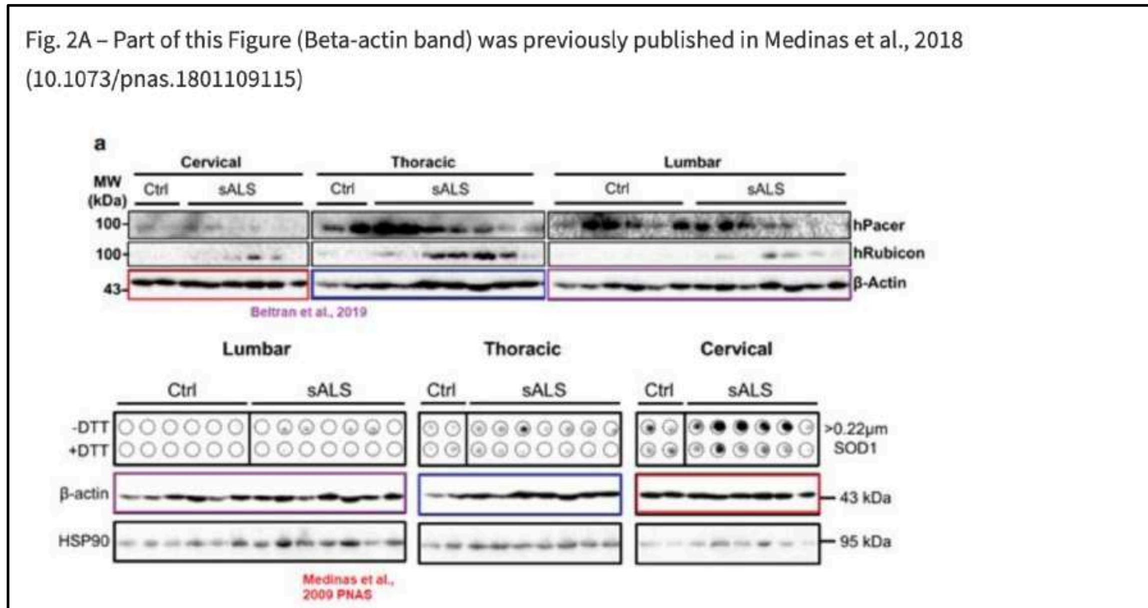


Fig. 2A – Part of this Figure (Beta-actin band) was previously published in Medinas et al., 2018 (10.1073/pnas.1801109115)

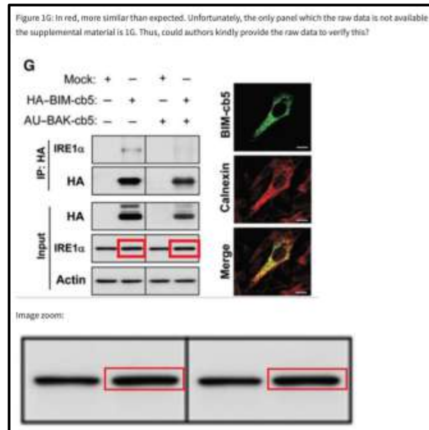
Answer:

We got permission from *PNAS* to use the blots and we disclosed that to *Molecular Neurodegeneration* upon submission.
The journals did not request indicating this in the figure legend. Because this material published after 2008, a copyright note is not required.

Since these rare human postmortem spinal cord samples of a rare disease, we had limited access and amount to run the blots and thus analyzed for both studies by the same authors.

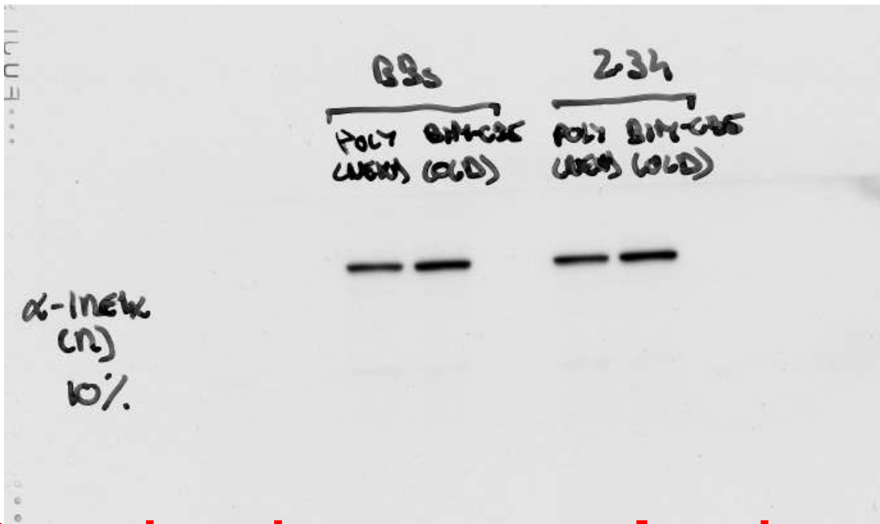
Actually there is more re-use. All actin blots are reused. Note that Dr Hetz doesn't post original gels. Important: those cannot be the same actin-controlled gels re-probed, because the proteins Rubicon, Pacer and HSP90 are all of similar size, around 90-100 kDa. For such close-range analyses, you always do separate gels, or you won't be able to trust your results. Then you would have separate actin loading controls also, but Dr Hetz doesn't. He used library controls.

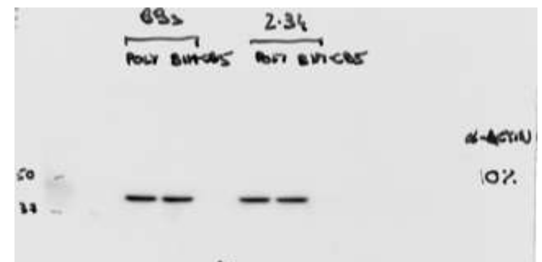
BH3-only proteins are part of a regulatory network that control the sustained signalling of the unfolded protein response sensor IRE1α.Rodriguez DA, Zamorano S, Lisbona F, Rojas-Rivera D, Urra H, Cubillos-Ruiz JR, Armisen R, Henriquez DR, Cheng EH, Letek M, Vaisar T, Irrazabal T, Gonzalez-Billault C, Letai A, Pimentel-Muiños FX, Kroemer G, Hetz C.EMBO J. 2012 May 16;31(10):2322-35.



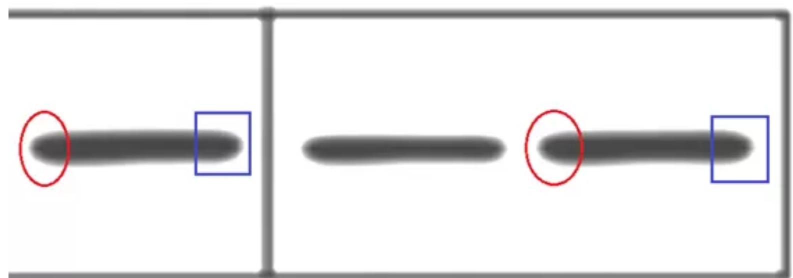
Answer:

Dr Felipe Pimentel-Muiños provided original images with high resolution , and althought they were very similar (not identical), they were correct.

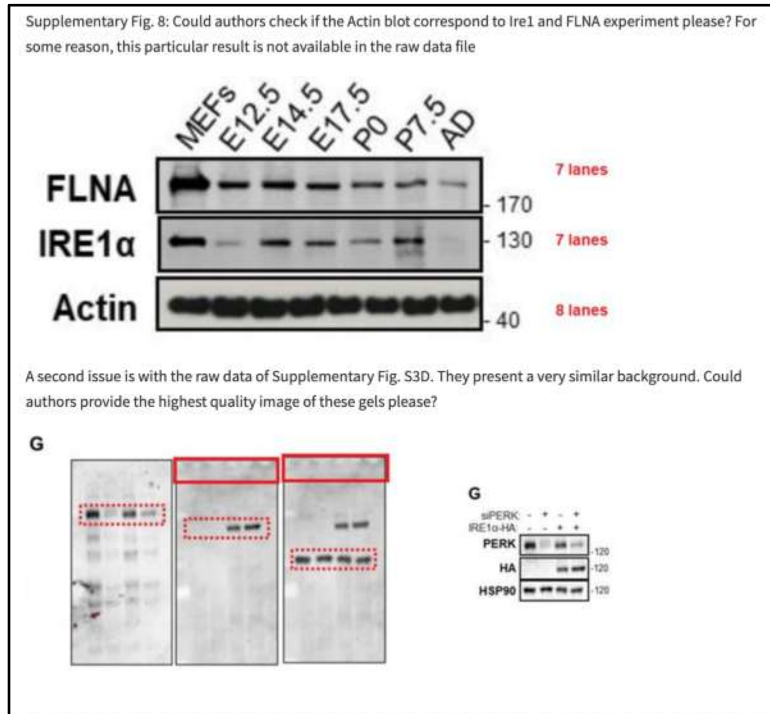




It may have been a wrong alarm here, but the Pubpeer evidence is also very convincing:



IRE1α governs cytoskeleton remodelling and cell migration through a direct interaction with filamin A. Urra H, Henriquez DR, Cánovas J, Villarroel-Campos D, Carreras-Sureda A, Pulgar E, Molina E, Hazari YM, Limia CM, Alvarez-Rojas S, Figueroa R, Vidal RL, Rodriguez DA, Rivera CA, Court FA, Couve A, Qi L, Chevet E, Akai R, Iwawaki T, Concha ML, Glavic Á, Gonzalez-Billault C, Hetz C. Nat Cell Biol. 2018 Aug;20(8):942-953.



Answers:

Answers: Yes, in fact when we prepared the full scan file for NCB we could not find the original data from figure S8. These experiments with embryos were done by the first author of the paper in a collaboration with a lab that is actually shut down years ago. We disclosed this problem to the editor and proposed to eliminate this figure from the paper since it was not fundamental (see email exchange). However, no further actions were required.
Regarding the fact that Actin blots contain 8 lanes is a mistake of the figure. We apologize for such a mistake since the last lane should not be part of the figure and correspond to extra controls.

Regarding figure S3D, these are re-blots of the same membrane. First, we blot for PERK, then HA to detect IRE1a and then loading control Actin. The antibody to detect HA tag is very strong and the signal is hard to remove, thus also appears in the next reblot.

These two blots are basically identical, except one has four extra bands. This is scientifically impossible. Dr Hetz explains this with re-probing, but this is extremely unlikely, if not impossible. All the tiny backgrounds, spots, shadows are identical in both blot images, after several washes and re-probing. This cannot happen. This is only possible if it's the same picture, the same gel and somebody manipulated it in Photoshop.

Claudio Hetz <claudio.hetz@gmail.com>

final requests

To: "Hetz, Claudio" <chetz@hsph.harvard.edu>

Thu, Jun 7, 2018 at 12:00 PM

Dear Claudio,

Thank you for providing most of the original scans. It's fine not to include the full scans that are missing.

I have only a few questions now. You say that the cropping of 4D was not mentioned in the legend. I still can't see that this is mentioned so, can you please clarify what the line means in the legend.

Also, it would be great if you could modify the Integrated Supplementary document so that full scans are added as Supplementary Figure 9. They will take up more than one page, but you can add several figures that are labelled, Supplementary Figure 9, continued, subsequent to the first one. Please also combine the full scans of figure 2/3 , S1/S3 and S6/S7 on one page to save some space.

Legends for tables and videos should follow after Supplementary Figure 9.

Thanks in advance,

[Quoted text hidden]

Pathogenic role of BECN1/Beclin 1 in the development of amyotrophic lateral sclerosis. Nassif M, Valenzuela V, Rojas-Rivera D, Vidal R, Matus S, Castillo K, Fuentealba Y, Kroemer G, Levine B, Hetz C. Autophagy. 2014 Jul;10(7):1256-71

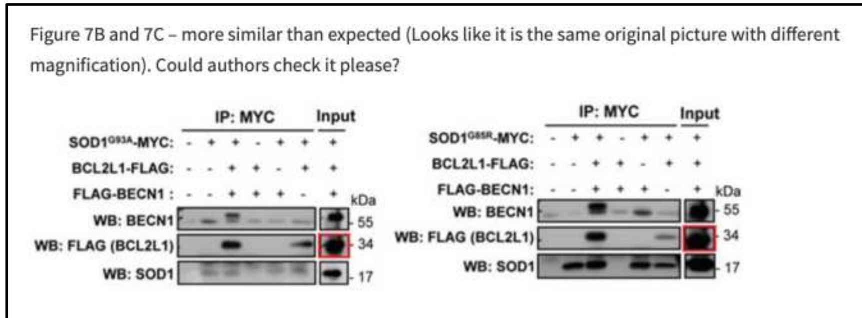
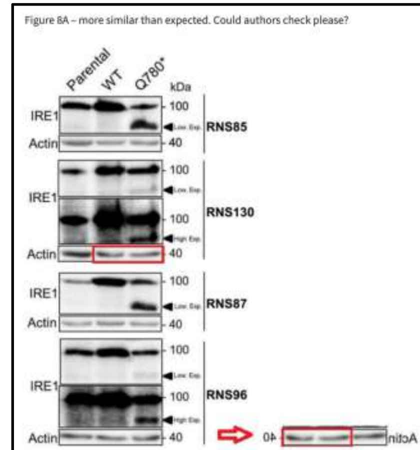


Figure 7B and 7C – more similar than expected (Looks like it is the same original picture with different magnification). Could authors check it please?

Answers:

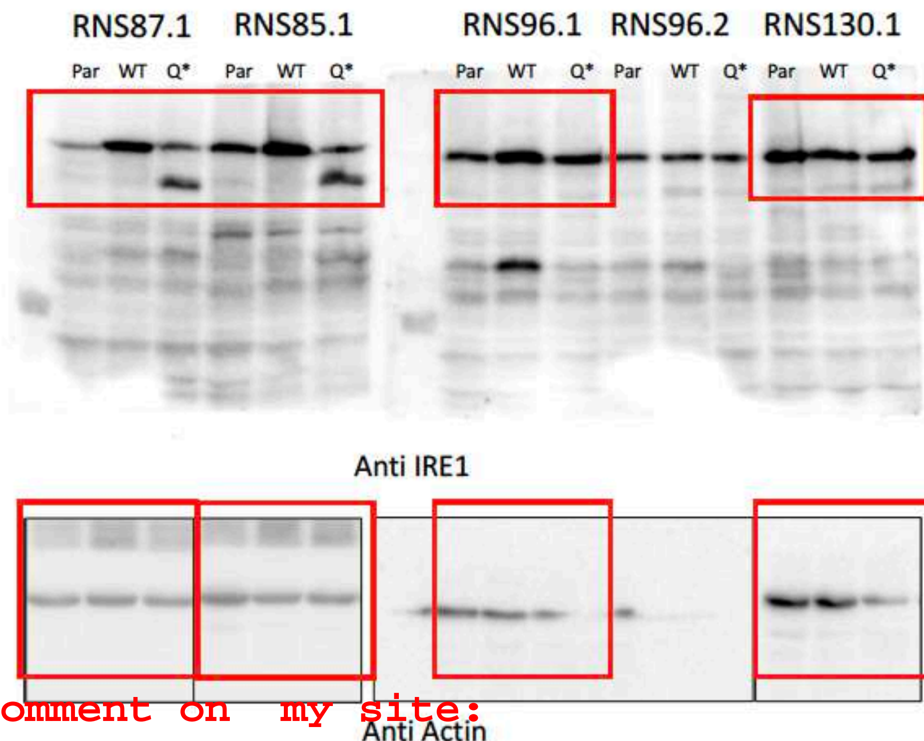
Full scanns were provided by Melissa Calegaro and experiments were properly performed. Inputs (controls for antibodies) were saturated.

Dual IRE1 RNase functions dictate glioblastoma development. Lhomond S, Avril T, Dejeans N, Voutetakis K, Doultsinos D, McMahon M, Pineau R, Obacz J, Papadodima O, Jouan F, Bourien H, Logotheti M, Jégou G, Pallares-Lupon N, Schmit K, Le Reste PJ, Etcheverry A, Mosser J, Barroso K, Vauléon E, Maurel M, Samali A, Patterson JB, Pluquet O, **Hetz C**, Quillien V, Chatziioannou A, Chevet E. EMBO Mol Med. 2018 Mar;10(3):e7929.



Answer:

The editorial office requested original data from Dr Chevet, which showed that the experiments were properly presented. No correction was needed.



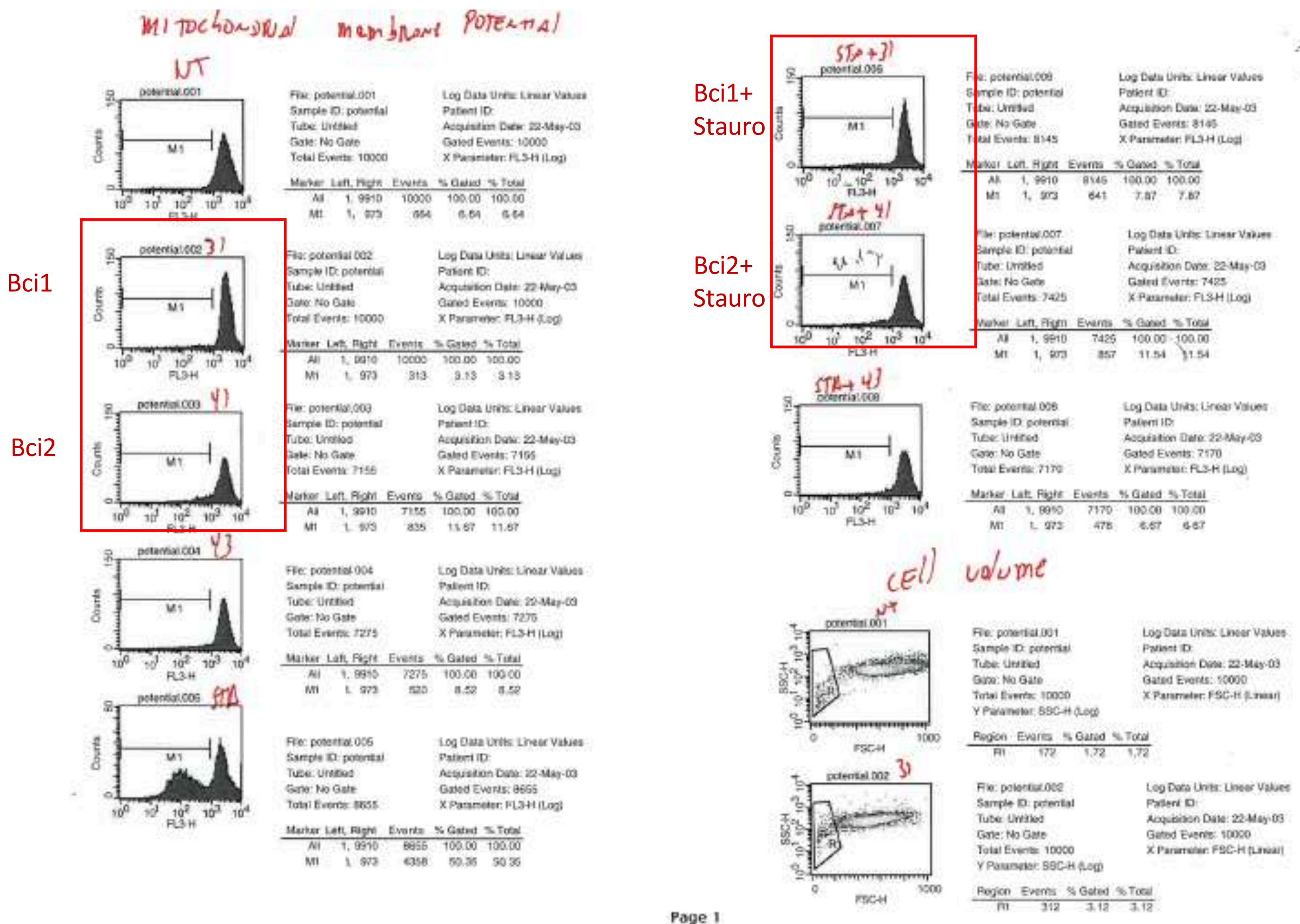
here is a comment on my site:
"Page 12 of this document. The raw data does NOT match their data for RNS96 and RNS130????"
What Dr Hetz posted as Actin blots is indeed something else of what he published.

Bax channel inhibitors prevent mitochondrion-mediated apoptosis and protect neurons in a model of global brain ischemia.**Hetz C**, Vitte PA, Bombrun A, Rostovtseva TK, Montessuit S, Hiver A, Schwarz MK, Church DJ, Korsmeyer SJ, Martinou JC, Antonsson B.J Biol Chem. 2005 Dec 30;280(52):42960-70.

Answer:

When we **filled the correction** we did not have the original FACS data. The SPRI institute moved to a new building in Geneva and data was stored. After the lab of Bruno Antonsson at MERK-Serono was closed, he actually found the **originals which are presented here for fig 5a and b**.

For all the western blots screened by Pubpeer, all of them were performed by Bruno Antonsson´s lab and another team at Serono doing brain ischemia and drug discovery. I requested Bruno the full scans as backup but it was not a priority (see email exchanges). The institute was shut down and sold to Merck years ago.



The concerns on PubPeer (from December 2019) are about falsified western blots in Figures 3A-B, 5A, 4G, 8A-B and microscopy in 5C. Afterwards, concern about FACS data in Figure 5A was also raised, and Dr Hetz addresses only that.

He achieved a correction for Fig 5A only and declared: "*This error does not change the interpretation of the results presented in this figure or any of the conclusions of the paper.*"

This is beyond shameful.

Gmail

Claudio Hetz <claudio.hetz@gmail.com>

Bax figure

> Fri, Feb 22, 2013 at 7:50 AM
To: claudio.hetz@gmail.com

Hi Claudio,

While cleaning out all my old stuff I found some old images from your FACS work with the inhibitors. This appears to be the images used in the paper. Unfortunately a bit to late for the correction.

Best regards,

Bruno

info

> Fri, Oct 19, 2012 at 4:59 AM
To: Claudio Hetz <claudio.hetz@gmail.com>

Hi Claudio,

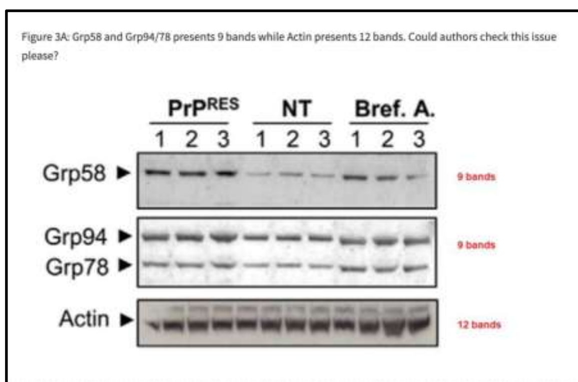
I'm sorry if my message was too direct, I just wanted to understand the full story. As you might understand there are a lot of issues and problems over here at the moment and start to dig into experiments that were performed about 10 years ago and find the data is not so easy. The note books, if still available, are somewhere in an archive, the archives are already being moved to Darmstadt in Germany, so I'm not sure if it is still in Geneva. Second, convincing the persons responsible for the archive that finding an old note book, I suppose we don't know exactly which, do you have the number??, is high priority at this moment, doesn't appear the easiest. The motivation is not on the top for the people that will be kicked out in the next few months.

I have written a short letter, I would suggest to send that to the journal and see what they say. Could you please have a look and make any changes or suggestions. I do not see that we'll be able to identify the original images. If you gave me paper copies I discarded most old papers related to already published papers when we moved to this new building, since I have much less storage space here. Then 2-3 years ago I moved in the building again, and then again my storage space was reduced by about half, so a new cleaning.

Redo the experiment is not that easy Claudio. I can't do any experiments at the moment and I'm not even sure we still have the compounds, and even if, I don't think I can get a material transfer agreement for this set up now, most people in the legal apparently have already left, and I'm not sending anything out without permission, I don't need more problems. Unless you already have some of the compounds, can't remember if I ever sent some to you. Anyway,

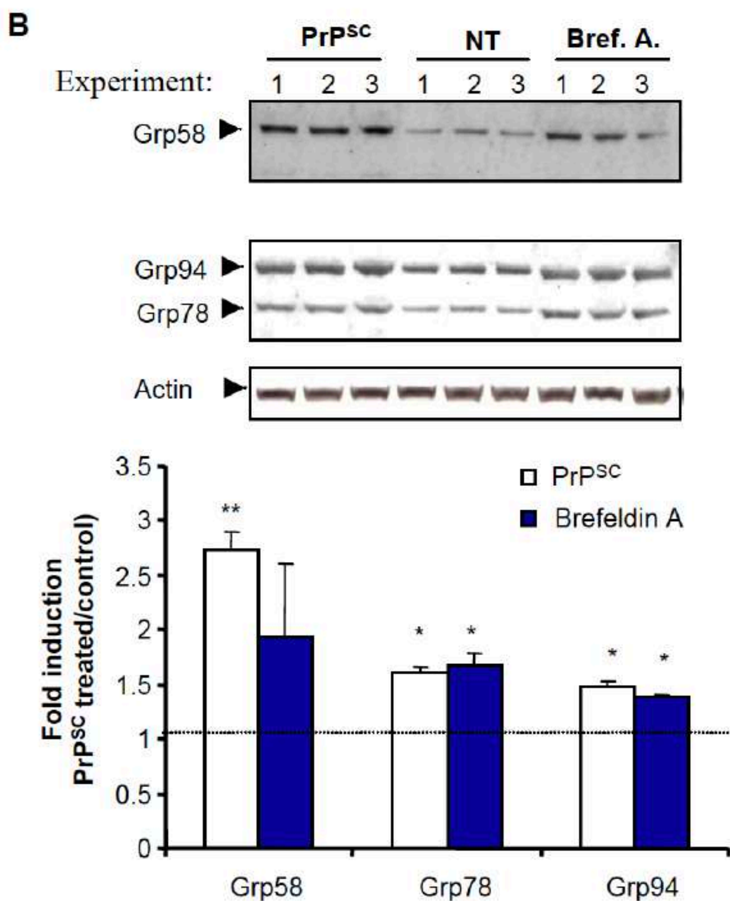
Best regards,

Prion protein misfolding affects calcium homeostasis and sensitizes cells to endoplasmic reticulum stress. Torres M, Castillo K, Armisén R, Stutzin A, Soto C, Hetz C. PLoS One. 2010 Dec 29;5(12):e15658.



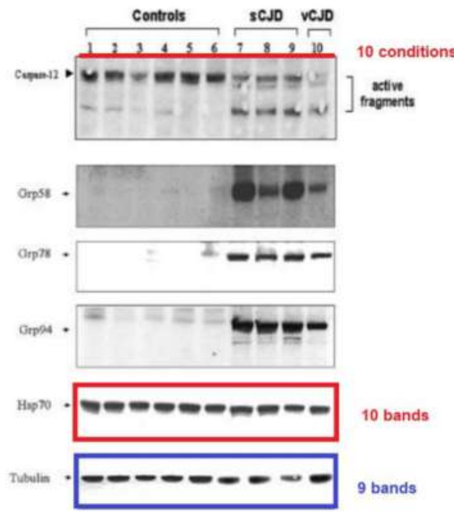
Answer:

Here we made an unintentional mistake, since we mix up loading controls. The problem was generated during the generation of new versions of the paper since initial figures were correct as indicated here (7 years to publish the data). The message does not change. We thank Pubpeer for noticing this error.

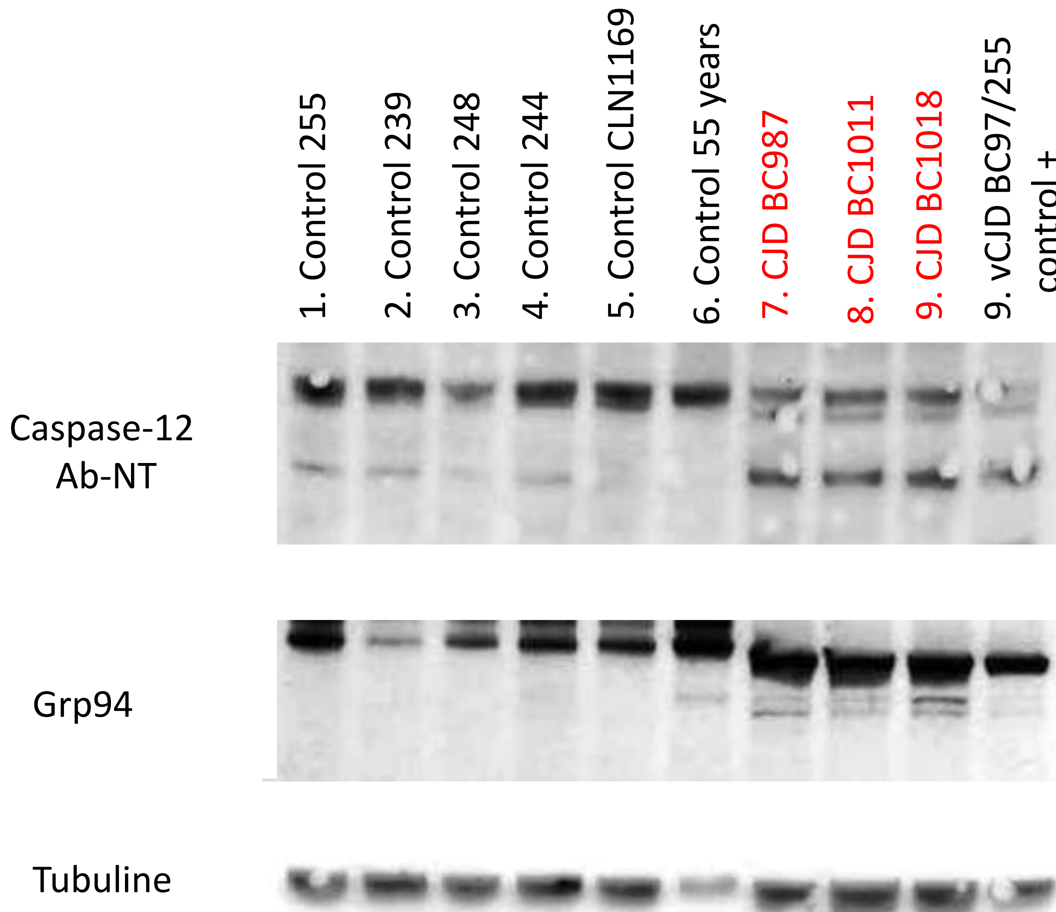


Again, the joke with mismatched lanes. Where is the raw data though?

Caspase-12 and endoplasmic reticulum stress mediate neurotoxicity of pathological prion protein. **Hetz C**, Russelakis-Carneiro M, Maundrell K, Castilla J, Soto C. EMBO J. 2003 Oct 15;22(20):5435-45



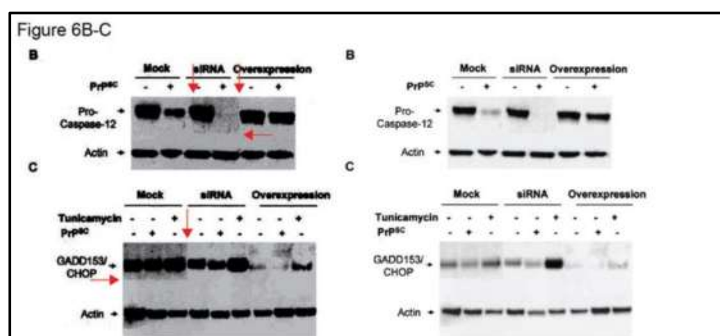
Answer:
We apologize for this error. We realized that the loading control presented corresponded from a different run of the human samples. Here we provide the original tubulin blot that actually shows the pattern corresponding to this particular run. We apologize for this unintentional error.



Again, the joke with mismatched lanes. The raw data for Tubulin seems not to match the published figure.

The disulfide isomerase Grp58 is a protective factor against prion neurotoxicity. **Hetz C**, Russelakis-Carneiro M, Wälchli S, Carboni S, Vial-Knecht E, Maundrell K, Castilla J, Soto C. J Neurosci. 2005 Mar 16;25(11):2793-802.

Some of immunoblots seem to have inconsistent backgrounds. Perhaps, the authors can provide the original uncropped blots.

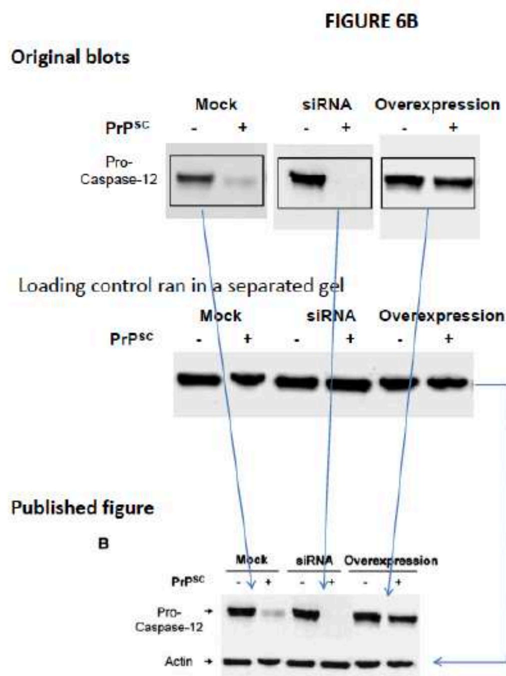


Answer:

The experiment described in figure 6B was carried out in various independent clones to check reproducibility. For simplicity, the results obtained with only one of the cell lines for each condition is shown and spliced to remove the lanes showing the other clones.
Nevertheless, the relevant comparison is for each cell line between treatment and no treatment done in the same blot and with the same exposition.

As for the actin control, this was done in a separate gel, loading the same volume from the same tubes used to load the gel for the pro-caspase-12 measurement. This gel was made after we have decided which lanes would be used to prepare the figure, so only the selected tubes were ran in the gel. This is the reason why the actin blot was not spliced.

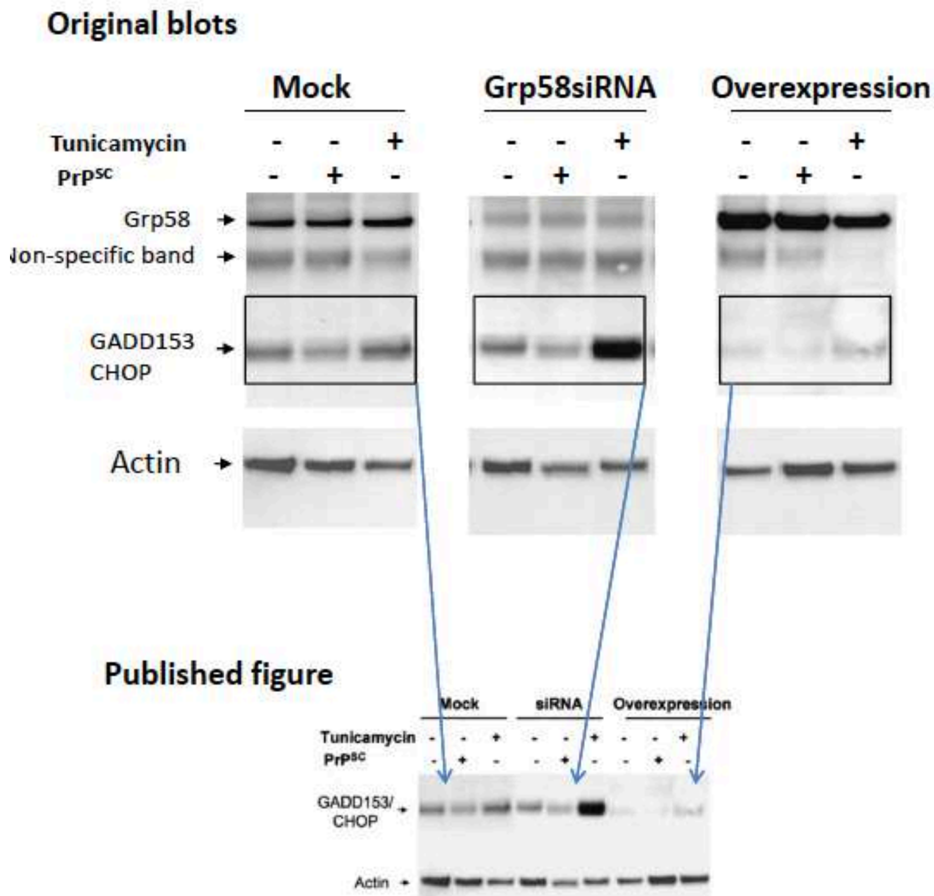
Because of similar molecular weight, it is a frequent practice to run separate gels for the loading control (pro-caspase-12 and actin are very similar (38-45 Kda versus 42-45 KDa, respectively).



Answer:
In the original experiment, all cell lines were treated also with a second positive control (another molecule causing endoplasmic reticulum stress), which in order to simplify the figure. For this reason the blots were spliced.
The relevant comparison is for each cell line between treatment and no treatment. The blots were developed simultaneously with Grp58 and CHOP antibodies to make sure that the cells used indeed expressed high levels or low levels of Grp58. As for the actin loading control, these blots were spliced in the same manner as the experimental blot.

Actions:
The Journal of Neuroscience requested in 2013 requested original data and this issue was clarified without the need of a correction (email in the next page).

FIGURE 6C



Dr Hetz admits digital data manipulation. He also admits to run loading controls on separate gels (Fig 6B), which is bad practice at best. In such experiments like in this figure, if you don't do loading control on the same gel, you can trash everything. The only thing which shocks me is that the Society of Neuroscience did not mind at all.

Claudio Hetz <claudio.hetz@gmail.com>

Re: Message from SfN Ethics Committee

Tue, Sep 17, 2013 at 8:37 PM

To: Claudio Soto <Claudio.Soto@uth.tmc.edu>, "Hetz, Dr Claudio" <chetz@hsph.harvard.edu>

Dear Drs. Soto and Hetz,

Thank you for providing your explanation and your report. The Ethics Committee agrees that no data misrepresentation occurred. Therefore this matter is now considered closed.

I do want to bring to your attention that JN instituted a new policy in December 2012 in which all gel splices are clearly demarcated with lines.

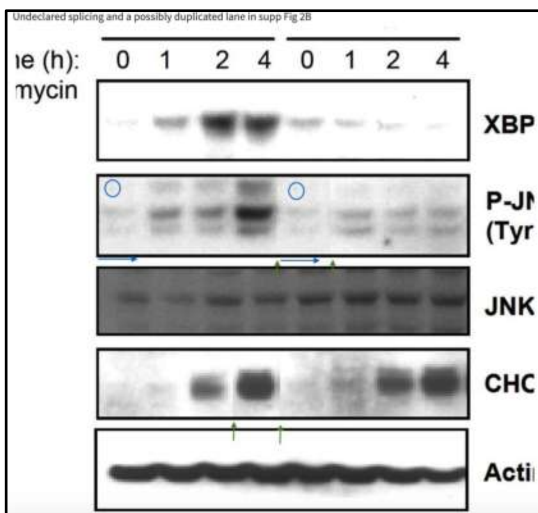
[Quoted text hidden]

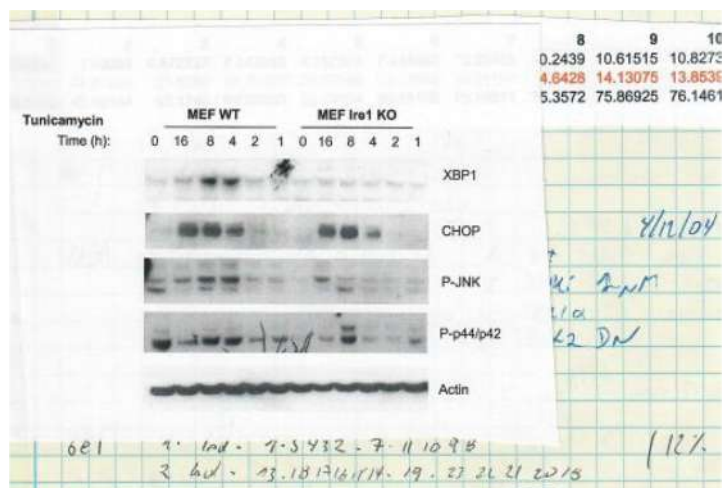
Proapoptotic BAX and BAK modulate the unfolded protein response by a direct interaction with IRE1alpha.**Hetz C**, Bernasconi P, Fisher J, Lee AH, Bassik MC, Antonsson B, Brandt GS, Iwakoshi NN, Schinzel A, Glimcher LH, Korsmeyer SJ.Science. 2006 Apr 28;312(5773):572-6

General comment:

Here we provided data for experiments and raw data from lab book. Most gels indicated were indeed spliced to improve clarity or simplify the results. In this process some times we were not accurate unintentionally, but these errors did not alter the conclusion of the experiments.

Folders with original films were stored at HSPH and then transferred to Cornel University when the lab moved to New York. Data was stored for 7 years (NIH policy) and then all boxes were eliminated. We contacted the lab to scan all primary data as backup, however this was no longer possible.

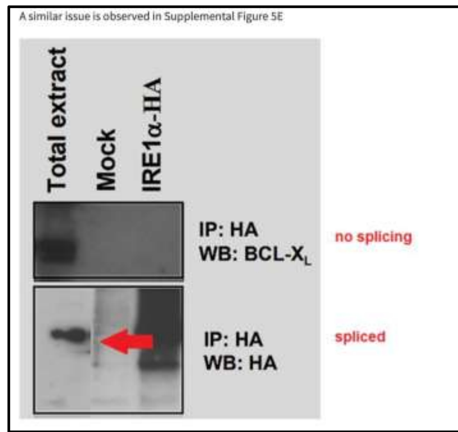




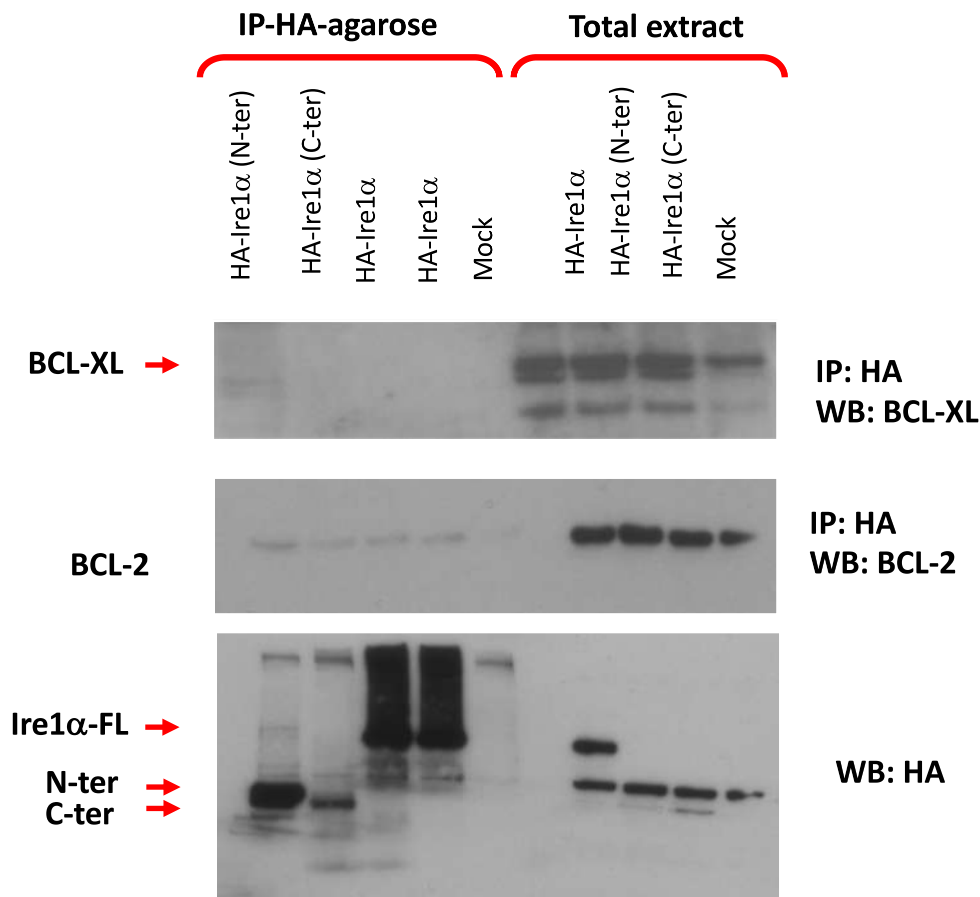
Answer:

Suppl Fig 2B, a kinetic was run in the opposite direction (order of cell harvest) and included more time points, we spliced and reoriented the kinetic. This is the raw data. There was a mistake in in the precision of cutting the gel to built the figure. We apologize for this mistake. This experiment was a simple control to validate the cell lines (published before many times).

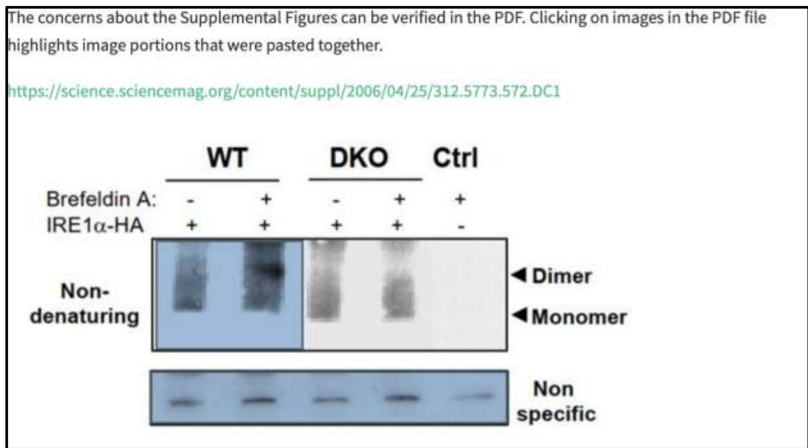
Dr Hetz can't deny manipulation, so he apologizes. But: there is a reason he duplicated the P-JNK band at time point 0. In his own original gel, the two "0(h)" values provide completely opposite results. The CHOP signals don't match (eg, compare 2h values),at 4h a CHOP band was excised and replaced. XBP1 bands were used with wrong legends, without any re-arrangement (16, 6, 4h became 1,2,4h). Dr Hetz selected the results which fit his hypothesis, but this experiment is utterly useless and should never been used. He falsified it instead.



Answer:
Original IP contained more meassurments and was simplified.

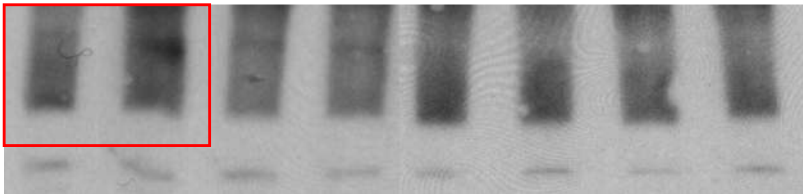


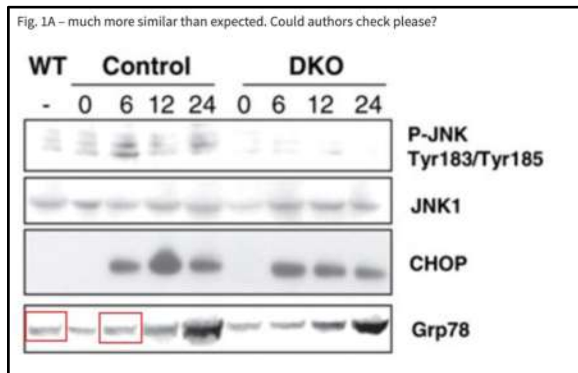
For the problematic WB:HA Dr Hetz shows a different gel from the published figure. The first band in the figure does not exist anywhere on the "raw data" image.



Answer:

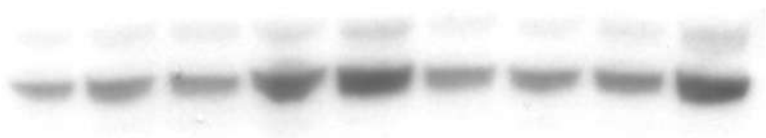
Many samples and conditions were performed, two separate gels were run containing controls plus minus brefeldin A (direct comparison) and other stimuli and time poitns. An error was performed when different versions of the figure were generated and mix up the non specific bands. The non-specific bands are presneted here. Experiment was properly performed.





Answer:

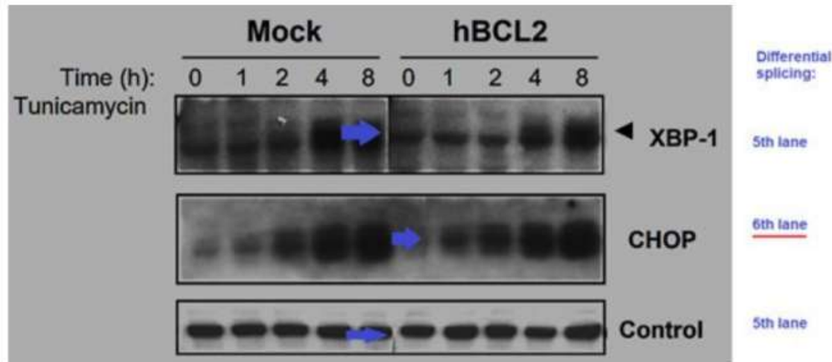
This is the non processed image without modifications to align bands. We applied too much contrast to the bands.



Above: Dr Hetz admits to have combined two gels to look like it was one.
He then provides raw data for Figure 1A (with duplicated band) but it doesn't match. It shows a different gel.
Below: For Figure 2A Dr Hetz shows no raw data at all.

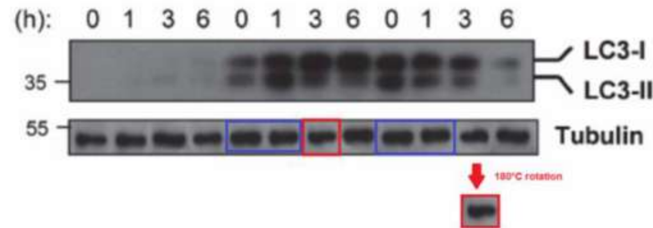
Other figures possibly contain differential splicing. Could authors provide an explanation, please?

Supplemental Figure 2A – Differential splicing. Could authors check it please?



Answer:
This blot contained more samples in the bidle and were excluded genersting this mistake. Data was negative.

BAX inhibitor-1 regulates autophagy by controlling the IRE1α branch of the unfolded protein response.Castillo K, Rojas-Rivera D, Lisbona F, Caballero B, Nassif M, Court FA, Schuck S, Ibar C, Walter P, Sierralta J, Glavic A, **Hetz C.**EMBO J. 2017 Jun 1;36(11):1640

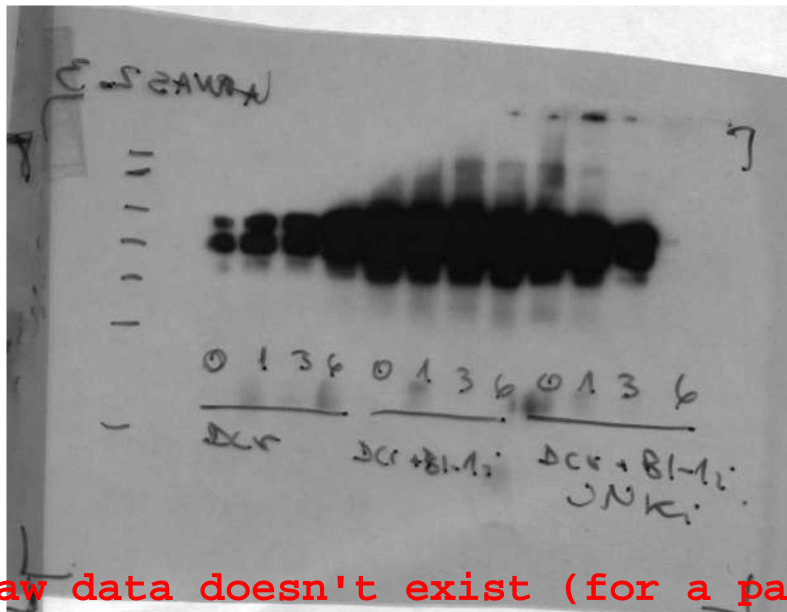


Answers:

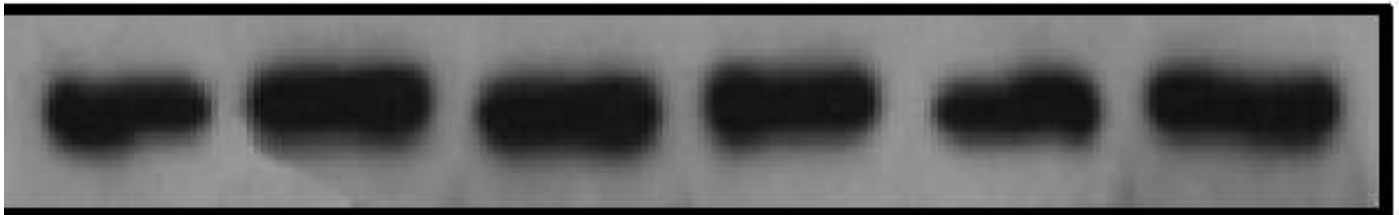
We were unable to find the original scan of the loading control. We found higher expossure of the LC3 blot that confirm that all wells have protein. Tubuline blot is presented with higher magnification that well confirming that all bands are different.

Action:

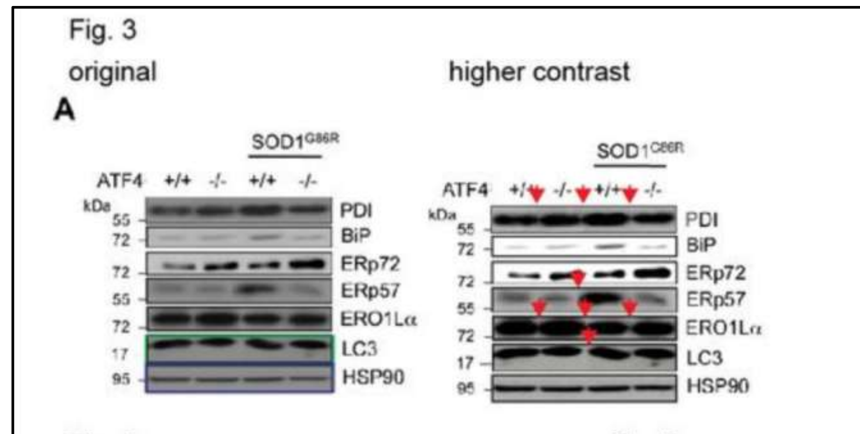
when we performed the correction because of an image duplication in the same fly experiments, the Journal screened the paper as a routine protocol and requested additional full scans to detect undiscusued splicing and all data was ok.



Dr Hetz admits raw data doesn't exist (for a paper from 2017!), shows something else instead, but it brings no additional insights. He then denies band duplication completely.



Functional contribution of the transcription factor ATF4 to the pathogenesis of amyotrophic lateral sclerosis.Matus S, Lopez E, Valenzuela V, Nassif M, Hetz C.PLoS One. 2013 Jul 18;8(7):e66672.

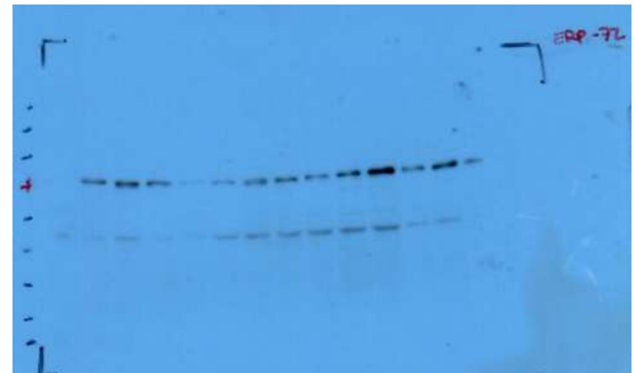


Answer:
Many independent animals were run in the same gel, an an average animal was selected for each protein marker. Also animal groups were run on a different order. See example of full scans. Not always the same animal was chosen, but the same genotype. Clearly this was not the best comparison but the loading controls were very similar between animals.

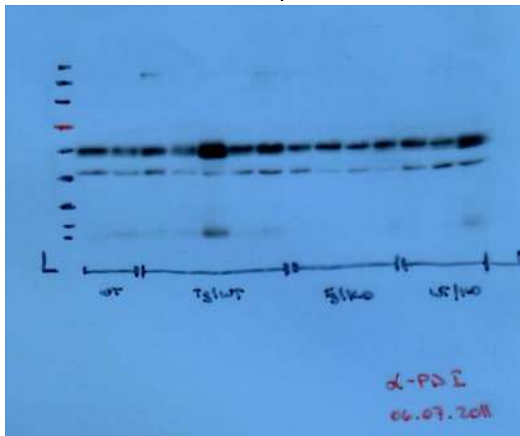
Anti-BiP, Spinal Cord.

WT/WT TG/WT TG/KO WT/KO

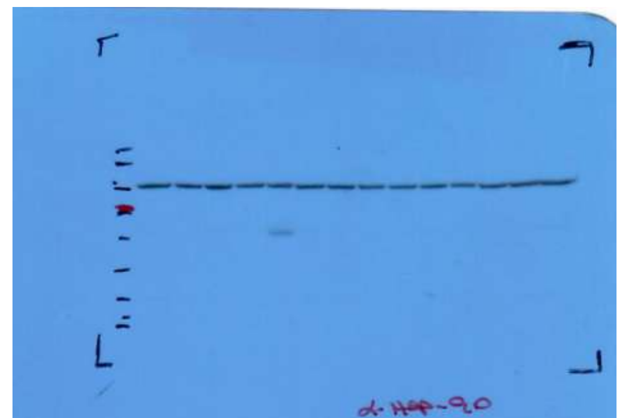
Anti-ERp72, Spinal Cord.



Anti-PDI, Spinal Cord.



Anti-HSP90, Spinal Cord.



PDI labeling is not clear, which band belongs where? Important: Original gel images for the problematic, spliced panels ERp57 and ER01La are not provided at all.