1. Publication

<u>Torres M</u>, Castillo K, Armisén R, Stutzin A, Soto C, <u>Hetz C</u>. **Prion protein misfolding affects calcium homeostasis and sensitizes cells to endoplasmic reticulum stress. PLoS One.** 2010 Dec 29;5(12):e15658. doi: 10.1371/journal.pone.0015658. Erratum in: PLoS One. 2011;6(3). doi: 10.1371/annotation/4087ecad-630d-4436-a301-257b13441156. PMID: 21209925; PMCID: PMC3012133.

2. Pubpeer Comments

Figure 3A: Grp58 and Grp94/78 presents 9 bands while Actin presents 12 bands. Could authors check this issue please?



3. Answer

We thank readers for noticing this error. Here we made a mistake when assembling the figure. These experiments were part of the PhD thesis of Claudio Hetz and the error was generated when figures were prepared by Mauricio Torres (PhD student). The problem was generated during the generation of new versions of the paper since initial figures were correct as indicated here (took 7 years to publish the data). Importantly, the biological conclusion of the experiment is not altered by this mistake. All blots were spliced to eliminated additional treatments and controls that were not shown in the experiments (Figure 1). Importantly, data was properly presented in the PhD thesis 2004 (Figure 2). We think that the image uncropped when the figures were prepared. **Data was provided to the editors and they accepted the submission of a correction.** We have posted raw data and explanations to PubPeer.



Figure 1. Data presentation and figure correction. Selection (Red rectangle) of the bands cropped that corresponds to the original figure.



Figure 2. Correct figure presented in PhD thesis 2004 from Claudio Hetz, University of Chile, including quantification of the experiment.

Email to editor PloS One, submission of a correction.

Correo de Universidad de Chile - about our article Torres et al., 2010 PloS One



Universidad de Chile

Claudio Hetz <chetz@uchile.cl>

about our article Torres et al., 2010 PloS One

Claudio Hetz <chetz@uchile.cl> Para: plosone@plos.org Cc: Mauricio Torres <mauriciotorresg@gmail.com>, Claudio Hetz <chetz@uchile.cl> 23 de febrero de 2021, 11:07

Dear editorial board

I am contacting you because we found a Pubpeer comment on one of our articles published in PloS One (PMID: 21209925). We have posted a response that is attached here to explain the error. Basically, one image uncropped during figure preparation adding extra lines to the loading control. This error does not alter the conclusions of the experiment. Importantly the correct figure was presented in a PhD thesis in 2004 (included here). For transparency we are now submitting the information. We apologize for this error.

Sincerely yours,

Claudio Hetz and Mauricio Torres

Claudio Hetz

Director Biomedical Neuroscience Institute (BNI) Faculty of Medicine, ICBM University of Chile



about our article Torres et al., 2010 PloS One

plosone <plosone@plos.org> Para: "chetz@uchile.cl" <chetz@uchile.cl>

Dear Dr. Hetz,

Thank you for your patience as we reviewed your initial email.

We kindly ask that you help in our assessment by completing the attached document with your responses and comments, and by providing the requested data files to support your published results. For section I, please outline the issues raised on PubPeer and any comments you may have, and respond to the prompts in sections II and III.

Please reply to confirm receipt of this message by March 12th and provide your full response and supporting files by March 30th. If you have any questions, you can reach me directly by responding to this message. Please note that after we receive your response this case will be reviewed by a member of PLOS' Publication Ethics Editorial team. We may contact you with additional queries depending on the outcome of that assessment.

Thank you in advance for your cooperation as we work to resolve this matter. I look forward to hearing from you soon.

Best regards, [El texto citado está oculto]

Correo de Universidad de Chile - about our article Torres et al., 2010 PloS One

Universidad de Chile

Claudio Hetz <chetz@uchile.cl>

14 de marzo de 2021, 20:39

about our article Torres et al., 2010 PloS One

Claudio Hetz <chetz@uchile.cl> Para: plosone <plosone@plos.org>, Mauricio Torres <mauriciotorresg@gmail.com>

Dear Evan

Please find enclosed the author Query and the backup data. Many thanks in advance. Please let us know how to proceed.

Sincerely yours,

claudio Hetz and Mauricio Torres.

[El texto citado está oculto]

2 adjuntos

gels Figure 3A.pptx 1084K

Author Query Form [pone.0015658] Hetz and Torres 2010.docx 60K

Data was provided to the editors and they accepted the correction of the article:

https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0262628

14-03-21 20:41

Claudio Hetz <chetz@uchile.cl>

9 de marzo de 2021, 18:52

24-03-21 13:35

CORRECTION

Correction: Prion Protein Misfolding Affects Calcium Homeostasis and Sensitizes Cells to Endoplasmic Reticulum Stress

Mauricio Torres, Karen Castillo, Ricardo Armisén, Andrés Stutzin, Claudio Soto, Claudio Hetz

An error was made in preparing the Actin panel of Fig 3A of this article [1]: lanes 1, 11, and 12 from the original blot should not have been included. An updated Fig 3 is provided here in which this has been corrected. Lanes 2–10 of the Actin blot were loaded with the same volumes of the same protein samples as those included in lanes 1–9 of other panels in the figure. Parallel blots with the same samples were probed with the indicated antibodies. The available quantitative data underlying graphs in Fig 3 are in S1 File of this notice. The original blots for Fig 3 and the data supporting some other results reported in the article [1] are no longer available.

The authors apologize for the $\underline{Fig 3A}$ error that was generated during figure preparation. The conclusions of the experiment are not altered by this error.



Citation: Torres M, Castillo K, Armisén R, Stutzin A, Soto C, Hetz C (2022) Correction: Prion Protein Misfolding Affects Calcium Homeostasis and Sensitizes Cells to Endoplasmic Reticulum Stress. PLoS ONE 17(1): e0262628. https://doi.org/10.1371/journal.pone.0262628

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Fig 3. Role of ER calcium release after acute exposure to purified PrP^{RES} **from scrapie-infected brains.** (A) Neuro2a cells were treated for 27 h with brain derived PrP^{RES} (50 nM) or brefeldin A (12 μ M), and the levels of Grp58, Grp78, and Grp94 were determined by Western blot. Three independent experiments are presented. Actin levels were monitored as loading control. Right panel: The protein band intensities were quantified and normalized with the expression of actin and the fold induction is presented in comparison with the average signal of non-treated cells. Values correspond to the mean and standard deviation. Student t-test was used to analyze statistical significance with control non-treated cells (** p<0.01, * p<0.05) (**B**) Neuro2a cells were stably transfected with an expression vector for SERCA, and its expression levels were determined by Western blot analysis. Two different cell clones and a control line transfected with empty pcDNA3.1 vector (Mock) are presented. (**C**) As control, the cell lines described in (A) were loaded with Fluo-4, and the release of ER calcium was monitored over time after addition of 300 nM A23187 (arrow) in the absence of extracellular calcium. Arbitrary units of fluorescence are shown (AU). (D) Cell lines expressing different amounts of SERCA pump and the control cell line (Mock) were treated with indicated concentrations of purified PrP^{RES} from 139A-scrapie infected brains. *After* 48 h of incubation, cell viability was analyzed with the MTS assay. Data represent mean and standard deviation of three experiments. *p* values were calculated with parametric t-test (**E**) Neuro2a cells were loaded with

Fluo-4 and then pre-incubated with 10 μ M ryanodine or 10 μ M xestospongin C for 2 hours or left untreated. Calcium fluorescence was measured after 5 min of the addition of 200 nM of purified PrP^{RES}. All determinations were performed in the absence of extracellular calcium. Data represent mean and standard deviation of three determinations. Student t-test was used to analyze statistical significance with control non-treated cells (* p<0.05).

https://doi.org/10.1371/journal.pone.0262628.g001

Supporting information

S1 File. Quantitative data underlying Fig 3A, 3C, 3D, and 3E. Individual-level data for Fig <u>3D</u> are no longer available. For Fig <u>3E</u>, raw data are provided only for two replicates although the graph in Fig <u>3E</u> reports data from three replicates. (XLSX)

Reference

 Torres M, Castillo K, Armisén R, Stutzin A, Soto C, Hetz C (2010) Prion Protein Misfolding Affects Calcium Homeostasis and Sensitizes Cells to Endoplasmic Reticulum Stress. PLoS ONE 5(12): e15658. https://doi.org/10.1371/journal.pone.0015658 PMID: 21209925