

## Ferroptosis induces membrane blebbing in placental trophoblasts

Kazuhiro Kajiwara, Ofer Beharier, Choon-Peng Chng, Julie P. Goff, Yingshi Ouyang, Claudette M. St Croix, Changin Huang, Valerian E. Kagan, K. Jimmy Hsia and Yoel Sadovsky  
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Editor: David Stephens

### Review timeline

Original submission:	14 October 2020
Editorial decision:	7 December 2020
First revision received:	11 December 2020
Accepted:	15 December 2020

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### Original submission

#### First decision letter

MS ID#: JOCES/2020/255737

MS TITLE: Ferroptosis Induces Membrane Blebbing in Placental Trophoblasts

AUTHORS: Kazuhiro Kajiwara, Ofer Beharier, Choon-Peng Chng, Julie P Goff, YINGSHI OUYANG, Claudette M St Croix, Changin Huang, Valerian E. E Kagan, K. Jimmy Hsia, and Yoel Sadovsky  
ARTICLE TYPE: Research Article

We have now reached a decision on the above manuscript.

To see the reviewers' reports and a copy of this decision letter, please go to: <https://submit-jcs.biologists.org> and click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, the reviewers gave highly favourable reports and raised only a few minor points that will require amendments to your manuscript. I hope that you will be able to carry these out, because I would like to be able to accept your paper. You will see there are no new experiments requested.

*We are aware that you may be experiencing disruption to the normal running of your lab that makes experimental revisions challenging. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.*

Please ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion.

I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. Please attend to all of the reviewers' comments. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1*Advance summary and potential significance to field*

The manuscript by Kajiwara, Beharier, Chng et al., describes that ferroptosis is accompanied by macro-blebbing of the plasma membrane, and that these blebs are devoid of cellular organelles. This study follows up previous observations from the same lab of macro-blebs forming in trophoblasts undergoing ferroptosis. In this study they also show that blebs transferred to naive cells does not induce ferroptosis. The study adds some novel fundamental cell biology to what is a fairly new area of research.

*Comments for the author*

The manuscript is very nicely written and beautifully presented - it really was a joy to review. The experiments seem well thought through and logical in design order and repeats and statistics appear appropriate. I do not suggest any additional experiments.

## Minor points:

Figure 1G - are the intensities the same for both panels of figure 1G? It looks like the RSL3 treated image is much higher? Or have the RSL3 cells permeabilised and the CellMask is staining intracellularly? Perhaps a small sentence would help clarify this.

'Lysotracker' is spelt wrongly in Figure 3 image.

Reviewer 2*Advance summary and potential significance to field*

This is very well written and thorough manuscript describing the Ferroptosis on placental trophoblasts. The method of analysis are rigorous and the findings are not overinterpreted.

Whether this process is physiologically relevant and if there is a function of these macroblebs is uncertain and was not uncovered by these studies; however, the authors directly point this out to their credit.

While most studies with uncertain relevance are not published, the rigor and depth of the studies herein and the admission that the function of this process or the blebs remains uncertain, I believe warrants publication as the process of ferroptosis is verifiably real as confirmed by these studies.

*Comments for the author*

Choon-Peng Chng correct spelling in the title?

**First revision**Author response to reviewers' comments

## Response to Reviewers

Ferroptosis Induces Membrane Blebbing in Placental Trophoblasts  
Kazuhiro Kajiwara, et al, JOCES/2020/255737/Rev1

## Reviewer 1 Comments:

The manuscript is very nicely written and beautifully presented - it really was a joy to review. The experiments seem well thought through and logical in design, order and repeats and statistics appear appropriate. I do not suggest any additional experiments.

Response: Thank you for your supportive, kind comments.

Minor points:

1. Figure 1G - are the intensities the same for both panels of figure 1G? It looks like the RSL3 treated image is much higher? Or have the RSL3 cells permeabilized and the CellMask is staining intracellularly? Perhaps a small sentence would help clarify this.

We thank the reviewer for the comment. The apparent enhancement of the intracellular CellMask signal in Fig. 1G likely represents ferroptotic cell shrinkage and membrane collapse. These dramatic changes in cell architecture during the final stages of ferroptosis in trophoblasts are also observed in Movie 1, and in Fig. 3 and 5G. We added a note regarding these changes on page 6, bottom of the first paragraph.

2. 'Lysotracker' is spelt wrongly in Figure 3 image.

Thank you, and we apologize. This is now corrected.

Reviewer 2 Comments:

This is very well written and thorough manuscript describing the Ferroptosis on placental trophoblasts. The method of analysis are rigorous and the findings are not overinterpreted. Whether this process is physiologically relevant and if there is a function of these macroblebs is uncertain and was not uncovered by these studies; however, the authors directly point this out to their credit. While most studies with uncertain relevance are not published, the rigor and depth of the studies herein and the admission that the function of this process or the blebs remains uncertain, I believe warrants publication as the process of ferroptosis is verifiably real as confirmed by these studies.

Response: Thank you for your thoughtful comments. We also hope that our studies will be validated by others, and that we or others define the physiological relevance.

1. Choon-Peng Chng correct spelling in the title?

Thank you. Yes, the name is spelled correctly

Please also note that we have updated the "Funding" section to include NIH grant support to Dr Kagan, which was mistakenly not included in the first draft.

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### Second decision letter

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ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Journal of Cell Science, pending standard ethics checks. Thank you for sending your work to JCS.