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Measuring mitochondrial Ca^{2+} efflux in isolated mitochondria and permeabilized cells

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Reviewer 1

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*Only major points from review and responses included.

Reviewer 1

The present technical communication by Serna et al. aims at proposing a protocol to measure mitochondrial Ca^{2+} efflux in different preparations (isolated mitochondria and permeabilized cells). Authors describe a new method to quantify Na+-sensitive and insensitive mitochondrial Ca^{2+} efflux. Experiments seem to be carefully conducted and I only have a few comments authors might consider.

Unless I missed it, I could not find the number of experiments (e.g. number of independent cultures, number of livers, hearts, etc… in figures 3-7) performed when quantifications are provided. Related to that, it seems that there was no quantification in INS-1E cells as only original recordings are provided. Does that allow authors to conclude about "validation" of the protocol as stated in their part number 4 on page 13? This same heading also mentions "reproducibility", but I couldn't find data to quantify reproducibility.

Authors

Thank you for your careful assessment of our work and useful comments. INS-1E data (Fig. 8) were not quantified, as they represent two replicates. Our quantified data are presented as bar graphs, with individual scatter dots for the results of each separate biological replicate. These demonstrate the number of repetitions made, and clearly show that the results are highly reproducible. We now mention that the scatter plots represent individual biological replicates in the figure legends throughout the manuscript.

Reviewer 1

Figure 1 is entitled "mitochondrial Ca^{2+} transport pathways" but authors mostly focused on inner membrane Ca^{2+} transport, as for instance only MCU is mentioned for Ca^{2+} uptake but not VDAC. I suggest to alter either the title or the figure.

Authors

We have changed the title accordingly (page 2).

Reviewer 1

Out of curiosity, have authors tried their protocol with skeletal muscle permeabilized cells?

Authors

We have not, but believe it should work, given the robust Ca^{2+} uptake in mitochondria from this tissue.