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

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Reviewer 3

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*Only major points from review and responses included.

Reviewer 3

The phosphate, ADP, and magnesium are nowhere mentioned; yet, we know that these compounds severely affect the Ca^{+} uptake/retention/release capacity.

Authors

Thank you for your kind comments. We discuss the effects of adenine nucleotides on mitochondrial calcium transport in the text (page 4, first paragraph), and specify how their absence in different tissues can severely impair Ca^{2+} uptake. This, however, is not the case in the preparations used in this manuscript (which did not include ADP). Phosphate and magnesium concentrations are specified in the detailed media composition (Table 4, page 20).

Reviewer 3

It is all about liver (and a bit of heart). But the species (mice, rats?) not mentioned). Yet, there is a difference between these species, and the cells type, of course.

Authors

We now included the mouse strain (page 5, under heading 2.), and agree that there is significant variation between species, strains and cell types.

Reviewer 3

The expression of these Ca^{2+} transporters in different relevant tissues (brain, heart, liver, kidneys, muscle... whatever else) is not mentioned.

Authors

Unfortunately, there is no consensus about the molecular nature of the calcium/proton exchanger (CHE), precluding quantification data, although there are new preprints which appear to have a strong candidate. NCLX abundance at the mRNA level is higher in mouse livers compared to heart and brain. But mRNA does not, of course, represent protein abundance. Very little is available about protein abundance, probably due to quality issues of the antibodies. As a result, we find it hard to discuss the relative expression of these pathways in different tissues, and hope more can be uncovered about their biology by contributing with this structured activity assay for mitochondrial Ca^{2+} release.