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# Magnesium Green for fluorometric measurement of ATP production does not interfere with mitochondrial respiration

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# **Reviewer 1**

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### **Reviewer 1**

The shown experiments investigate an important question relevant to any study measuring mitochondrial oxygen consumption in the presence of Magnesium Green TM. The text is well-written and organized, the interpretation of the data and the conclusions are clear. It would be nice to increase the number of experiments (as the authors point out as well), and because in the references cited variable MgG concentrations were used, testing more concentrations of the dye in future experiments would also be of significance.

#### Authors

In the new version of the manuscript, we added on page 13: "Further experiments are necessary to investigate and compare  $P_{\nu}/O_2$  ratios, which is beyond the aim of this technical communication". We consider the replicates sufficient to show the effect of MgG on mitochondrial respiration, which is the main aim of this technical communication.

Regarding the different concentrations used in the literature, it would certainly be interesting to analyse whether MgG in higher concentrations could affect mitochondrial respiration (in the literature cited, up to 5  $\mu$ M). However, we consider this testing out of the scope of the present work, as our aim was to assess the impact of MgG under conditions that allow measurement of ATP production – we further clarified this in the reviewed manuscript, last paragraph of page 2: "The experimental MgG concentration was chosen for simultaneous assessment of ATP production and mitochondrial respiration" and first paragraph of page 11: "Similar controls should be applied in studies of mitochondria from other species and tissues or cells, and under different experimental conditions including media with different composition and different MgG concentrations".