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Dear Editor,

Please find enclosed a revised version of the manuscript entitled: "Oxidation of KCNB1 K^+ channels in central nervous system and beyond", ESPS # 7134, by myself, Xilong Wu and Shuang Liu.

We want to thank the reviewers for the enthusiasm and valuable suggestions. In accordance with reviewers' advice we have corrected a few typos, completely redrawn the figure and amended its legend. The revised figure was prepared by Dr. Shuang Liu and therefore we have added her name in the authors' list. Finally, we have added a core-tip section. The specific criticisms of the reviewers are addressed below:

Reviewers #1 and #4

These reviewers only pointed out a few typos which we have corrected.

Reviewer #2

In the figure, the signs apparently indicating non-phosphorylated and phosphorylated KCNB1 should be clearly defined.

We have incorporated the signs in the new figure.

The role of phosphorylated KCNB1 in the cytoplasm and on the membrane should be discussed in the figure legend.

The role of phosphorylated KCNB1 channels in the membrane in the context of apoptosis is not known. Therefore we have added this sentence to the legend of the figure: "It is not known whether Src and p38 phosphorylation directly act to increase KCNB1 current".

The abstract states that the "oxidized KCNB1 channels can directly promote

apoptosis". Neither the oxidized KCNB1 nor the roles of the oxidized KCNB1 are shown in the diagram or mentioned in the figure legend.

We have re-phrased the sentence in the abstract with: "oxidized KCNB1 channel can directly activate pro-apoptotic signaling pathways". In addition in the revised figure we show oxidized KCNB1 channels (in the forms of oligomers) and the pro-apoptotic signaling pathway that they activate.

Adding another figure showing the protective role of KCNB1 and the potential actions of some inhibitors discussed in the text would be very helpful.

We thank the reviewer for this suggestion but we deem a single figure sufficient to this review article.

Reviewer #3

Title: The title reflects the significant of KCNB1 K+ channels oxidation in the CNS; however, this role (oxidation of the channel) has been strengthened by data from other tissues (pulmonary arteries). This should be clearly reflected in the title.

The title states .."in the nervous system and beyond" meaning other tissues such as pulmonary arteries for example. In addition, according to WJBC guidelines, the title should not be longer than 12 words and our current title is 11 words.

The proposed scheme (Fig.1) is not clearly explained in the text nor in the legend. For example, it is not clear: i- whether and at what level the channel acts as pro- or anti-apoptotic. ii- the role of ROS in this mechanism. In other words, ROS induce oxidation, which inhibits the channel and thus has a protective effect. However, ROS, also induce apoptosis. This should be clarified. iii- ROS induction and the link with the channels. iv- the clock inserted above the arrow v- which scheme is for pro and which one is for protection.

We thank the reviewer for this important point. However there is no evidence that ROS induce protection by inhibiting the channel. Rather, it is likely that the current may not be the primary cause of the apoptotic effect of the channel. In the revised manuscript we discuss this concept in the text.

The link at the mitochondrial level (which is the main site for ROS production) between O2 and H2O2 level is not correct. The reduced state of the respiratory chain (i.e. the increase in the electron leakage due to defect in the electron flow in respiratory chain is correlated to the superoxide production which is consequently leading to H2O2 production.

This reviewer is absolutely right, mitochondria would not directly produce hydrogen peroxide. However, multiple sources of ROS appear to be involved in the pathways activated by KCNB1. Since it is likely that hydrogen peroxide is not the only reactive oxygen specie involved, and the details of how these ROS are produced have not been completely elucidated, in the figure, we represent these mechanisms by arrows.

Reviewer #5

it would be very informative to present a model of KCNB1 spatial structure with indication of the positions of the residues discussed.

We thank the reviewer for this suggestion but unfortunately the crystal structure of KCNB1 is not know. However, the approximate position of these residues in the channel has been indicated in the revised figure.

This work is unpublished and I am pleased to share it. Thank you for considering this manuscript for publication.

Sincerely