Response to Reviewers’ comments

Please note that our responses to the reviewers’ comments were shown in blue below. Our changes in the manuscript text were made using the “Track Changes” function.

This manuscript reports roles for subtypes of IP3R in the HEK cell IP3R triple KO cell line. The authors demonstrate that ER calcium leakage, SERCA pump-dependent calcium reuptake and SOCE are all partially controlled by the presence of IP3Rs. IP3R3 subtype plays a dominant role in controlling Orai1 protein levels and SOCE through NEDD4L-dependent degradation of the channel protein. The experiments seem well carried out and the findings are clear. The paper needs language editing in places as the text is occasionally ahrd to follow.

We sincerely thank the reviewer for stating that our “experiments seem well carried out and the findings are clear”. We have followed the review’s suggestion and asked a faculty friend in USA to help us with the language editing.

What the paper also lacks is a mechanistic explanation of the observations. How does the absence of IP3Rs slow down SERCA activity? And how does the IP3 R3 regulate NEDD4L activity? These points should be discussed. The authors show that over expression of IP3Rs can rescue ER calcium homeostasis but does this require calcium release activity? Is SOCE rescued for example when a pore-dead IP3R is put back instead? These are simple experiments to do but would provide valuable insight into mechanism.

We thank the reviewer for the suggestion of adding discussions of possible mechanistic explanations for our observations. We have now added the following sentences “We do not know how the expression of IP3Rs would affect the function of SERCA and Ca\textsuperscript{2+} leak channels. IP3Rs may exert their effects via direct physical interactions, or through IP3Rs-dependent transcriptional changes, or by some post-translational modifications. Further mechanistic studies are needed to get a better understanding on this.” (At line 213-217). “The mechanistic underpinning of the linked expression between IP3R3 and NEDD4L is yet to be established. It is likely that some IP3R-dependent transcriptional factors might get involved and further investigations are needed to elucidate this.” (At line 327-330).

We also thank the reviewer for suggesting the generation of pore-dead IP3Rs to better understand how IP3Rs affect SOCE responses. IP3R 1/3 constructs with either pore-dead or IP3-binding deficiencies are in the process of being made. We will then combine these tools together with other techniques to get mechanistic understandings of the effects of IP3R-expression on SOCE responses, as well as alterations in SERCA pumping and NEDD4L activities. We feel that these investigations themselves stand alone as a separate project, thus they are beyond the scope of the current manuscript. We sincerely hope the reviewer would agree with us on this.