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PONE-D-23-00791
"Reassessing the adrenomedullin scavenging function of ACKR3 in lymphatic endothelial cells"
Original Submission

[redacted] (Reviewer 1)

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5. Review Comments to the Author Please use the space provided to explain your answers to the questions above. You may also include additional comments for the author, including concerns about dual publication, research ethics, or publication ethics. (Please upload your review as an attachment if it exceeds 20,000 characters)	This manuscript from the Halin laboratory has reassessed the established notion (Klein et al. Dev Cell 2014) that ACKR3 acts as a scavenger of the opioid peptide adrenomedullin (AM) in LECs. They have convincingly demonstrated that, in their hands, ACKR3 does not have scavenging activity on AM at physiologically relevant concentrations either in primary LECs (from multiple sources) or transfected cell lines. They have used a combination of approaches to demonstrate this, including knock down of ACKR3 and an ACKR3-specific inhibitor. The experiments are generally well controlled and unequivocal. The manuscript is well-written, appropriately discussed and the data generally are well presented. There are a few concerns regarding statistical analysis, rigour and data interpretation that should be addressed prior to publication however. 1) Figure 1A: data is from a single experiment. This should be done more than once at a minimum. 2) Figure 1A and 1B: quantification and statistical analysis should be provided here. 3) Figure 2C: a direct statistical comparison between the MFI of the '37C AM-AF568' condition between the unstim and TNFa/IFNg groups should be provided to support the claim made on line 354 of the results. 4) Figure 4E and 4F: To show that the signal measured for AM/chemokine uptake in these assays is specific, a negative control should be provided (e.g. cells at 4C) 5) Figure 4H and 4I: the 50nM group depicted in these plots appears to be identical to the data shown in Figure 2C. This group should be removed from 4H and 4I to avoid this duplication. 6) It is notable that the data shown in Figure 4D and 4E appear to suggest that the KD of ACKR3 using shRNA 'C' has actually inhibited AM-induced LEC proliferation. While this is clearly the opposite of what would be expected based on the data published by Klein et al. Dev Cell 2014, the authors should discuss what might underly this. 7) Some of the p values being reported in the manuscript do not seem to match the distribution of data points as presented. Figure 4F (Ctrl: Ctrl v AM p=0.0010); Supp Figure 4C (Ctrl 37C v shRNA C 37C p=0.0004). Please clarify how these values have been computed. 8) Statistical tests and p values should be provided in Supp Fig 1B and SF1G to support claims made in the manuscript. Minor: human gene names should be italicised all caps not lower case for annotation of genes in qPCR
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