Author's response to reviews

Title: Palm is expressed in both developing and adult mouse lens and retina

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Author's response to reviews: see over
To Whom It May Concern:

We are re-submitting a manuscript entitled “Palm is expressed in both developing and adult mouse lens and retina” for consideration for publication in *BMC Ophthalmology*. We thank the reviewers for their careful reading of the manuscript and thoughtful critiques. We have addressed the reviewer’s comments by numerous changes in the manuscript and feel that the resulting product is clearer than the prior submission. We address the reviewer comments point by point below:

**Reviewer one:**

We thank the reviewer for their positive assessment of our manuscript. We address the reviewer’s comments below:

1) We had not presented negative controls for immunostaining to conserve space. We have added the experimental design of our negative controls to the methods section and show two negative control panels in Figure 2.

2) We have italicized gene names throughout the manuscript.

3) We agree with the reviewer that our use of the word “related” to compare the cellular anatomy of lens epithelial and fiber cells to be misleading. We have reorganized this paragraph to better develop our idea here.

4) We have re-evaluated the expression of Palm in the embryonic RPE, and see very little signal for Palm as early as 11.5 dpc (see figure 1) and it is still relatively low at 12.5 dpc. We believe that the reviewer is noting expression in the peri-ocular mesenchyme which is also positive for Palm staining. We have re-written this section of the manuscript to clarify the issue.

5) The reviewer questions whether Palm D expression levels are high enough to be biologically relevant? This is always a difficult question to answer when only gene
expression data is available. In an attempt to partially clarify this issue, we have provided C\textsubscript{T} values for each gene obtained from the quantitative rt-PCR experiments to give the reader another measure of the relative expression levels of these genes.

6) The reviewer asks if we intend to show Palm in the cytoplasm as well as at the cell membrane in retinal cultures. Yes, as stated in the manuscript we see both membrane staining and staining in intracellular puncta.

**Reviewer 2.**

1) We are sorry that our writing was less than clear. Our justification for our study is that we have already shown that PALM levels elevate in the lens in response to increased Pax6 levels in lens fiber cells. We wanted to look at PALM expression more carefully during eye development to determine whether it was likely that the Pax6 site found in the PALM promoter contributes significantly to its expression pattern in vivo. We have rewritten the introduction in an attempt to clarify these points.

2) We did not consider the dorso-ventral gradient of Pax6 expression in our discussion of this study since the sections that we routinely use for immunohistochemistry are transverse sections through the head and do not contain the extremes of the dorsal/ventral surfaces of the retina. However, even with the dorso-ventral gradient, Pax6 is still expressed in all retinal ganglion cells throughout adulthood, however, PALM levels seem to transiently up-regulate during periods of rapid neurite outgrowth. So, although PALM is expressed at highest levels by neurons that express Pax6, and may be a true Pax6 responsive gene, it is clear that other mechanisms must be involved in fine tuning its expression level.

3) The reviewer is correct in stating that the previous version of the manuscript did not show a functional connection between Pax6 and Palm. We strengthened this portion of the manuscript by including transfection data functionally testing the Pax6 binding site we previously identified in the 5’ flanking sequence of the PALM gene. This data shows
that the Pax6 site identified previously by bioinformatics methods is capable of conferring Pax6 responsiveness on a heterologous promoter.

4) We are sorry that our writing is less than clear. PALM is primarily a protein found on the cytoplasmic face of plasma membranes due to its prenyl/palmitoyl anchor. Thus, the protein is membrane associated, but not a membrane protein. We have attempted to clarify that point in the manuscript.

5) We of course agree with the reviewer that expression studies do not prove functional connections. However, as we move closer to creating PALM knockout mice it is important to know what related genes are normally expressed by tissues of interest in order to address each gene’s contribution to tissue function. In response to the reviewer’s concern, we have re-written this portion of the manuscript to soften our language.

6) We had initially omitted a detailed description of our quantitative rt-PCR methods since we were using standard approaches for our data analysis. However, we appreciate that this is still a relatively new technique and we have complied with the reviewer’s request to elaborate on this point. In addition, we elaborated on the significance of the lack of the exon 8 containing splice variant in relation to the known molecular weight of Palm protein in the lens.

7) We mentioned the Palm2-AKAP connection in the manuscript because most of the literature on Palm2 addresses this issue. We have shortened this because we agree with the reviewer that this information is not strictly relevant to the present study.

Minor points:

a) We have changed the wording to “from retinal progenitor” as suggested.

b and c) We have spelled out PBS and provided its pH in the manuscript.

d) We have added a list of abbreviations to the beginning of the manuscript.
e) We have italicized in vivo and in vitro throughout the manuscript.

f) We have reiterated the specificity of the RT-97 antibody as against neurofilament in the results.

g) We have added retina to the list on Page 12, paragraph 2.

h) We have changed the wording to “data are”.

We hope that these changes will make this manuscript acceptable for publication in *BMC Ophthalmology*. If any questions or problems should arise concerning this manuscript, please do not hesitate to contact me.

Sincerely,

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