

Author Response: Comment on “Identification of Novel G Protein–Coupled Receptor 143 Ligands as Pharmacologic Tools for Investigating X-Linked Ocular Albinism”

Thank you for the opportunity to respond to the letter by McKay and colleagues,¹ which argued that the studies in our paper² failed to consider a critical design flaw since the culture medium in which our cells were grown contained tyrosine, complicating interpretation of our data.

Prior to performing the reported experiments,² we compared the effect of tyrosine in the culture medium. Heterologous expression in COS cells resulted in intracellular localization of GPR143, with no difference regardless of whether cells were cultured in the absence or presence of tyrosine (Fig. 1). Furthermore, Palmisano et al.³ found that there was increased expression of heterologous GPR143 in the absence of tyrosine, but that this effect was not limited to tyrosine starvation, but could also be elicited by removal of methionine or cysteine from the culture medium, suggesting a nonspecific effect. Our data similarly demonstrate increased GPR143 levels when tyrosine-free culture medium is used (Fig. 1).

The authors of the letter further demonstrate, using a Ca^{2+} FLIPR assay, that L-DOPA is able to elicit a response from heterologously expressed GPR143 in Chinese hamster ovary (CHO) cells, suggesting that it is a ligand; however, we note that there is no positive control for comparison, making it difficult to determine the magnitude of response. It has been reported⁴ that L-DOPA-promoted intracellular Ca^{2+} concentration increases in retinal pigment epithelium cells but not in CHO cells transfected to express GPR143. These experiments were carried out in the presence of tyrosine. Our studies confirm that L-DOPA does not

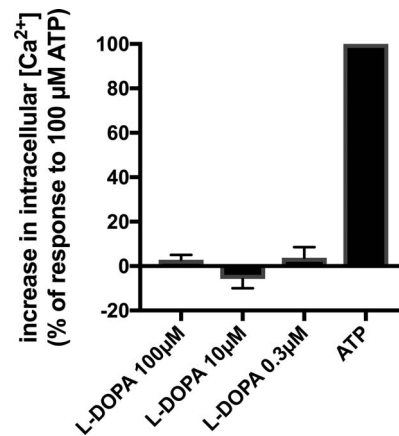


FIGURE 2. Calcium mobilization assay with CHO cells expressing GPR143. CHO cells were grown in DMEM without tyrosine. Assay was performed as described previously.²

elicit a Ca^{2+} response following addition of L-DOPA either in the presence (Fig. 2b seen in De Filippo et al.²) or absence of tyrosine (Fig. 2). Furthermore, no response to L-DOPA could be observed in cells grown with or without tyrosine in the medium as determined by a β -arrestin assay (Fig. 3).

While we concur that increased expression of GPR143 in the absence of tyrosine in the culture medium may indeed improve sensitivity of our assays, given the data presented here (particularly that we observe no change in both the Ca^{2+} and β -arrestin assays between cells cultured in the presence or absence of tyrosine [Figs. 2, 3]). Our data further demonstrate that tyrosine in the medium does not promote the constitutive

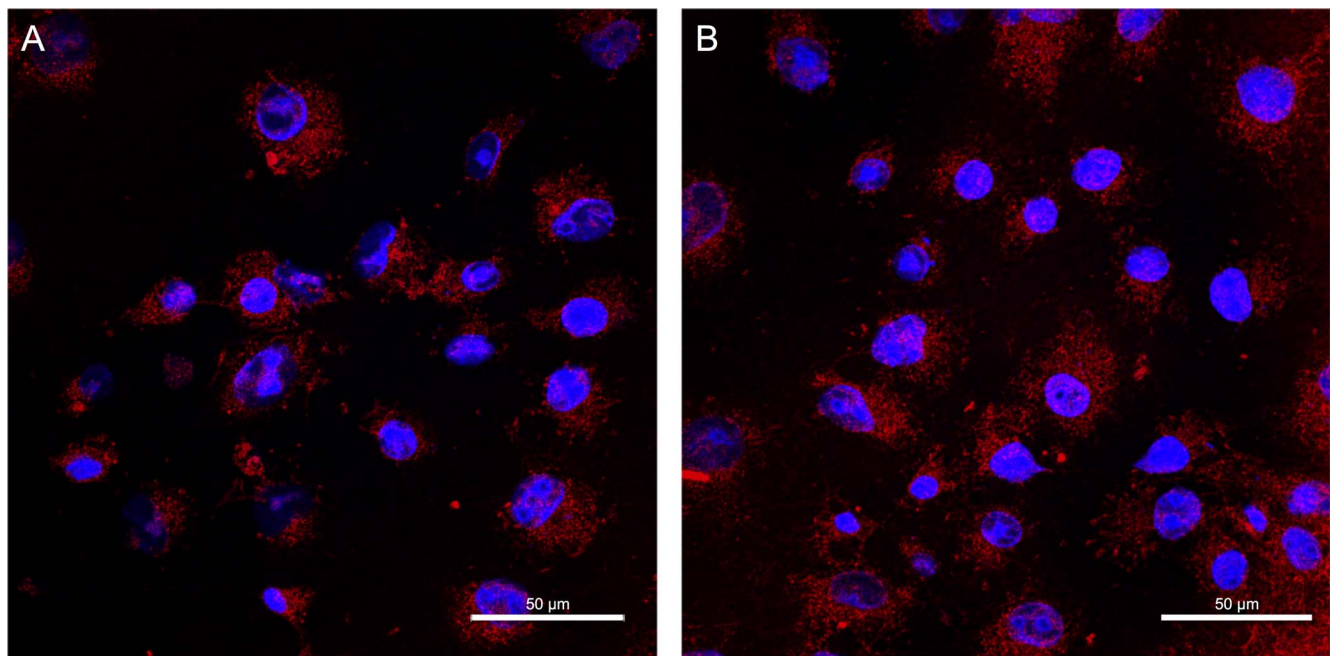


FIGURE 1. Effect of tyrosine on localization of human GPR143 in COS7 cells. COS7 cells transfected with human GPR143 with C-terminal ProLink tag were cultivated in Dulbecco's modified Eagle's medium (DMEM) (A) or DMEM without tyrosine (B) for 2 days. Cells were then fixed and stained with anti-ProLink (first) and anti-mouse Alexa 594 (second) antibodies, and nuclei were stained with 4',6-diamidino-2-phenylindole as reported previously.⁵

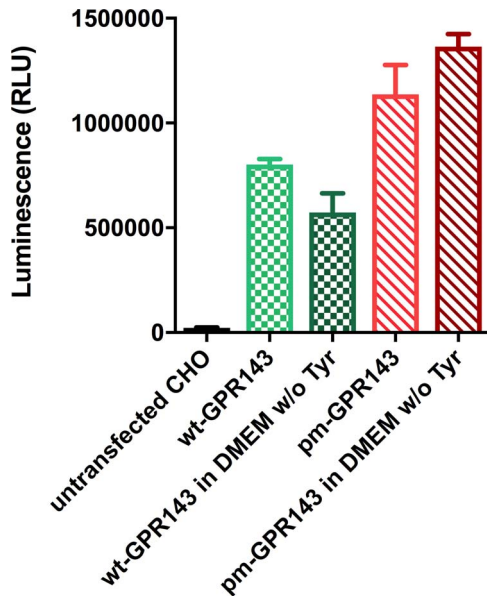


FIGURE 3. β -arrestin recruitment assay of CHO cells transfected with wt-GPR143 or pm-GPR143. CHO cells were cultivated either in DMEM or DMEM without tyrosine. The assay was performed as previously described.²

GPCR activity we observe in the β -arrestin assay (Fig. 3). In addition, the agents identified by our screen were found to have a significant impact on melanin production in melanocytes, even in the presence of tyrosine,² validating the results of our assay.

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