Dear Dr. Péter Ferdinandy

We are pleased to submit our manuscript entitled "Cholecystokinin-type neuropeptides do not act as ligands for GPR173-type receptors (SREBs): the importance of an evolutionary approach to receptor deorphanisation" for consideration in the Journal of British Pharmacology.

In this study, we investigate the recent proposal that the cholecystokinin octapeptide CCK8 acts as a ligand for the orphan receptor GPR173. We find that the use of mammalian cell lines to test this hypothesis is confounded by the endogenous expression of CCK receptors, precluding definitive conclusions. To overcome this, we have adopted a novel comparative evolutionary approach, testing species-specific CCK-type peptides on GPR173 orthologs from the starfish *Asterias rubens* and octopus *Octopus vulgaris* expressed in CHO-K1 cells.

Our key findings demonstrate that:

- CCK-type peptides from both invertebrate species do not activate their respective GPR173-type receptors, or the endogenous CCK receptors in CHO-K1 cells.
- Positive controls confirm the activation of species-specific CCK-type receptors by their cognate CCK-type peptides in these model systems.

These results challenge the previous identification of CCK8 as a ligand for GPR173 and instead support the notion that CCK-type peptides and GPR173-type receptors do not share an evolutionary history of ligand-receptor pairing and co-evolution. As such, the receptors GPR173 should still be considered orphan and the efforts to identify ligands for these receptors should continue.

We believe that the evolutionary approach presented in this study is of broad interest to the readership of the Journal of British Pharmacology, as it highlights the importance of considering ligand-receptor co-evolution when investigating candidate ligands for orphan receptors, particularly in the context of GPCR deorphanisation. Moreover, the use of invertebrate models offers a valuable strategy for overcoming the challenges associated with endogenous receptor expression in mammalian cell lines.

This manuscript has not been submitted to any other journal and all authors have approved the submission. We have no conflicts of interest to declare.

We suggest the following potential reviewers, which have expertise in GPCR characterization/deorphanisation.

- Isabel beets (isabel.beets@kuleuven.be)

- Dan Larhammar (dan.larhammar@uu.se)
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Thank you for your time and consideration of our manuscript. We look forward to your response.

Sincerely,

Luis Alfonso Yanez-Guerra and Maurice R. Elphick.