nature research

Peer Review Information

Journal: Nature Structural and Molecular Biology
Manuscript Title: Structural identification of lysophosphatidylcholines as activating ligands for orphan receptor GPR119
Corresponding author name(s): Professor H. Eric Xu, Dr Yi Jiang , Professor Xin Xie

Reviewer Comments & Decisions:

Decision Letter, initial version:

Apr 20, 2022

Dear Eric,

Thank you again for submitting your manuscript "Structural identification of lysophosphatidylcholines as activating ligands for orphan receptor GPR119". I apologize sincerely for the delay in responding. As you know, this resulted from the difficulty in obtaining a full set of suitable referee reports. Nevertheless, we now have comments (below) from the 3 reviewers who evaluated your paper. In light of those reports, we remain interested in your study and would like to see your response to the comments of the referees, in the form of a revised manuscript.

I hope you will be pleased to see that the reviewers are generally supportive of the work, but they have some helpful suggestions for improving it. Please be sure to address all concerns of the referees in full in a point-by-point response and highlight all changes in the revised manuscript text file. If you have comments that are intended for editors only, please include those in a separate cover letter.

We are committed to providing a fair and constructive peer-review process. Do not hesitate to contact us if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome.

We expect to see your revised manuscript within 6 weeks. If you cannot send it within this time, please contact us to discuss an extension; we would still consider your revision, provided that no similar work has been accepted for publication at NSMB or published elsewhere.

As you already know, we put great emphasis on ensuring that the methods and statistics reported in our papers are correct and accurate. As such, if there are any changes that should be reported, please submit an updated version of the Reporting Summary along with your revision.

Please follow the links below to download these files:

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When submitting the revised version of your manuscript, please pay close attention to our href="https://www.nature.com/nature-research/editorial-policies/image-integrity">Digital Image Integrity Guidelines.

Finally, please ensure that you retain unprocessed data and metadata files after publication, ideally archiving data in perpetuity, as these may be requested during the peer review and production process or after publication if any issues arise.

If there are additional or modified structures presented in the final revision, please submit the corresponding PDB validation reports.

SOURCE DATA: we urge authors to provide, in tabular form, the data underlying the graphical representations used in figures. This is to further increase transparency in data reporting, as detailed in this editorial (http://www.nature.com/nsmb/journal/v22/n10/full/nsmb.3110.html). Spreadsheets can be submitted in excel format. Only one (1) file per figure is permitted; thus, for multi-paneled figures, the source data for each panel should be clearly labeled in the Excel file; alternately the data can be provided as multiple, clearly labeled sheets in an Excel file. When submitting files, the title field should indicate which figure the source data pertains to. We encourage our authors to provide source data at the revision stage, so that they are part of the peer-review process.

Data availability: this journal strongly supports public availability of data. All data used in accepted papers should be available via a public data repository, or alternatively, as Supplementary Information. If data can only be shared on request, please explain why in your Data Availability Statement, and also in the correspondence with your editor. Please note that for some data types, deposition in a public repository is mandatory - more information on our data deposition policies and available repositories can be found below:
https://www.nature.com/nature-research/editorial-policies/reporting-standards#availability-of-data

We require deposition of coordinates (and, in the case of crystal structures, structure factors) into the Protein Data Bank with the designation of immediate release upon publication (HPUB). Electron microscopy-derived density maps and coordinate data must be deposited in EMDB and released upon publication. To avoid delays in publication, dataset accession numbers must be supplied with the final accepted manuscript and appropriate release dates must be indicated at the galley proof stage.

While we encourage the use of color in preparing figures, please note that this will incur a charge to partially defray the cost of printing. Information about color charges can be found at http://www.nature.com/nsmb/authors/submit/index.html#costs

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This URL links to your confidential home page and associated information about manuscripts you may have submitted, or that you are reviewing for us. If you wish to forward this email to co-authors, please delete the link to your homepage.

We look forward to seeing the revised manuscript and thank you for the opportunity to review your work.

Kind regards,
Florian

Florian Ullrich, Ph.D.
Associate Editor
Nature Structural & Molecular Biology
ORCID 0000-0002-1153-2040

Referee expertise:

Referee #1: cryo-EM

Referee #2: GPCRs

Referee #3: GPCR pharmacology

Reviewers' Comments:

Reviewer #1:
Remarks to the Author:

The authors present a structure-function analysis of the orphan GPCR GPR119. It has been known for a while that LPCs are agonists and may be endogenous ligands, and this work describes the binding pocket for LPC. They also determine structures with the drug candidate APD668 that binds in a similar fashion as LPC. Further, computational docking is performed for OEA and Gordonoside F with mutational analyses. Their work will help rational drug design to target GRP119.

I am supportive of the work for publication at NSMB. The cryoEM maps seem to be of high quality, and their structure-based mutations make sense.

I only have minor suggestions:

1. Page 6 lines 142-143: indicate the Cys residues in the figure 1D.
2. Page 7 line 169- add reference where "ref" is indicated.
3. The data for W265A is not shown in Fig 4d, but is talked about in the text.
4. For Fig 6e, they talk of an interaction between I107 and L388 (pg. 10, lines 245-246), but they look really far in the way it has been shown.
5. The Gordonoside F modeling, although supported by mutations, is speculative. The paper is informative even without it.

Reviewer #2:
Remarks to the Author:
This manuscript describes the electron microscopic complex of GPR119-Gs combined with LPC, and further identified LPC with an acyl tail of 18 carbons (18:0 or 18:1) through mass spectrum analyses. In addition, through the analysis of mutational studies, it is further confirmed these two types of lipids LPC and LPE induced GPR119 activation with comparable activities.

The manuscript also identified the unique pocket of GPR119 combined with the LPC. LPC is docked into an extended shallow pocket formed by all seven transmembrane helices and ECL2, which is not recognized in other GPCRs. Through a series of mutations to clarify the detailed binding of LPC, which explains why GPC119 has high constitutive activity. In addition, the out of the registration of the extracellular half of TM5 at P1765.50 suggests that the unique TM5 structure of GPR119, which maybe evolved for specific binding of LPC. What is more attractive is the water molecule seems to stabilize the toggle switch W2386.48 at a position near the ligand.

This study further obtained the complex of a clinical stage drug candidate APD668 binding to GPR119-Gs, and identified that the binding pocket of APD668 is similar to that of LPC. Superposition of the two structures shows that the different ligand-binding modes near the hydrophilic pocket cause different conformations in residues W2657.39 and R2627.36 of the receptor, indicating that the ligand-binding pocket of GPR119 can perform different conformations to adopt the binding of different ligands. Additionally, the toggle switch residue W2386.48 of GPR119 is pulled by the ligand toward the TM3, is a relatively novel activation mode.

From the scientific perspective, the structure is of reasonable quality to draw the conclusions in the manuscript. The mutagenesis and signalling experiments are robust. There are a number of corrections required (see below).

Major points:

The binding of LPC includes a hydrophilic pocket and a hydrophobic pocket, but APD668 only occupies the hydrophobic pocket where LPC is located, so how to explain the affinity of APD668 is much higher than LPC. Moreover, whether the APD668-bound GPR119-Gs complex sample can classify LPC-bound GPR119-Gs complex particles during the data processing.

Although mass spectrometry identified four different types of LPCs with the acyl tail of 18:1, 18:0, 16:1, and 20:0, and suggests that the LPC of 18:1 is the preferred ligand of GPR119. However, different lysophospholipids can induce GPR119, and the functional data of activation show that LPE and LPC have similar effects, so how to exclude the density of pocket is LPC, but not LPE?

It is mentioned in the text that LPC is docked into an extended shallowed pocket formed by all seven transmembrane helices and ECL2, but only ECL2 and TM3, 4, 5, 6, 7 are mutated in fig3. The amino acids involved in the interaction on TM1 and TM2 also need to be confirmed by function assay.

Minor points.

Line 63: Mass spectrometry is not abbreviated at first appearance

Line 78-80: An overall structures comparison is needed to describe that GPR119 is similar to β2AR and D1R.

Line 120: The combined pockets of LPE and LPC are similar. Then, whether the mutation point in Fig. 3 also affects the activation of LPE?

Line 123: It is not shown in Fig.1a that "the hydrophobic pocket of GPR119 is located between TM5/6/7 and TM3 and is covered by ECL2".

Line 134: The comparison of GPR119 with other lipid-binding receptor should be added in the supplementary figure to show its unique features of the GPR119 pocket. The PDB number of the lipid-binding receptor structures should be given.

Line 153: These mentioned receptors need the full name.

Line 160: "including β2AR, D1R, FFA1R, and GPBAR."References need to be added.

Line 169: References need to be added.

Line198: RMSD needs to give the full name.

For Fig.2, 3, 4, Extended Data Fig. 2, 6, 9, 10, the n value of the function experiments need to be given in the Figure legend.

Fig.2d, e: The title of Y-axis "Emax...." in Fig. 2e should be in fig. 2d.

Fig 6e: R1303.50 is too big compared to other marks.

Reviewer #3:
Remarks to the Author:
I was asked to focus on the biology and pharmacology of GPR119; not the structural part.

The manuscript mainly focusses on the structural data, but also describes superficially some biological and pharmacological properties of GPR119. This part can be sharpened and more in accordance to the IUPHAR guidelines regarding GPR119
https://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=126.

Author Rebuttal to Initial comments

Title: Structural identification of lysophosphatidylcholines as activating ligands for orphan receptor GPR119

We thank the editor and the reviewers for their valuable time in reviewing our manuscript and the very constructive suggestions. We carefully addressed all points that have been raised in the revised version of our manuscript. Please find below the point-by-point response to the reviewers' comments (in **black**), with our responses in **blue**.

Referees' comments:

Referee #1 (Remarks to the Author):

The authors present a structure-function analysis of the orphan GPCR GPR119. It has been known for a while that LPCs are agonists and may be endogenous ligands, and this work describes the binding pocket for LPC. They also determine structures with the drug candidate APD668 that binds in a similar fashion as LPC. Further, computational docking is performed for OEA and Gordonoside F with mutational analyses. Their work will help rational drug design to target GRP119.

I am supportive of the work for publication at NSMB. The cryoEM maps seem to be of high quality, and their structure-based mutations make sense.

Response: We appreciate the reviewer for his/her positive comments.

I only have minor suggestions:

1) Page 6 lines 142-143: indicate the Cys residues in the figure 1D.

Response: We thank the referee for his/her helpful suggestion. We have revised the manuscript accordingly by adding the Cys residues in Fig. 1d.

2) Page 7 line 169- add reference where "ref" is indicated.

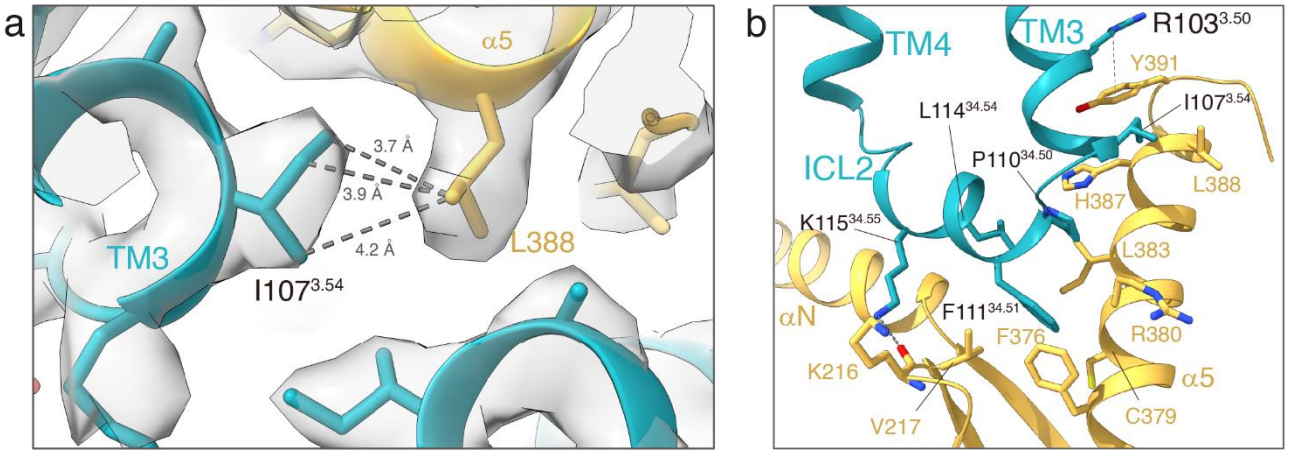
Response: We thank the reviewer for pointing out our mistake and have added the corresponding reference in the revised manuscript.

3) The data for W265A is not shown in Fig 4d, but is talked about in the text.

Response: We apologize for our miss to show this data and have added the data for W265A in Fig. 4d.

4) For Fig 6e, they talk of an interaction between I107 and L388 (pg. 10, lines 245-246), but they look really far in the way it has been shown.

Response: We thank the comment and checked the distance between the residue I107 of the receptor and residue L338 of Gαs. The distance of these two residues is 3.7 Å, suggesting they forms hydrophobic and van de Waal interactions (Response Figure 1a). To better show the interactions, we revised the figure by slightly rotated the perspective view to make the interactions of I107 and L388 more apparent in the figure (Response Figure 1b, see also Figure 6e).



Response Fig. 1 | The interactions between receptor I107 and Gαs L388. a, The model and the EM map of GPR119-Gs-APD668 shows the receptor residue I107 forms hydrophobic interaction with the Gαs residue L388. b, The interactions of ICL2 with Gαs.

5) The Gordonoside F modeling, although supported by mutations, is speculative. The paper is informative even without it.

Response: We thank the referee for the comment. Since the Gordonoside F has been found to suppress appetite and specifically target GPR119. We became interested in the molecular mechanism of the interaction between the Gordonoside F and GPR119. However, there were problems in sample preparation, and the structure of the Gordonoside F recognition of GPR119 fail to obtain at present, so we used molecular docking to obtain possible binding pockets and carried out related functional studies. It is hoped that the new specificity pocket will provide new ideas for the design of weight-loss drugs.

Referee #2 (Remarks to the Author):

This manuscript describes the electron microscopic complex of GPR119-Gs combined with LPC, and further identified LPC with an acyl tail of 18 carbons (18:0 or 18:1) through mass spectrum analyses. In addition, through the analysis of mutational studies, it is further confirmed these two types of lipids LPC and LPE induced GPR119 activation with comparable activities.

The manuscript also identified the unique pocket of GPR119 combined with the LPC. LPC is docked into an extended shallow pocket formed by all seven transmembrane helices and ECL2, which is not recognized in other GPCRs. Through a series of mutations to clarify the detailed binding of LPC, which explains why GPC119 has high constitutive activity. In addition, the out of the registration of the extracellular half of TM5 at P1765.50 suggests that the unique TM5 structure of GPR119, which maybe evolved for specific binding of LPC. What is more attractive is the water molecule seems to stabilize the toggle switch W2386.48 at a position near the ligand.

This study further obtained the complex of a clinical stage drug candidate APD668 binding to GPR119-Gs, and identified that the binding pocket of APD668 is similar to that of LPC. Superposition of the two structures shows that the different ligand-binding modes near the hydrophilic pocket cause different conformations in residues W2657.39 and R2627.36 of the receptor, indicating that the ligand-binding pocket of GPR119 can perform different conformations to adopt the binding of different ligands. Additionally, the toggle switch residue W2386.48 of GPR119 is pulled by the ligand toward the TM3, is a relatively novel activation mode.

From the scientific perspective, the structure is of reasonable quality to draw the conclusions in the manuscript. The mutagenesis and signalling experiments are robust. There are a number of corrections required (see below).

Response: We sincerely appreciate the reviewer for the positive assessment of the quality and importance of this work.

Major points:

The binding of LPC includes a hydrophilic pocket and a hydrophobic pocket, but APD668 only occupies the hydrophobic pocket where LPC is located, so how to explain the affinity of APD668 is much higher than LPC. Moreover, whether the APD668-bound GPR119-Gs complex sample can classify LPC-bound GPR119-Gs complex particles during the data processing.

Response: We thank the reviewer for the comment. Although APD668 binds less volume of pocket than LPC, APD668 has multiple rigid ring groups. These hydrophobic ring groups provide APD668 with more and stronger interactions with GPR119 and allow APD668 to bind to

the GPR119 pocket in a more compact pattern, which stabilizes the binding of APD668 to the receptor. In the contrast, the hydrophobic group of LPC is a structurally flexible acyl tail, which makes LPC have a lower affinity for GPR119. In the data processing of GPR119-Gs-APD668 complexes, we did not obtain the classification of LPC-bound complexes, we assume that this is due to the originally bound LPC in almost all complexes being competitively displaced by APD668 during protein purification.

Although mass spectrometry identified four different types of LPCs with the acyl tail of 18:1, 18:0, 16:1, and 20:0, and suggests that the LPC of 18:1 is the preferred ligand of GPR119. However, different lysophospholipids can induce GPR119, and the functional data of activation show that LPE and LPC have similar effects, so how to exclude the density of pocket is LPC, but not LPE?

Response: We thank the referee for the comment. It is difficult to distinguish LPC and LPE by the density at this level of resolution. However, as the reviewer mentioned, we only identified LPC but not LPE from the mass spectrometry results, so we assume that only LPC is bound to the receptor, which may suggest that LPC is relatively abundant in the membrane.

It is mentioned in the text that LPC is docked into an extended shallowed pocket formed by all seven transmembrane helices and ECL2, but only ECL2 and TM3, 4, 5, 6, 7 are mutated in fig3. The amino acids involved in the interaction on TM1 and TM2 also need to be confirmed by function assay.

Response: We thanks for the comment. Although TM1 and TM2 are involved in shaping the ligand-binding pocket, at the edge of the pocket, the structure shows that the residues of TM1 and TM2 does not form interactions with LPC. To clear the statement and avoid ambiguity, we do not generalize TM1 and TM2 as part of the LPC pocket in the revised manuscript.

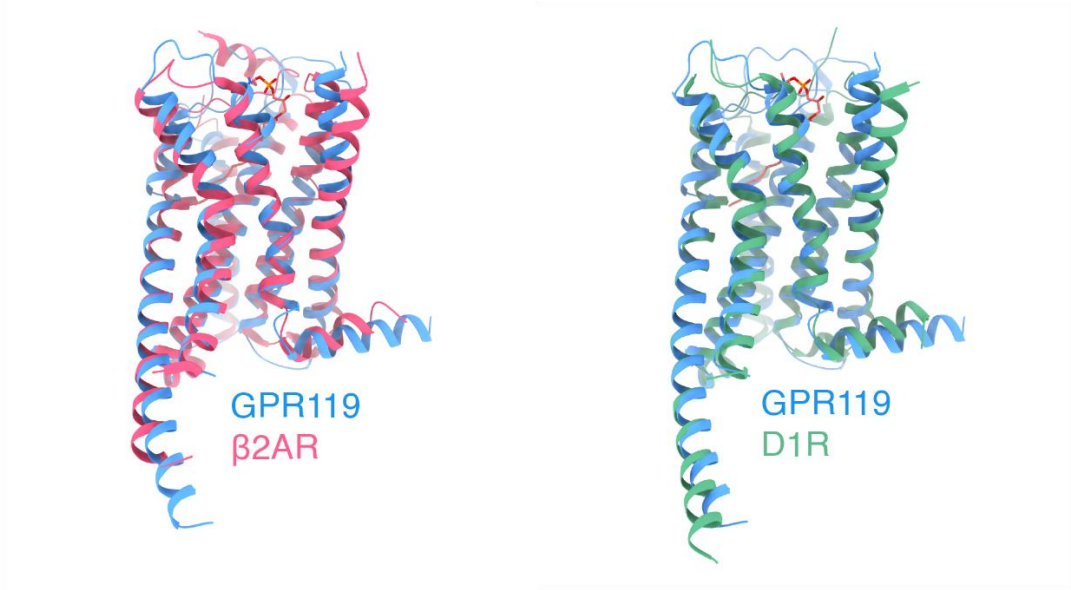
Minor points.

Line 63：Mass spectrometry is not abbreviated at first appearance

Response: We thank the referee for the comment and have added the abbreviation.

Line 78-80: An overall structures comparison is needed to describe that GPR119 is similar to β2AR and D1R.

Response: We agree and have compared GPR119 with β2AR and D1R in the revised manuscript (Response Fig.2, see also Extended Data Fig. 2a).



Response Fig.2 | Structural comparison of GPR119 with β2AR (PDB: 3SN6) and D1R (PDB: 7JVQ).

Line 120: The combined pockets of LPE and LPC are similar. Then, whether the mutation point in Fig. 3 also affects the activation of LPE?

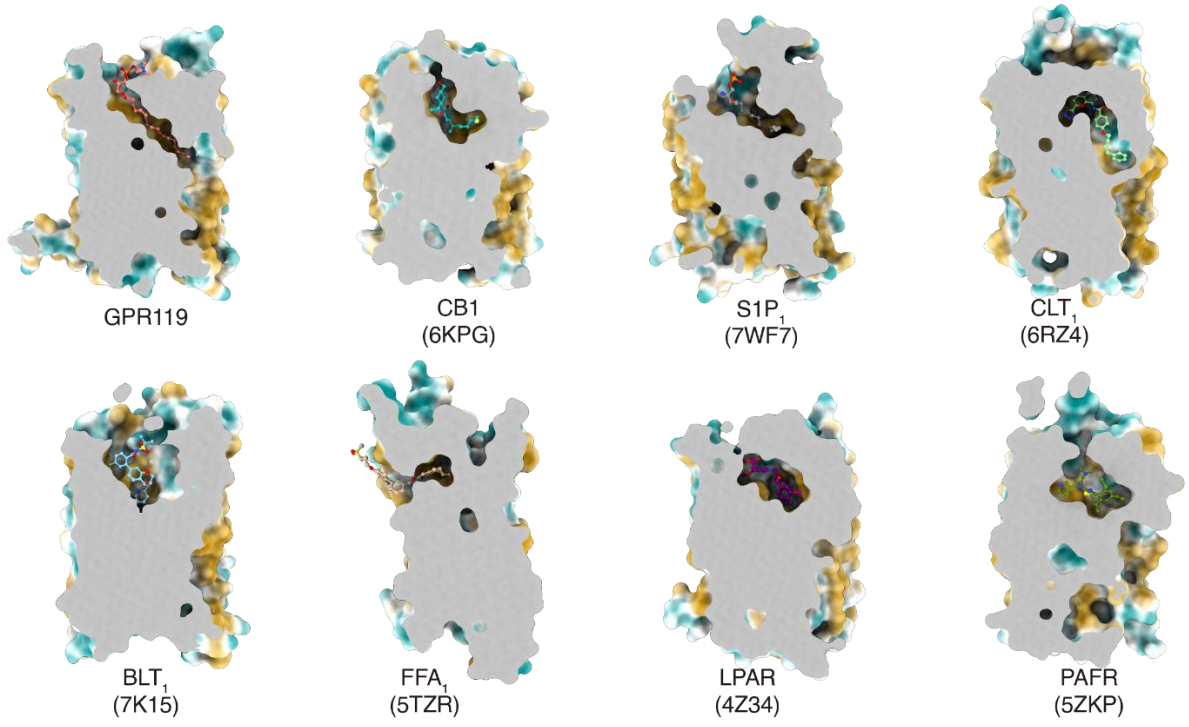
Response: Although the combined pockets of LPE and LPC are similar, the MS analysis has only identified LPC. We thus in this paper only focus on LPC instead of LPE. In addition, LPC and LPE are very similar in their chemical structure groups, with only difference in their head group of ethanolamine verse choline, we envision that the mutations will have similar effects for LPC or LPE mediated activation.

Line 123: It is not shown in Fig.1a that "the hydrophobic pocket of GPR119 is located between TM5/6/7 and TM3 and is covered by ECL2".

Response: The statement should be referred by Fig. 1d. We have revised the manuscript to make the description consistent with the Fig. 1d.

Line 134: The comparison of GPR119 with other lipid-binding receptor should be added in the supplementary figure to show its unique features of the GPR119 pocket. The PDB number of the lipid-binding receptor structures should be given.

Response: We thank the reviewer for the constructive suggestion. We have added the figure to compared GPR119 with other lipid-binding receptors, including CB1, $S1P_1$, CLT_1, BLT_1, FFA_1, LPAR, and PAFR in the revised manuscript (Response Fig.3, see also Extended Data Fig.4a). We also given the PDB codes in figures and legends.



Response Fig.3 | Comparison of the ligand-binding pocket for GPR119 with other lipid-binding GPCRs, including CB1 (PDB: 6KPG), $S1P_1$ (PDB: 7WF7), CLT_1 (PDB: 6RZ4), BLT_1 (PDB: 7K15), FFA_1 (PDB: 5TZR), LPAR (PDB: 4Z34), and PAFR (PDB: 5ZKP).

Line 153: These mentioned receptors need the full name.

Response: We thanks for the suggestion and have added the full name of the receptors.

Line 160: “including β2AR, D1R, FFA1R, and GPBAR.” References need to be added.

Response: We thank the comment and have added the corresponding reference in the revised manuscript.

Line 169: References need to be added.

Response: We have added the corresponding reference in the revised manuscript.

Line198： RMSD needs to give the full name.

Response: We have added the full name of the RMSD.

For Fig.2, 3, 4, Extended Data Fig. 2, 6, 9, 10, the n value of the function experiments need to be given in the Figure legend.

Response: We have added the corresponding statement in the Figure legend.

Fig.2d, e: The title of Y-axis “Emax....” in Fig. 2e should be in fig. 2d.

Response: We thank the comment and have made the change.

Fig 6e： R1303.50 is too big compared to other marks.

Response: We have made the change.

Referee #3 (Remarks to the Author):

I was asked to focus on the biology and pharmacology of GPR119; not the structural part.

The manuscript mainly focusses on the structural data, but also describes superficially some biological and pharmacological properties of GPR119. This part can be sharpened and more in accordance to the IUPHAR guidelines regarding GPR119 https://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=126

Response: We thank the referee for his/her helpful suggestion. According to the IUPHAR guidelines regarding GPR119, we have revised the introduction related to the biology and pharmacology of GPR119. We have refined the statement about the classification and the function of GPR119, which will substantially improve the quality of the paper.

GPR119 is an orphan GPCR that is abundantly expressed in pancreatic beta cells and gastrointestinal enteroendocrine cells[1,2]. Activated GPR119 is coupled with stimulatory G protein (Gs) to promote cAMP production[3]. GPR119 has been provisionally classified into a family with GPR18 and GPR55 because they respond to the analogs of cannabinoid receptor ligands, although showing little structural similarity to cannabinoid receptors[4]. It has been reported that GPR119 play roles in maintenance of glycaemic control, which activation can promote glucose-dependent insulin release[1] in the pancreas and secretion of incretins in the intestine[5,6], such as glucagon-like peptide-1 (GLP-1)[6]. In addition, GPR119 has been suggested to regulate satiety. Hence, GPR119 has received considerable attention as a drug target for type 2 diabetes and obesity in recent years[5,7].

Reference

1 Chu, Z.-L. *et al.* A role for β-cell-expressed G protein-coupled receptor 119 in glycemic control by enhancing glucose-dependent insulin release. *Endocrinology* **148**, 2601-2609 (2007).

2 Chu, Z.-L. *et al.* A role for intestinal endocrine cell-expressed g protein-coupled receptor 119 in glycemic control by enhancing glucagon-like Peptide-1 and glucose-dependent insulinotropic Peptide release. *Endocrinology* **149**, 2038-2047 (2008).

3 Hothersall, J. D. *et al.* Sustained wash-resistant receptor activation responses of GPR119 agonists. *Eur J Pharmacol* **762**, 430-442 (2015).

4 Alexander, S. P. *et al.* Hydrolases (version 2019.5) in the IUPHAR/BPS Guide to Pharmacology Database. *IUPHAR/BPS Guide to Pharmacology CITE* **2019** (2019).

5 Overton, H., Fyfe, M. & Reynet, C. GPR119, a novel G protein - coupled receptor target for the treatment of type 2 diabetes and obesity. *Brit J Pharmacol* **153**, S76-S81 (2008).

6 Lauffer, L. M., Iakoubov, R. & Brubaker, P. L. GPR119 is essential for oleoylethanolamide-induced glucagon-like peptide-1 secretion from the intestinal enteroendocrine L-cell. *Diabetes* **58**, 1058-1066 (2009).
7 Yang, J. W., Kim, H. S., Choi, Y. W., Kim, Y. M. & Kang, K. W. Therapeutic application of GPR119 ligands in metabolic disorders. *Diabetes, Obesity and Metabolism* **20**, 257-269 (2018).

Decision Letter, first revision:

Our ref: NSMB-A45882A

6th May 2022

Dear Dr. Xu,

Thank you for submitting your revised manuscript "Structural identification of lysophosphatidylcholines as activating ligands for orphan receptor GPR119" (NSMB-A45882A). It has now been seen by the original referees and their comments are below. The reviewers find that the paper has improved in revision, and therefore we'll be happy in principle to publish it in Nature Structural & Molecular Biology, pending minor revisions to satisfy the referees' final requests and to comply with our editorial and formatting guidelines.

We are now performing detailed checks on your paper and will send you a checklist detailing our editorial and formatting requirements in about a week. Please do not upload the final materials and make any revisions until you receive this additional information from us.

Thank you again for your interest in Nature Structural & Molecular Biology Please do not hesitate to contact me if you have any questions.

Sincerely,

Carolina Perdigoto, PhD
Chief Editor
Nature Structural & Molecular Biology
orcid.org/0000-0002-5783-7106

Final Decision Letter:

4th Jul 2022

Dear Eric,

We are now happy to accept your revised paper "Structural identification of lysophosphatidylcholines as activating ligands for orphan receptor GPR119" for publication as a Article in Nature Structural & Molecular Biology.

Acceptance is conditional on the manuscript's not being published elsewhere and on there being no announcement of this work to the newspapers, magazines, radio or television until the publication date in Nature Structural & Molecular Biology.

Over the next few weeks, your paper will be copyedited to ensure that it conforms to Nature Structural & Molecular Biology style. Once your paper is typeset, you will receive an email with a link to choose the appropriate publishing options for your paper and our Author Services team will be in touch regarding any additional information that may be required.

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Note the policy of the journal on data deposition:
http://www.nature.com/authors/policies/availability.html.

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