Supplementary Material

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**Supplementary Figure 1. Cryo­-EM data processing of APD597-GPR119-Gs complex.**

**(A)** Representative 200kV cryo-electron microscope particle detection diagram, the scale is 50nm. **(B)** Cryo-electron microscopy image processing flow chart of APD597-GPR119-Gs complex. **(C)** Fourier shell correlation function curve of cryo-electron microscope. **(D)** Local resolution map of 3D classification model density map.

**Table S1.** **Structure statistics.**

|  |  |
| --- | --- |
| **Structure name** | **APD597-GPR119-G**s**-Nb35** |
| **Data collection and processing** |  |
| Magnification | 105,000 |
| Voltage (kV) | 300 |
| Electron exposure (e-/Å2) | 54 |
| Defocus range (μm) | -1.0 ~ -1.5 |
| Pixel size (Å) | 0.851 |
| Symmetry imposed | C1 |
| Initial particle projections (no.) | 2,868,242 |
| Final particle projections (no.) | 930,794 |
| Map resolution (Å) | 2.80 |
| FSC threshold | 0.143 |
| Map resolution range (Å) | 2.36 ~ 41.01 |
| **Refinement** |  |
| Initial model used | 7WCM |
| Model resolution (Å) | 2.87 |
| FSC threshold | 0.5 |
| Map sharpening B factor (Å2) | -130.8 |
| Model composition |  |
| Non-hydrogen atoms | 8,233 |
| Protein residues | 1,047 |
| Ligand | 1 |
| *B*-factors (Å2) |  |
| Protein | 1.91/97.74/33.87 |
| Ligand | 42.06/43.06/43.06 |
| R.m.s. deviations |  |
| Bond lengths (Å) | 0.003 |
| Bond angles (o) | 0.738 |
| Validation |  |
| MolProbity score | 1.35 |
| Clashscore | 3.24 |
| Rotamer outliers (%) | 0.00 |
| Ramachandran plot |  |
| Favored (%) | 96.41 |
| Allowed (%) | 3.59 |
| Disallowed (%) | 0.00 |



**Supplementary Figure 2. cAMP accumulation assay of GPR119.**

Amount of cAMP accumulation in GPR119 mutants induced by GPR119 relative to wild-type receptor, data are from at least three independent experiments performed in triplicate.

**Table S2. APD597 induced cAMP accumulation assays of GPR119.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Mutants** | **APD597** | | | | | | **Expression** | |
| **EC50**  **(nM)** | **PEC50** | | **Span** | | **n** |
| **mean ±s.e.m.** | ***P* value** | **% of WT** | ***P* value** | **% of WT** | ***P* value** |
| Wild type | 41.46 | 7.38±0.13 |  | 100±5 |  | 3 | 100 |  |
| Bril-GPR119 | 102.7 | 6.98±0.08 | 0.9955 | 120±4 | 0.073 | 3 | 124±15 | 0.9847 |
| Q652.64V | 48.70 | 7.31±0.19 | 0.9998 | 116±10 | 0.2242 | 3 | 62±15 | 0.5560 |
| V853.32A | 18.65 | 7.73±0.43 | 0.9827 | 38±7\*\*\* | ＜0.0001 | 3 | 55±8 | 0.2784 |
| T863.33A | ND | ND | ND | ND | ND | 3 | 85±10 | >0.9999 |
| T863.33G | ND | ND | ND | ND | ND | 3 | 40±11 | 0.0360 |
| L943.41D | ND | ND | ND | ND | ND | 3 | 27±7\* | 0.0045 |
| I1364.56A | 53.79 | 7.27±0.43 | 0.9997 | 21±4\*\*\* | ＜0.0001 | 3 | 65±11 | 0.6906 |
| F157 ECL2A | ND | ND | ND | ND | ND | 3 | 73±18 | 0.9467 |
| W2386.48A | ND | ND | ND | ND | ND | 3 | 69±20 | 0.8468 |
| F2416.51A | 9.84 | 8.00±0.73 | 0.6926 | 15±5\*\*\* | ＜0.0001 | 3 | 61±20 | 0.5113 |
| E2617.35A | 272.1 | 6.56±0.44 | 0.3875 | 49±10\*\*\* | ＜0.0001 | 3 | 59±18 | 0.4256 |
| R2627.36A | ND | ND | ND | ND | ND | 3 | 102±7 | >0.9999 |
| W2657.39A | ND | ND | ND | ND | ND | 3 | 83±17 | 0.9997 |

Data are from at least three independent experiments performed in triplicate. \*\*\*P < 0.0001 performed by one-way ANOVA and Dunnett’s post-test, compared with the data of the WT. ND refers to the response value is too low to detect.



**Supplementary Figure3. Chemical structure of APD597 derivatives.**

**(A-J)** The dotted boxes are the different motifs of the derivatives, the EC50 are derived from other literature reports.



**Supplementary Figure4. Structural comparison of APD597-GPR119 and GPR119(predicted by AlphaFold).**

**(A)** Overall structural comparison of APD597-GPR119 and GPR119(predicted by AlphaFold), APD597-GPR119 in violet, GPR119(predicted by AlphaFold) in blue. **(B)** The conformational changes of ligand binding pocket residues between APD597-GPR119 with GPR119(predicted by AlphaFold). **(C-D)** The conformational changes of conserved motifs between APD597-GPR119 with GPR119(predicted by AlphaFold).



**Supplementary Figure5. Interaction between GPR119 and Gβs protein.**

The interaction between GPR119 and Gβs protein was analyzed by LigPlot+. GPR119 and 5HT4R residues are located above the dashed black line, and Gβs residues below the line. Hydrophobic interactions are illustrated by pink (GPR119) or red (Gβs) arcs. Amino acids involved in salt bridge and H-bonds are shown in atomic detail with salt bridge shown as dashed red lines and H-bonds shown as dashed green lines.



**Supplementary Figure6. Chemical structure formula of GPR119 synthesis agonists.**

**(A)** six-membered heterocyclic core agonists. **(B)** five-membered heterocyclic core agonists. **(C)** double-ring fusion core agonists. **(D)** linear connection core agonists. The blue boxes are the core skeleton, the black dotted circles are the different motifs of the synthesis agonists, the EC50 are derived from other literature reports(Jones et al., 2009; Shah and Kowalski, 2010; Buzard et al., 2012; Li et al., 2021; Qian et al., 2022).

**Table S3. Docking scores of synthetic agonists to GPR119.**

|  |  |  |
| --- | --- | --- |
| **Ligand number** | **Docking Score** | **EC50** |
| 1.1 | -11.69 | 5.8nM |
| 1.2 | -12.95 | 14nM |
| 1.3 | -12.02 | 6.9nM |
| 1.4 | -11.6 | 4nM |
| 1.7 | -11.24 | 46nM |
| 1.8 | -11.34 | 1.2µM |
| 1.9 | -10.84 | 2nM |
| 1.10 | -11.47 | 14nM |
| 2.1 | -10.03 | 1.9µM |
| 2.2 | -8.42 | 8.4µM |
| 2.3 | -11.85 | 0.4µM |
| 3.1 | -12.67 | 40nM |
| 3.2 | -13.14 | 3nM |
| 3.4 | -12.1 | 43nM |
| 4.1 | -8.51 | 0.5µM |
| 4.2 | -11.07 | 0.5µM |
| 4.3 | -10.63 | 0.3µM |