

Title

Structural insight into lysophosphatidylserine binding and $G\alpha_{12/13}$ -coupling selectivity of P2Y₁₀

Authors/Affiliations

Han Yin^{1,4}, Nozomi Kamakura^{2,4}, Yu Qian¹, Manae Tatsumi², Tatsuya Ikuta², Jiale Liang¹, Zhenmei Xu¹, Ruixue Xia¹, Anqi Zhang¹, Changyou Guo¹, Asuka Inoue^{2,3*}, Yuanzheng He^{1,5*}

¹Laboratory of Receptor Structure and Signaling, HIT Center for Life Sciences, School of Life Science and Technology, Harbin Institute of Technology, Harbin 150001, China

²Graduate School of Pharmaceutical Sciences, Tohoku University, 6-3, Aoba, Aramaki, Aoba-ku, Sendai, Miyagi, 980-8578 Japan

³Graduate School of Pharmaceutical Sciences, Kyoto University, 46-29 Yoshida-Shimo-Adachi-cho, Sakyo-ku, Kyoto 606-8501 Japan

⁴These authors contributed equally to this work

⁵Lead contact

* Corresponding authors: iaska@tohoku.ac.jp (A. I.) and ajian.he@hit.edu.cn (Y.H.)

SUMMARY

The lysophosphatidylserine (LysoPS) receptor P2Y₁₀, also known as LPS₂, plays crucial roles in the regulation of immune responses and holds promise for the treatment of autoimmune diseases. Here, we report the cryo-EM structure of LysoPS-bound P2Y₁₀ in complex with engineered G₁₃ heterotrimeric protein. The structure and a mutagenesis study highlight the predominant role of a comprehensive polar network in facilitating the binding and activation of the receptor by LysoPS. This interaction pattern is preserved in GPR174, but not in GPR34. Moreover, our structural study unveils the essential interactions that underlie the G α_{13} engagement of P2Y₁₀ and identifies key determinants for G α_{13} coupling, whose mutations selectively disrupt G α_{12} engagement while preserving the intact coupling of G α_{13} . Together, our structural insights serve as a foundational basis for comprehending LysoPS signaling through P2Y₁₀.

Keywords

Lysophosphatidylserine; Lysophosphatidylserine receptor; P2Y₁₀; G $\alpha_{12/13}$ coupling; G α_{13} coupling selectivity

SIGNIFICANCE

Among the three LysoPS receptors, P2Y₁₀ was the last subtype to be structurally determined. In this study, we have determined the cryo-EM structure of LysoPS-bound P2Y₁₀ in complex with an engineered G₁₃, revealing a conserved ligand-binding mode shared with GPR174, but not with GPR34. Additionally, we identified key determinants of G_{α12}-versus-G_{α13}-coupling for P2Y₁₀. These findings provide a foundation for understanding P2Y₁₀ ligand recognition and signaling.

Highlights

- Cryo-EM structure of LysoPS-bound P2Y₁₀ in complex with G_{α13}
- A conserved binding mode of LysoPS in P2Y₁₀ and GPR174
- Key determinant of G_{α12}-vs.-G_{α13}-coupling selectivity

eTOC blurb

In this study, Yin et al. unveil the cryo-EM structure of LysoPS-bound P2Y₁₀ in complex with G₁₃, elucidating the binding mode of LysoPS and the key determinants of G_{α13} coupling with P2Y₁₀. Their findings lay a foundation for a deeper understanding of LysoPS signaling.

INTRODUCTION

Lysophosphatidylserine (LysoPS) is a bioactive lysophospholipid and involved in various physiological functions. LysoPS contains the glycerol backbone with the

phosphoserine head group and one fatty acid at either *sn*-1 or *sn*-2 position^{1,2}. It was first recognized as a bioactive lipid that significantly amplifies histamine release triggered by IgE in rodent mast cells. Experimental studies on cells have demonstrated that LysoPS enhances mast cell degranulation and mast cell-related anaphylactic shock, promoting inflammatory responses³. LysoPS also functions as a lipid mediator that supports macrophages in engulfing apoptotic neutrophils, facilitating the resolution of inflammation. As a result, LysoPS has a dual role in both promoting inflammation and its resolution. Additionally, LysoPS inhibits the proliferation of T cells while stimulating the migration of glioma cells⁴⁻⁶.

Many of the LysoPS functions are known to be mediated through G-protein-coupled receptors (GPCRs) including GPR34 (also known as LPS₁), P2Y10/LPS₂, and GPR174/LPS₃^{1,7,8}. These receptors for LysoPS are primarily found in immune cells and trigger various immunological functions by activating downstream G α proteins³. GPR34 is implicated in mast cell degranulation and anaphylaxis, appearing crucial for a robust immune response upon antigen and pathogen challenges⁷. GPR174, a G α_s - and G α_{13} -coupled GPCR, exhibits exclusive expression in immune-related tissues and cell types, particularly T and B cells^{9,10}. P2Y10 is expressed in immune-related organs, such as the spleen, bone marrow, and lymphoid tissues^{1,3}. Structure-activity relationship studies have unveiled that P2Y10 agonists, including LysoPS and its analogues, promote the formation of actin stress fibers in P2Y10-expressing mammalian cells¹¹. While GPR34 functions through G α_i coupling, P2Y10 exhibits a strong preference for G $\alpha_{12/13}$ downstream signaling¹². In T cells, G $\alpha_{12/13}$ signaling serves to dampen cellular function, and the genetic inactivation of G α_{12} and G α_{13} genes has been shown to enhance T cell proliferation and adhesive properties¹³.

Recent advancements in understanding the structures of the LysoPS receptor GPR174 in complex with the G α_s protein^{14,15} and GPR34 in complex with G α_i protein¹⁶ have shed light on the ligand's recognition process. Despite a considerable homology shared between GPR174 and P2Y10, the binding of LysoPS to these two receptors results in distinct signaling pathways: G α_s and G α_{13} for GPR174 and G $\alpha_{12/13}$ for P2Y10. A critical question in LysoPS signaling concerns how the same ligand can initiate different signaling cascades.

Given the significance of P2Y₁₀ signaling in the immune system, we resolved the cryo-EM structure of human P2Y₁₀ complexed with LysoPS, revealing its interaction with the Gα₁₃ protein and unveiling the selective coupling mechanism of P2Y₁₀ to Gα₁₃.

RESULTS

Overall architecture of P2Y₁₀

Human P2Y₁₀ was co-expressed with a mini-G_{13/i} protein¹⁷ encompassing the N-terminus of Gα_{i1} (amino acids 1-23, details see Methods), along with Gβ₁, Gγ₂, and a single-chain variable fragment (scFv16) of an antibody specifically targeting the N-terminus of Gα_{i1}, within *Spodoptera frugiperda* (Sf9) insect cells. To enhance the stability of the receptor–G-protein complex, we fused the C-terminus of P2Y₁₀ with a large fragment (LgBiT) of the NanoBiT split luciferase, while the C-terminus of Gβ₁ was fused with the high-affinity version of a small fragment (HiBiT) of NanoBiT¹⁸. Employing a conventional membrane protein purification protocol developed in our laboratory¹⁹ (Figure S1 and details see Methods), we isolated the complex.

The structure of the P2Y₁₀ signaling complex bound with LysoPS and mini-G_{13/i} (hereafter simply referred to as P2Y₁₀–G₁₃ or P2Y₁₀–Gα₁₃ when solely describing the interface between P2Y₁₀ and the engineered Gα protein) was resolved through single-particle analysis of cryo-EM at a resolution of 3.06 Å, adhering to the gold standard criterion of FSC = 0.143 (Figure S1 and Table S1). The general architecture of the P2Y₁₀–G₁₃ complex closely resembles the canonical GPCR–G-protein complex, with the receptor primarily engaging with the Gα subunit of the heterotrimeric G₁₃ protein (Figure 1). An analysis of local resolution indicates that the core of the Gβ₁ subunit, the transmembrane domain of the receptor, and the Gα₁₃ interface of scFv16 exhibit the highest resolution. The decent density map of the receptor enables the unequivocal identification of residues 24 to 314 of P2Y₁₀, encompassing the typically invisible intracellular loop 3 (ICL3). The endogenous ligand, LysoPS, is distinctly visible through the lateral entry formed by transmembrane helices 3, 4, and 5 (TM3, TM4, and TM5) (Figure 1). In addition, we also identified two cholesterol molecules on the transmembrane side of P2Y₁₀. One is on the lower part of the

TM4-TM5 groove, beneath LysoPS; the other is on the TM2 side (Figure S3A). It is intriguing to note that the positions of these cholesterol molecules resemble those found in GPR174, suggesting evolutionary conserved binding sites (Figure S3B). These cholesterol molecules might aid in stabilizing the receptor.

LysoPS binding

LysoPS is well resolved in the ligand binding pocket. Similar to GPR174^{14,15}, the lower section of ligand binding pocket in P2Y10 exhibits a marked hydrophobic property, whereas the upper region is rich in hydrophilic elements and carries a significant charge (Figures S2A and S2B). The pocket's lower extremity is open and connects with the hydrophobic region of the plasma membrane, enabling a lateral entry of lipid ligand (Figure S2C).

The LysoPS head, encompassing L-serine and the phosphate group, is enveloped within a pocket composed of polar amino acid residues, including Y114^{3,33}, K113^{3,32}, R90^{2,60}, Y41^{1,39}, Y94^{2,64}, Y37^{1,35}, H287^{7,35}, Y266^{6,58}, Y187^{ECL2}, T267^{6,59}, and R171^{ECL2} (Figure 2A). The presence of positively charged residues such as K113^{3,32}, R90^{2,60}, H287^{7,35}, and R171^{ECL2} imparts a positive charge to the upper pocket, which aligns well with the negatively charged LysoPS head. On the whole, the LysoPS head is securely anchored within the pocket through an intricate network of polar interactions involving Y37^{1,35}, Y94^{2,64}, R90^{2,60}, Y114^{3,33}, and R171^{ECL2} (Figure 2A).

We next compared the polar interactions of P2Y10 with those of the other LysoPS receptors. When we compared our P2Y10 structure with previously reported GPR174 structures^{14,15}, we found highly conserved interactions with LysoPS that are formed by residues R90^{2,60}, Y94^{2,64}, Y114^{3,33}, and R171^{ECL2}, though slight variations, such as Y246^{6,51}, K257^{6,62}, and F169^{ECL2} in GPR174, and Y37^{1,35} in P2Y10, introducing subtle differences (Figure 2B). In contrast to GPR174, a comparison with the recently resolved GPR34 structure¹⁶ revealed marked differences. Firstly, the phosphate serine head of LysoPS in GPR34 is not deeply embedded into the pocket close to TM1, TM2, and TM7. Secondly, the acyl tail of LysoPS in GPR34 tilts toward TM4 (Figure S2D). A detailed examination of the head group interaction reveals that the phosphate serine group of LysoPS forms a polar network primarily on the TM5/TM6 side of the GPR34 ligand-binding pocket,

whereas the polar network is mainly situated on the TM1/TM2 side in P2Y10 (Figure 2C). In the alignment of the LysoPS family sequences, it is evident that the conserved polar network is present in GPR174 and P2Y10, but not in GPR34 (Figure 2D), aligning with the higher homology observed between GPR174 and P2Y10. The only consensus residue shared by the two polar networks is Y^{3.33} (Y144^{3.33} for P2Y10, Y135^{3.33} for GPR34). Beside the head and tail differences, significant disparities exist in the position and orientation of the extracellular sides of TM4 and TM5, resulting in notable differences in the binding of the acyl chain of LysoPS. In GPR34, TM4 stands upright (perpendicular to the cell membrane surface), whereas in P2Y10, TM4 is tilted towards the receptor's center. Conversely, TM5 in P2Y10 is shifted outward. Consequently, the acyl tail of LysoPS is directed towards TM3 in GPR34, while in P2Y10, it is directed towards the receptor's center (Figure S2E).

To confirm our structural observation, we performed a mutagenesis study. We constructed five alanine-substituted mutants including Y37^{1.35}A, R90^{2.60}A, Y94^{2.64}A, Y114^{3.33}A and R171^{ECL2}A. Flow cytometry analysis showed that the expression levels of Y37^{1.35}A, R90^{2.60}A and R171^{ECL2}A were comparable to those of the wild-type (WT) P2Y10 whereas Y94^{2.64}A and Y114^{3.33}A showed a slight reduction in expression, comparable to half the volume of WT plasmid (Figure 2E). Receptor response to LysoPS was measured by the TGF α shedding assay²⁰ using a chimeric G $\alpha_{q/13}$ protein containing substitution with the entire $\alpha 5$ helix (Methods) as well as by the NanoBiT-G-protein dissociation assay using a NanoBiT-G₁₃ sensor¹². In both assays, we found that all of the five mutants demonstrated diminished signaling responses to LysoPS with R171^{ECL2}A showing the most significant reduction, followed by Y37^{1.35}A and R90^{2.60}A (Figure 2F, G). The impact on Y94^{2.64}A and Y114^{3.33}A was relatively mild. Collectively, these mutant experiment supports the importance of the residues of Y37^{1.35}, R90^{2.60}, Y94^{2.64}, Y114^{3.33}, and R171^{ECL2} in recognition of LysoPS, although the extent of contributions varies among these individual residues.

Receptor activation

A comparison between the antagonist BPTU-bound P2Y1²¹ and the LysoPS-bound P2Y10 reveals significant structural changes. The most notable alteration is the outward shift of transmembrane helix 6 (TM6) on the intracellular side in the active P2Y10 configuration. This movement enables the accommodation of the helix 5 (α H5) of $G\alpha_{13}$ to establish a contact with the intracellular cavity of P2Y10 (Figure 3A), a distinctive hallmark of GPCR activation²². Furthermore, a subtle inward movement of TM7 is observed on the receptor's intracellular side.

The binding of LysoPS to the ligand binding pocket of P2Y10 has impacts on Y266^{6.58} and Y259^{6.51} of TM6, which pushes the extracellular side of TM6 outward. Then the perturbation of the extracellular side of TM6 generates a twist effect at F256^{6.48} of the micro-switch motif, which tilts the intracellular side of TM6 toward cytoplasm. Then the interaction is passing to Y305^{7.53} of the NPxxY motif and R131^{3.50} of the DRY motif, leading the fully opening of the intracellular for G-protein to engage the receptor (Figures 3B). In the context of Family-A GPCR activation, these changes are part of coordinated motion involving the conserved motifs, such as N^{7.49}P^{7.50}xxY^{7.53} and D^{3.49}R^{3.50}Y^{3.51}. Specifically, within the DRY motif, a swaying motion of R131^{3.50} towards the expanded TM6 is noticeable in the active P2Y10 state. This allows R131^{3.50} to form polar interactions with the tip of α H5 (Figures 3C and 4A). In the NPxxY motif, a prominent adjustment involves the movement of Y305^{7.53} towards the central region (Figure 3D), a phenomenon frequently observed during the activation of most Family A GPCRs²³.

$G\alpha_{13}$ engagement

The interaction between P2Y10 and $G\alpha_{13}$ involves a combination of both hydrophobic and hydrophilic interactions. Specifically, notable hydrogen bond interactions are observed, such as K240^{6.32} and R131^{3.50} forming bonds with the backbone carboxyl groups of Q230^{G.H5.26} and L227^{G.H5.23} (superscript denotes the common $G\alpha$ numbering system²⁴), respectively. Additionally, R238^{6.30} and K66^{ICL1} establish ionic interactions with D217^{DG.H5.13} and D222^{G.H5.18}, respectively (Figure 4A). Hydrophobic interactions are facilitated by L225^{5.65}, which engages in significant interactions with L220^{G.H5.16} and L224^{G.H5.20}. On the ICL2 side, R142^{ICL2} forms a hydrogen bond interaction with the

backbone carboxyl group of R32^{G.hns1.03} of the α N-terminal helix (α N), and the phenol ring of F139^{ICL2} fits into a hydrophobic cavity formed by V79^{G.S3.01}, F81^{G.S3.03}, F212^{G.H5.08}, V215^{G.H5.11}, and I219^{G.H5.15} of $G\alpha_{13}$ (Figure 4B). A comparative analysis was also conducted between the P2Y10– $G\alpha_{13}$ engagement and the engagement between the sphingosine-1-phosphate receptor 2 (S1PR2) and $G\alpha_{13}$ ²⁵. This comparison reveals the α H5 in the P2Y10– $G\alpha_{13}$ complex is shifted more to the cytoplasm side than in other $G\alpha_{13}$ complexes (S1PR2– $G\alpha_{13}$, CD97– $G\alpha_{13}$ and GPR35– $G\alpha_{13}$) (Figure 4C).

To examine the importance of the observed interactions in the P2Y10– $G\alpha_{13}$, we performed a mutant study. Specifically, we evaluated the impact of mutations in seven P2Y10 residues (K66^{ICL1}, R131^{3.50}, F139^{34.51}, R142^{34.54}, L225^{5.65}, R238^{6.30} and K240^{6.32}) that interact with non- α H5 regions of the $G\alpha_{13}$ subunit. Flow cytometry analysis found that, except for K66^{ICL1}A and R131^{3.50}A mutants, which were expressed similarly to WT (1:5), the other mutants showed equal or higher expression compared to WT (1:1) (Figure 4D). The TGF α shedding assay revealed that, aside from the K66^{ICL1}A mutant, the remaining six mutants displayed either partial loss-of-function (R142^{34.54}A and R238^{6.30}A) or were almost completely defective in $G\alpha_{13}$ coupling (R131^{3.50}A, F139^{34.51}A, L225^{5.65}A, and K240^{6.32}A) (Figure 4E, Figure S2F). The NanoBiT-G-protein dissociation assay confirmed the selective reduction of $G\alpha_{12}$ coupling in the R142^{34.54}A and the R238^{6.30}A mutants (Figure 4F, Figure S2G). These findings experimentally confirm the functional significance of interactions outside of the α H5 region in activating G proteins.

$G\alpha_{13}$ -vs.- $G\alpha_{12}$ -coupling selectivity

P2Y10 almost exclusively couples with $G\alpha_{12/13}$ proteins, whereas the closely related GPR174 primarily interacts with $G\alpha_s$ protein and has the potential to couple with $G\alpha_{13}$ protein^{1,3,26,27}. To elucidate these interactions, we focused on comparing the coupling of P2Y10 with $G\alpha_{13}$ against the coupling of GPR174 with $G\alpha_s$ ¹⁴. The most notable differences between P2Y10– $G\alpha_{13}$ and GPR174– $G\alpha_s$ interactions are observed in positioning and penetration depth of the α H5 tip. Within the context of P2Y10's engagement with $G\alpha_{13}$, the tip (C-terminus) of α H5 extends outward of TM6, with the insertion of the tip being

240 relatively shallow. Conversely, in GPR174's interaction with $G\alpha_s$, the α H5 tip delves
241 deeply into the intracellular cavity, orienting towards TM5 and TM3 (Figure 5A).

242 There are two notable residues in α H5 that may account for the $G\alpha_{12}$ selectivity
243 between P2Y10 and GPR174. The $G\alpha$ residue G.H5.23 (L374 in $G\alpha_{13}$ and I378 in $G\alpha_{12}$)
244 interacts with the GPCR residue 3.53 (Figure 5B). In P2Y10, F134^{3.53} seems to be flexible
245 and can accommodate both Leu and Ile of G.H.5.23 whereas F119^{3.53} in GPR174 is
246 sterically immobilized by the adjacent F126^{34.53} (Figure 5B). It is worth noting that Leu
247 (G.H.5.23) plays a pivotal role in $G\alpha_{13}$ recognition, and a solitary substitution of Leu with
248 Ile abolishes $G\alpha_{13}$ coupling in GPR35²⁸. In the P2Y10- $G\alpha_{13}$ structure, the $G\alpha$ residue
249 G.H5.17 (H368 in $G\alpha_{13}$ and Q372 in $G\alpha_{12}$) does not have interaction. However,
250 superimposition of the GPR174- $G\alpha_s$ structure onto the P2Y10- $G\alpha_{13}$ structure suggests that
251 GPR174 has a different conformation at the beginning of TM6 and D221^{6.26} in GPR174
252 forms a polar interaction with H368^{G.H5.17} of $G\alpha_{13}$ (Figure 5B). The D221^{6.26} in GPR174
253 may disfavor the Q372^{G.H5.17} of $G\alpha_{12}$. The difference is likely caused by the presence of
254 G234^{6.26} in P2Y10, which disrupts the stability of a helical structure in that region. We also
255 used ColabFold²⁹, a variation of AlphaFold³⁰, to predict the GPR174- $G\alpha_{13}$ complex. The
256 predicted model shows that D221^{6.26} is facing H368^{G.H5.17} in a proximity of 4.0 Å, a
257 reasonable distance to form a direct interaction between these two residues (Figure S3C).

258 To validate the importance of the two sites in the $G\alpha_{12}$ recognition, we performed a
259 mutagenesis study. For this, we constructed A141^{34.53}F and G234^{6.26}D P2Y10 mutants. We
260 reasoned that the A141^{34.53}F mutant would let F134^{3.53} to flip out from α H5 and favor
261 interaction with $G\alpha_{13}$ but not $G\alpha_{12}$. Similarly, the G234^{6.26}D mutant would disfavor $G\alpha_{12}$
262 coupling. An expression level of the G234^{6.26}D was found to be similar to that of WT while
263 the A141^{34.53}F mutant was expressed at a level equivalent to that of a half volume of WT
264 plasmid transfection (Figure 5C). We then assessed $G\alpha_{12}$ -vs.- $G\alpha_{13}$ coupling by the TGF α
265 shedding assay using the $G\alpha_{q/12}$ and $G\alpha_{q/13}$ chimeras. At first, we confirmed that GPR174
266 minimally coupled with $G\alpha_{q/12}$ (Figure 5D). In A141^{34.53}F and G234^{6.26}D mutants, $G\alpha_{q/12}$
267 coupling was greatly diminished as compared with expression-matched P2Y10-WT
268 conditions (WT (1:2) and WT (1:1), respectively) while the levels of $G\alpha_{q/13}$ coupling were
269 only modestly reduced (Figures 5D and S2F). The NanoBiT-G-protein dissociation assay

also showed the shift of coupling preference toward $G\alpha_{13}$ in these mutants (Figure 5E). Collectively, these data strongly support that the dual coupling of $G\alpha_{12}$ and $G\alpha_{13}$ is attributed to the flexible nature of P2Y10 in the regions involving A141^{34,53} and F134^{3,53} as well as G234^{6,26}.

DISCUSSION

The $G\alpha_{12/13}$ subfamily stands out as distinctive members among the four $G\alpha$ subfamilies. Upon GPCR-mediated activation, $G\alpha_{12/13}$ primarily associate with RhoGEF to activate downstream effectors such as RhoA, and tightly correlate with cell migration activity³¹. Despite sharing common signaling features, gene deficiency studies in mice reveal that $G\alpha_{12}$ and $G\alpha_{13}$ are not interchangeable³². In terms of $G\alpha_{12/13}$ coupled receptors, it is noteworthy that certain receptors exhibit a preference for either $G\alpha_{13}$ or $G\alpha_{12}$ to carry out their physiological functions. For example, CD97 (ADGRE5) has been demonstrated to promote dendritic cell homeostasis through $G\alpha_{13}$ coupling but not $G\alpha_{12}$ ³³, underscoring functional distinctions between $G\alpha_{13}$ and $G\alpha_{12}$.

In spite of recent advancements involving the structural elucidation of the first LysoPS receptor, GPR174 in complex with G_s heterotrimer¹⁴, and the subsequent GPR34 in complex with G_i heterotrimer¹⁶, significant inquiries regarding the G-protein selectivity of LysoPS receptors, particularly the $G\alpha_{12}$ -vs- $G\alpha_{13}$ selectivity, remain unanswered. In this study, we present the cryo-EM structure of LysoPS-bound P2Y10 in complex with an engineered G_{13} heterotrimer. This structure not only offers insights into the mode of LysoPS recognition within P2Y10 but also unveils key determinants of $G\alpha_{13}$ engagement. Building upon these structural insights, we have identified mutants that selectively disrupt $G\alpha_{12}$ signaling while maintaining the activity of $G\alpha_{13}$ coupling. The insights derived from our study significantly contribute to the understanding of LysoPS-mediated signaling via P2Y10 and provide valuable clues for the design of LysoPS modulators that can selectively activate $G\alpha_{13}$ signaling.

297

298 **ACKNOWLEDGMENTS**

299 We thank Kayo Sato, Shigeko Nakano and Ayumi Inoue at Tohoku University for their
300 assistance in plasmid preparation, the flow cytometry analysis and the NanoBiT-G-protein
301 assay. This work was supported by the Startup Funds of HIT Center for Life Sciences; the
302 National Natural Science Foundation of China (32070048 to Y.H.). A.I. was funded by
303 KAKENHI JP21H04791, JP21H05113 and JP21H05037 from the Japan Society for the
304 Promotion of Science (JSPS); JP22ama121038 and JP22zf0127007 from the Japan Agency
305 for Medical Research and Development (AMED); JPMJFR215T, JPMJMS2023 and
306 22714181 from the Japan Science and Technology Agency (JST).

307

308 **AUTHOR CONTRIBUTIONS**

309 H.Y. made the expression constructs, purified the proteins, prepared and screened grids for
310 cryo-EM, analyzed data and prepared the figures. N.K. made mutants, performed
311 functional assays, analyzed data. Y.Q. made initial constructs and set the purification
312 method. M.T. and T.I design and made mutants. J.L., Z.X. and R.X. set the experiments,
313 prepared plasmids and cultured cells. A.I. designed and supervised functional study,
314 analyzed data and edited the manuscript. Y.H. designed experiments, analyzed data, solved
315 the structures, wrote the manuscript and supervised the whole project. All authors
316 contributed to data interpretation and preparation of the manuscript.

317

318 **DECLARATION OF INTERESTS**

319 The authors declare no competing interests.

320

321

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-DYKDDDDK monoclonal antibody	FujiFilm Wako Pure Chemicals	Cat# 012-22384
Anti-mouse IgG secondary antibody conjugated with Alexa Fluor 488	Thermo Fisher Scientific	Cat# A11001
Bacterial and virus strains		
<i>E. coli</i> cells DH5 α	Invitrogen	Cat# 18265017
<i>E. coli</i> cells DH10Bac	Invitrogen	Cat# 10361012
Biological samples		
Fetal bovine serum	Thermo Fisher Scientific	Cat# 10270-106
Chemicals, peptides, and recombinant proteins		
DMEM	Thermo Fisher Scientific	Cat# D6546
DMEM	Nissui	Cat #05919
Opti-MEM	Thermo Fisher Scientific	Cat# 31985-070
Insect Cell Culture medium	Expression Systems	Cat# ESF921
LysoPS	Avanti Polar Lipids	Cat# 858143
EDTA solution	Thermo Fisher Scientific	Cat# 15575-020
Bovine serum albumin	SERVA Electrophoresis	Cat# 11945
Coelenterazine	Angene	N/A
Fetal bovine serum (FBS)	HAKATA	Cat# HB-FBS-500
Protease Inhibitor Cocktail	APExBIO	Cat# K1007
Lauryl Maltose Neopentyl Glycol (LMNG)	Anatrace	Cat# NG310
Glyco-diosgenin (GDN)	Anatrace	Cat# GDN101
Cholesterol hemisuccinate TRIS Salt (CHS)	Anatrace	Cat# C6512
X-tremeGENE HP DNA Transfection Reagent	Roche	Cat# 06366546001
Critical commercial assays		
Phusion Plus DNA Polymerase	Thermo Fisher Scientific	Cat# F630S
Deposited data		
LysoPS-bound P2Y ₁₀ -G ₁₃ complex	This study	PDB: 8KGG
Cryo-EM map of LysoPS-bound P2Y ₁₀ -G ₁₃ complex	This study	EMDB: EMD-37220
Experimental models: cell lines		
Insect cell line Sf9	Expression Systems	N/A
HEK293 cells	Nat Methods 2012 ²⁰	N/A

$\Delta G\alpha_{q/11/12/13}$ -deficient HEK293 cells	Cell 2019 ¹²	
Oligonucleotides		
P2Y10-F: TTCTGCCTGGTATTCGCCATGGCCAACCTGG ACAAGTAC	This study	N/A
P2Y10-R: ATCTTCGAGTGTGAAGACTCCGATCATGCT GCTGCCGCT	This study	N/A
Recombinant DNA		
pFastBac-CD97(1-339) -lgBit-TEV-MBP	This paper	N/A
pFastBac-(mini-)G $\alpha_{13/i}$	This paper	N/A
pFastBac-G β_1	This paper	N/A
pFastBac-G γ_2	This paper	N/A
pFastBac-scFv16	This paper	N/A
pCAGGS-ssHA-FLAG-P2Y10	This paper	N/A
pCAGGS-ssHA-FLAG-GPR174	This paper	N/A
pCAGGS-AP-TGF α	Nat Methods 2012 ²⁰	N/A
pcDNA3.1-G $\alpha_{q/12}$	Sci Rep 2024 ³⁴	N/A
pcDNA3.1-G $\alpha_{q/13}$	Sci Rep 2024 ³⁴	N/A
pcDNA3.1-G α_{12} fused with LgBiT	Cell 2019 ¹²	N/A
pcDNA3.1-G α_{13} fused with LgBiT	Cell 2019 ¹²	N/A
pcDNA3.1-G β_1	Cell 2019 ¹²	N/A
pCAGGS-G γ_2 N-terminally fused with SmBiT	Cell 2019 ¹²	N/A
pCAGGS-G γ_2 (C68S) N-terminally fused with SmBiT	Cell 2019 ¹²	N/A
pCAGGS-RIC8A	Cell 2019 ¹²	N/A
Software and algorithms		
PyMOL 2	Schrödinger	https://pymol.org/2/
Prism 8, Prism 9, Prism 10	GraphPad Software	https://www.graphpad.com/scientific-software/prism/
Flow Jo 10	BD Biosciences	https://www.flowjo.com/solutions/flowjo
CryoSparc 3.0	Punjani, A, et al., 2017	https://cryosparc.com/
PHENIX 1.18.2	Adams, P. D. <i>et al.</i> 2010	https://www.phenix-online.org/
COOT	(Emsley and Cowtan, 2004)	https://www2.mrc-lmb.cam.ac.uk/personal/pemsley/coot/
CHIMERA	Pettersen et al., 2004	http://www.cgl.ucsf.edu/chimera

Relion3.0	(Zivanov et al., 2018)	https://www2.mrc-lmb.cam.ac.uk/relion
MotionCor2	(Zheng et al., 2017)	https://emcore.ucsf.edu/ucsf-software
Other		
6-cm culture dish	Greiner Bio-One	Cat# VTC-P6
6-well plate	Greiner Bio-One	Cat#
24-well plate	Greiner Bio-One	Cat# 662970
48-well plate	Greiner Bio-One	Cat# 677980
96-well plate	Greiner Bio-one	Cat# 651970
Superdex 200 Increase column 10/300	GE healthcare	Cat# 28990944
Protein chromatography purification system	Cytiva	Cat# AKTA pure 25
96-well white microplate assay plate	Corning	Cat# 3917
96-well white microplate assay plate	Greiner Bio-one	Cat# 655083
EnVision 2015	PerkinElmer	Cat# 2105-0010

323

324

325 **Resource Availability**

326 **Lead contact**

327 Further information and requests for resources and reagents should be directed to and will
328 be fulfilled by the lead contact, Yuanzheng He (ajian.he@hit.edu.cn) or Asuka Inoue
329 (iaska@tohoku.ac.jp).

330 **Materials availability**

331 All reagents generated in this study are available from the lead contact with a completed
332 materials transfer agreement.

333 **Data and code availability**

• The cryo-EM density maps and atomic coordinates have been deposited in the Electron Microscopy Data Bank (EMDB) and Protein Data Bank (PDB) under accession numbers EMD-37220 and 8KGG for the LysoPS-bound P2Y₁₀/Gα₁₃ complex.

• This paper does not report original code.

• Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Insect cell culture

Spodoptera frugiperda (Sf9) insect cells were cultured and maintained in ESF 921 insect cell culture medium (Expression System) at 27°C, the sex of sf9 cells is unknown.

Mammalian cell culture

HEK293 cells were cultured in DMEM medium with 10% FBS at 27°C with 5% CO₂.
HEK293 cells were originated from female fetus.

Escherichia coli cell culture

E. coli strain BL21 was cultured in LB Broth with continuous shaking (130 rpm) or on LB agar plates (LB Broth, Miller medium with 15 g agar per liter) at 37°C.

METHOD DETAILS

Constructs

The codon-optimized human *P2Y10* gene (residues 1-339) with an N-terminal haemagglutinin (HA)-signal peptide sequence was cloned in pFastBac1 baculovirus expression vector, and a LgBiT fusion was fused to the C-terminus of P2Y10, followed by a tobacco etch virus (TEV) cleavage site and 2× maltose-binding protein (MBP). The C-terminus HiBiT fusion of wild-type human Gβ₁ was cloned into pFastBac1 plasmid as the VIP1R paper¹⁸. The mini-Gα₁₃ construct¹⁷ which has a mini-Gα_s backbone and the Gα₁₃-derived residues (FRDVKDTILHDNLKQLMLQ) in the α5 helix was adopted from the latrophilin receptor 3/Gα₁₃ complex paper¹⁷. The Gα sequences was codon-optimized and synthesized by Langjing Biotech, Shanghai, and inserted into pFastBac1 plasmid. The wild-type human Gγ₂ and scFv16 that encodes the single-chain variable fragment of mAb16 were cloned into pFastBac1 plasmid.

Protein expression and purification

For the Gα₁₃ complex assembling, baculovirus encoding the P2Y10, Gα (mini-Gα_{13/i}), Gβ₁, Gγ₂, and scFv16 were co-infected into the *Spodoptera frugiperda* (Sf9) cells (2× 10⁶ cells per ml) at a ratio of 1:100 (virus volume vs. cells volume) and cells were harvested 48 h post infection. Cell pellets were resuspended in 20 mM Hepes buffer, 150 mM NaCl, 10 mM MgCl₂, 20 mM KCl, 5 mM CaCl₂, pH 7.5, with 0.5 mU/ml apyrase, and homogenized by douncing ~30 times. LysoPS (18:0) at 1 μM was added all through the purification process. After 1 h incubation of the lysis at room temperature, 0.5% (w/v) lauryl maltose neopentyl glycol (LMNG, Anatrace) and 0.1% (w/v) cholesteryl hemisuccinate TRIS salt (CHS) were added to solubilize the membrane at 4°C for 2 h. Then the lysis was ultracentrifuged at 45,000 rpm at 4°C for 40 min. The supernatant was incubated with amylose column for 2 h then washed with a buffer of 20 mM Hepes, pH 7.5, 150 mM NaCl and 0.01% LMNG/0.002% CHS, and eluted with the same buffer plus 10 mM maltose. The elution was concentrated and cutted with home-made TEV overnight at 4°C, then separated on a Superdex 200 Increase 10/300 GL (GE health science) gel infiltration column with a buffer of 20 mM Hepes, pH 7.5, 150 mM NaCl, 0.00075% (w/v) LMNG, 0.00025% glycol-diosgenin (GDN) and 0.0002% (w/v) CHS (Anatrace). The P2Y10/G-protein complex corresponding peak was concentrated at about 10 mg/ml and snap-frozen for later cryo-EM grid preparation.

Grid preparation and cryo-EM data collection

A 3-5 μl protein complex sample (~ 10 mg/ml) was loaded to a glow-charged quantifoil R1.2/1.3 Cu holey carbon grids (Quantifoil GmbH), followed by vitrification in liquid ethane on a Vitrobot Mark IV (Thermo Fisher Scientific) instrument at setting of blot force 10, blot time 5 seconds, humidity 100%, temperature 4°C. Grids with evenly distributed particles in thin ice were loaded to a FEI 300 kV Titan Krios TEM with a Gatan Quantum energy filter. Images were taken by a Gatan K2 Summit direct electron detector with a super-resolution counting model at pixel size of 0.55 Å (magnification 64,000 $\times$). The energy filter slit was set to 20 eV. Each image contains 40 frames with a total exposure time of 7.3 seconds at a dose rate of 1.5 e/Å²/s (total dose 60 e/Å²), nominal defocus value varies from -1.2 to -2.2 μm .

Data processing

The initial raw movies were subjected to one round of binning (1.1 Å) and subsequently underwent motion correction using MotionCor2³⁵. The estimation of contrast transfer function (CTF) parameters was accomplished using CTFFIND 4.1³⁶. To identify particles, the crYOLO³⁷ software was employed, followed by reference-free two-dimensional (2D) classification carried out in RELION³⁸. The clearly defined features extracted from this classification were utilized for the generation of an initial model, employing cryoSPARC's ab initio method³⁹. This generated model was utilized as a reference for subsequent refinement stages conducted within RELION. A three-dimensional (3D) classification procedure yielded 3 to 4 classes. The most favorable class, exhibiting distinct secondary structure attributes, was chosen for Non-uniform Refinement within cryoSPARC. This was followed by an unaligned 3D classification performed in RELION, employing 6 to 10 classes and applying a complex-specific mask. Further steps involved Bayesian polishing⁴⁰ and additional rounds of Non-uniform Refinement to enhance the quality of the resulting map. The final map's resolution was determined using the gold standard Fourier Shell Correlation (FSC) criteria at FSC = 0.143. Estimates of local resolution were computed utilizing an integrated program within cryoSPARC.

414

415 **Model building**

416 We initiated the process of model rebuilding against the electron microscopy map by
417 utilizing AlphaFold predictions³⁰ for human P2Y10 (AF- O00398-v1) as initial models.
418 These models were employed as guides for the docking process into the density map, which
419 was executed using UCSF Chimera⁴¹. Subsequent to this, a series of iterative manual
420 adjustments were conducted in Coot⁴² to enhance the precision of the models. To further
421 refine the structural accuracy, Rosetta cryoEM refinement⁴³ and Phenix real space
422 refinement⁴⁴ steps were performed. For the purpose of visualizing and preparing structural
423 figures, we employed UCSF ChimeraX⁴⁵ and PyMOL (<https://pymol.org/2/>).

424

425 **Plasmids**

426 The full-length human P2Y10 was inserted into the pCAGGS expression vector with an
427 N-terminal fusion of the hemagglutinin-derived signal sequence (ssHA), FLAG epitope tag
428 and a flexible linker (MKTIIALSYIFCLVFA-DYKDDDDK-GGSGGGGSGGSSSSGGG;
429 the FLAG epitope tag is underlined). The resulting construct was named as ssHA-FLAG-
430 P2Y10. Similarly, the full-length human GPR174 was fused with the ssHA-FLAG
431 sequence.

432

433 **TGF α shedding assay**

434 TGF α shedding assay was performed as described previously^{12,34} with minor
435 modifications. G $\alpha_{q/11/12/13}$ -deficient HEK293 cells were seeded in a 12-well culture plate
436 (Greiner Bio-One) at a concentration of 2.5×10^5 cells/mL (1 mL per well hereafter) in
437 DMEM (Nissui Pharmaceutical) supplemented with 5% (v/v) FBS, glutamine, penicillin,
438 and streptomycin, one day before transfection. The transfection solution was prepared by
439 combining 3 μ L of 1 mg/ml polyethylenimine Max solution (Polysciences) and a plasmid
440 mixture consisting of 250 ng alkaline phosphatase (AP)-tagged TGF- α (AP-TGF α ; human
441 codon-optimized), 100 ng ssHA-FLAG-tagged P2Y10 construct and 50 ng chimeric G α_q

construct with a substitution of the last 26 amino acids (G.H.5.01 to G.H.5.26) with those of $G\alpha_{12}$ (named as $G\alpha_{q/12}$ chimera) or $G\alpha_{13}$ ($G\alpha_{q/13}$ chimera) in 100 μ L of Opti-MEM I Reduced Serum Medium (Thermo Fisher Scientific). One day after incubation, the transfected cells were harvested by trypsinization, neutralized with DMEM containing 5% (v/v) FCS and penicillin–streptomycin, washed once with HBSS containing 5 mM HEPES (pH 7.4), and resuspended in 3 ml of the HEPES-containing HBSS. The cell suspension was seeded into a 96-well plate at a volume of 80 μ L (per well hereafter) and incubated for 30 minutes in a CO₂ incubator. LysoPS (1-oleyl LysoPS; Avanti Polar Lipids) was serially diluted in assay by 3.2-fold (final concentrations ranging from 0.32 nM to 1 μ M) and added to the plate with 10 μ L per well. As a GPCR-independent TGF α -shedding control, phorbol 12-myristate 13-acetate (FujiFilm Wako Pure Chemicals) was added at a final concentration of 1 μ M. After 1 h incubation, the cell plate was centrifuged and conditioned media (80 μ L) was transferred to an empty 96-well plate. AP reaction solution (10 mM *p*-nitrophenylphosphate (*p*-NPP), 120 mM Tris–HCl (pH 9.5), 40 mM NaCl, 10 mM MgCl₂) was dispensed into the cell culture plates and plates containing conditioned media (80 μ L). Absorbance at 405 nm was measured before and after a 2 h incubation at room temperature using a microplate reader (SpectraMax 340 PC384; Molecular Devices). For each mutant receptor condition, LysoPS-induced AP-TGF α release was calculated by setting vehicle-treated and TPA-stimulated AP-TGF α release signal as a baseline and 100%, respectively. Using Prism 9 software (GraphPad Prism), AP-TGF α release signals were fitted with a four-parameter sigmoidal concentration-response curve, with the setting of absolute *HillSlope* values less than 2.

NanoBiT-G-protein dissociation assay

LysoPS-induced G-protein dissociation was measured by the NanoBiT-G-protein dissociation assay¹², in which interaction between the $G\alpha$ subunit and the $G\beta\gamma$ subunit was monitored by the NanoBiT enzyme complementation system (Promega). Specifically, the NanoBiT-G protein consisting of $G\alpha$ subunit fused with the large fragment (LgBiT) in the alpha helical domain (inserted at the position 115–116 in the $G\alpha_{12}$ and the position 132–133 in the $G\alpha_{13}$) and the $G\gamma_2$ subunit with the small fragment (SmBiT) at the N-terminus

was expressed along with untagged G β ₁ subunit, RIC8A and a test GPCR. HEK293A cells were seeded in a 6-well culture plate and transfected with a mixture of plasmids (100 ng LgBiT-containing G α ₁₂, 500 ng G β ₁, 500 ng SmBiT-fused G γ ₂ and 200 ng P2Y₁₀ construct; 100 ng LgBiT-containing G α ₁₃, 500 ng G β ₁, 500 ng SmBiT-fused G γ ₂ (C68S) and 200 ng P2Y₁₀ construct) by following the procedures described in the “TGF α shedding assay” section. After incubation for one day, transfected cells were harvested with 0.5 mM EDTA-containing Dulbecco’s PBS, centrifuged and suspended in 2 ml of HBSS containing 0.01% bovine serum albumin (BSA; fatty acid-free grade; SERVA) and 5 mM HEPES (pH 7.4) (assay buffer). The cell suspension was dispensed in a white 96-well plate at a volume of 80 μ l per well and loaded with luciferase substrate of 20 μ L of 50 μ M coelenterazine (Angene) diluted in the assay buffer. After 2-h incubation at room temperature, the plate was measured for baseline luminescence (SpectraMax L, Molecular Devices) and a titrated ligand was manually added. The plate was immediately read at room temperature for the following 5 min at a measurement interval of 20 sec with an accumulation time of 0.17 sec per read. The luminescence counts over 5–10 min after ligand addition were averaged and normalized to the initial count. The fold-change values were further normalized to those of vehicle-treated samples. Using Prism 10 software (GraphPad Prism), the NanoBiT-G-protein dissociation signals were fitted to a four-parameter sigmoidal concentration-response curve.

Flow cytometry

Plasmid transfection for the ssHA-FLAG-P2Y₁₀ and the AP-TGF α sensor was performed according to the same procedure as described in the “TGF α shedding assay” section. One day after transfection, the cells were collected by adding 100 μ l (hereafter per well in 12-well plate) of 0.53 mM EDTA-containing Dulbecco’s PBS (D-PBS), followed by 100 μ l of 5 mM HEPES (pH 7.4)-containing Hank’s Balanced Salt Solution (HBSS). The cell suspension was transferred to a 96-well V-bottom plate and fluorescently labeled with an anti-FLAG epitope (DYKDDDDK) tag monoclonal antibody (Clone 1E6, FujiFilm Wako Pure Chemicals, cat no. 012-22384; 10 μ g per ml diluted in 2% goat serum- and 2 mM EDTA-containing D-PBS (blocking buffer)) and a goat anti-mouse IgG secondary

antibody conjugated with Alexa Fluor 488 (Thermo Fisher Scientific, cat no. A11001; 10 µg per ml diluted in the blocking buffer). After washing with D-PBS, the cells were resuspended in 200 µl of 2 mM EDTA-containing-D-PBS and filtered through a 40-µm filter. The fluorescent intensity of single cells was quantified by an EC800 flow cytometer equipped with a 488 nm laser (Sony). The fluorescent signal derived from Alexa Fluor 488 was recorded in an FL1 channel, and the flow cytometry data were analyzed with the FlowJo software (FlowJo). Live cells were gated with a forward scatter (FS-Peak-Lin) cutoff at the 390 setting, with a gain value of 1.7. Values of mean fluorescence intensity (MFI) from approximately 20,000 cells per sample were used for analysis. For each experiment, we normalized an MFI value of the mutants by that of WT performed in parallel and denoted relative levels.

QUANTIFICATION AND STATISTICAL ANALYSIS

Cell based TGFα shedding assay, NanoBit assay were analyzed in GraphPad Prism. For these functional assays, bars and error bars represent the mean and S.E.M., respectively. Dots show individual data of three independent experiments, each performed in triplicate. The exact sample number and statistical analysis please see figure legends of Figure 2E-F; Figure 4E-F; Figure 5C-E; , 5E; and 5F; Figure S3F-G.

REFERENCES

1. Makide, K., Uwamizu, A., Shinjo, Y., Ishiguro, J., Okutani, M., Inoue, A., and Aoki, J. (2014). Novel lysophospholipid receptors: their structure and function. *J Lipid Res* 55, 1986-1995. 10.1194/jlr.R046920.
2. Grzelczyk, A., and Gendaszewska-Darmach, E. (2013). Novel bioactive glycerol-based lysophospholipids: new data -- new insight into their function. *Biochimie* 95, 667-679. 10.1016/j.biochi.2012.10.009.

- 530 3. Yaginuma, S., Omi, J., Uwamizu, A., and Aoki, J. (2023). Emerging roles of
531 lysophosphatidylserine as an immune modulator. *Immunol Rev* 317, 20-29.
532 10.1111/imr.13204.
- 533 4. Lee, S.Y., Lee, H.Y., Kim, S.D., Jo, S.H., Shim, J.W., Lee, H.J., Yun, J., and Bae, Y.S. (2008).
534 Lysophosphatidylserine stimulates chemotactic migration in U87 human glioma cells.
535 *Biochem Biophys Res Commun* 374, 147-151. 10.1016/j.bbrc.2008.06.117.
- 536 5. Lourenssen, S., and Blennerhassett, M.G. (1998). Lysophosphatidylserine potentiates
537 nerve growth factor-induced differentiation of PC12 cells. *Neurosci Lett* 248, 77-80.
538 10.1016/s0304-3940(98)00275-4.
- 539 6. Martin, T.W., and Lagunoff, D. (1979). Interactions of lysophospholipids and mast cells.
540 *Nature* 279, 250-252. 10.1038/279250a0.
- 541 7. Liebscher, I., Muller, U., Teupser, D., Engemaier, E., Engel, K.M., Ritscher, L., Thor, D.,
542 Sangkuhl, K., Ricken, A., Wurm, A., et al. (2011). Altered immune response in mice
543 deficient for the G protein-coupled receptor GPR34. *J Biol Chem* 286, 2101-2110.
544 10.1074/jbc.M110.196659.
- 545 8. Kitamura, H., Makide, K., Shuto, A., Ikubo, M., Inoue, A., Suzuki, K., Sato, Y., Nakamura,
546 S., Otani, Y., Ohwada, T., and Aoki, J. (2012). GPR34 is a receptor for
547 lysophosphatidylserine with a fatty acid at the sn-2 position. *Journal of biochemistry*
548 151, 511-518. 10.1093/jb/mvs011.
- 549 9. Sugita, K., Yamamura, C., Tabata, K., and Fujita, N. (2013). Expression of orphan G-
550 protein coupled receptor GPR174 in CHO cells induced morphological changes and
551 proliferation delay via increasing intracellular cAMP. *Biochem Biophys Res Commun* 430,
552 190-195. 10.1016/j.bbrc.2012.11.046.
- 553 10. Shinjo, Y., Makide, K., Satoh, K., Fukami, F., Inoue, A., Kano, K., Otani, Y., Ohwada, T.,
554 and Aoki, J. (2017). Lysophosphatidylserine suppresses IL-2 production in CD4 T cells
555 through LPS3/GPR174. *Biochem Biophys Res Commun* 494, 332-338.
556 10.1016/j.bbrc.2017.10.028.
- 557 11. Ikubo, M., Inoue, A., Nakamura, S., Jung, S., Sayama, M., Otani, Y., Uwamizu, A., Suzuki,
558 K., Kishi, T., Shuto, A., et al. (2015). Structure-activity relationships of
559 lysophosphatidylserine analogs as agonists of G-protein-coupled receptors GPR34,
560 P2Y10, and GPR174. *J Med Chem* 58, 4204-4219. 10.1021/jm5020082.
- 561 12. Inoue, A., Raimondi, F., Kadji, F.M.N., Singh, G., Kishi, T., Uwamizu, A., Ono, Y., Shinjo, Y.,
562 Ishida, S., Arang, N., et al. (2019). Illuminating G-Protein-Coupling Selectivity of GPCRs.
563 *Cell* 177, 1933-1947 e1925. 10.1016/j.cell.2019.04.044.
- 564 13. Herroeder, S., Reichardt, P., Sassmann, A., Zimmermann, B., Jaeneke, D., Hoeckner, J.,
565 Hollmann, M.W., Fischer, K.D., Vogt, S., Grosse, R., et al. (2009). Guanine nucleotide-
566 binding proteins of the G12 family shape immune functions by controlling CD4+ T cell
567 adhesiveness and motility. *Immunity* 30, 708-720. 10.1016/j.immuni.2009.02.010.
- 568 14. Liang, J., Inoue, A., Ikuta, T., Xia, R., Wang, N., Kawakami, K., Xu, Z., Qian, Y., Zhu, X.,
569 Zhang, A., et al. (2023). Structural basis of lysophosphatidylserine receptor GPR174
570 ligand recognition and activation. *Nature communications* 14, 1012. 10.1038/s41467-
571 023-36575-0.
- 572 15. Nie, Y., Qiu, Z., Chen, S., Chen, Z., Song, X., Ma, Y., Huang, N., Cyster, J.G., and Zheng, S.
573 (2023). Specific binding of GPR174 by endogenous lysophosphatidylserine leads to high
574 constitutive G(s) signaling. *Nature communications* 14, 5901. 10.1038/s41467-023-
575 41654-3.

- 576 16. Xia, A., Yong, X., Zhang, C., Lin, G., Jia, G., Zhao, C., Wang, X., Hao, Y., Wang, Y., Zhou, P.,
577 et al. (2023). Cryo-EM structures of human GPR34 enable the identification of selective
578 antagonists. *Proc Natl Acad Sci U S A* 120, e2308435120. 10.1073/pnas.2308435120.
- 579 17. Barros-Alvarez, X., Nwokonko, R.M., Vizurraga, A., Matzov, D., He, F., Papasergi-Scott,
580 M.M., Robertson, M.J., Panova, O., Yardeni, E.H., Seven, A.B., et al. (2022). The tethered
581 peptide activation mechanism of adhesion GPCRs. *Nature*. 10.1038/s41586-022-04575-
582 7.
- 583 18. Duan, J., Shen, D.D., Zhou, X.E., Bi, P., Liu, Q.F., Tan, Y.X., Zhuang, Y.W., Zhang, H.B., Xu,
584 P.Y., Huang, S.J., et al. (2020). Cryo-EM structure of an activated VIP1 receptor-G protein
585 complex revealed by a NanoBIT tethering strategy. *Nature communications* 11, 4121.
586 10.1038/s41467-020-17933-8.
- 587 19. Xia, R., Wang, N., Xu, Z., Lu, Y., Song, J., Zhang, A., Guo, C., and He, Y. (2021). Cryo-EM
588 structure of the human histamine H1 receptor/Gq complex. *Nature communications* 12,
589 2086. 10.1038/s41467-021-22427-2.
- 590 20. Inoue, A., Ishiguro, J., Kitamura, H., Arima, N., Okutani, M., Shuto, A., Higashiyama, S.,
591 Ohwada, T., Arai, H., Makide, K., and Aoki, J. (2012). TGFalpha shedding assay: an
592 accurate and versatile method for detecting GPCR activation. *Nature methods* 9, 1021-
593 1029. 10.1038/nmeth.2172.
- 594 21. Zhang, D., Gao, Z.G., Zhang, K., Kiselev, E., Crane, S., Wang, J., Paoletta, S., Yi, C., Ma, L.,
595 Zhang, W., et al. (2015). Two disparate ligand-binding sites in the human P2Y1 receptor.
596 *Nature* 520, 317-321. 10.1038/nature14287.
- 597 22. Rasmussen, S.G., DeVree, B.T., Zou, Y., Kruse, A.C., Chung, K.Y., Kobilka, T.S., Thian, F.S.,
598 Chae, P.S., Pardon, E., Calinski, D., et al. (2011). Crystal structure of the beta2 adrenergic
599 receptor-Gs protein complex. *Nature* 477, 549-555. 10.1038/nature10361.
- 600 23. Deupi, X., and Standfuss, J. (2011). Structural insights into agonist-induced activation of
601 G-protein-coupled receptors. *Current opinion in structural biology* 21, 541-551.
602 10.1016/j.sbi.2011.06.002.
- 603 24. Flock, T., Ravarani, C.N.J., Sun, D., Venkatakrishnan, A.J., Kayikci, M., Tate, C.G.,
604 Veprintsev, D.B., and Babu, M.M. (2015). Universal allosteric mechanism for Gα
605 activation by GPCRs. *Nature* 524, 173-179. 10.1038/nature14663.
- 606 25. Chen, H., Chen, K., Huang, W., Staudt, L.M., Cyster, J.G., and Li, X. (2022). Structure of
607 S1PR2-heterotrimeric G(13) signaling complex. *Sci Adv* 8, eabn0067.
608 10.1126/sciadv.abn0067.
- 609 26. Barnes, M.J., and Cyster, J.G. (2018). Lysophosphatidylserine suppression of T-cell
610 activation via GPR174 requires Gas proteins. *Immunology and cell biology* 96, 439-445.
611 10.1111/imcb.12025.
- 612 27. Wolf, E.W., Howard, Z.P., Duan, L., Tam, H., Xu, Y., and Cyster, J.G. (2022). GPR174
613 signals via Galphas to control a CD86-containing gene expression program in B cells.
614 *Proc Natl Acad Sci U S A* 119, e2201794119. 10.1073/pnas.2201794119.
- 615 28. Mackenzie, A.E., Quon, T., Lin, L.C., Hauser, A.S., Jenkins, L., Inoue, A., Tobin, A.B.,
616 Gloriam, D.E., Hudson, B.D., and Milligan, G. (2019). Receptor selectivity between the G
617 proteins Gα(12) and Gα(13) is defined by a single leucine-to-isoleucine variation. *FASEB J*
618 33, 5005-5017. 10.1096/fj.201801956R.
- 619 29. Mirdita, M., Schütze, K., Moriwaki, Y., Heo, L., Ovchinnikov, S., and Steinegger, M.
620 (2022). ColabFold: making protein folding accessible to all. *Nature methods* 19, 679-682.
621 10.1038/s41592-022-01488-1.
- 622 30. Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O.,
623 Tunyasuvunakool, K., Bates, R., Zidek, A., Potapenko, A., et al. (2021). Highly accurate

protein structure prediction with AlphaFold. *Nature* 596, 583-589. 10.1038/s41586-021-03819-2.

31. Guo, P., Tai, Y., Wang, M., Sun, H., Zhang, L., Wei, W., Xiang, Y.K., and Wang, Q. (2022). Gα(12) and Gα(13): Versatility in Physiology and Pathology. *Frontiers in cell and developmental biology* 10, 809425. 10.3389/fcell.2022.809425.

32. Yang, Y.M., Kuen, D.S., Chung, Y., Kurose, H., and Kim, S.G. (2020). Gα(12/13) signaling in metabolic diseases. *Experimental & molecular medicine* 52, 896-910. 10.1038/s12276-020-0454-5.

33. Liu, D., Duan, L., Rodda, L.B., Lu, E., Xu, Y., An, J., Qiu, L., Liu, F., Looney, M.R., Yang, Z., et al. (2022). CD97 promotes spleen dendritic cell homeostasis through the mechanosensing of red blood cells. *Science* 375, eabi5965. 10.1126/science.abi5965.

34. Tatsumi, M., Cruz, C., Kamakura, N., Kuwabara, R., Nakamura, G., Ikuta, T., Abrol, R., and Inoue, A. (2024). Identification of Gα(12)-vs-Gα(13)-coupling determinants and development of a Gα(12/13)-coupled designer GPCR. *Scientific reports* 14, 11119. 10.1038/s41598-024-61506-4.

35. Zheng, S.Q., Palovcak, E., Armache, J.P., Verba, K.A., Cheng, Y., and Agard, D.A. (2017). MotionCor2: anisotropic correction of beam-induced motion for improved cryo-electron microscopy. *Nature methods* 14, 331-332. 10.1038/nmeth.4193.

36. Rohou, A., and Grigorieff, N. (2015). CTFFIND4: Fast and accurate defocus estimation from electron micrographs. *Journal of structural biology* 192, 216-221. 10.1016/j.jsb.2015.08.008.

37. Wagner, T., Merino, F., Stabrin, M., Moriya, T., Antoni, C., Apelbaum, A., Hagel, P., Sitsel, O., Raisch, T., Prumbaum, D., et al. (2019). SPHIRE-crYOLO is a fast and accurate fully automated particle picker for cryo-EM. *Commun Biol* 2, 218. 10.1038/s42003-019-0437-z.

38. Fernandez-Leiro, R., and Scheres, S.H.W. (2017). A pipeline approach to single-particle processing in RELION. *Acta Crystallogr D Struct Biol* 73, 496-502. 10.1107/S2059798316019276.

39. Punjani, A., Rubinstein, J.L., Fleet, D.J., and Brubaker, M.A. (2017). cryoSPARC: algorithms for rapid unsupervised cryo-EM structure determination. *Nature methods* 14, 290-296. 10.1038/nmeth.4169.

40. Zivanov, J., Nakane, T., and Scheres, S.H.W. (2019). A Bayesian approach to beam-induced motion correction in cryo-EM single-particle analysis. *IUCrJ* 6, 5-17. 10.1107/S205225251801463X.

41. Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C., and Ferrin, T.E. (2004). UCSF Chimera--a visualization system for exploratory research and analysis. *J Comput Chem* 25, 1605-1612. 10.1002/jcc.20084.

42. Emsley, P., and Cowtan, K. (2004). Coot: model-building tools for molecular graphics. *Acta Crystallogr D Biol Crystallogr* 60, 2126-2132. 10.1107/S09074444904019158.

43. Wang, R.Y., Song, Y., Barad, B.A., Cheng, Y., Fraser, J.S., and DiMaio, F. (2016). Automated structure refinement of macromolecular assemblies from cryo-EM maps using Rosetta. *eLife* 5. 10.7554/eLife.17219.

44. Adams, P.D., Afonine, P.V., Bunkoczi, G., Chen, V.B., Davis, I.W., Echols, N., Headd, J.J., Hung, L.W., Kapral, G.J., Grosse-Kunstleve, R.W., et al. (2010). PHENIX: a comprehensive Python-based system for macromolecular structure solution. *Acta Crystallogr D Biol Crystallogr* 66, 213-221. 10.1107/S09074444909052925.

45. Pettersen, E.F., Goddard, T.D., Huang, C.C., Meng, E.C., Couch, G.S., Croll, T.I., Morris, J.H., and Ferrin, T.E. (2021). UCSF ChimeraX: Structure visualization for researchers,

672 educators, and developers. Protein science : a publication of the Protein Society 30, 70-
673 82. 10.1002/pro.3943.
674

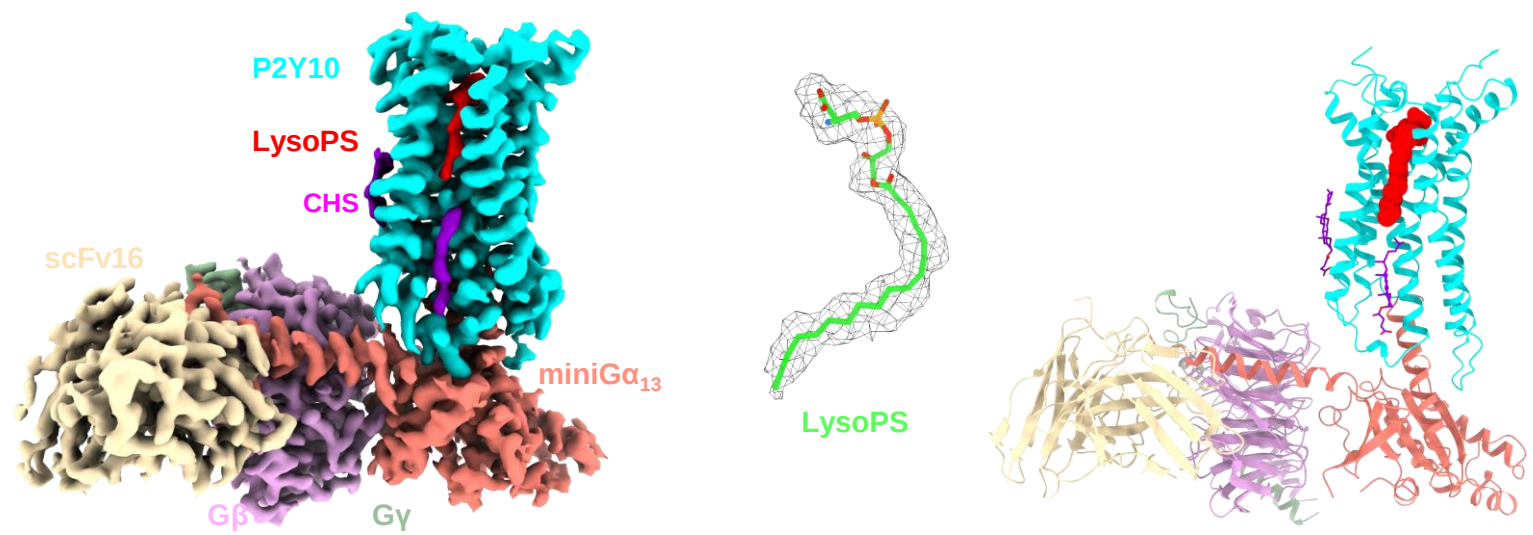


Figure 1. The overall structure of P2Y10–miniGα₁₃ complex

Left panel, orthogonal view of the cryo-EM density map; right panel, model of the complex in the same view and color scheme as shown in the left panel; middle panel, cryo-EM density map of LysoPS.

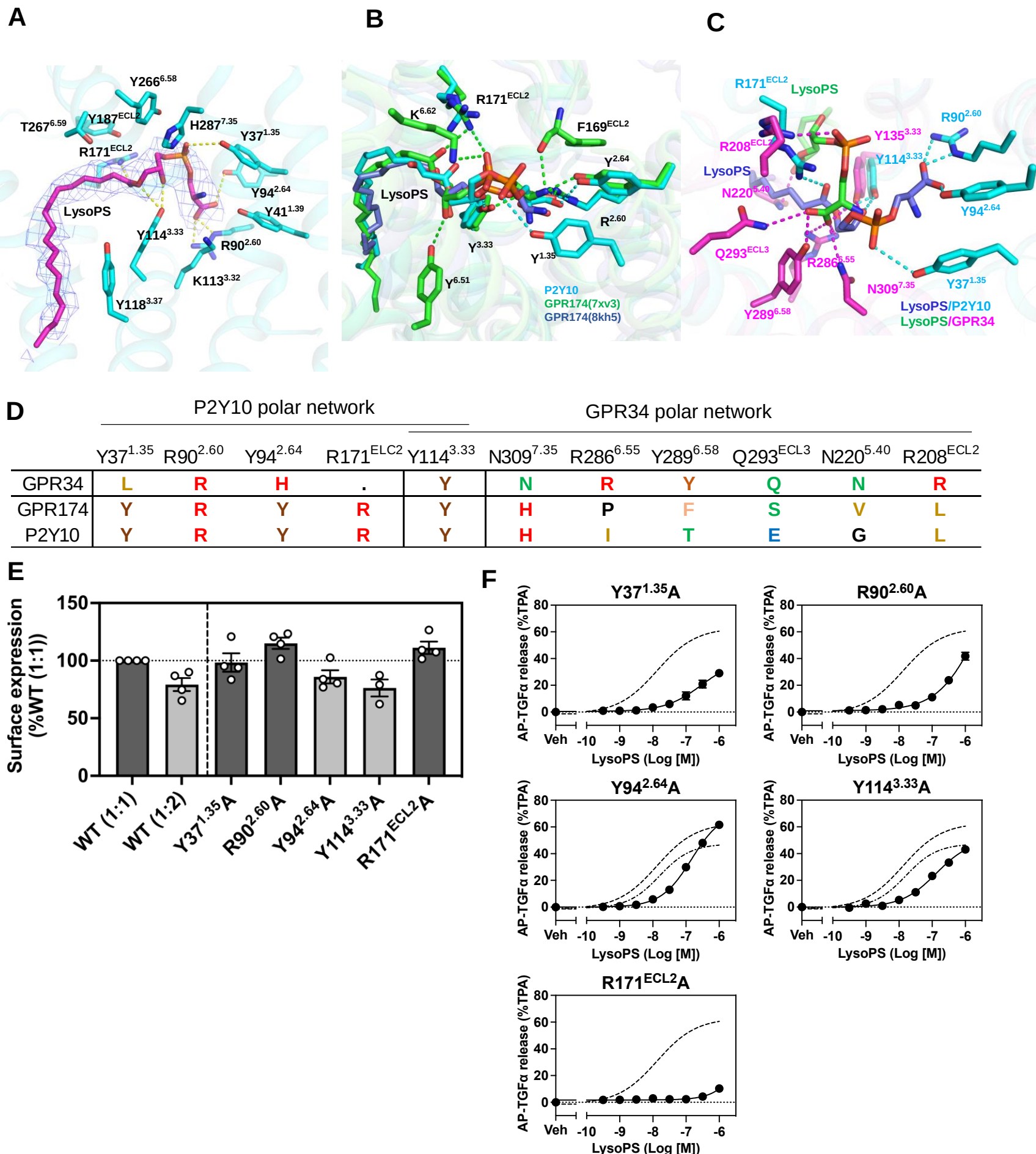


Figure 2. Ligand binding of P2Y10

(A) The ligand binding pocket of P2Y10. Density map of the LysoPS (blue mesh) is set at a contour level of 5.0 in pymol.

(B) A comparison of LysoPS binding modes between P2Y10 and GPR174 (PDB: 7vx3, 8kh5).

(C) A comparison of LysoPS binding modes between P2Y10 and GPR34 (PDB: 8sai).

(D) Sequence alignment of the polar interaction network for LysoPS binding among LysoPS receptors.

(E) Cell-surface expression of the ligand-binding-pocket P2Y10 mutants. Note that Y37^{1.35}A, R90^{2.60}A and R171^{ECL2}A mutants were expressed at a level equivalent to WT (1:1) while Y94^{2.64}A and Y114^{3.33}A mutants were equivalent to WT (1:2), a half volume of transfected wild-type P2Y10 plasmid. Bars and error bars indicate mean values and S.E.M., respectively, from four independent experiments (dots).

(F) TGFα shedding assay for the ligand-binding-pocket P2Y10 mutants. The dash line in each panel is identical and indicates WT (1:1), which was assessed in parallel with the mutants. The alternate long and short dash line indicates the expression-level-matched WT (1:2). Symbols and error bars indicate mean values and S.E.M., respectively, from four independent experiments. Note that, in many datapoints, the sizes of error bars are smaller than the sizes of symbols.

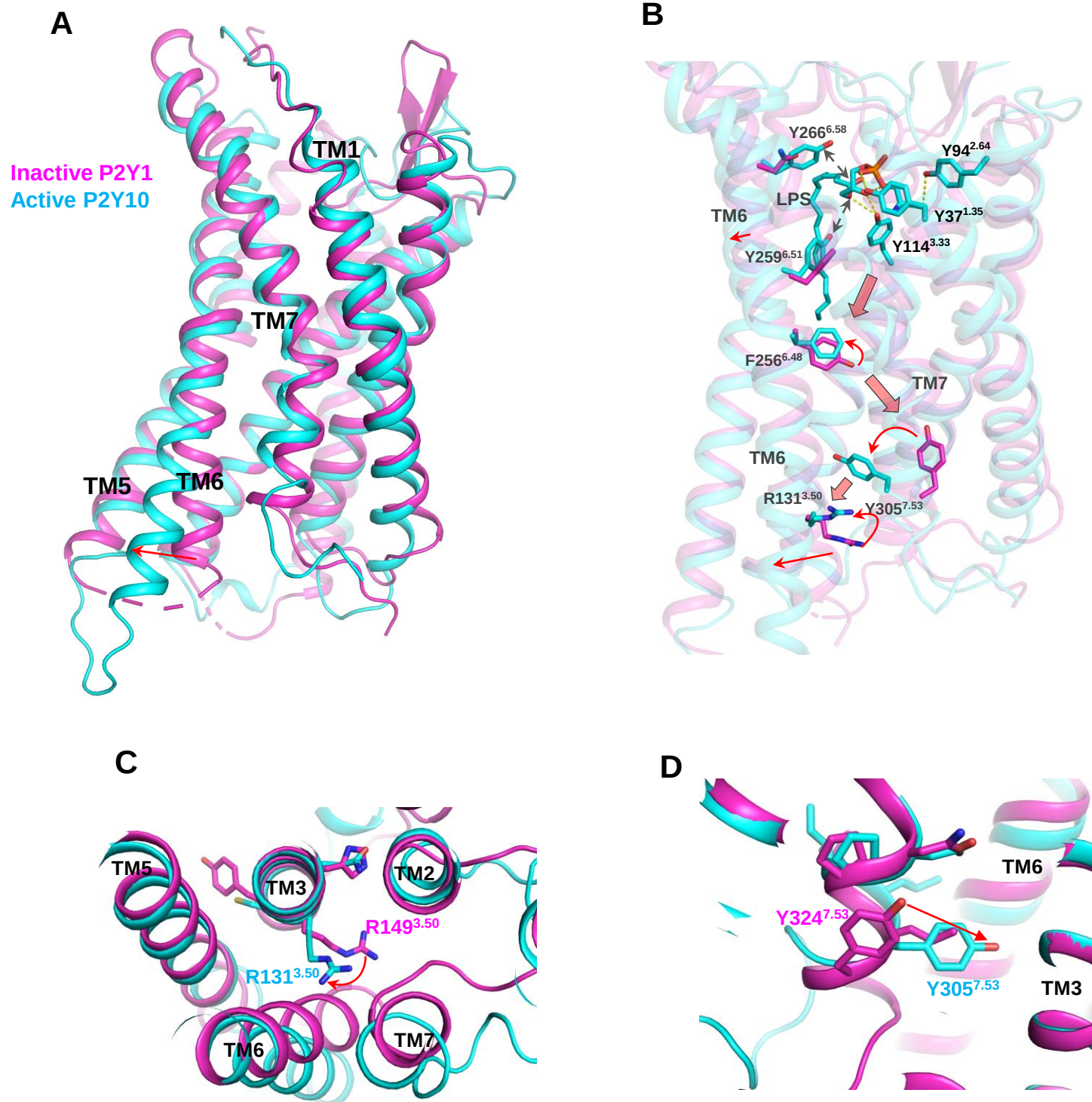


Figure 3. Activation of P2Y10

- (A) A comparison of the overall structure of the active P2Y10 (LysoPS-bound) with the inactive P2Y1 (BPTU-bound, pdb:4xnv).
- (B) Activation of P2Y10 from the top (ligand binding) to the bottom (intracellular cavity).
- (C) A comparison of the DRY motif between the active P2Y10 with the inactive P2Y1.
- (D) A comparison of the NPxxY motif between the active P2Y10 with the inactive P2Y1.

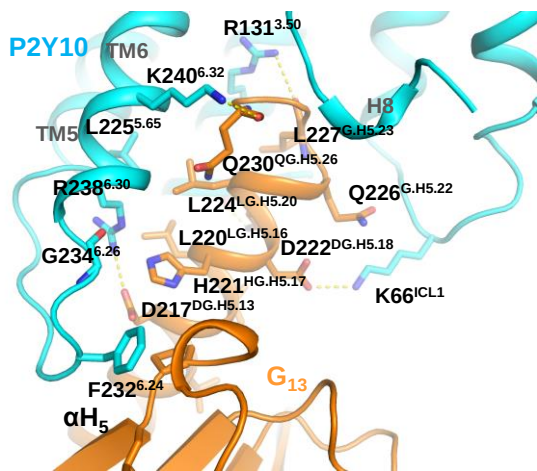
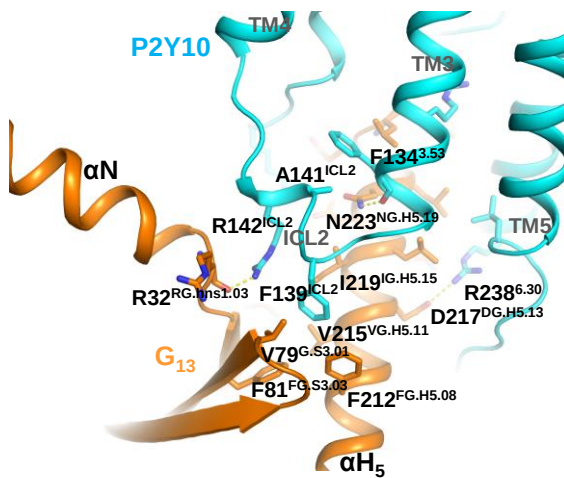
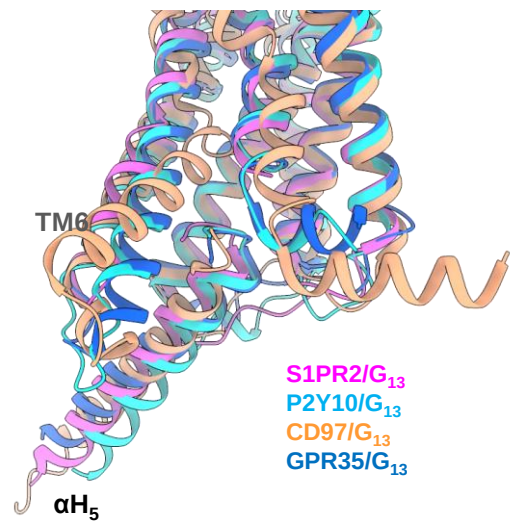
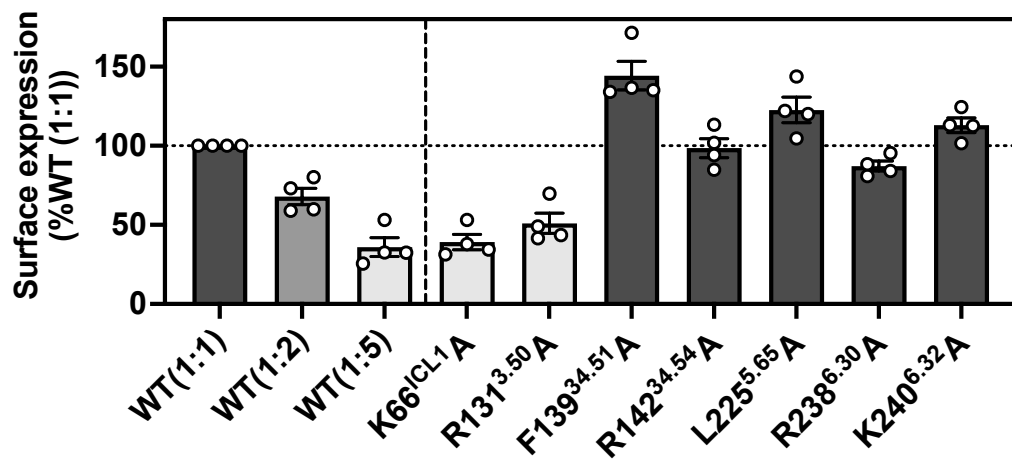
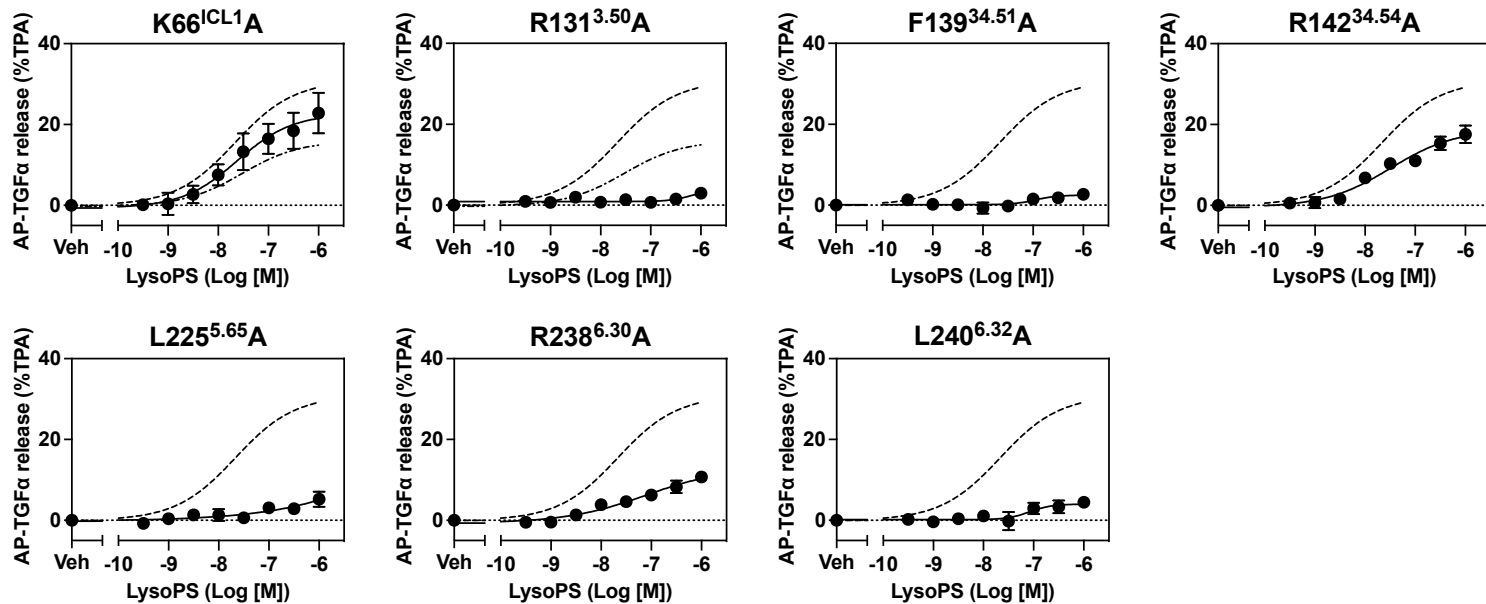
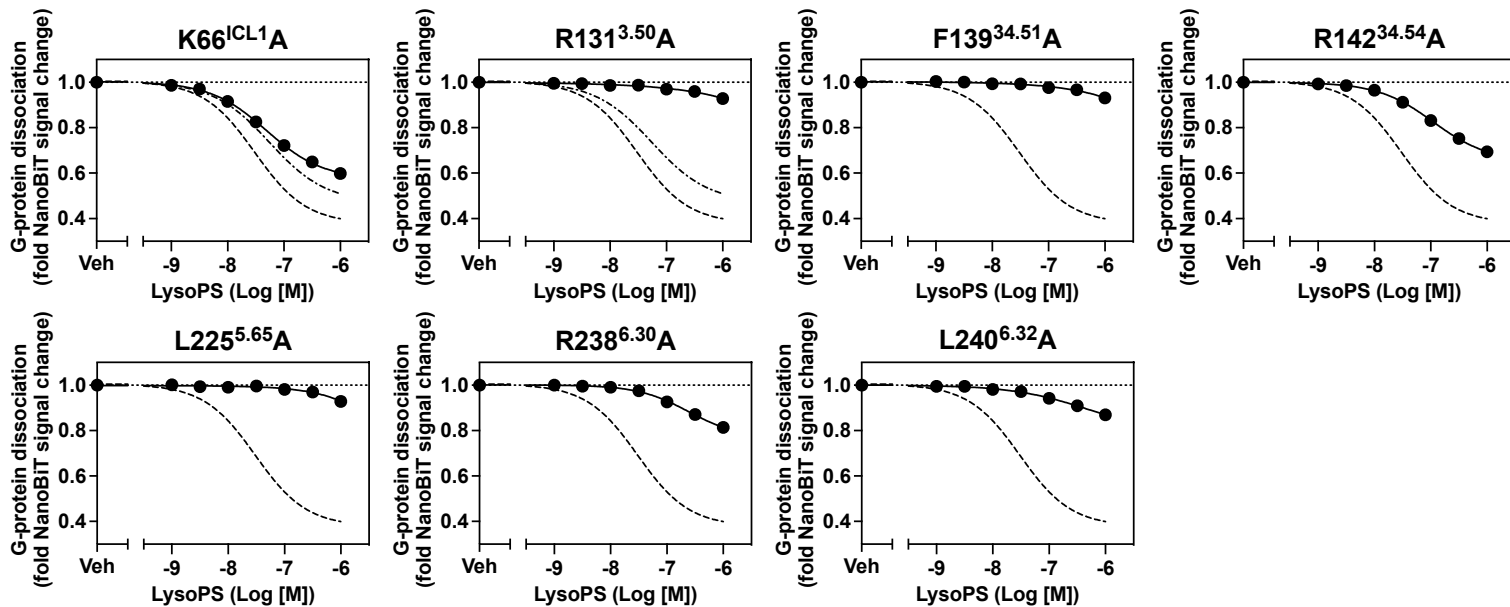
A**B****C****D****E****F**

Figure 4. The $G\alpha_{13}$ engagement of P2Y10

(A) The $G\alpha_{13}$ engagement of P2Y10, viewing from the TM6 H8 front.

(B) The $G\alpha_{13}$ engagement of P2Y10, viewing from the TM4-ICL2-TM3 side.

(C) A comparison of the overall $G\alpha_{13}$ engagements of various receptors. S1PR2 (PDB:7t6b); CD97 (PDB:7ydh); GPR35 (PDB:8h8j).

(D) Cell-surface expression of the $G\alpha_{13}$ -interface P2Y10 mutants. Note that F139^{34.51}A, R142^{34.54}A, L225^{5.65}A, R238^{6.30}A, and L240^{6.32}A mutants were expressed at a level equivalent to or higher than WT (1:1) while K66^{ICL1}A and R131^{3.50}A mutants were equivalent to WT (1:5), one fifth of volume of transfected wild-type P2Y10 plasmid. Bars and error bars indicate mean values and S.E.M., respectively, from three independent experiments (dots).

(E, F) Mutagenesis studies for the interface between $G\alpha_{13}$ and P2Y10. The TGF α shedding assay (E) and the NanoBiT-G-protein dissociation assay (F) were used to assess signaling activity of the P2Y10 mutants. The dash line in each panel is identical and indicates WT (1:1), which was assessed in parallel with the mutants. The alternate long and short dash line indicates the expression-level-matched WT (1:5). Symbols and error bars indicate mean values and S.E.M., respectively, from three (E) or four (F) independent experiments. Note that, in many data points, the sizes of error bars are smaller than the sizes of symbols (E and F).

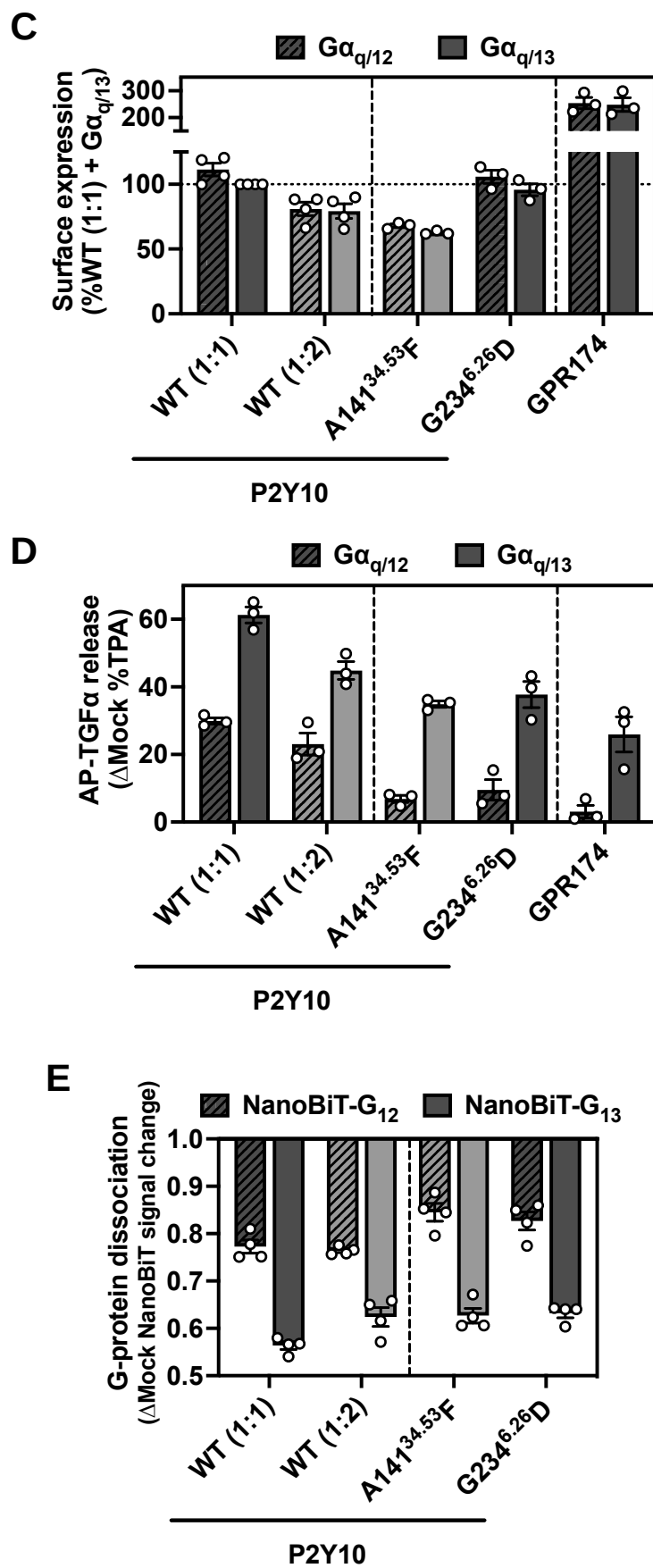
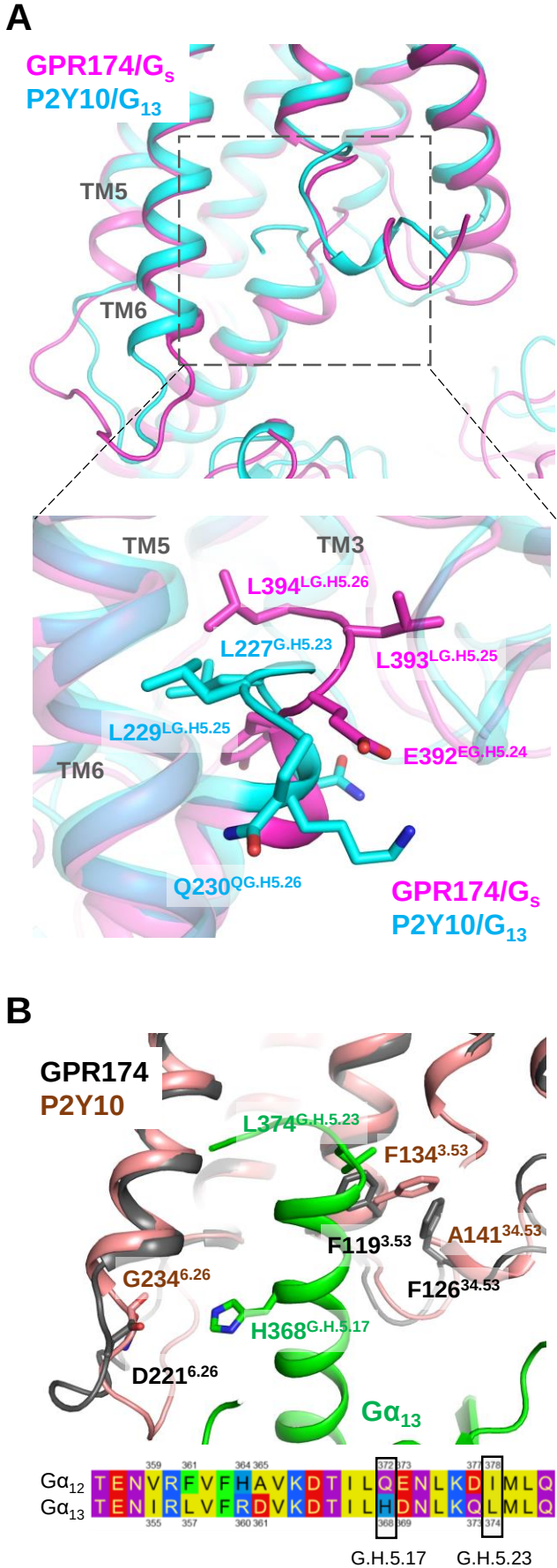


Figure 5. The structural insight into the Gα₁₃-coupling selectivity of P2Y10

(A) A comparison of the GPR174–Gα_s engagement (PDB:7xv3) with the P2Y10–Gα₁₃ engagement.

(B) Structural insight into Gα₁₃ coupling selectivity of P2Y10.

(C) Cell-surface expression of the Gα₁₂-vs.-Gα₁₃-selectivity P2Y10 mutants. Note that the G234^{6.26}D mutant were expressed at a level equivalent to WT (1:1) while the A141^{34.53}F mutant was equivalent to WT (1:2), a half of volume of transfected wild-type P2Y10 plasmid. Bars and error bars indicate mean values and S.E.M., respectively, from three or four independent experiments (dots). Note that the wild-type P2Y10 dataset was identical to Fig. 4D.

(D, E) Mutagenesis studies for the Gα₁₂-vs.-Gα₁₃-coupling selectivity of P2Y10. The TGFα shedding assay (D) and the NanoBiT-G-protein dissociation assay (E) were used to assess signaling activity of the P2Y10 mutants. Receptor-dependent AP-TGFα-release responses (D) and G-protein-dissociation responses (E) at 1 μM and 100 nM LysoPS stimulations, respectively, were plotted. Note that due to weak NanoBiT-G-protein-dissociation responses, GPR174 data were not included. Bars and error bars indicate mean values and S.E.M., respectively, from three independent or four experiments (dots). Note that the full concentration-response curves are shown in Supplementary Figure S2F and S2G.