

Supplementary Information

Structural mechanisms of potent lysophosphatidic acid receptor 1 activation by nonlipid basic agonists

Hiroaki Akasaka, Fumiya K. Sano, Wataru Shihoya, Osamu Nureki.

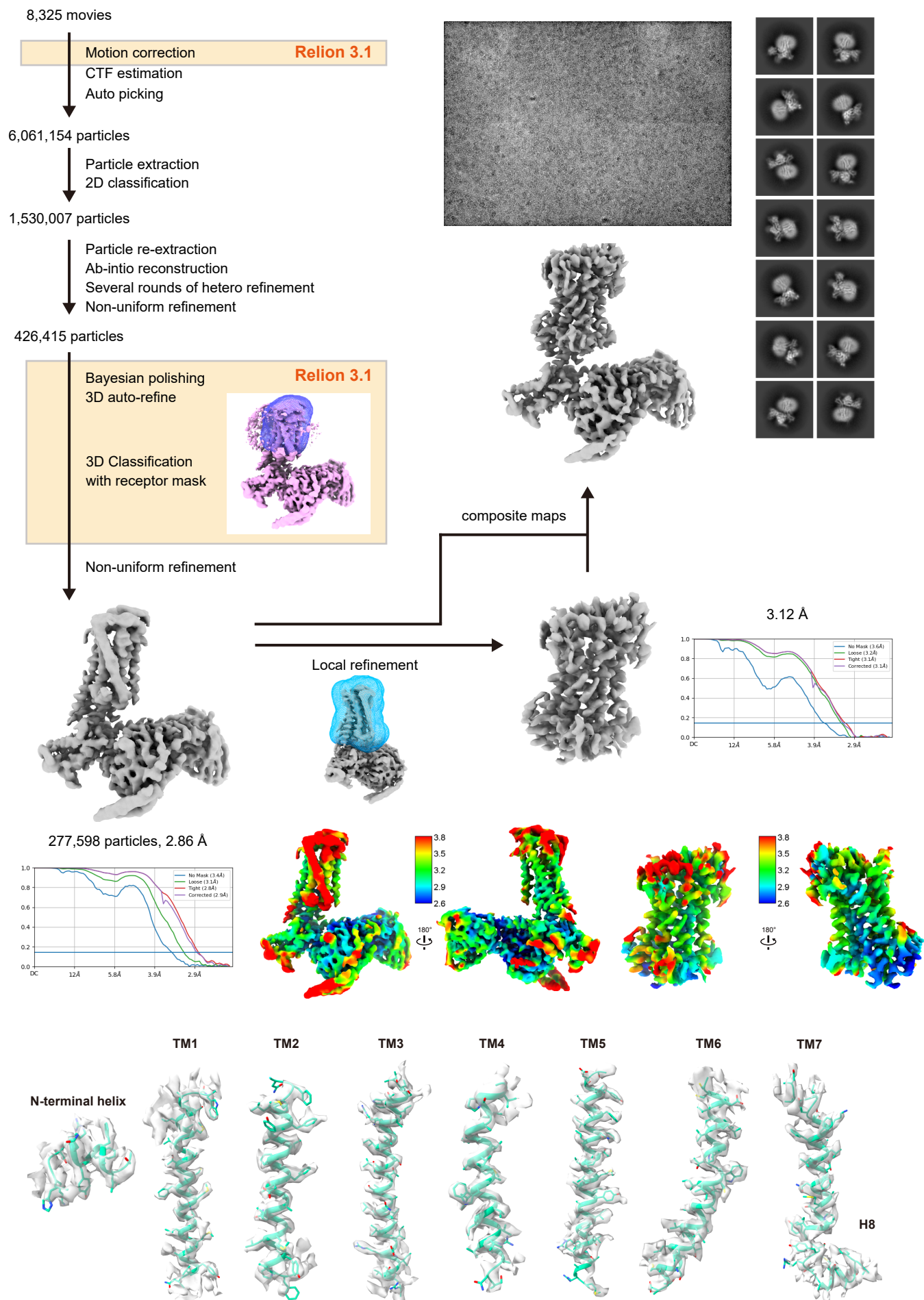
	WT			W210A		
	pEC ₅₀ ± s.e.m	EC ₅₀	E _{max}	pEC ₅₀ ± s.e.m	EC ₅₀	E _{max}
CpX	8.64 ± 0.04	2.28 nM	0.458	7.33 ± 0.15	49.5 nM	0.440
CpY	9.03 ± 0.5	0.95 nM	0.456	8.17 ± 0.12	7.21 nM	0.474
LPA	7.75 ± 0.12	19.6 nM	0.330	7.21 ± 0.10	64.8 nM	0.201
ONO-0740556	9.62 ± 0.08	0.25 nM	0.330	N/A	N/A	N/A

	pEC ₅₀ ± s.e.m	EC ₅₀	Expression
WT	9.03 ± 0.5	0.95 nM	1.000
WT(1:5)	8.95 ± 0.07	1.15 nM	0.513
D129A	N/A	N/A	1.054
W210A	8.17 ± 0.12	7.21 nM	0.855
L278A	7.32 ± 0.06	48.5 nM	1.042
L297A	7.40 ± 0.06	40.9 nM	0.765

Supplementary Table 1 | Mutant experoments

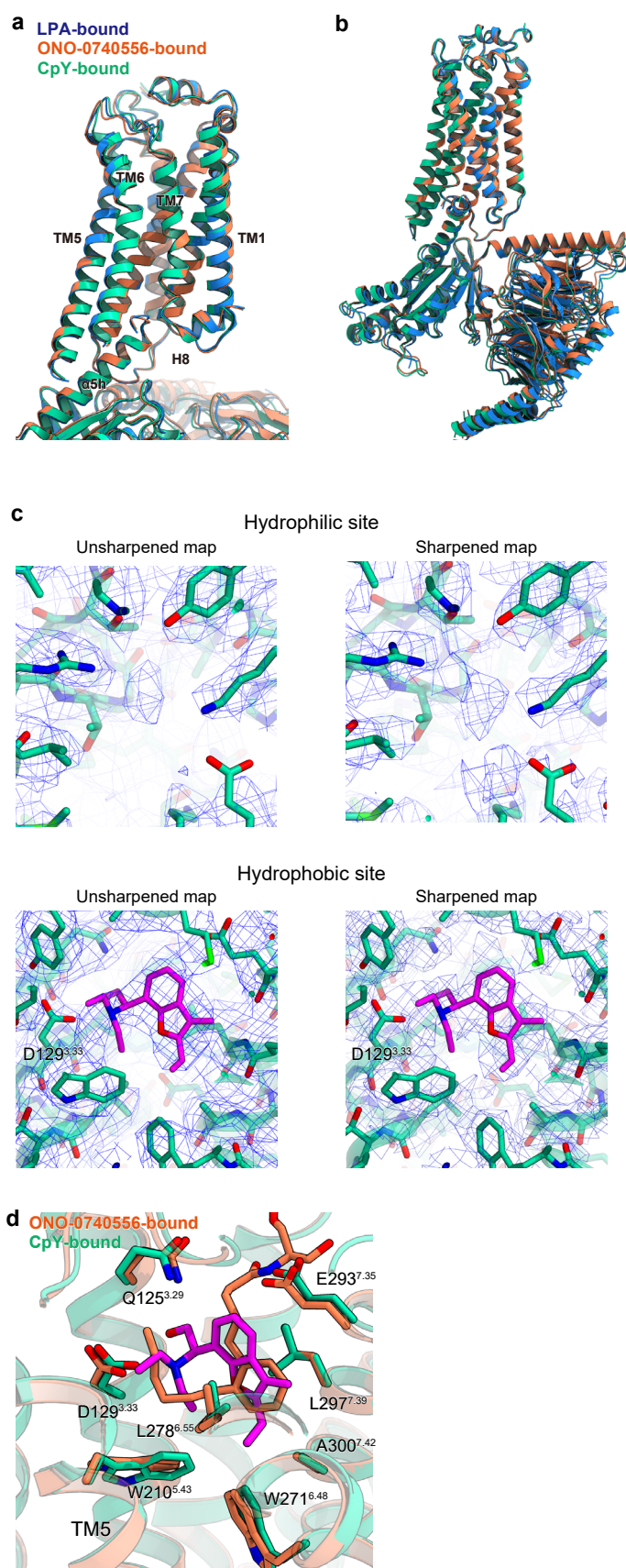
Microscope	Titan Krios (Thermo Fisher Scientific)	
Voltage (keV)	300	
Electron exposure (e ⁻ /Å ²)	50.362	48.405
Detector	Gatan K3 summit camera (Gatan)	
Magnification	×105,000	
Defocus range (μm)	-0.8–1.6	-0.6–1.6
Pixel size (Å/pix)	0.83	
Number of movies	5,013	3,312
Symmetry	C1	
Picked particles	3,667,591	2,393,563
Final particles	277,598	
Map resolution (Å)	2.86	
FSC threshold	0.143	
Model refinement		
Atoms	17,319	
R.m.s. deviations for ideal		
Bond lengths (Å)	0.0024	
Bond angles (°)	0.52	
Validation		
Clashscore	7.63	
Rotamers (%)	0	
Ramachandran plot		
Favored (%)	97.32	
Allowed (%)	2.68	
Outlier (%)	0	

Supplementary Table 2 | Cryo-EM data collection, refinement and validation statistics



Supplementary Figure 1 | Cryo-EM analysis.

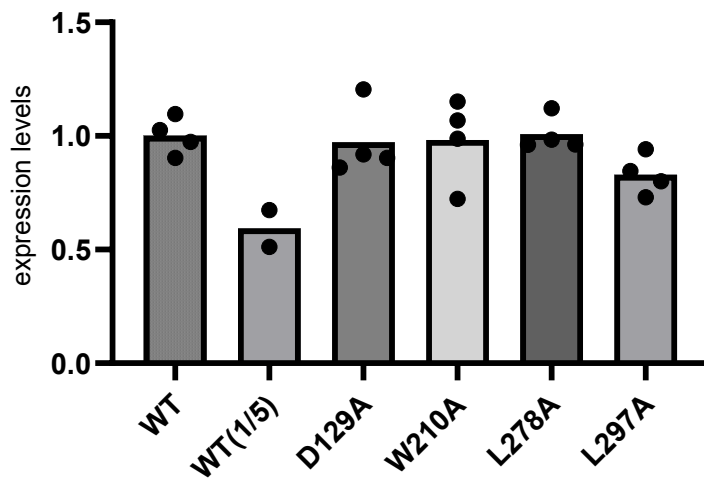
Flow chart of the cryo-EM data processing for the LPA₁-G_i complex, including particle projection selection, classification, and 3D density map reconstruction. The 3D density map was refined with a mask on the receptor and combined with the overall map. Details are provided in the Methods section.



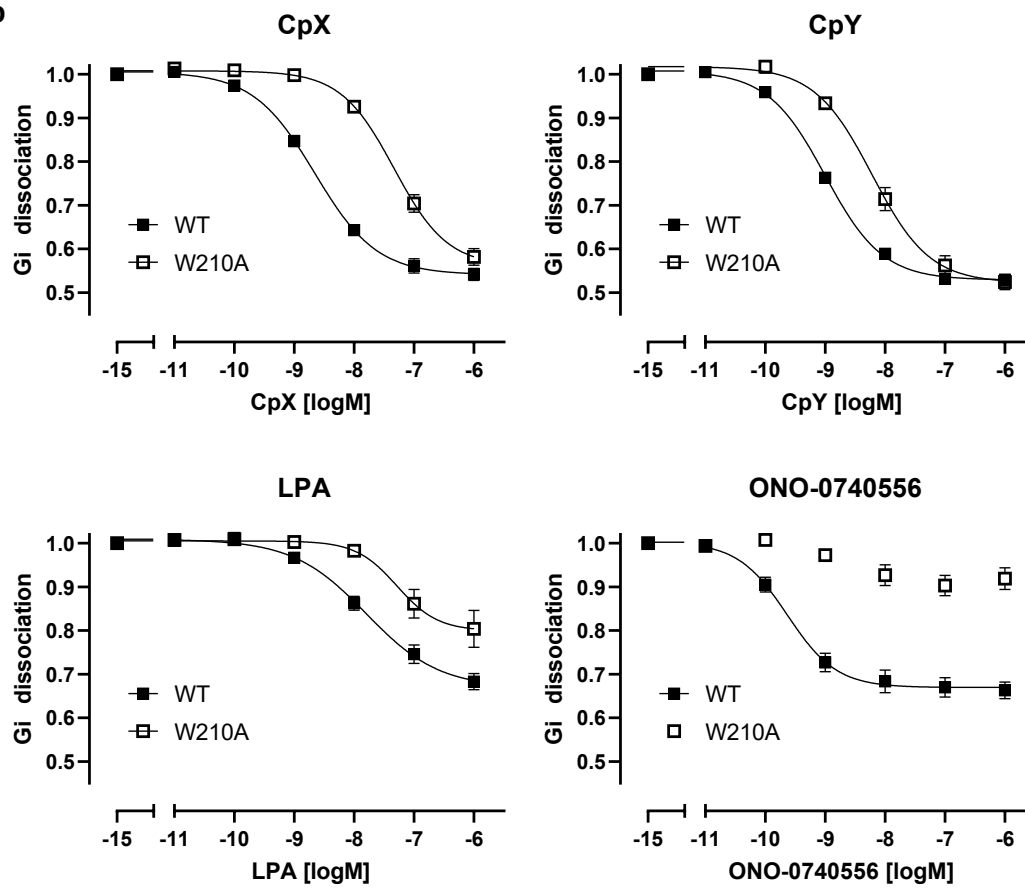
Supplementary Figure 2| Structure of LPA₁ and its ligand binding site.

a,b Superimposition of the CpY-, LPA-, and ONO-0740556-bound LPA₁ structures, **(a)** focused on the receptor and $\alpha 5h$, and **(b)** overall view of the complex. **c** The unsharpened and sharpened densities of the ligand binding site. On the hydrophilic side of the binding site, there is a density derived from water or anions, but it is noisy and not definitive. On the hydrophobic side, structural models of CpY are built within the density. The model was determined considering the strong density of benzofurans and the interaction of D129^{3.33} with amines, although the densities of some methylamines are not confirmed. **d** Superimposition of the CpY- and ONO-0740556-bound LPA₁ structures, focused on the binding site.

a



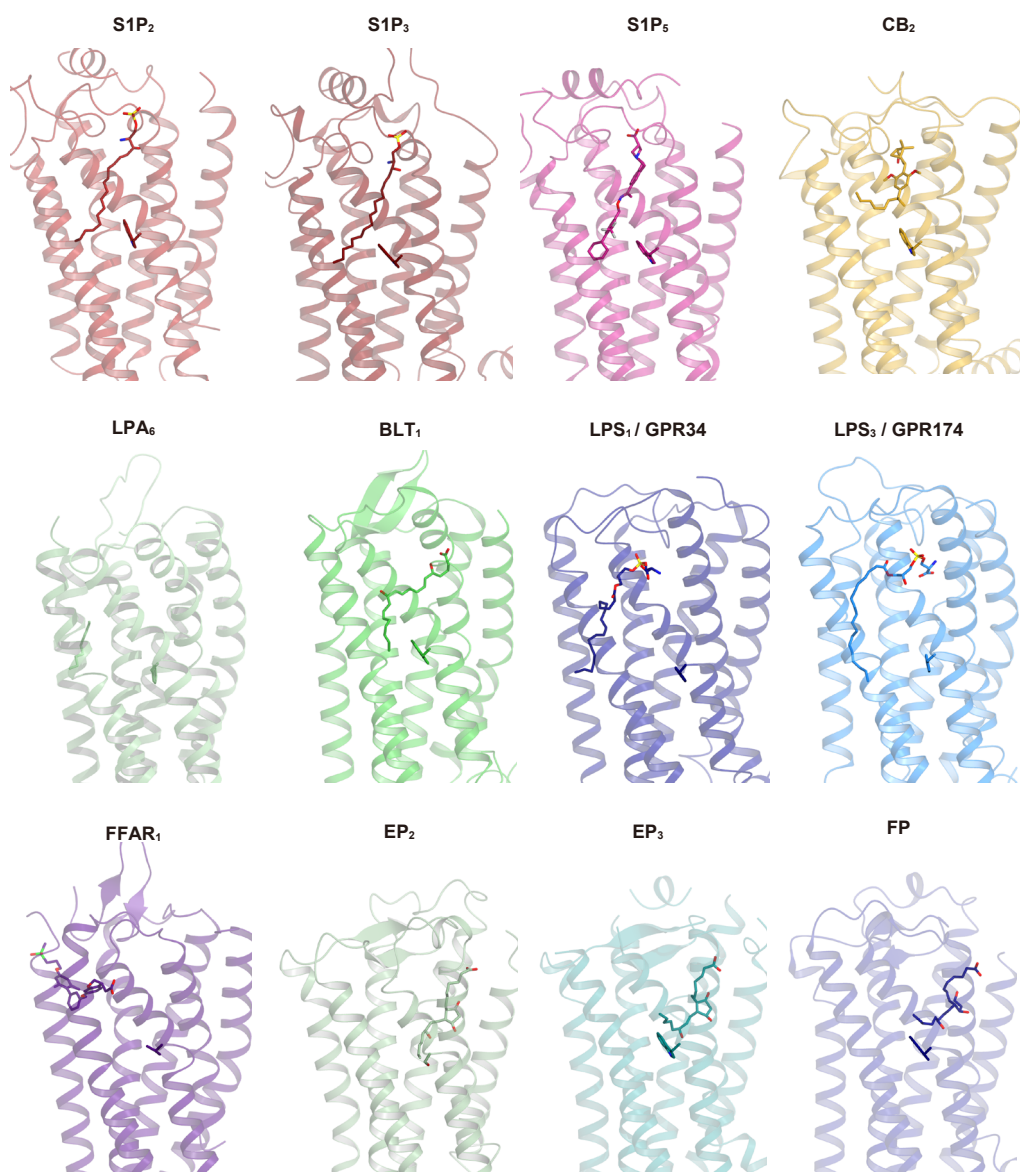
b



Supplementary Figure 3| Mutant experiments.

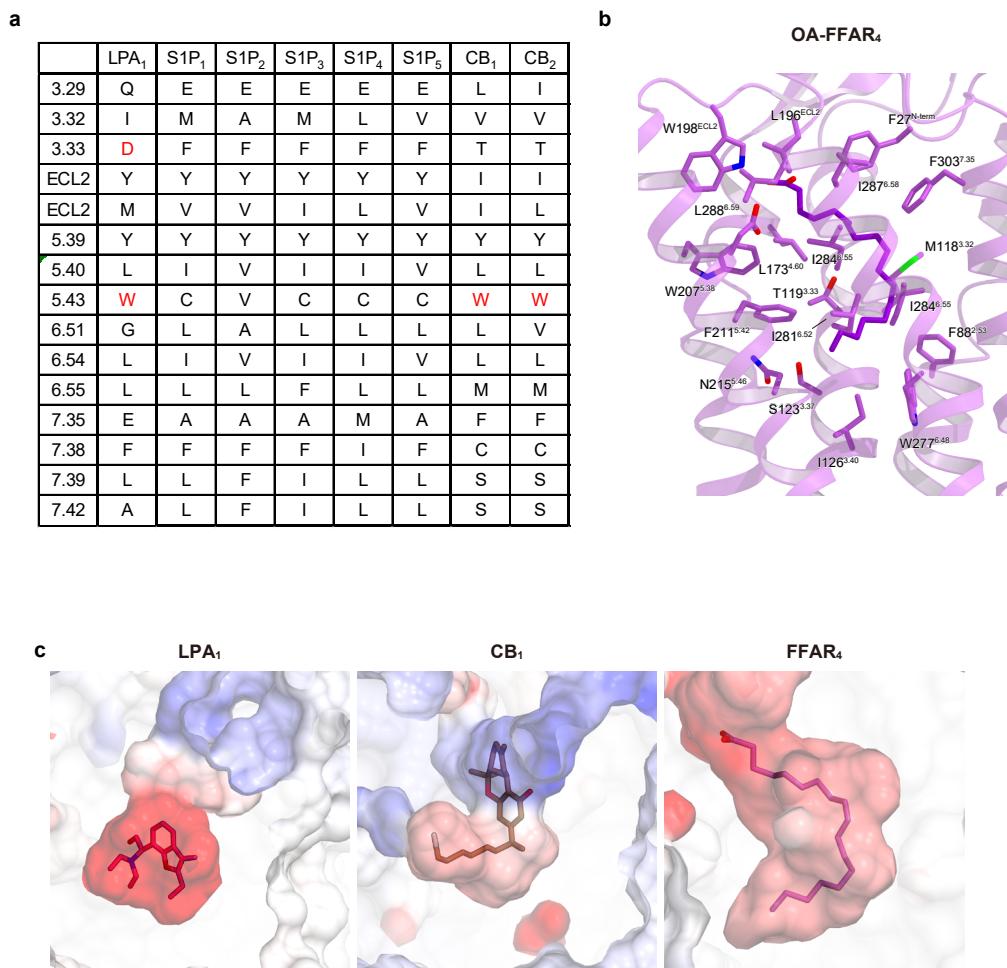
a Cell surface expression levels of the LPA₁-constructs. Cells transiently expressing the FLAG epitope-tagged LPA₁-constructs were labeled with an anti-FLAG HRP conjugate.

b NanoBIT-G-protein dissociation assays for LPA₁ and the W210A mutant. Concentration–response curves for agonist-dependent G-protein dissociation signals for LPA₁ are shown as means $\pm$ s.e.m. from three independent experiments.



Supplementary Figure 4| Binding site of lipid GPCRs.

The structures of previously determined agonist-bound lipid GPCRs. The agonist and the residue at position 6.48 are shown with sticks. In LPA₆, a monoolein molecule, rather than an agonist, is bound to the agonist binding site. These structures are acquired from S1P₂ (PDB 7T6B), S1P₃ (PDB 7C4S), S1P₅ (PDB 7EW1), CB₂ (PDB 8GUS), LPA₆ (PDB 5XSZ), BLT₁ (PDB 7VKT), LPS₁ / GPR34 (PDB 8SAI), LPS₃ / GPR174 (PDB 7XV3), FFAR₁ (PDB 8EJC), EP₂ (PDB 7CX2), EP₃ (PDB 6AK3), and FP (PDB 8ICK).



Supplementary Figure 5 | Conservation of the ligand binding site and surface charge.
a Conservation of the ligand binding site in LPA₁, S1P₁₋₅, and CB_{1,2}. **b** Binding site of OA in FFAR₄ (PDB: 8ID6). **c** Surface charge of the binding pocket. Positive charges are shown in blue and negative charges in red. LPA₁, has a stronger negatively charged pocket than CB₁ and FFAR₄.