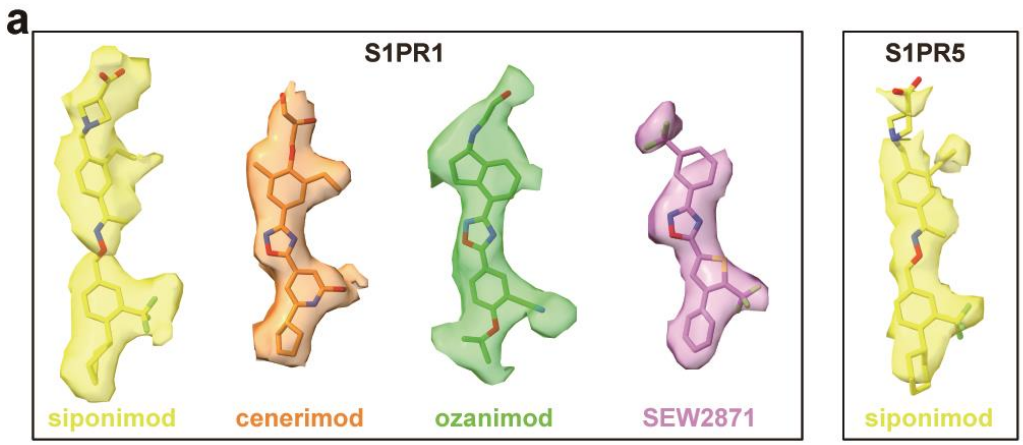
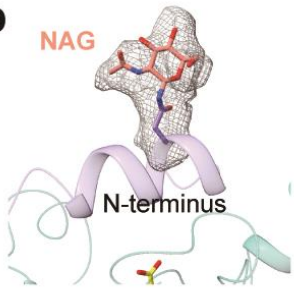
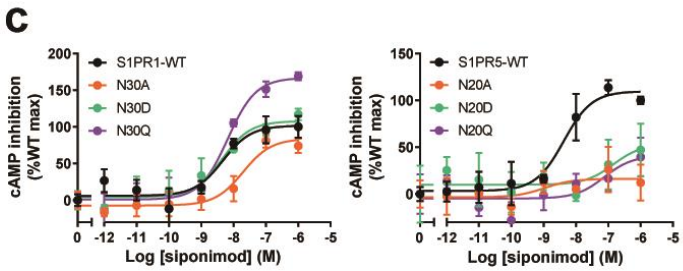
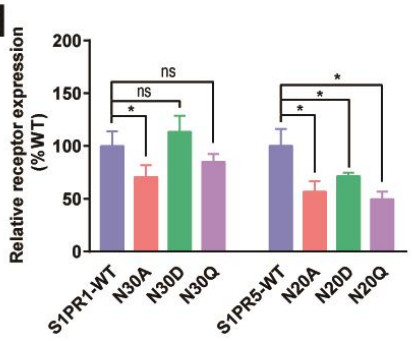
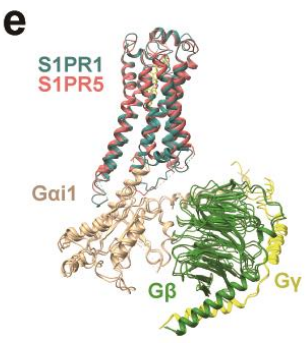
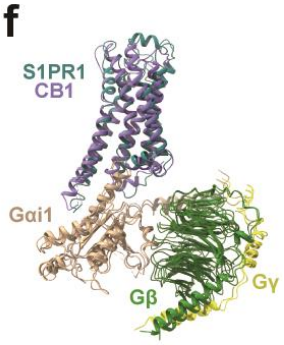

a
S1PR1
S1PR5
siponimod
cenerimod
ozanimod
SEW2871
siponimod

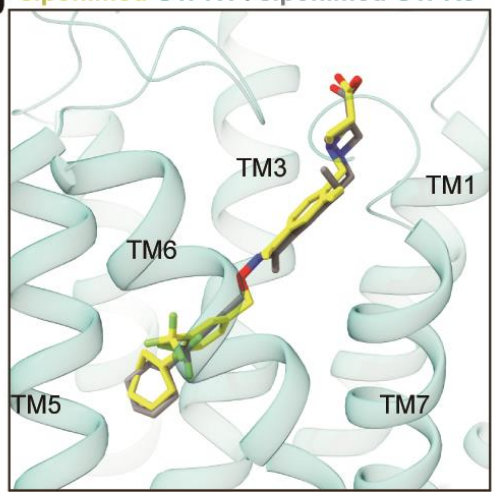

b
NAG
N-terminus


c
S1PR1-WT
N30A
N30D
N30Q
cAMP inhibition (%WT max)
Log [siponimod] (M)
S1PR5-WT
N20A
N20D
N20Q
cAMP inhibition (%WT max)
Log [siponimod] (M)


d
Relative receptor expression (%WT)
ns
ns
*
*
*
*
S1PR1-WT
N30A
N30D
N30Q
S1PR5-WT
N20A
N20D
N20Q


e
S1PR1
S1PR5
Gαi1
Gβ
Gγ


f
S1PR1
CB1
Gαi1
Gβ
Gγ


g
siponimod-S1PR1 / siponimod-S1PR5
TM3
TM1
TM6
TM5
TM7

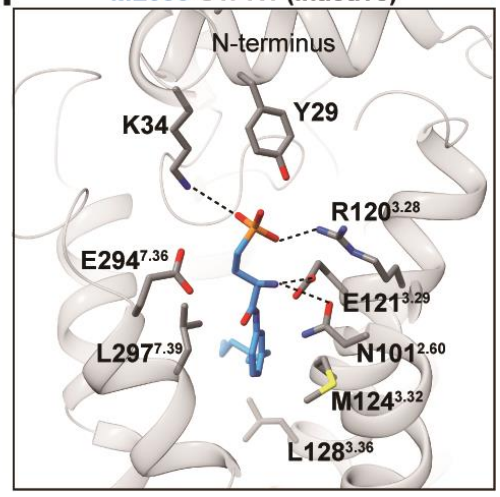

h
ML056-S1PR1 (Inactive)
N-terminus
K34
Y29
R120 3.28
E294 7.36
E121 3.29
L297 7.39
N101 2.60
M124 3.32
L128 3.36

Figure S4. Structural and functional comparison of S1PRs-Gi complexes with CB1-Gi complex or inactive S1PR1.

(a) The EM density of ligands in S1PR1 or S1PR5 allowed unambiguous identification of siponimod (yellow), cenerimod (dark orange), ozanimod (lime green), and SEW2871 (orchid) respectively. The density maps of the agonists are depicted at contour level of 0.206.

(b) The EM density of N-linked glycosylation modification was identified at residue N30 of S1PR1.

(c) Effects of the N30 mutations of S1PR1 and corresponding residue N20 mutations of S1PR5 on siponimod-induced cAMP inhibition.

(d) Cell surface expression levels of S1PR1 and S1PR5 wide-type and their mutations according to ELISA. Bar represents difference in calculated relative cell surface expression levels of mutations to WT. ns, no significance, *$p < 0.1$ (one-way analysis of variance [ANOVA] followed by the Dunnett's test, compared with the response of WT). Data represent mean ± SEM from three independent experiments performed in triplicate.

(e) Structural superposition of the S1PR1-Gi protein complex and the S1PR5-Gi protein complex.

(f) Structural superposition of the S1PR1-Gi protein complex and the CB1-Gi protein complex. Slate blue, CB1; tan, Gαi; forest green, Gβ; yellow, Gγ.

(g) Comparison of siponimod binding mode in S1PR1 with that in S1PR5. Yellow, siponimod bound S1PR1; gray, siponimod bound S1PR5.

(h) Detailed interactions between antagonist ML056 and S1PR1 (Inactive state, PDB code:3V2Y). The important interactions including hydrogen bonds and slat bridges are highlighted as black dashed lines.