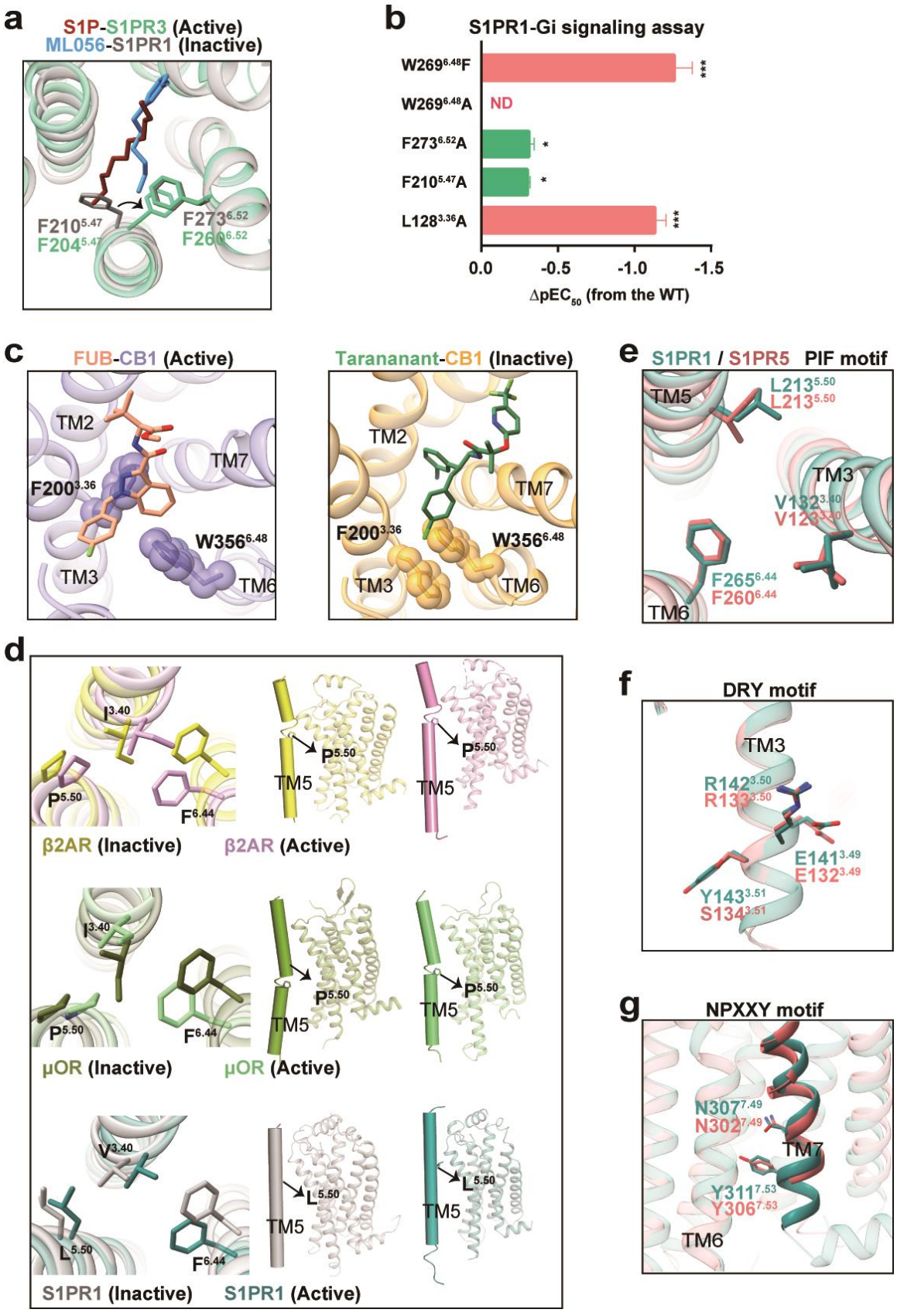
a
S1P-S1PR3 (Active)
ML056-S1PR1 (Inactive)
F210 5.47
F204 5.47
F273 6.52
F260 6.52
b
S1PR1-Gi signaling assay
W269 6.48 F
W269 6.48 A
F273 6.52 A
F210 5.47 A
L128 3.36 A
ND
***
*
*
***
0.0
-0.5
-1.0
-1.5
ΔpEC50 (from the WT)
c
FUB-CB1 (Active)
Tarananant-CB1 (Inactive)
TM2
TM7
F200 3.36
W356 6.48
TM3
TM6
e
S1PR1 / S1PR5 PIF motif
TM5
L213 5.50
L213 5.50
TM3
V132 3.40
V123 3.40
F265 6.44
F260 6.44
TM6
d
I 3.40
P 5.50
F 6.44
TM5
β2AR (Inactive)
β2AR (Active)
μOR (Inactive)
μOR (Active)
V 3.40
L 5.50
S1PR1 (Inactive)
S1PR1 (Active)
f
DRY motif
TM3
R142 3.50
R133 3.50
E141 3.49
E132 3.49
Y143 3.51
S134 3.51
g
NPXXY motif
N307 7.49
N302 7.49
TM7
Y311 7.53
Y306 7.53
TM6

Figure S6. Structural features in S1PR1 upon activation

(a) Structural superposition of S1P-bound activated S1PR3 and ML056-bound inactivated S1PR1. The side-chain of residue $F^{5.47}$ in SPR3 is observed to exhibit notable displacement whereas the $F^{6.52}$ in both structures adopt the same conformation. Medium spring greer, S1PR3; S1P, maroon; gray, S1PR1; dodger blue, ML056.

(b) Effects of the mutants of S1PR1 on siponimod-induced cAMP inhibition. Bars represents difference in calculated potency (pEC_{50}) of agonist for mutations relative to WT of S1PR1. Data are colored according to extent of effect. ND, not detected, *p < 0.1, ***p < 0.001 (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.

(c) Structural comparison MDMB-Fubinaca (FUB) bound CB1 (agonist, light salmon, PDB: 6N4B) with Taranabant bound CB1 (antagonist, sea green, PDB: 5U09) reveals the toggle switches $F^{3.36}$-$W^{6.48}$ activation mechanism in receptor.

(d) Structural rearrangement of $P^{5.50}$-$I^{3.40}$-$F^{6.44}$ motif in S1PR1(inactive PDB: 3V2Y, gray; active, teal), β_2AR (inactive PDB: 2RH1, yellow; active PDB: 3SN6, plum) and μOR (inactive PDB: 4DKL, dark olive green; active PDB: 6DDE, light green) upon activation. S1PR1 is absent of proline at the position 5.50, which is substituted by leucine.

(e-g) Superposition of microswitches between S1PR1 and S1PR5. $P^{5.50}$-$I^{3.40}$-$F^{6.44}$ motif (e); $E^{3.49}$-$R^{3.50}$-Y motif (f); N-$P^{7.50}$xx$Y^{7.53}$ motif (g).