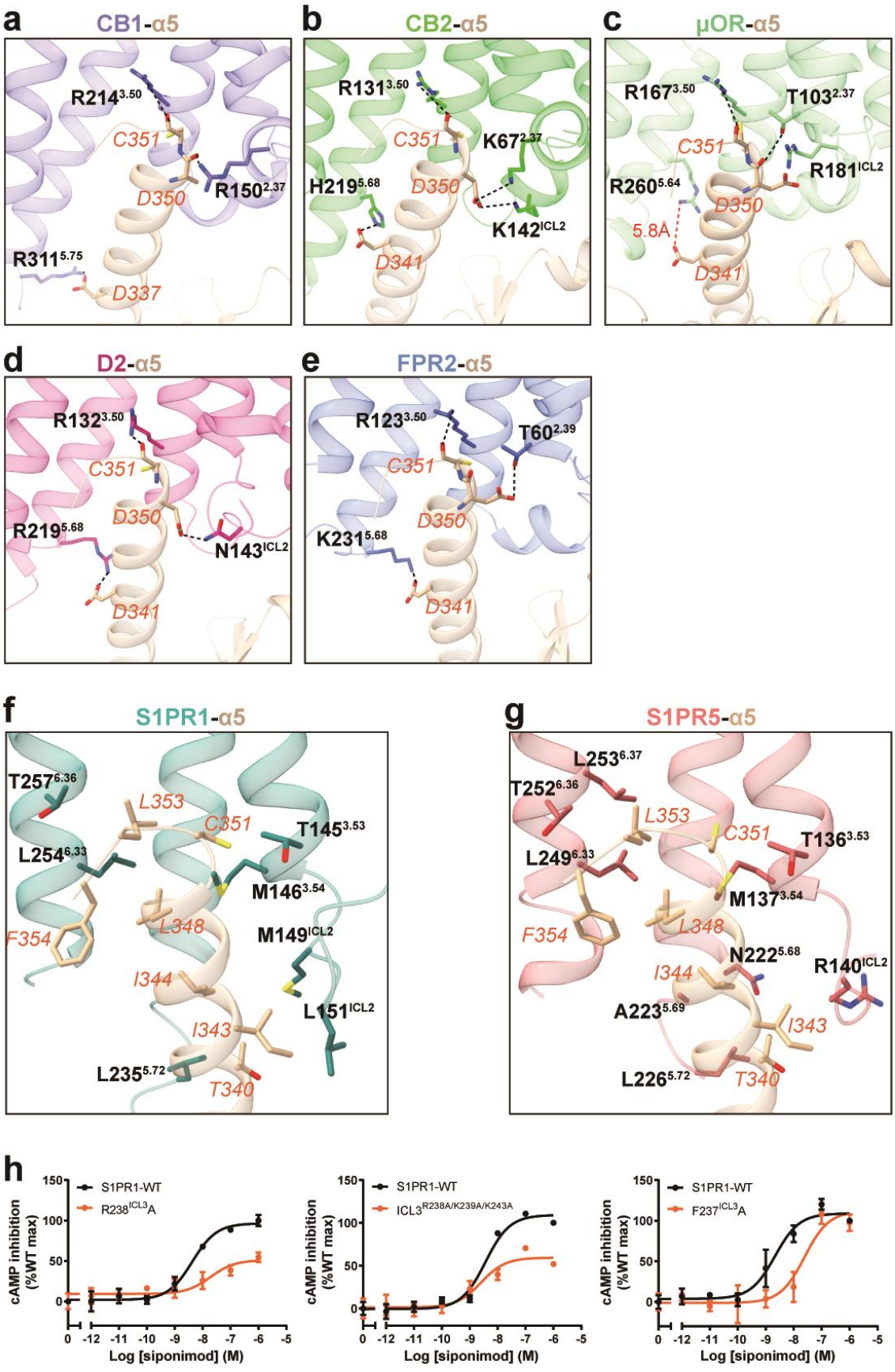
a
CB1-α5
R214^3.50
C351
R150^2.37
D350
R311^5.75
D337
b
CB2-α5
R131^3.50
C351
K67^2.37
H219^5.68
D350
K142^ICL2
D341
c
μOR-α5
R167^3.50
T103^2.37
C351
R181^ICL2
R260^5.64
D350
5.8Å
D341
d
D2-α5
R132^3.50
C351
D350
R219^5.68
N143^ICL2
D341
e
FPR2-α5
R123^3.50
T60^2.39
C351
D350
K231^5.68
D341
f
S1PR1-α5
T257^6.36
L353
C351
T145^3.53
L254^6.33
M146^3.54
F354
L348
M149^ICL2
I344
L151^ICL2
I343
L235^5.72
T340
g
S1PR5-α5
L253^6.37
T252^6.36
L353
C351
T136^3.53
L249^6.33
M137^3.54
F354
L348
N222^5.68
R140^ICL2
I344
A223^5.69
I343
L226^5.72
T340
h
S1PR1-WT
R238^ICL3A
cAMP inhibition (%WT max)
Log [siponimod] (M)
S1PR1-WT
ICL3^R238A/K239A/K243A
cAMP inhibition (%WT max)
Log [siponimod] (M)
S1PR1-WT
F237^ICL3A
cAMP inhibition (%WT max)
Log [siponimod] (M)

Figure S7. Comparison of receptor-Gi interface between S1PRs-Gi complex and previously reported receptors-Gi complexes.

(a-e) Polar interactions in complex structures of CB1 (slate blue)-Gαi, CB2 (lime)-Gαi, μOR (light green)-Gαi, D2 (deep pink)-Gαi and FPR2(royal blue)-Gαi. Residues from α5 are shown in orange font. The residues belonging to S1PR1 are shown in black font. Polar interactions are highlighted as black dashed lines.

(f-g) Hydrophobic pattern between S1PR1 (f) or S1PR5 (g) and the hydrophobic side of α5 helix from Gαi.

(h) Effects of the $R238^{ICL3}A$ and $ICL3^{R238A/K239A/K243A}$ and $F237^{ICL3}A$ mutants of S1PR1 on siponimod induced cAMP inhibition. Data are presented as the mean ± SEM of three independent experiments performed in triplicate.