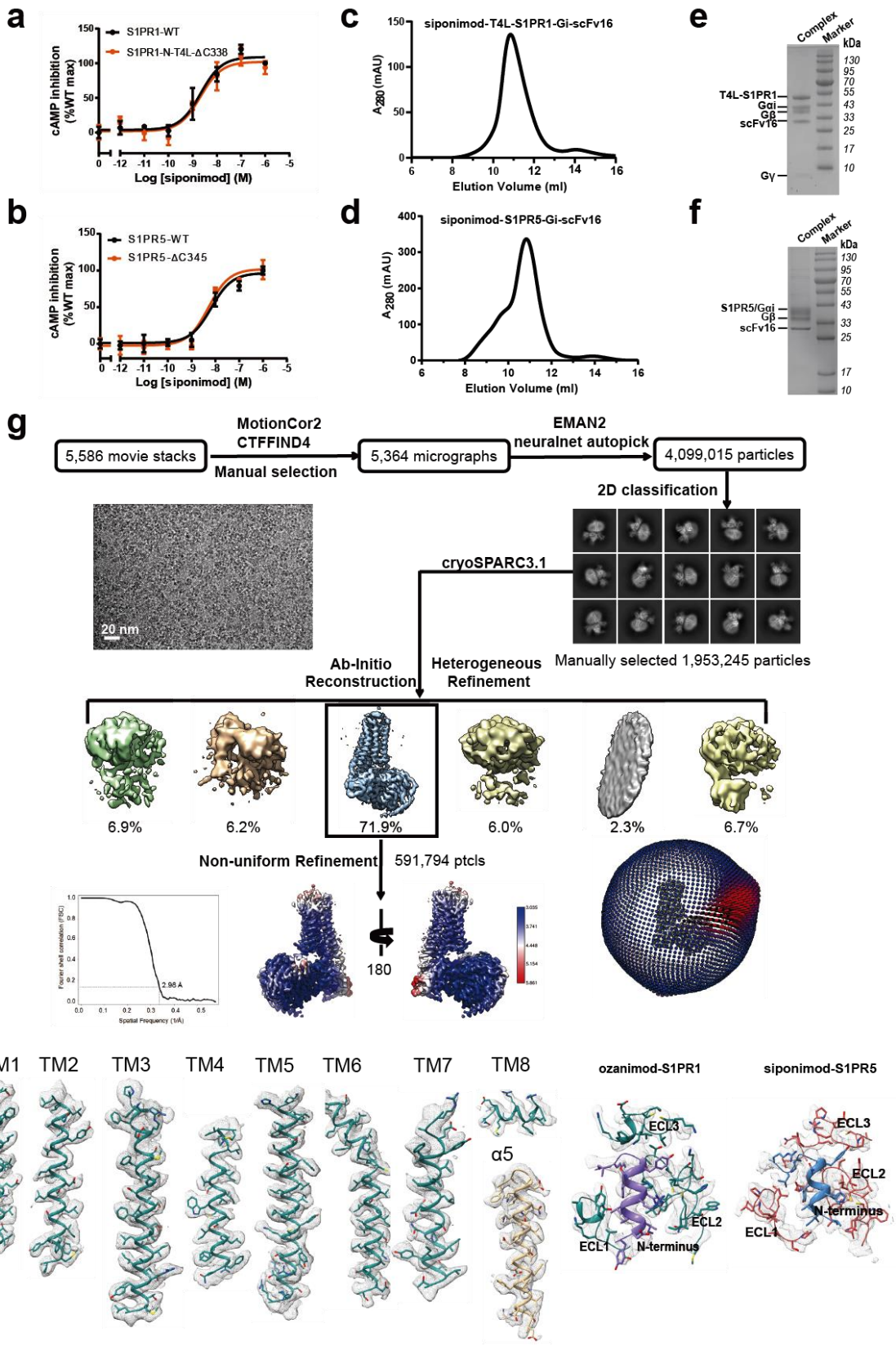
a
S1PR1-WT
S1PR1-N-T4L-ΔC338
cAMP inhibition (%WT max)
Log [siponimod] (M)
b
S1PR5-WT
S1PR5-ΔC345
cAMP inhibition (%WT max)
Log [siponimod] (M)
c
siponimod-T4L-S1PR1-Gi-scFv16
A280 (mAU)
Elution Volume (ml)
d
siponimod-S1PR5-Gi-scFv16
A280 (mAU)
Elution Volume (ml)
e
Complex
Marker
kDa
T4L-S1PR1
Gαi
Gβ
scFv16
Gγ
f
Complex
Marker
kDa
S1PR5/Gαi
Gβ
scFv16
g
5,586 movie stacks
MotionCor2
CTFFIND4
Manual selection
5,364 micrographs
EMAN2
neuralnet autopick
4,099,015 particles
2D classification
20 nm
cryoSPARC3.1
Manually selected 1,953,245 particles
Ab-Initio Reconstruction
Heterogeneous Refinement
6.9%
6.2%
71.9%
6.0%
2.3%
6.7%
Non-uniform Refinement
591,794 ptcls
2.98 Å
180
h
TM1
TM2
TM3
TM4
TM5
TM6
TM7
TM8
α5
ozanimod-S1PR1
ECL3
ECL2
ECL1
N-terminus
siponimod-S1PR5
ECL3
ECL2
N-terminus
ECL1

Figure S1. The siponimod bound S1PR-Gi-scFv16 complex purification, cryo-EM data collection, structure determination, and cryo-EM map quality.

(a) Comparison of siponimod induced Gi signaling potency between wild-type (WT) S1PR1 and T4L-S1PR1(1-337 aa).

(b) Comparison of siponimod induced Gi signaling potency between wild-type (WT) S1PR5 and S1PR5 (1-344 aa).

(c) Representative size-exclusion chromatography elution profile of the purified Siponimod-T4L-S1PR1(1-337 aa)-Gi-scFv16 complex using Superdex 200 10/300 Increase column.

(d) Representative size-exclusion chromatography elution profile of the purified Siponimod-S1PR5(1-344 aa)-Gi-scFv16 complex using Superdex 200 10/300 Increase column.

(e-f) SDS-PAGE analysis of siponimod bound S1PR1 (e) and S1PR5 (f) in complex with Gi and scFv16.

(g) Flow chart of cryo-EM single particle reconstruction of siponimod bound S1PR1-Gi complex. Single particle pipeline yields the final cryo-EM reconstruction with the FSC curve, corresponding angular distribution and local resolution map. FSC curve shows a 2.98 Å resolution according to the 0.143 cut-off. Particles (ptcls).

(h) Representative cryo-EM density map (contoured at 0.206) and models for TM1-7, Helix-8 of S1PR1, α5-helix of Gαi in S1PR1-Gi complex, as well as ECL1-3 and N-terminus of S1PR1-ozanimod (contoured at 0.3) and S1PR5-siponimod (contoured at 0.011).