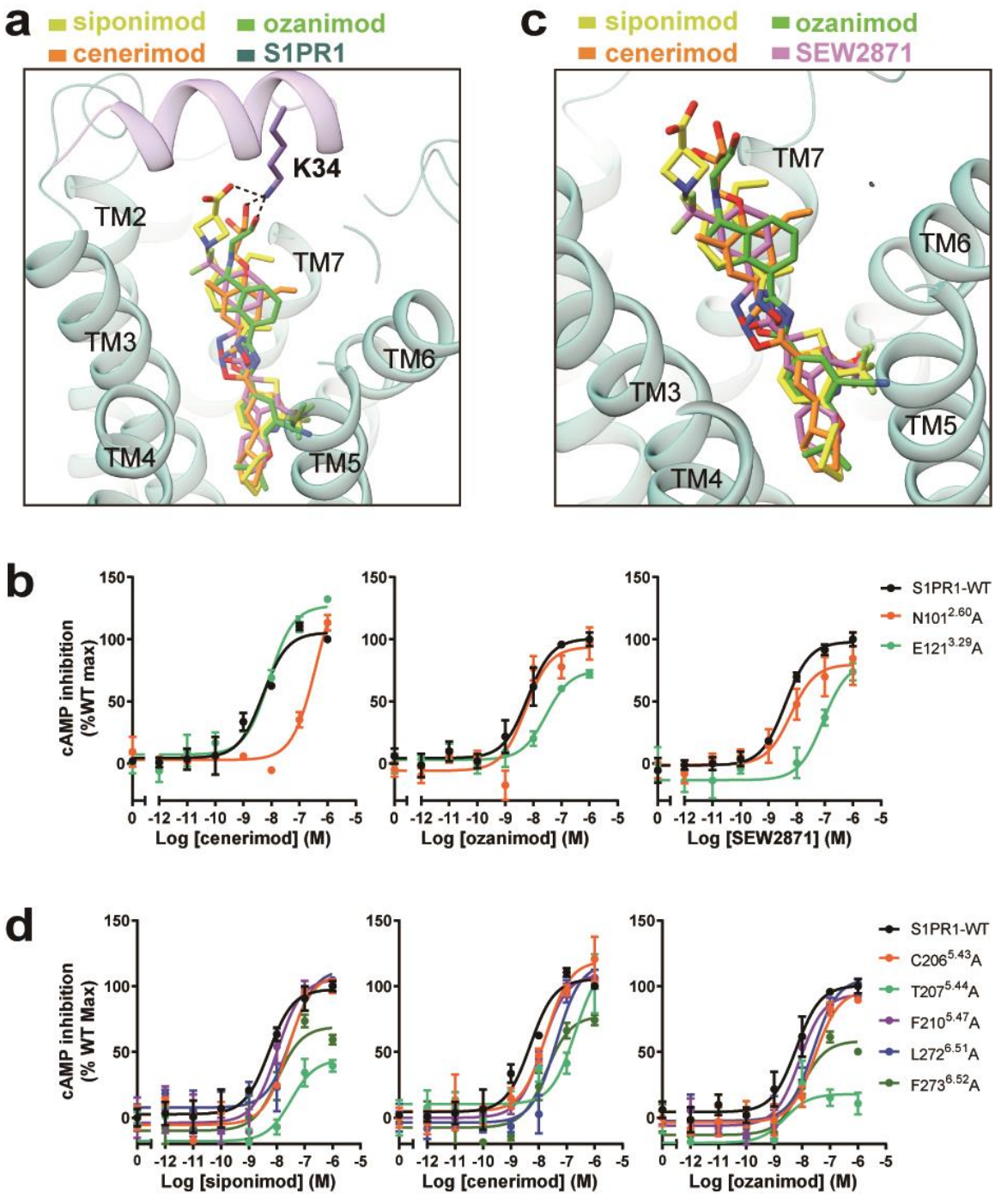
a
siponimod
ozanimod
cenerimod
S1PR1
K34
TM2
TM7
TM3
TM6
TM4
TM5
c
siponimod
ozanimod
cenerimod
SEW2871
TM7
TM6
TM3
TM5
TM4
b
cAMP inhibition (%WT max)
Log [cenerimod] (M)
Log [ozanimod] (M)
Log [SEW2871] (M)
S1PR1-WT
N101^2.60A
E121^3.29A
d
cAMP inhibition (% WT Max)
Log [siponimod] (M)
Log [cenerimod] (M)
Log [ozanimod] (M)
S1PR1-WT
C206^5.43A
T207^5.44A
F210^5.47A
L272^6.51A
F273^6.52A

Figure S5. Comparison of the ligand recognition among chemically distinct agonists bound S1PR1-Gi complexes

(a) The hydrogen bond interaction of the polar headgroups from siponimod (yellow), cenerimod (dark orange) and ozanimod (lime green) with the same residue $K34^{N\text{-}ter}$ of S1PR1 are observed in S1PR1-Gi complexes.

(b) Effects of the $N101^{2.60}A$, and $E121^{3.29}A$ mutations of S1PR1 on cenerimod, ozanimod, and SEW2871 induced cAMP inhibition. Data are presented as the mean ± SEM of three independent experiments performed in triplicate.

(c) Structural superposition of siponimod (yellow)-, cenerimod (dark orange)-, ozanimod (lime green)-, and SEW2871 (orchid)-bound S1PR1 reveals that the hydrophobic pattern of ligand inserted the nearly identical narrow hydrophobic pocket of receptor.

(d) Effects of the $C206^{5.43}A$, $T207^{5.44}A$, $F210^{5.47}A$, $F273^{6.52}A$ mutations of S1PR1 on siponimod, cenerimod and ozanimod induced cAMP inhibition. Data are presented as the mean ± SEM of three independent experiments performed in triplicate.