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Supplemental Information

Structures of the Human PGD_2 Receptor CRTH2

Reveal Novel Mechanisms for Ligand Recognition

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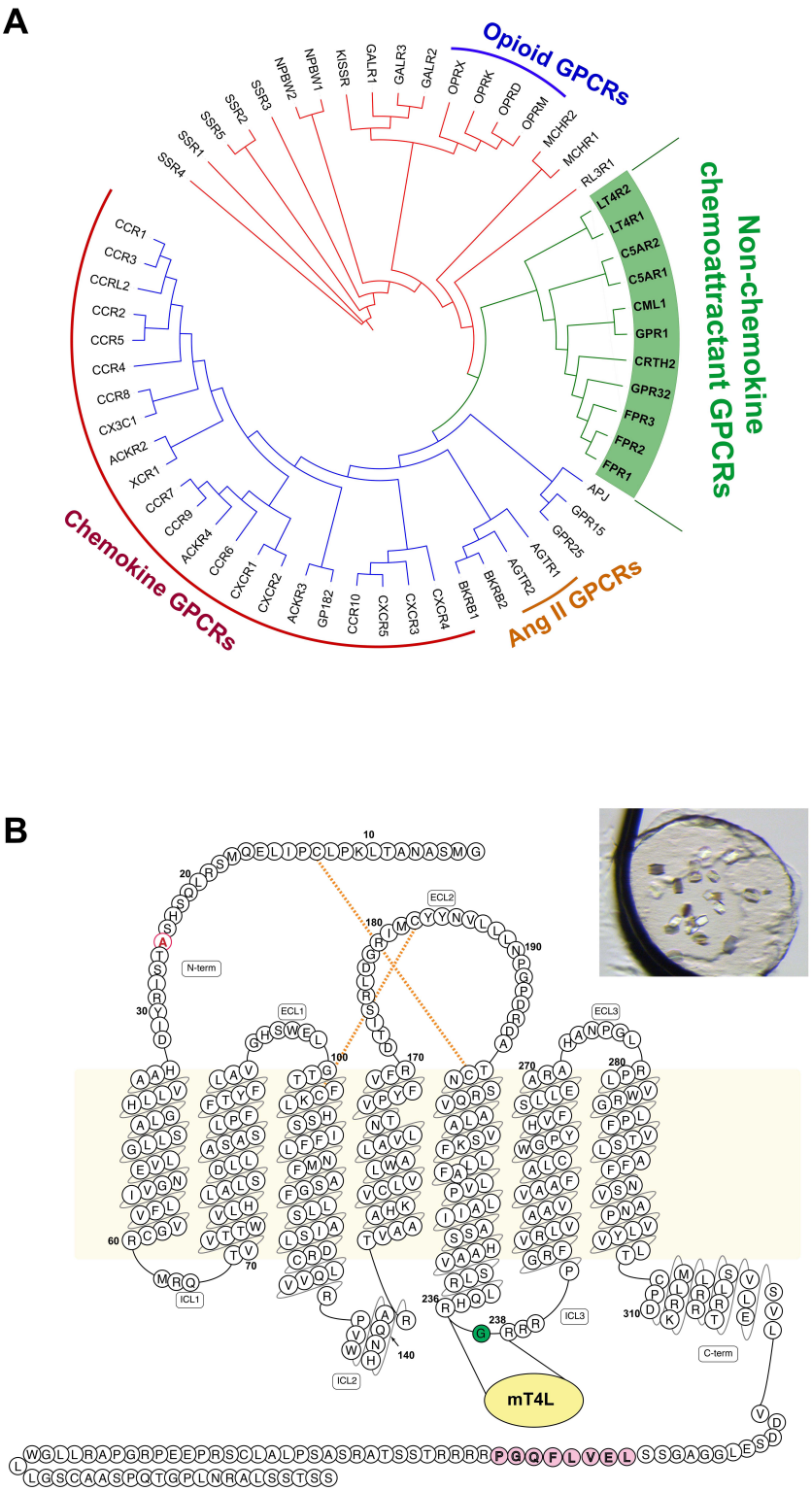
A
Opioid GPCRs
Non-chemokine
chemoattractant GPCRs
Chemokine GPCRs
Ang II GPCRs
NPBW2
NPBW1
KISSR
GALR1
GALR3
GALR2
OPRX
OPRK
OPRD
OPRM
MCHR2
MCHR1
RL3R1
SSR1
SSR4
SSR5
SSR2
SSR3
LT4R2
LT4R1
C5AR2
C5AR1
CML1
GPR1
CRTH2
GPR32
FPR3
FPR2
FPR1
CCR1
CCR3
CCRL2
CCR2
CCR5
CCR4
CCR8
CX3C1
ACKR2
XCR1
CCR7
CCR9
ACKR4
CCR6
CXCR1
CXCR2
ACKR3
GP182
CCR10
CXCR5
CXCR3
CXCR4
BKRB1
BKRB2
AGTR2
AGTR1
GPR25
GPR15
APJ
B
N-term
ECL1
ECL2
ECL3
ICL1
ICL2
ICL3
C-term
mT4L

Figure S1

Figure S1. Non-chemokine chemoattractant GPCRs and CRTH2 crystallization. Related to Figure 1 and Table 1. (A) Phylogenetic tree of the γ-subgroup of the rhodopsin-like GPCRs. This subgroup contains mainly peptide GPCRs, such as chemokine GPCRs (red arc), angiotensin II receptors (Ang II GPCRs, orange arc) and opioid GPCRs (blue arc). The non-chemokine chemoattractant GPCRs (green arc) also belong to γ-subgroup, which include receptors for anaphylatoxins, formyl peptides and eicosanoids. **(B)** Amino acid sequence of the engineered CRTH2 for our structural study. To aid the crystallization, we engineered human CRTH2 by replacing G237 (green) with mT4L. We also introduced a protease 3C site between S339 and R340 (pink) and cut off the C-terminal segment before crystallization. N25A mutation was introduced to remove the glycosylation site. The construct is referred as CRTH2-mT4L. Two disulfide bonds are shown as orange dashed lines. Crystals of CRTH2-mT4L with CAY10471 in lipidic cubic phase are shown in the upper right panel.

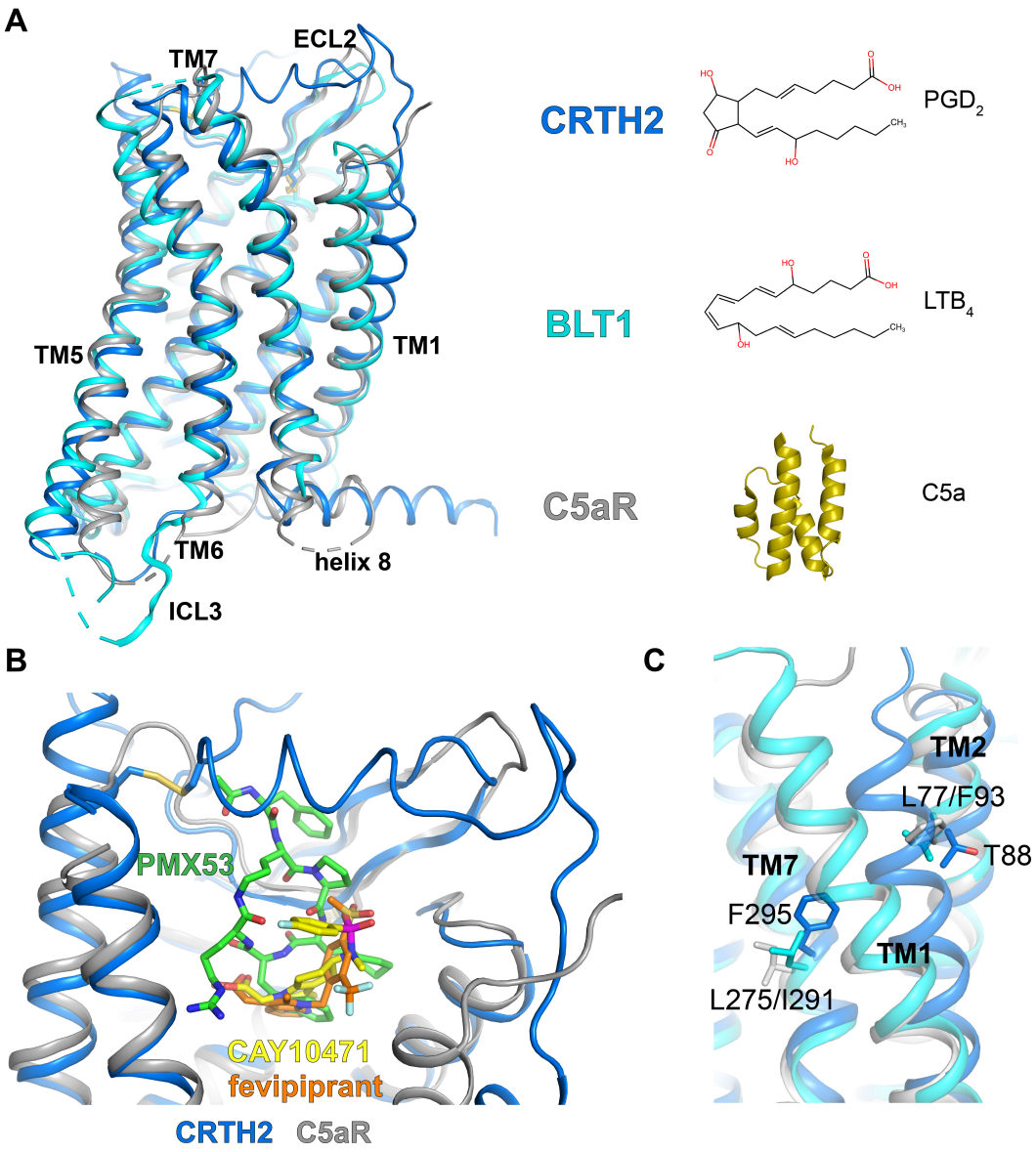
A
TM7
ECL2
TM5
TM1
TM6
helix 8
ICL3
CRTH2
PGD2
BLT1
LTB4
C5aR
C5a
B
PMX53
CAY10471
fevipiprant
CRTH2
C5aR
C
TM2
L77/F93
T88
TM7
F295
TM1
L275/I291

Figure S2

Figure S2. Structural comparison of CRTH2 to BLT1 and C5aR. Related to Figure 2. (A) Superimposition of the structures of three non-chemokine chemoattractant receptors, CRTH2 (in blue), C5aR (in grey, PDB ID 5O9H) and BLT1 (in cyan, PDB ID 5X33), and their ligands. The overall structures of these receptors are similar to each other except for the TM1 and the N-terminal region. The endogenous ligands, PGD_2 and LTB_4, for CRTH2 and BLT1 respectively, are both eicosanoids with similar chemical structures, while the endogenous ligand C5a for C5aR is a 74-amino acid small protein (PDB ID 4P3A). **(B)** Superimposition of ligands in C5aR and CRTH2. The CRTH2 antagonists CAY10471 and fevipiprant overlap with the lower part of the peptide antagonist PMX53 (green) for C5aR. **(C)** Conformation of TM1. The conformation of TM1 in CRTH2 compared to it in BLT1 and C5aR. The color scheme is the same as it in **A**. Two residues in CRTH2, $F295^{7.44}$ in TM7 and $T88^{2.61}$ in TM2, may partly account for the conformation of TM1. These residues are replaced by L275 and L77 in BLT1 and by I291 and F93 in C5aR, leading to a different conformation of TM1 in those two receptors.

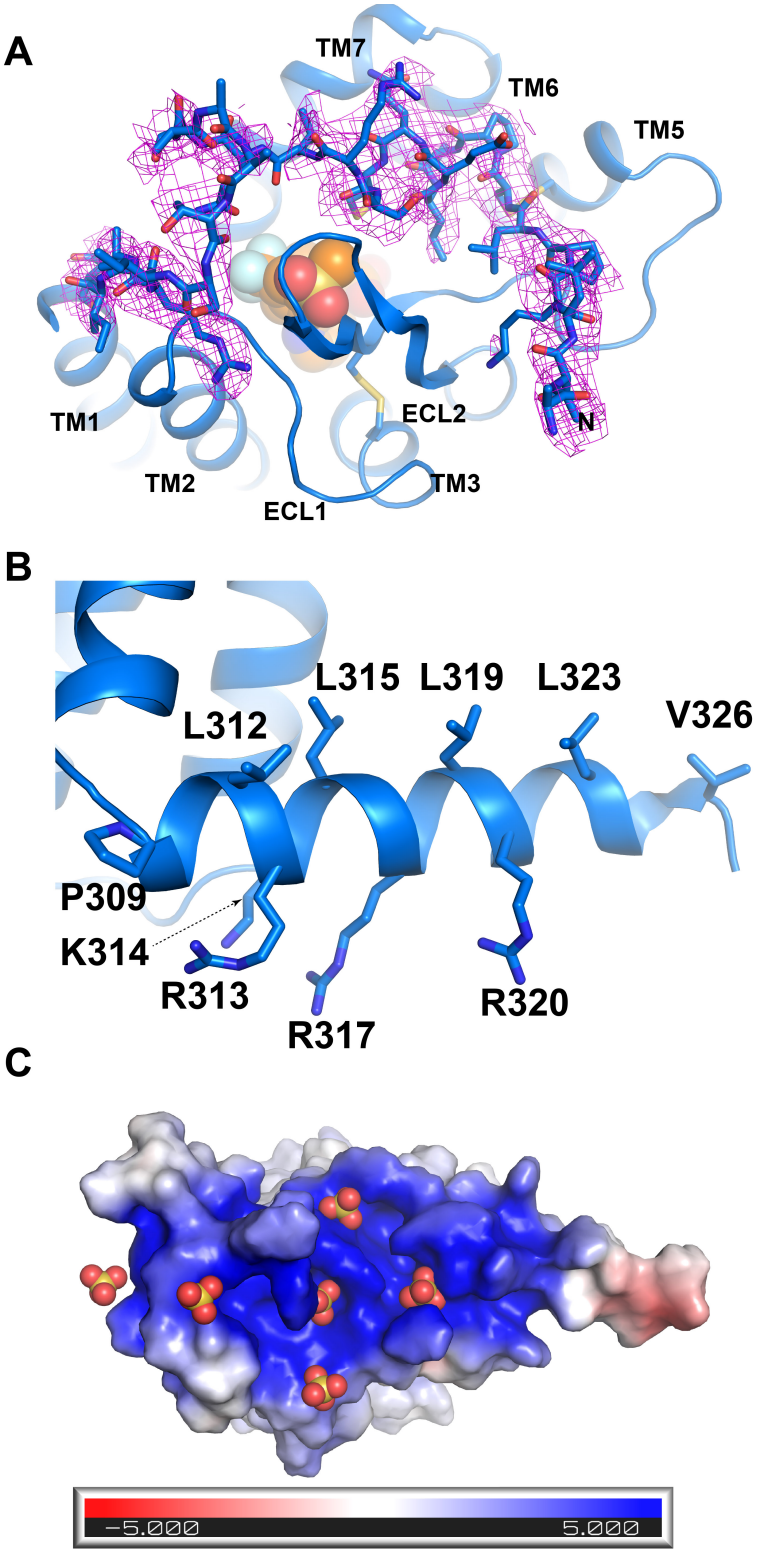
A
TM7
TM6
TM5
TM1
ECL2
N
TM2
TM3
ECL1
B
L315
L319
L323
V326
L312
P309
K314
R313
R317
R320
C
−5.000
5.000

Figure S3

Figure S3. Extracellular and cytoplasmic regions in CRTH2. Related to Figure 2. (A) Composite $2F_o$-F_c omit electron-density map for the N-terminal region from A5 to D32 calculated based on the structure of fevipiprant-CRTH2 contoured at 1.0 σ shown as magenta mesh. Fevipiprant is shown as spheres. **(B)** Helix 8 with hydrophobic residues facing the lipid biplayer and positively charged residues extending into the intracellular space. **(C)** Highly positively charged cytoplasmic surface with sulfate ions shown as spheres.

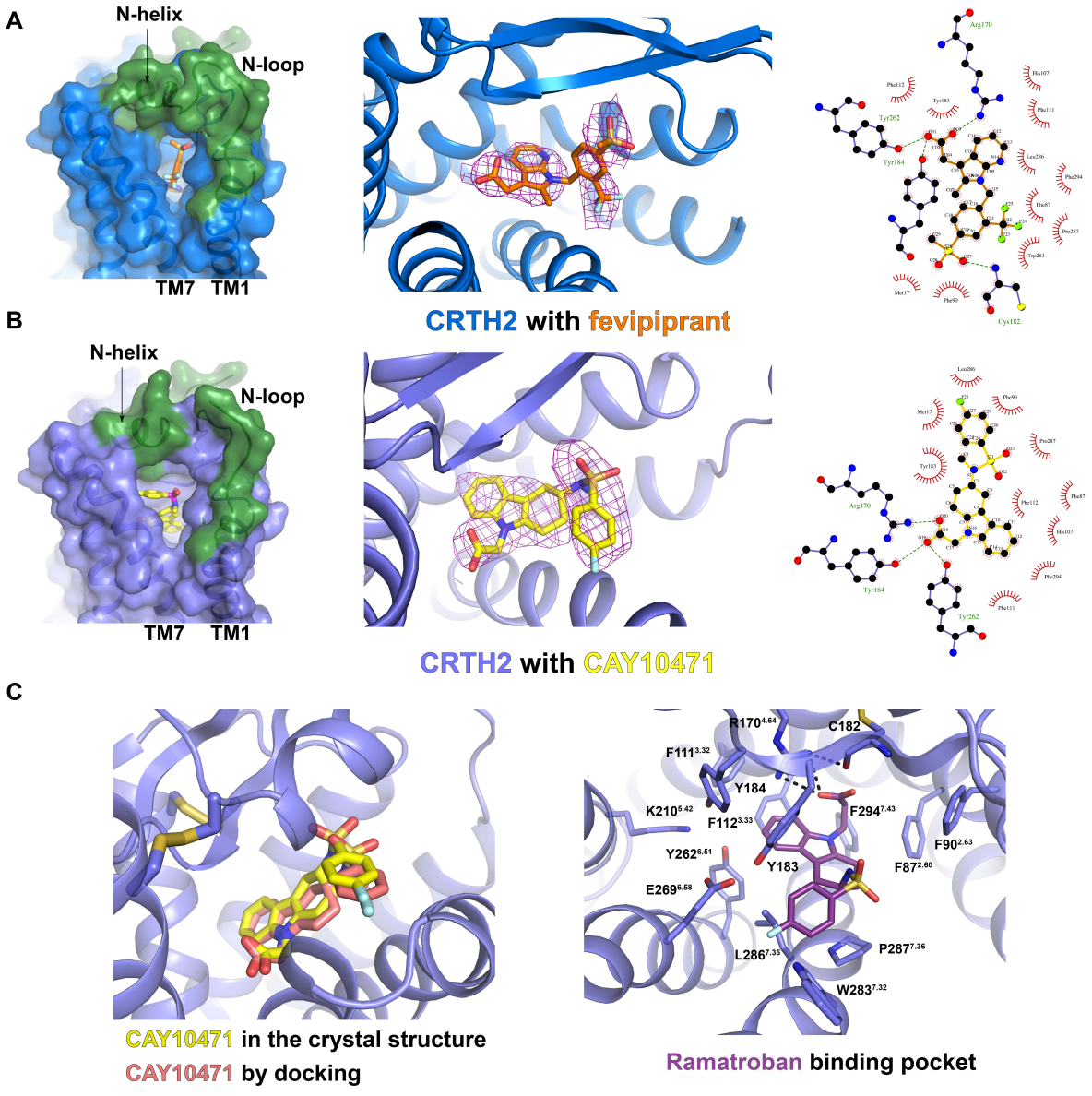
A
N-helix
N-loop
TM7
TM1
CRTH2 with fevipiprant
B
N-helix
N-loop
TM7
TM1
CRTH2 with CAY10471
C
CAY10471 in the crystal structure
CAY10471 by docking
R170^4.64
C182
F111^3.32
Y184
K210^5.42
F112^3.33
F294^7.43
F90^2.63
Y262^6.51
Y183
F87^2.60
E269^6.58
L286^7.35
P287^7.36
W283^7.32
Ramatroban binding pocket

Figure S4

Figure S4. Binding of CRTH2 antagonists. Related to Figure 3 and Figure 4. (A) Fevipiprant in orange and CRTH2 in blue. **(B)** CAY10471 in yellow and CRTH2 in slate. In A or B, the *left* panel shows the open end of the ligand binding pocket formed by N-helix, N-loop as well as the extracellular regions of TM1 and TM7; the *middle* panel shows the composite $2F_o$-F_c omit electron density map for each ligand as purple mesh contoured at 1.2 σ; the *right* panel shows the 2-D representation of ligand-receptor interactions generated using LigPlot$^+$ (https://www.ebi.ac.uk/thornton-srv/software/LigPlus/). In this panel, the bonds in fevipiprant and CAY10471 are colored in orange and yellow, respectively, while the bonds in the CRTH2 residues are colored in blue. The hydrogen bonds are shown as green dashed lines. The residues involved in the hydrophobic interactions are shown as red half-circles. **(C)** Docking of ramatroban.The *left* panel shows that docking CAY10471 back into the structure of CRTH2 with CAY10471 could reproduce the same binding pose, validating our docking methods. The *right* panel shows the details of the binding site for ramatroban by docking. The carboxylate group forms hydrogen-bonds only with R170$^{4.64}$. Most of the residues that form aromatic and hydrophobic interactions with CAY10471 also engage in similar interactions with ramatroban. The central tetracarcazole group sits in an unfavorable polar environment surrounded by E269$^{7.35}$ and the Y184-K210$^{5.42}$-Y262$^{6.51}$ cluster.

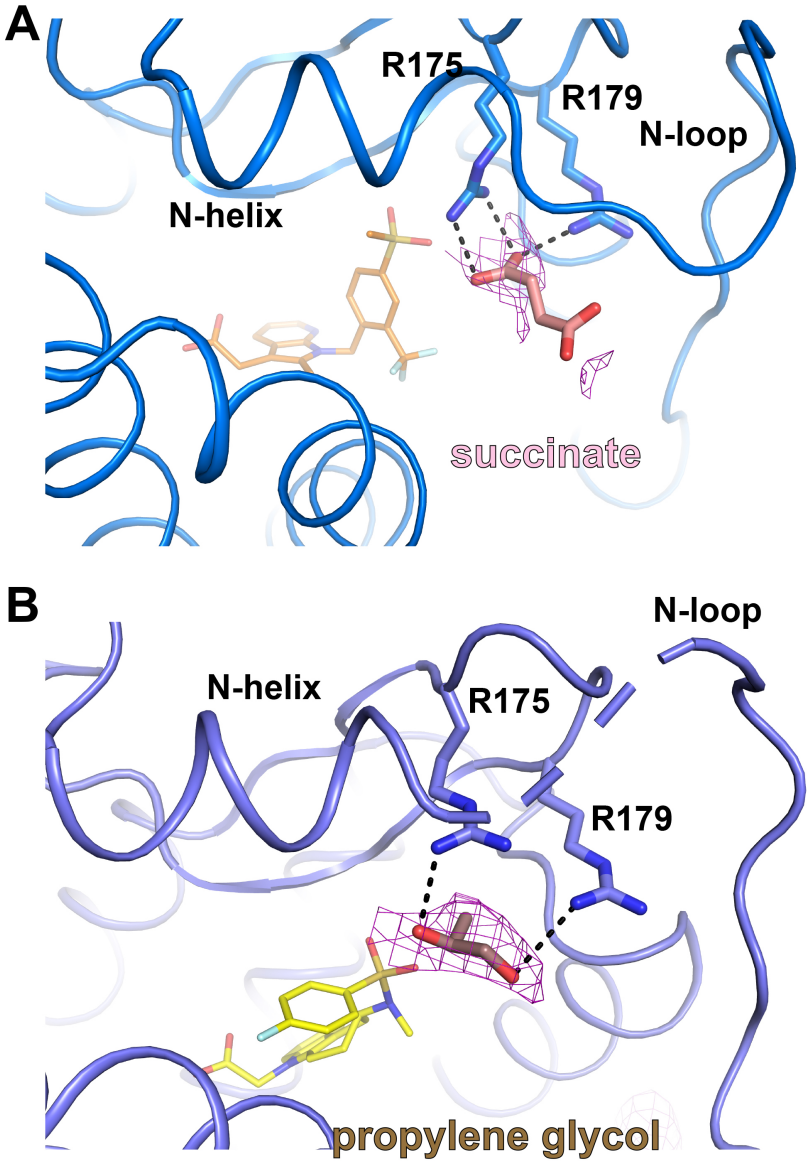
A
R175
R179
N-loop
N-helix
succinate
B
N-loop
N-helix
R175
R179
propylene glycol

Figure S5

Figure S5. Molecules modeled at the potential ligand access port. Related to Figure 5. **(A)** Succinate molecule modeled in the structure of fevipiprant-bound CRTH2 shown as pink sticks. The composite $2F_o$-F_c omit electron density map shown as purple mesh is contoured at 0.8 σ. **(B)** Propylene glycol molecule modeled in the structure of CAY10471-bound CRTH2 shown as brown sticks. The composite $2F_o$-F_c omit electron density map shown as purple mesh is contoured at 1.2 σ. Fevipirant and CAY10471 in **A** and **B** are shown as orange and yellow sticks, respectively. The density for succinate is weaker than the density for propylene glycol, presumably due to the low concentration of succinate in the crystallization conditions for the crystals of fevipiprant-bound CRTH2, or a higher structural flexibility of succinate compared to propylene glycol.

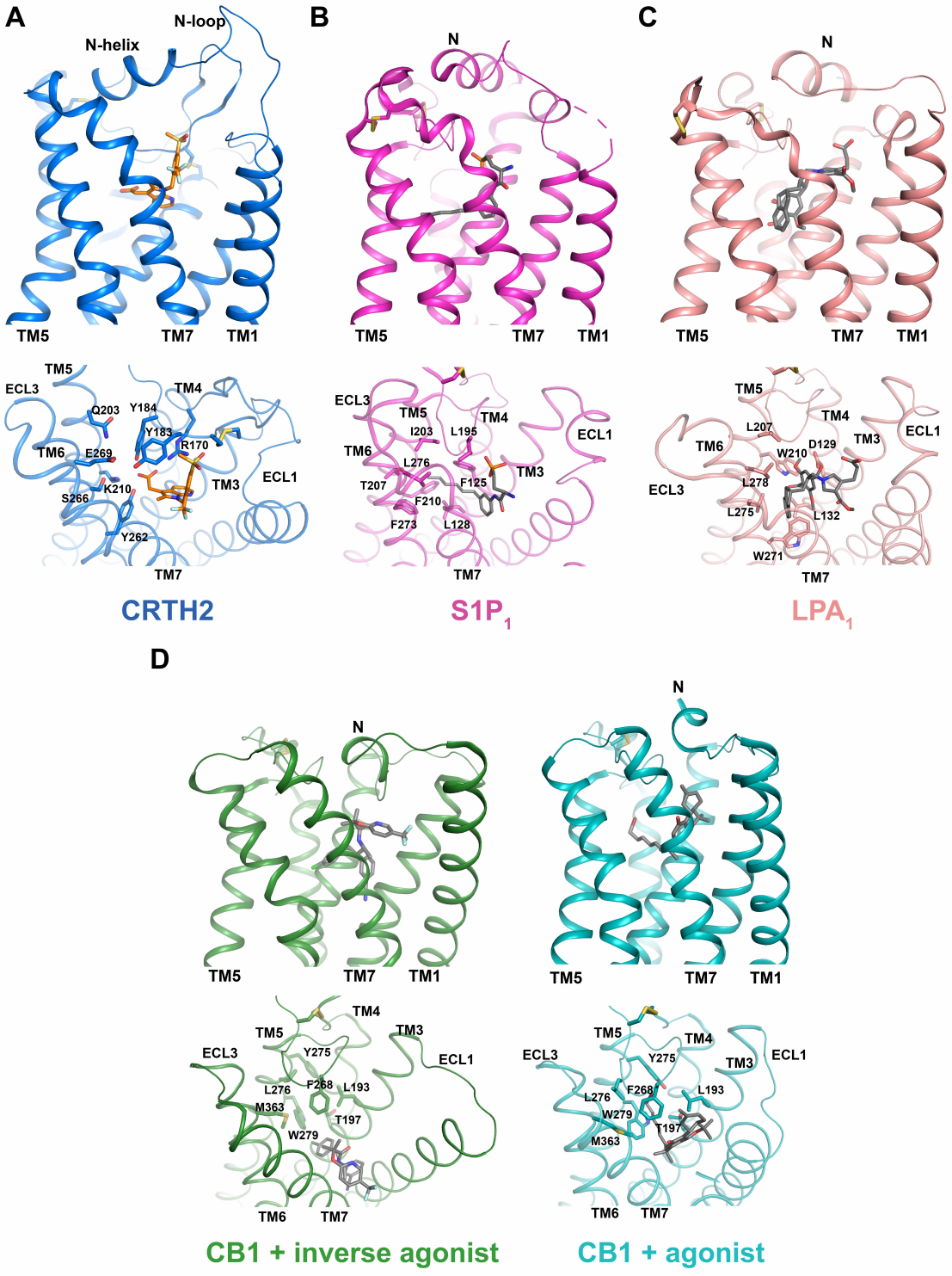
A
N-helix
N-loop
TM5
TM7
TM1
TM5
ECL3
TM4
Q203
Y184
Y183
R170
TM6
E269
K210
TM3
ECL1
S266
Y262
TM7
CRTH2
B
N
TM5
TM7
TM1
ECL3
TM5
TM4
I203
L195
ECL1
TM6
L276
TM3
T207
F125
F210
F273
L128
TM7
S1P1
C
N
TM5
TM7
TM1
TM5
TM4
L207
TM6
D129
TM3
ECL1
W210
ECL3
L278
L275
L132
W271
TM7
LPA1
D
N
TM5
TM7
TM1
N
TM5
TM7
TM1
TM4
TM5
TM3
ECL3
Y275
ECL1
L276
F268
L193
M363
T197
W279
TM6
TM7
CB1 + inverse agonist
TM5
TM4
ECL3
Y275
TM3
ECL1
L276
F268
L193
W279
T197
M363
TM6
TM7
CB1 + agonist

Figure S6

Figure S6. Comparison of the ligand-binding pockets in four lipid GPCRs. Related to Figure 6. (A) CRTH2 (blue) with fevipiprant (orange). **(B)** $S1P_1$ (magenta) with antagonist (grey), PDB ID 3V2W. **(C)** LPA_1 (pink) with antagonist (grey), PDB ID 4Z35. **(D)** *Left:* CB1 (green) with inverse agonist (grey), PDB ID 5U09. *Right:* CB1 (cyan) with agonist (grey), PDB ID 5XRA. In all figures, *upper* panels show the potential ligand access port in CRTH2 formed by N-loop, N-helix and the N-terminal region of TM1, and the corresponding regions in $S1P_1$, LPA_1 and CB1. *Lower* panels show the details of the distal end of the ligand-binding pocket. For $S1P_1$ and LPA_1, only antagonist-bound structures are available. In each structure, one end of the ligand is located at the occluded distal end of the ligand-binding pocket formed by residues from TM3-7, pointing to TM5. In $S1P_1$, LPA_1 and CB1, these areas are largely hydrophobic, while in CRTH2 the corresponding area is with a highly polar environment, indicating a different binding mode of PGD_2 for CRTH2 compared to the lipid ligands for the other lipid GPCRs.

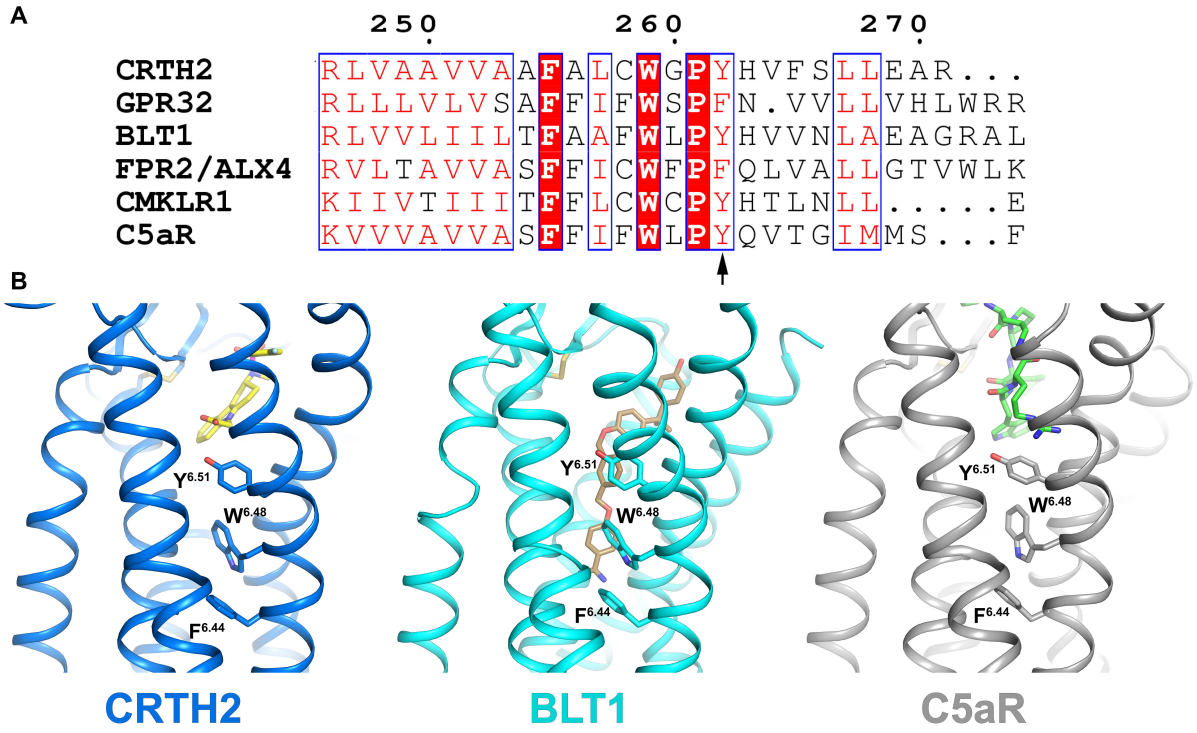
A
250
260
270
CRTH2 RLVAAVVAAFALCWGPYHVFSLLEAR...
GPR32 RLLLVLVSAFFIFWSPFN.VVLLVHLWRR
BLT1 RLVVLIILTFAAFWLPYHVVNLAEAGRAL
FPR2/ALX4 RVLTAVVASFFICWFPFQLVALLGTVWLK
CMKLR1 KIIVTIIITFFLCWCPYHTLNLL.....E
C5aR KVVVAVVASFFIFWLPYQVTGIMMS...F
B
Y6.51
W6.48
F6.44
CRTH2
BLT1
C5aR

Figure S7

Figure S7. Potentially conserved $F^{6.44}$-$W^{6.48}$-$Y/F^{6.51}$ motif in non-chemokine chemoattractant GPCRs. Related to Figure 2. (A) Sequence alignment of six members of the non-chemokine chemoattractant GPCRs from human. The highly conserved residues $F^{6.44}$, $W^{6.48}$ and $P^{6.50}$ are shown with a red background. The arrow points to a relatively conserved residue $Y/F^{6.51}$. **(B)** Conserved $F^{6.44}$-$W^{6.48}$-$Y^{6.51}$ motif. Three residues, $F^{6.44}$, $W^{6.48}$ and $Y^{6.51}$, line on TM6 in the structures of CRTH2, BLT1 (PDB ID 5X33) and C5aR (PDB ID 6C1R). $Y^{6.51}$ interacts with the ligand and packs with $W^{6.48}$ in each structure. The antagonists of CRTH2, BLT1 and C5aR are shown as yellow, brown and green sticks respectively.