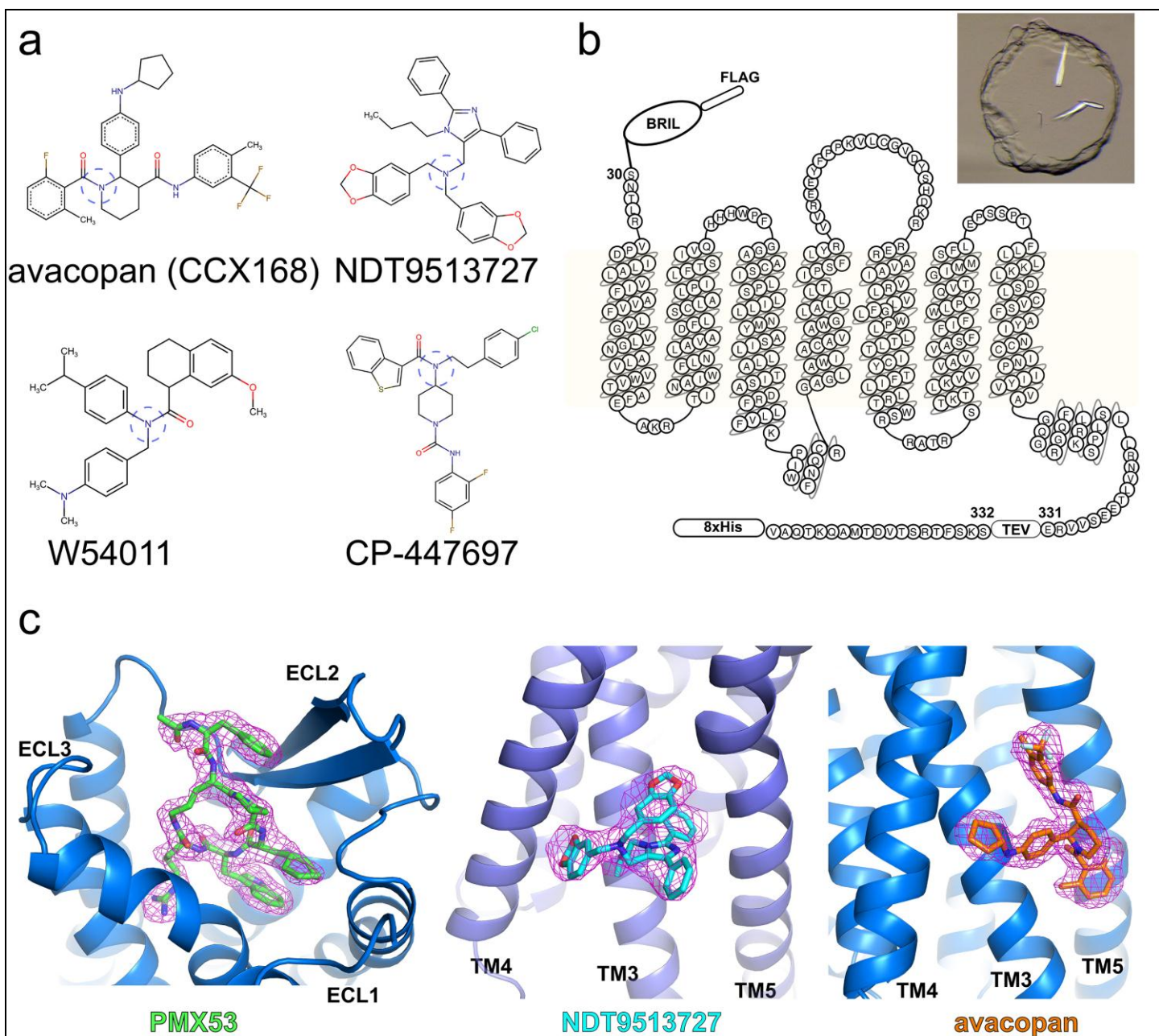


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Orthosteric and allosteric action of the C5a receptor antagonists

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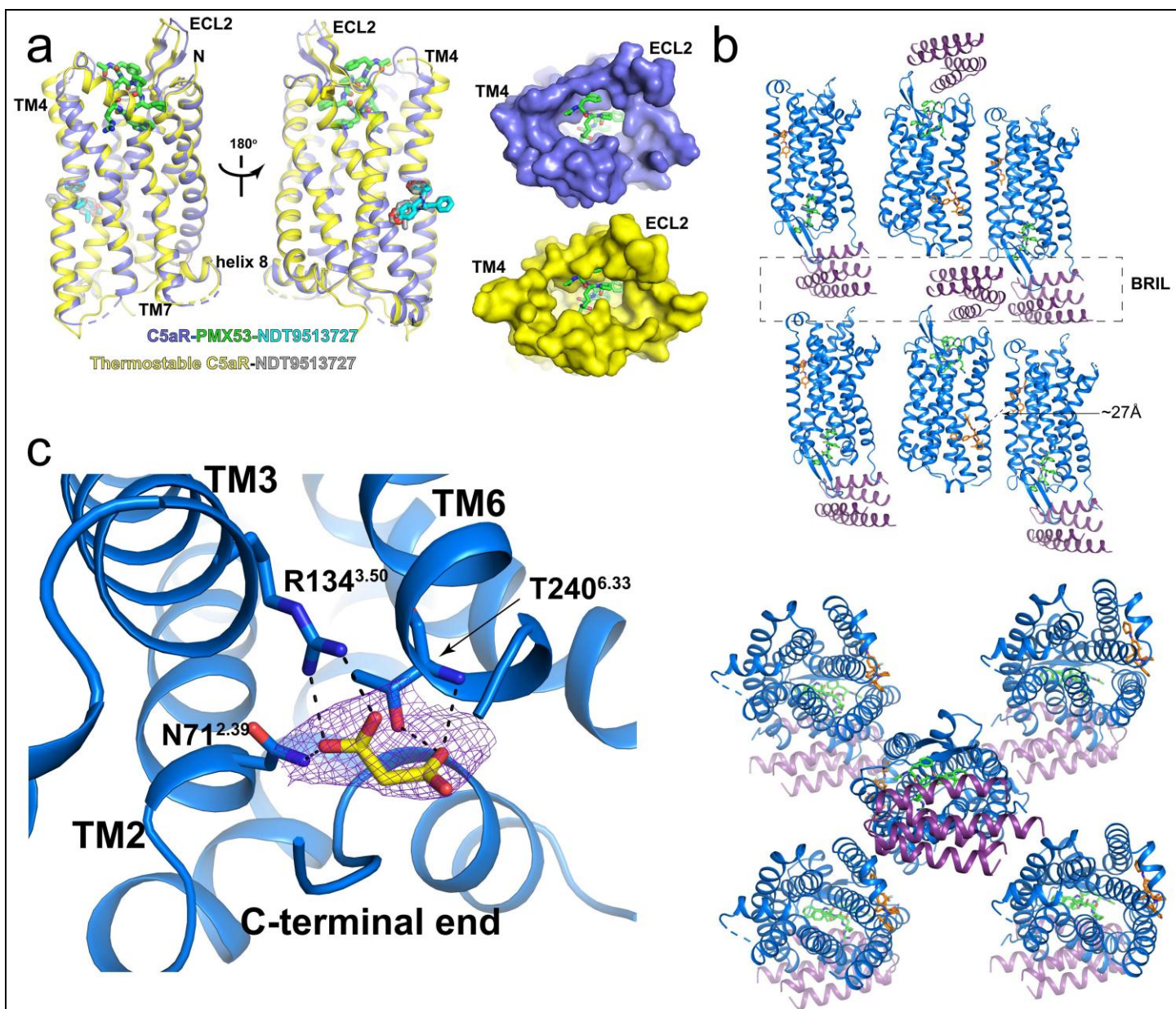
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Supplementary Figure 1

Chemically diverse non-peptide C5aR antagonists and C5aR crystallization.

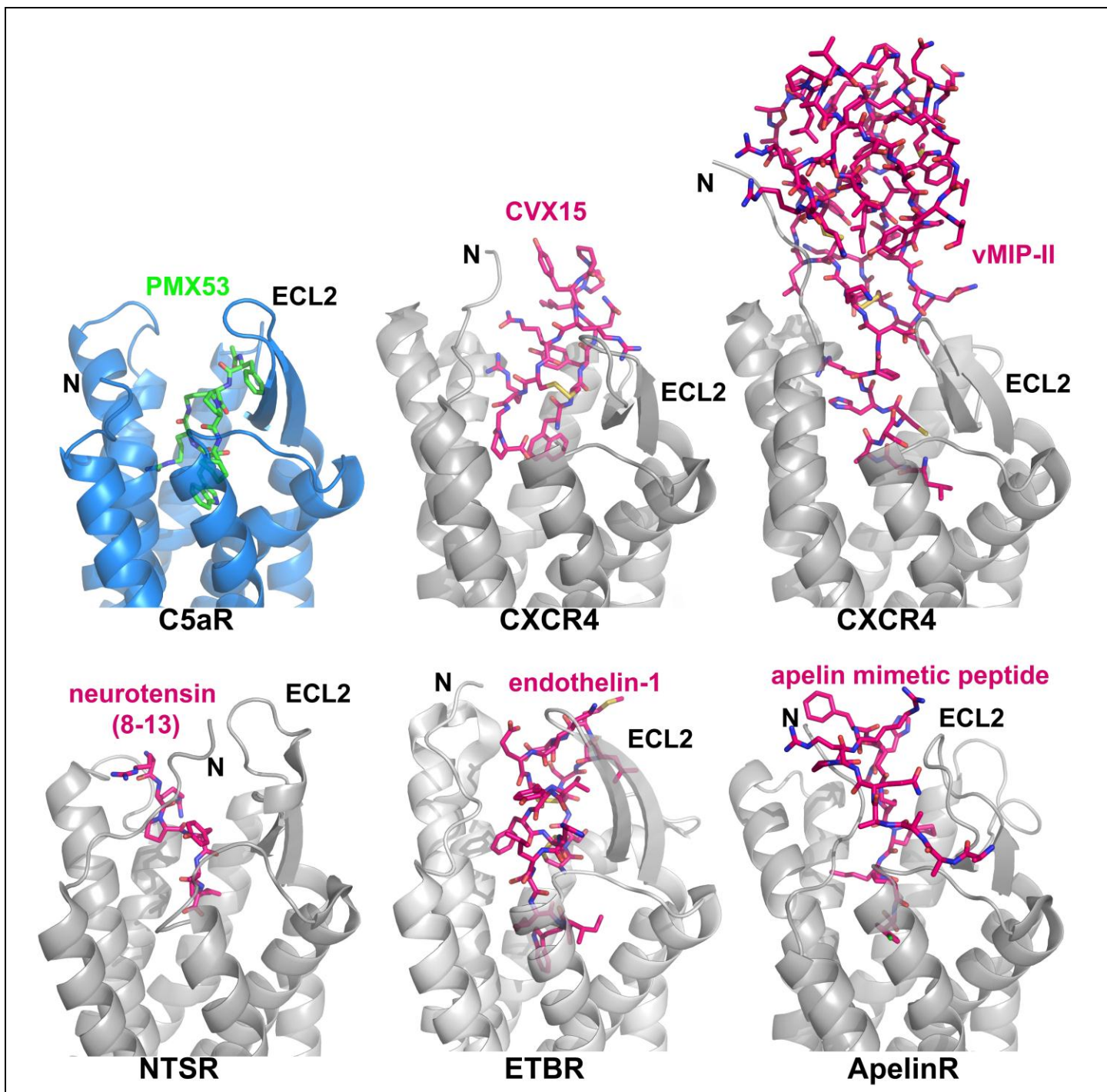
(a) Chemical structures of four C5aR non-peptide antagonists: avacopan (CCX168), NDT9513727, W54011 and CP-447697. The central tertiary nitrogen group is circled. **(b)** To aid the crystallization, we engineered human wtC5aR by replacing the N-terminal residues 1-29 with cytochrome b₂₆₂ RIL (BRIL). We also introduced a TEV protease site between 331 and 332 and cut off the C-terminal segment 332-350 before crystallization by TEV protease. The construct is referred as C5aR-BRIL. Crystals of C5aR with PMX53 and avacopan in lipidic cubic phase are shown in the upper right corner. **(c)** The $F_o - F_c$ omit electron-density maps for PMX53 (green), NDT9513727 (cyan) and avacopan (orange) contoured at 2σ are shown as purple mesh.



Supplementary Figure 2

Structural comparison with thermostable C5aR, crystal packing and ligand binding at the intracellular surface.

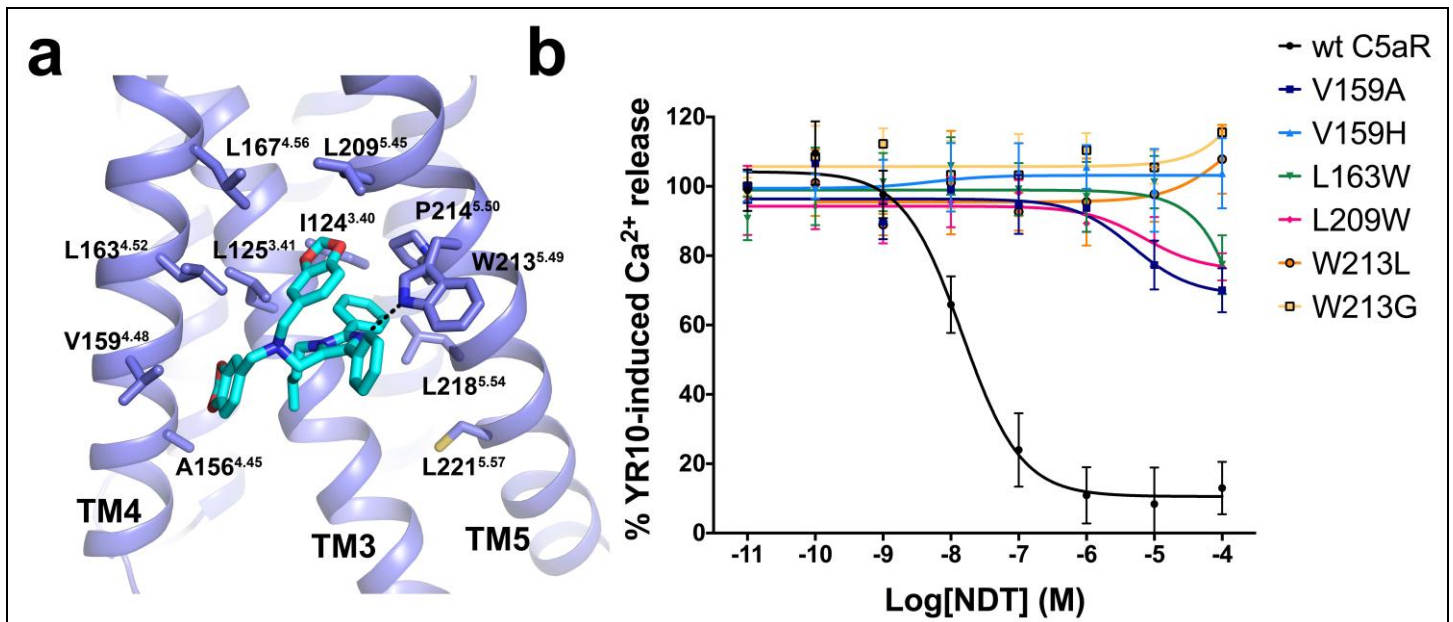
(a) Left: Superimposition of the C5aR-PMX53-NDT9513727 structure with the structure of thermostable C5aR-NDT9513727. Right: The surfaces of these two structures viewed from the extracellular side. The large differences are observed for ECL2 and the extracellular end of TM4. The unique conformation of helix 8 is observed in both structures. **(b)** Top: Lattice packing in C5aR-PMX53-avacopan crystals. Receptor is shown in blue and BRIL is shown in purple. PMX53 and avacopan are shown in green and orange respectively. In any two C5aR molecules that form crystal-packing interactions, the allosteric sites are far away from each other with a distance of ~27Å (measured between two W255 residues in the sites). Bottom: In each layer of the crystal lattice, one receptor packs against the surrounding receptor molecules in an anti-parallel manner, excluding the possibility of forming physiological C5aR dimers in the crystals. **(c)** The malonate molecule (yellow) bound at the cytoplasmic surface of C5aR-PMX53-avacopan (blue). An $F_o - F_c$ omit electron-density map for the malonate contoured at 2σ is shown as purple mesh. Residues N71^{2.39}, R134^{3.50} and T240^{6.33} that form hydrogen-bonding interactions with the malonate are shown as sticks.



Supplementary Figure 3

Comparison of C5aR structure to the structures of other GPCRs with peptide ligands.

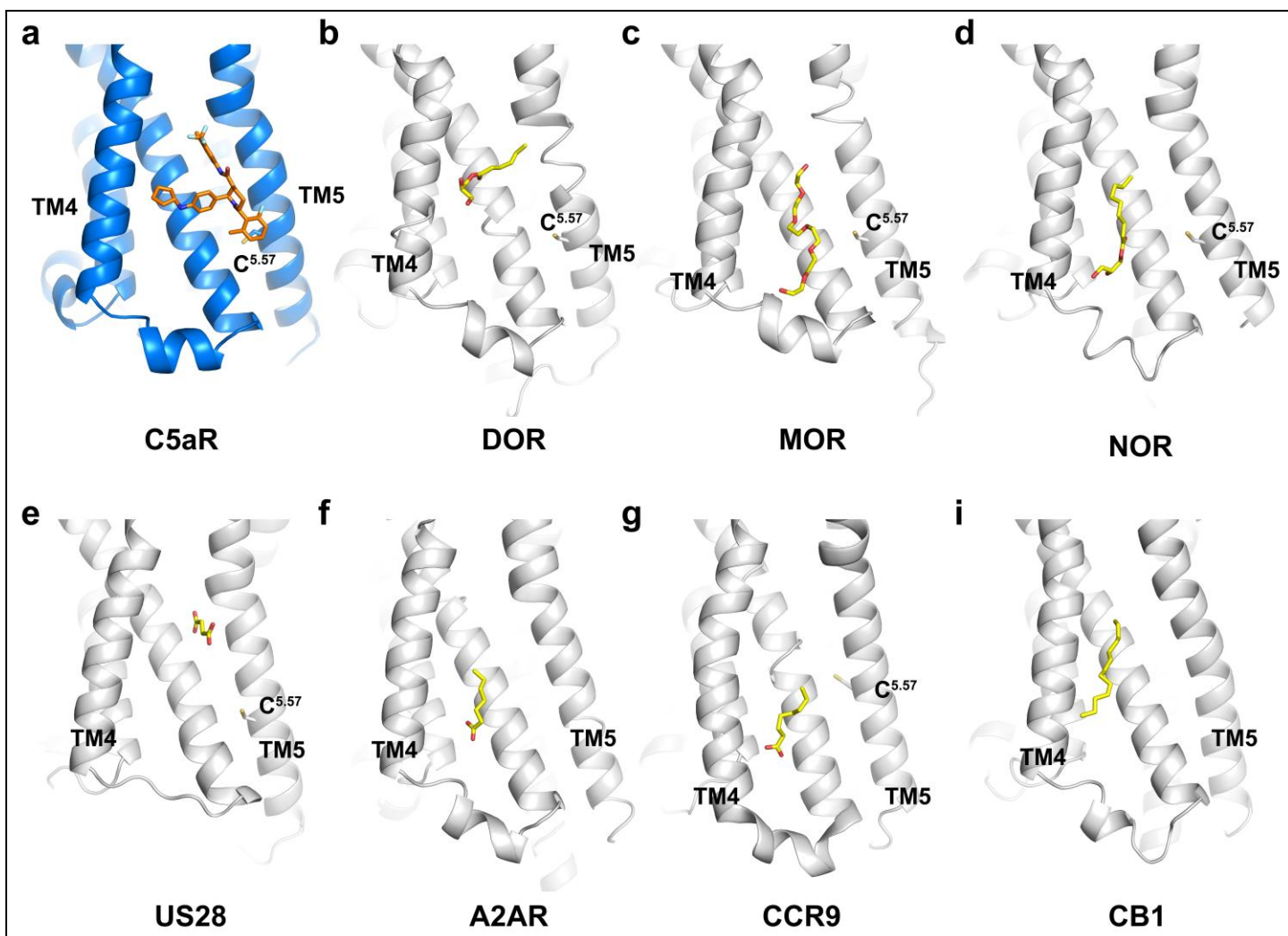
Crystal structures of GPCRs bound to the peptide ligands: C5aR with PMX53; CXCR4 with CVX15 (PDB ID 3OE0); CXCR4 with mVIP-II (PDB ID 4RWS); neurotensin receptor 1 (NTSR) with neurotensin (8-13) (PDB ID 4GRV); endothelin receptor type-B (ETBR) with endothelin-1 (PDB ID 5GLH); apelin receptor (Apelin R) with an apelin mimetic peptide (PDB ID 5VBL). C5aR is colored in blue and other GPCRs are colored in grey. PMX53 is colored in green and other peptide ligands are colored in red. PMX53 interacts with ECL2 but not N-terminus of C5aR, while other peptide ligands all interact with the N-termini and ECL2s of their receptors.



Supplementary Figure 4

Allosteric site for NDT9513727.

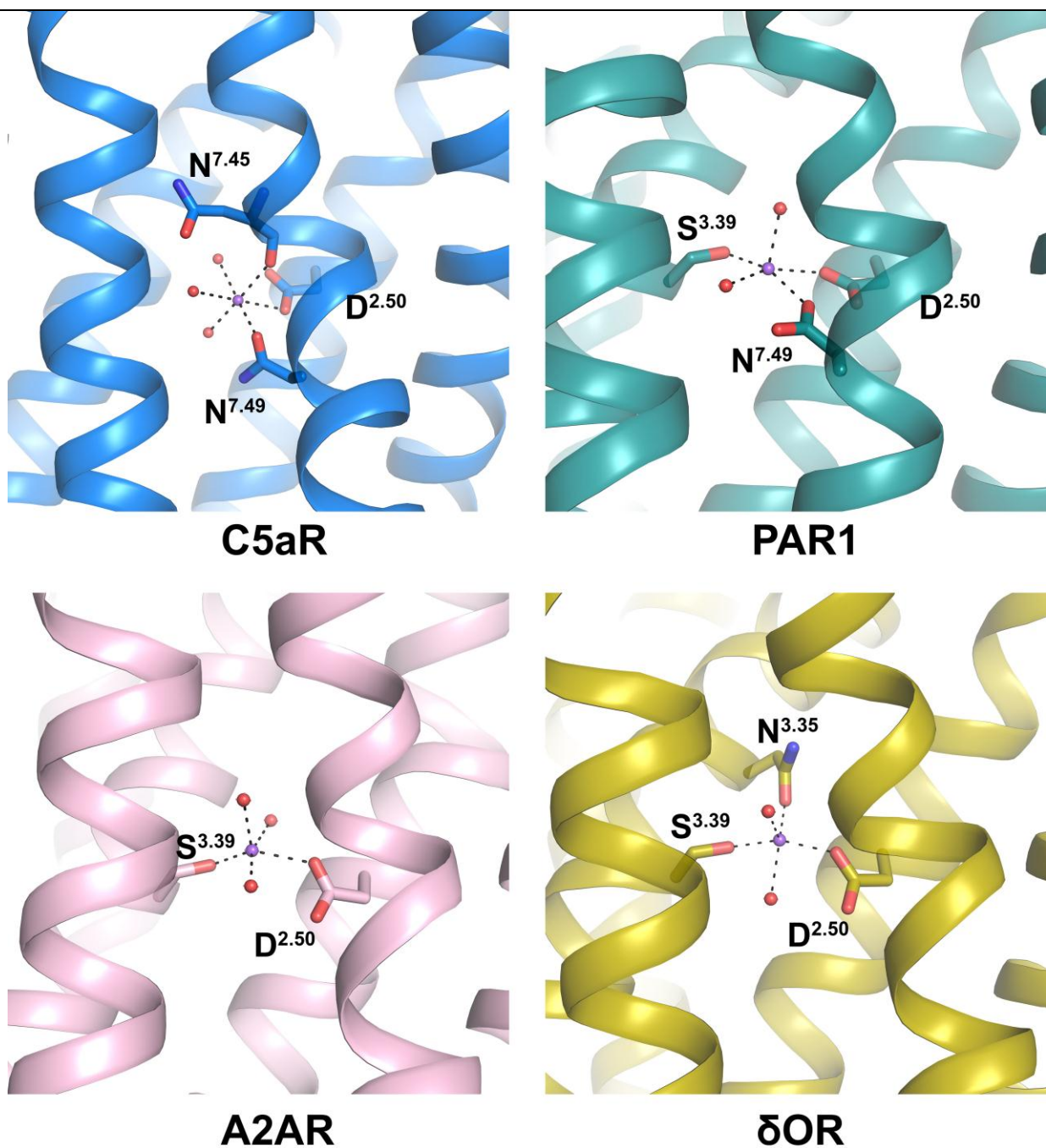
(a) Allosteric binding site for NDT9513727 (cyan) in our structure. All the residues that interact with NDT9513727 are in the same conformation as observed in the structure of thermostable C5aR. However, the residue A156^{4.45} was mutated to leucine in the thermostable C5aR, which may provide additional hydrophobic interactions for the binding of NDT9513727. **(b)** The effects of 6 mutations V159A, V159H, L163W, L209W, W213L and W213G on the antagonistic action of NDT9513727 measured by an agonist YR10-induced Ca^{2+} release assay. The cellular calcium level was determined by measuring the fluorescence of fluo-4 in HEK293T cells stably expressing wtC5aR. 10 μM YR10 was used to stimulate the receptor. Data points are presented as the mean values $\pm$ SEM, $n=3$.



Supplementary Figure 5

Conservation of the allosteric site in other GPCRs.

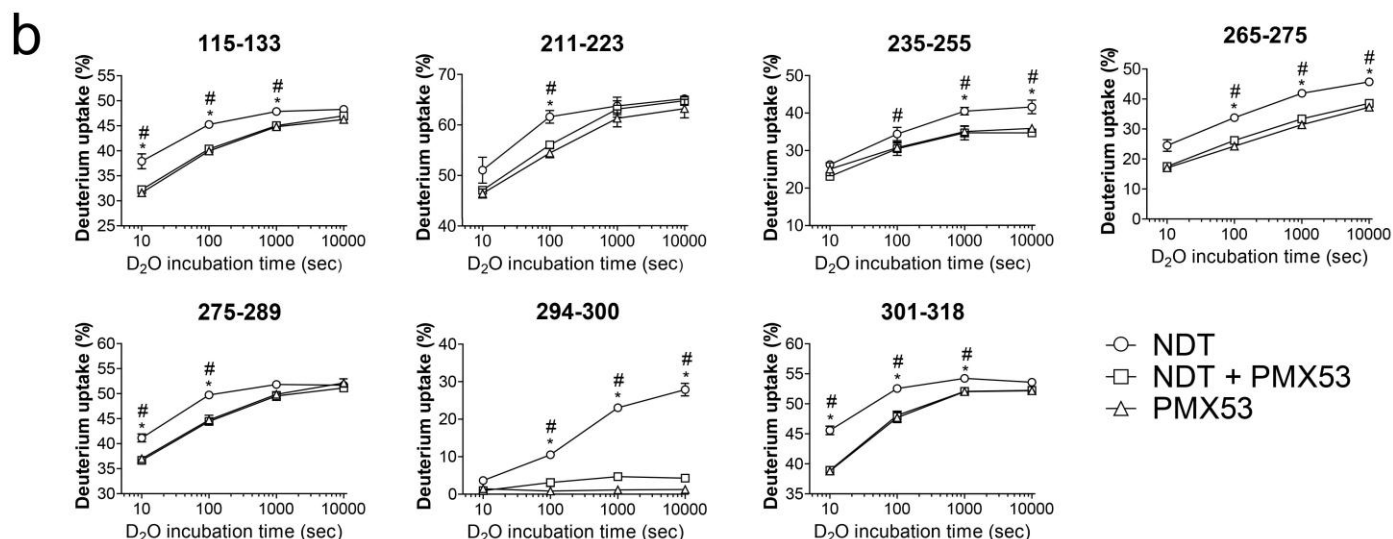
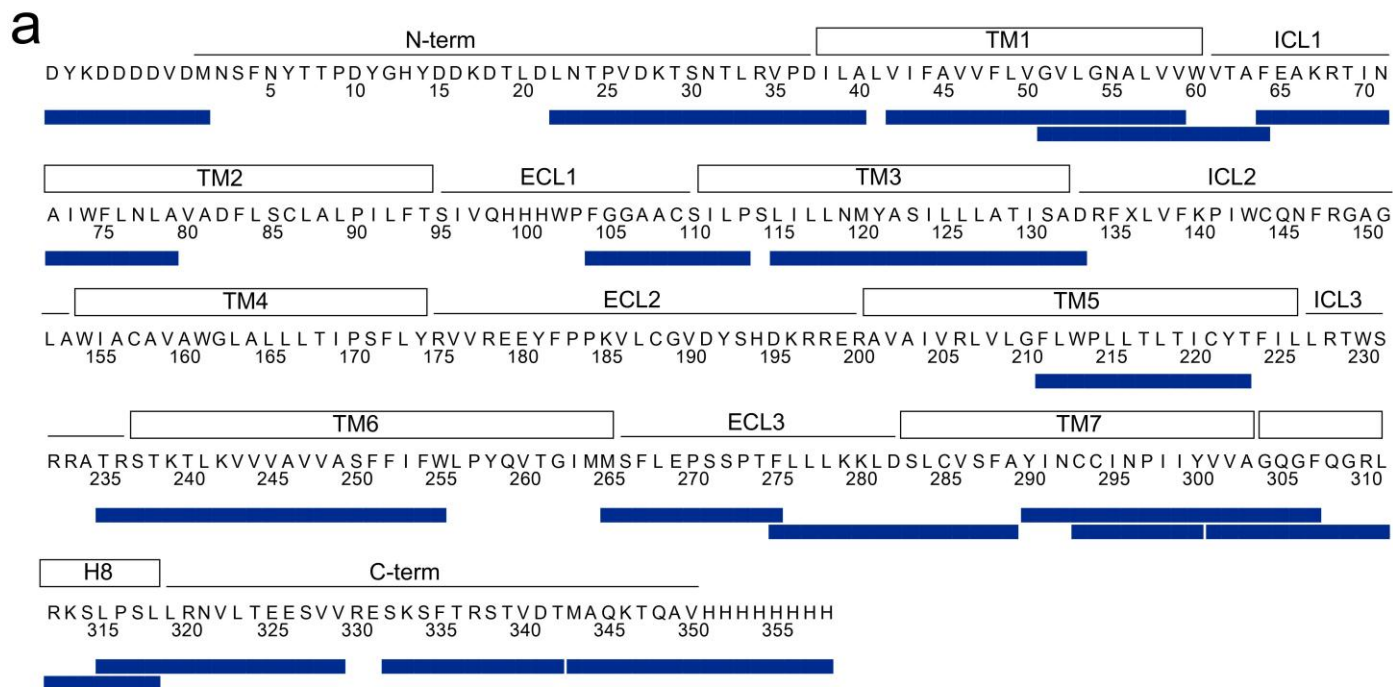
Crystal structures of GPCRs with small compounds bound to the similar site as identified in C5aR and GPR40 for the allosteric modulators (PDB IDs shown in the brackets): **(a)** C5aR with avacopan; **(b)** δ -opioid receptor with 1-oleoyl-R-glycerol (DOR, 4N6H); **(c)** μ -opioid receptor with a PEG400 molecule (MOR, 5C1M); **(d)** N/OFQ opioid receptor with 1-oleoyl-R-glycerol (NOR, 4EA3); **(e)** viral GPCR US28 with a succinic acid molecule (US28, 4XT1); **(f)** A2a adenosine receptor with an oleic acid molecule (A2AR, 5K2D); **(g)** chemokine receptor CCR9 with an oleic acid molecule (CCR9, 5LWE); **(i)** cannabinoid receptor CB1 with an oleic acid molecule (CB1, 5TGZ). The cysteine residue at 5.57 position C^{5.57} is conserved in all of these GPCRs except A2AR and CB1.



Supplementary Figure 6

Different sodium coordination sites in four GPCRs.

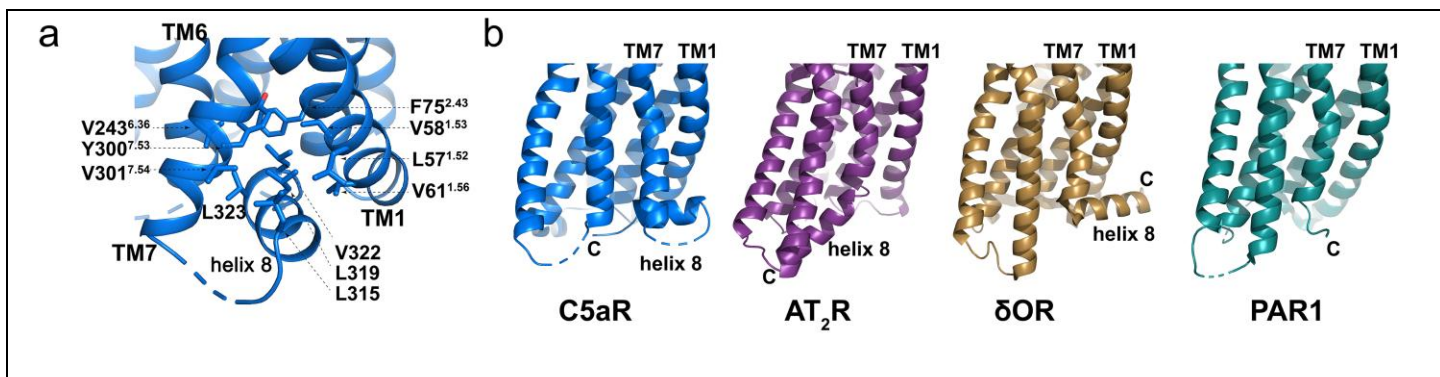
The sodium coordination sites in four GPCRs: C5aR, protease-activated receptor 1 (PAR1, PDB ID 3VW7), A_{2A} adenosine receptor (A2AR, PDB ID 4EIY) and δ -opioid receptor (δ OR, PDB ID 4N6H) are shown. The sodium ion (shown as a purple sphere) interacts with different sets of residues at the cytoplasmic side of these GPCRs and water molecules (shown as red spheres).



Supplementary Figure 7

HDX-MS sequence coverage map and deuterium uptake plots.

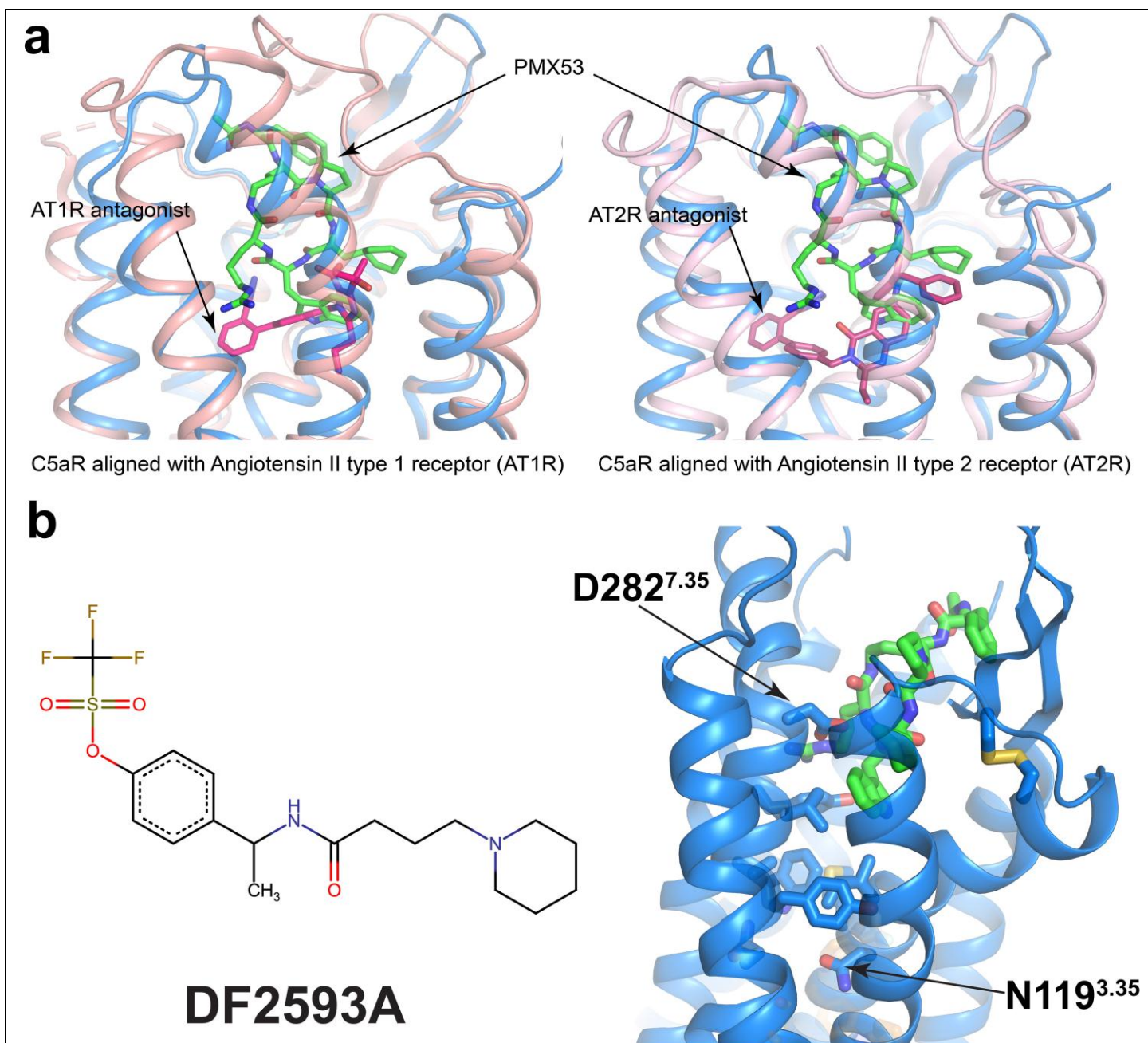
(a) Bars indicated the peptic peptides in different regions of C5aR for HDX analysis. **(b)** Deuterium uptake plots of those regions in C5aR are represented. The results of three samples, C5aR bound to NDT9513727 (NDT), C5aR bound to PMX53 (PMX53) and C5aR bound to both ligands (NDT+PMX53), are compared. There is no significant difference between the results of PMX53 and NDT+PMX53. Results are means $\pm$ S.E. for at least three independent experiments. * $p < 0.05$: NDT-bound state vs. PMX53-bound state, # $p < 0.05$: NDT-bound state vs. NDT and PMX53-bound state.



Supplementary Figure 8

Unique helix 8 in C5aR.

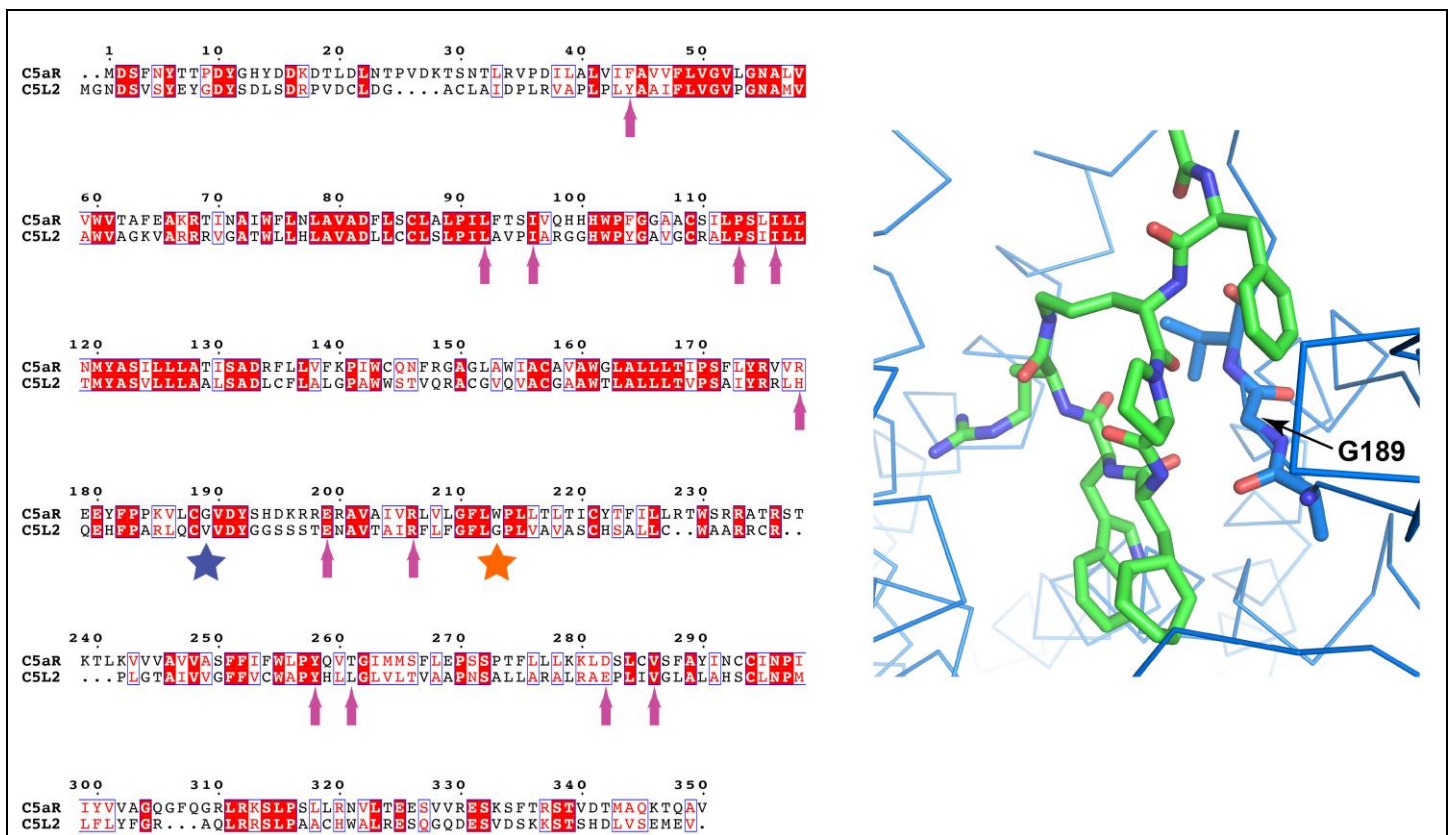
(a) Hydrophobic interactions that stabilize the conformation of helix 8. In both C5aR structures, residues L315, L319, V322 and L323 on helix 8 form extensive hydrophobic interactions with the surrounding residues L57^{1.52}, V58^{1.53}, V61^{1.56}, F75^{2.43}, V243^{6.36}, Y300^{7.53} and V301^{7.54} from TM1, TM2, TM6 and TM7, respectively. **(b)** The conformation of helix 8 in four GPCRs: C5aR, angiotensin II receptor 2 (AT₂R, PDB ID 5UNF), δ-opioid receptor (δOR, PDB ID 4N6H) and protease-activated receptor 1 (PAR1, PDB ID 3VW7). In nearly all solved structures of rhodopsin-like GPCRs, either there is no well-folded helix 8 as seen in PAR1 or the helix 8 adopts a similar conformation as that in δOR with the C-terminus extending away from the helical bundle. In AT₂R, the helix 8 adopts a unique conformation to interact with the cytoplasmic surface, which can sterically block the binding of signaling effectors and result in the lacking of signaling responses for AT₂R. In C5aR, the helix 8 is in a reversed orientation compared to that in δOR and inserts between TM1 and TM7. Such conformation has not been observed in other GPCRs so far.



Supplementary Figure 9

Extracellular sites for potential non-peptide C5aR antagonist.

(a) Alignment of C5aR structure with the structures of two angiotensin II receptors AT1R (pink, PDB ID 4ZUD) and AT2R (light pink, PDB ID 5UNF). PMX53 is colored in green and the antagonists of AT1R and AT2R are colored in red. The binding sites for angiotensin antagonists overlap with the bottom region of the PMX53 binding site, suggesting the feasibility of developing non-peptide C5aR antagonists that target this region. **(b)** Extracellular allosteric antagonist DF2593A. Two residues, D282^{7.35} and N119^{3.35}, were proposed to form direct polar interactions with DF2593A. However, there is not enough space in our structures around these two residues to accommodate DF2593A, even with the absence of PMX53 (green sticks). It is likely that DF2593A binding is associated with large conformational changes of the extracellular region, resulting in a distinct binding pocket compared to PMX53.



Supplementary Figure 10

Sequence alignment of C5aR and C5L2.

The purple arrows point to the residues in C5aR that are involved in the interactions with PMX53, including F44, L92, I96, P113, I116, R178, E199, R206, Y178, T261, D282 and V286. Except for F44, R178 and T261, all other residues are conserved in C5L2. However, the substitution of G189 in C5aR, as indicated by the blue star, by V187 in C5L2 may introduce steric clash with PMX53. The right panel shows the position of G189 relative to PMX53 (green sticks) in the structure of C5aR (blue). The distance between G189 and the Pro residue in PMX53 is too short to tolerate any substitution of G189. The orange star indicates the residue W213 in C5aR, which is critical for the binding of non-peptide antagonists. It is substituted by G211 in C5L2.