Electronic Supplementary Material (ESI) for Chemical Science.

Exploring a new ligand binding site of G protein-coupled receptors

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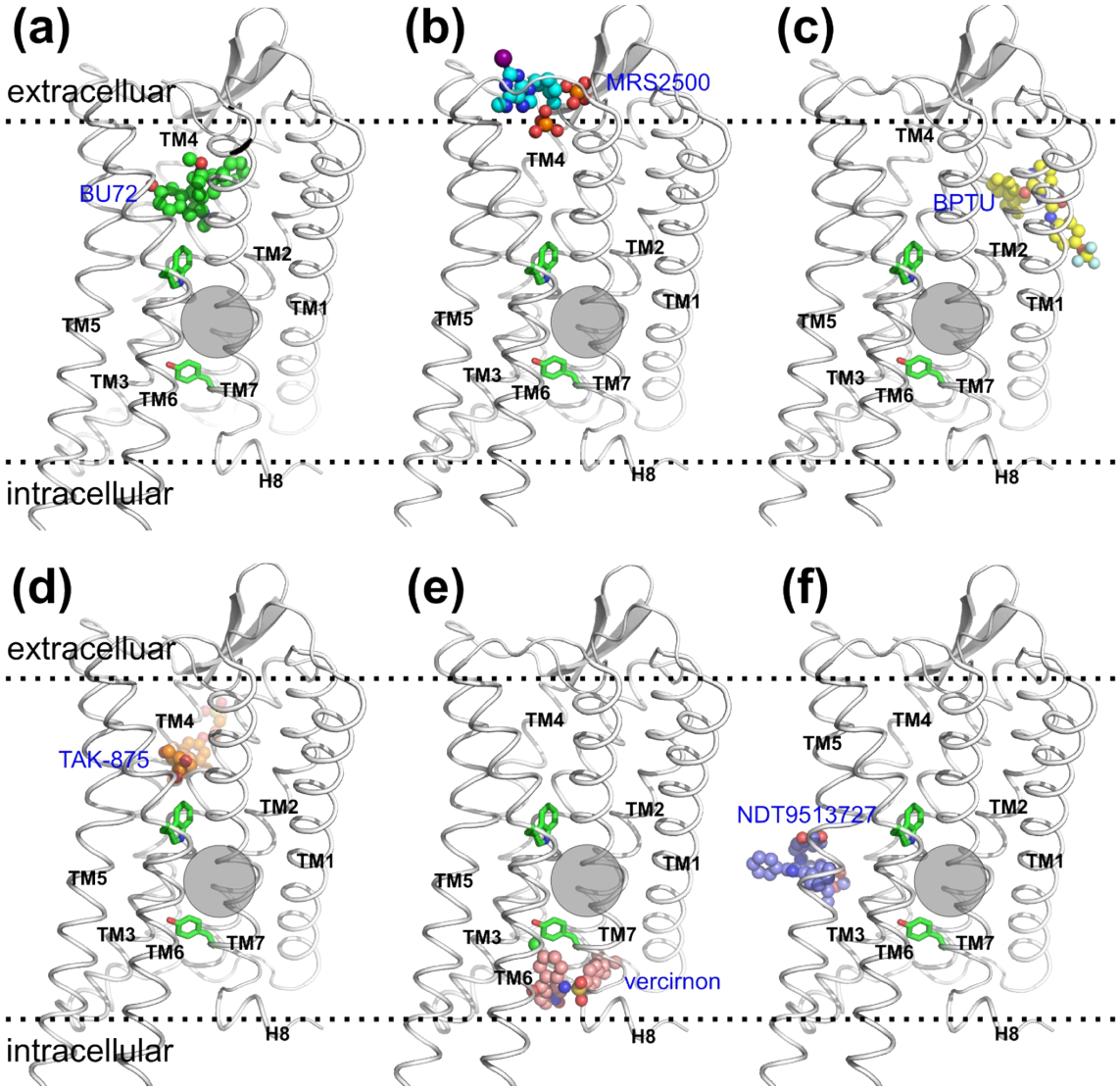


Fig. S1 The locations of ligands observed in crystal structures for (a) μOR in complex with BU72 (pdb: 5C1M), (b) P2Y1R in complex with MRS2500 (pdb: 4XNW), (c) P2Y1R in complex with BPTU (pdb: 4XNV), (d) GPR40 in complex with TAK-875 (pdb: 4PHU), (e) CCR9 in complex with vercirnon (pdb: 5LWE) and (f) C5aR in complex with NDT9513727 (pdb: 5O9H). To facilitate the comparisons, all receptors are represented by μOR (pdb: 5C1M) after superimposing their positions. Green stick in TM6: the highly conserved residue $W^{6.48}$. Green stick in TM7: the highly conserved residue $Y^{7.53}$. Grey circle: the new ligand binding site discovered in this work.

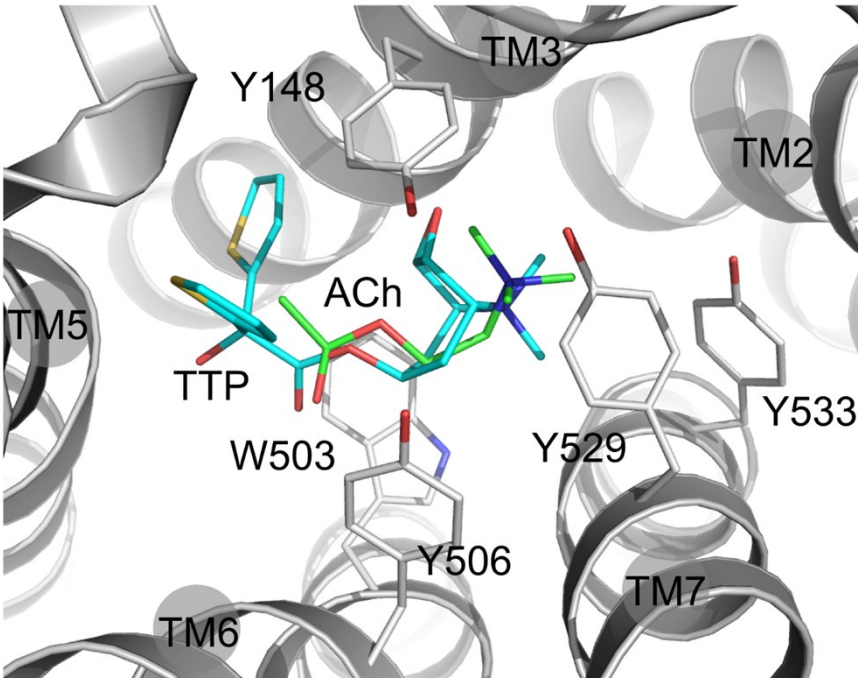


Fig. S2 The superimposed representative position of ACh in the M3 receptor at 100 ns MD simulation with antagonist TTP observed in crystal structure (pdb: 4DAJ). Cyan stick: TTP molecule; Green stick: ACh molecule; white stick: sidechains of M3 receptor.

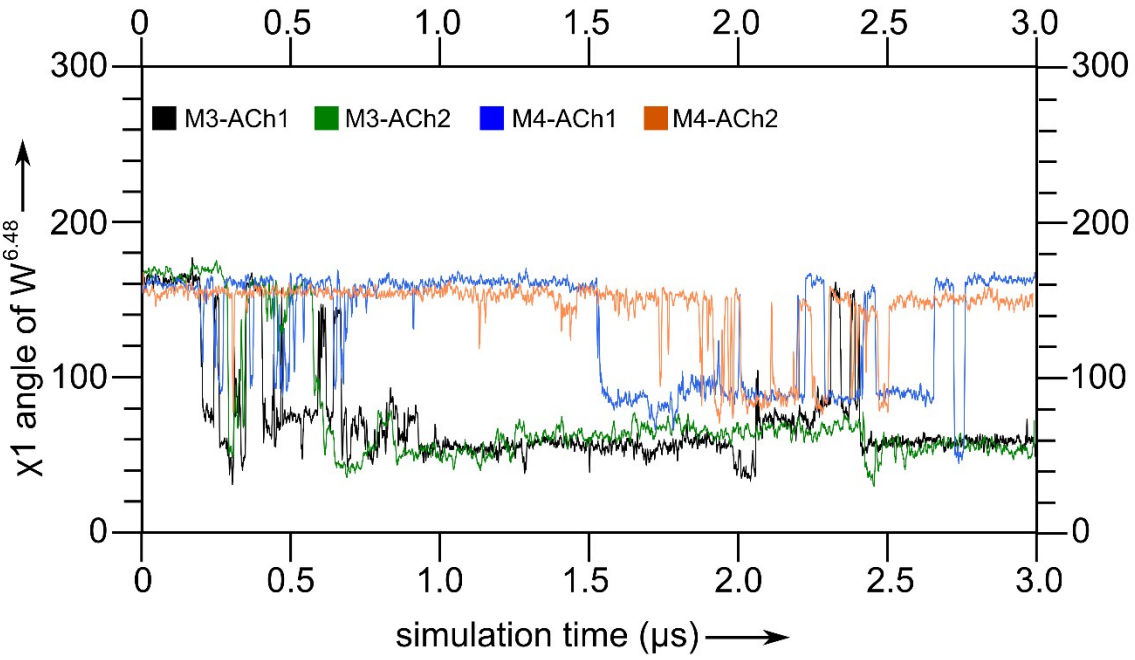


Fig. S3 The χ1 angle switching of the highly conserved $W^{6.48}$ for M3 (black and green) and M4 receptors (blue and orange) during the long-time scale MD simulations.

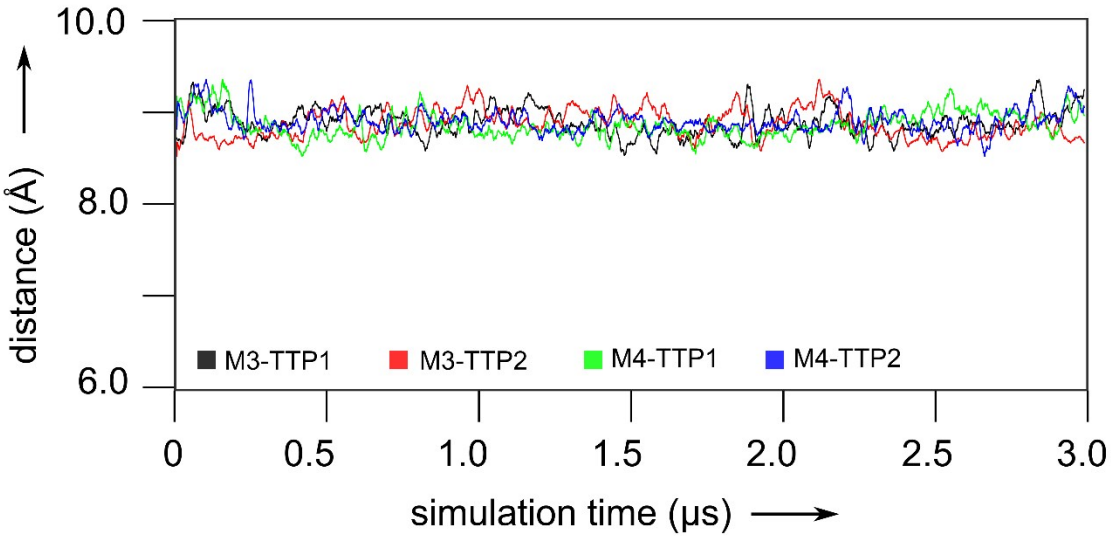


Fig. S4 Distances between mass centers of the bound antagonist TTP and a carboxyl group of $D^{2.50}$ in M3 and M4 receptors. Black and red: two independent MD simulations of M3-TTP. Green and blue: two independent MD simulations of M4-TTP.

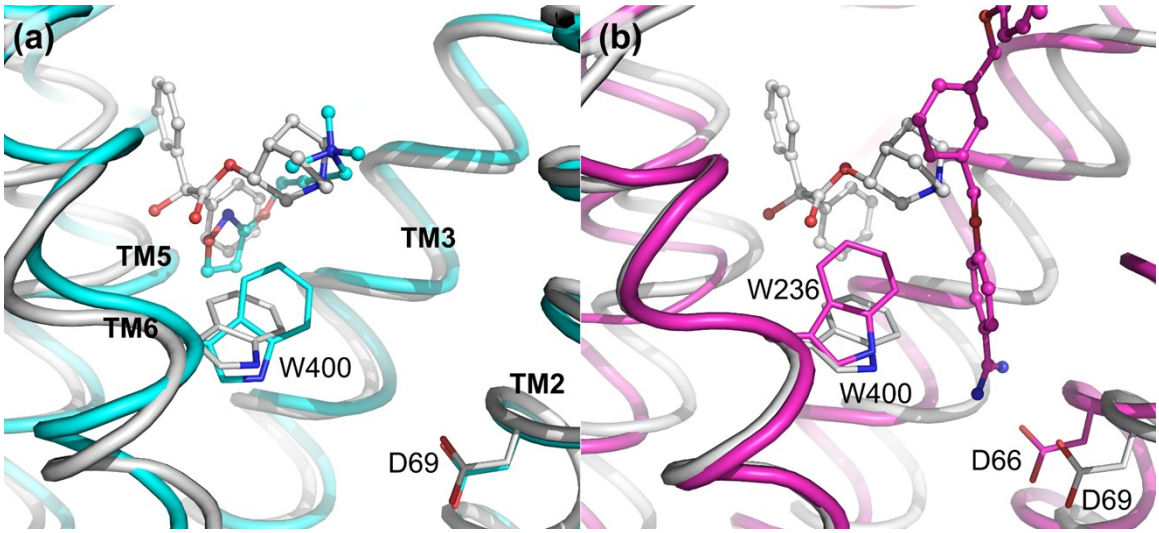


Fig. S5 The molecular switching of $W^{6.48}$ observed in crystal structures. (**a**) Molecular switching of $W^{6.48}$ observed in M2 receptors. White: antagonist bound M2 receptor (pdb: 3UON). Cyan: agonist bound M2 receptor (pdb: 4MQS). (**b**) Molecular switching of $W^{6.48}$ observed in BIIL260-bound LTB4 receptor. White: antagonist bound M2 receptor (pdb: 3UON). Purple: inverse agonist BIIL260-bound receptor (pdb: 5X33). The benzamidine moiety of BIIL260 occupies newly discovered ligand binding site in this work, binding to $D66^{2.50}$ through an ionic interaction.

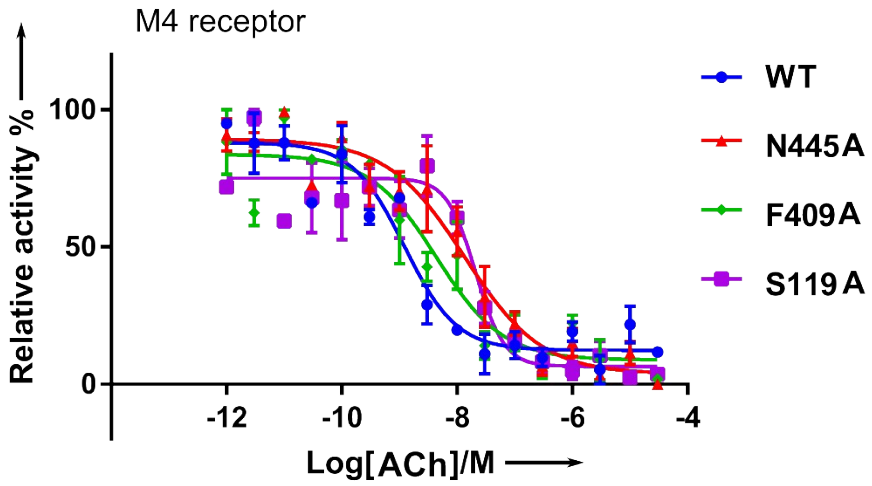


Fig. S6 The Gi protein signaling assay of M4 variants as a function of ACh concentration.

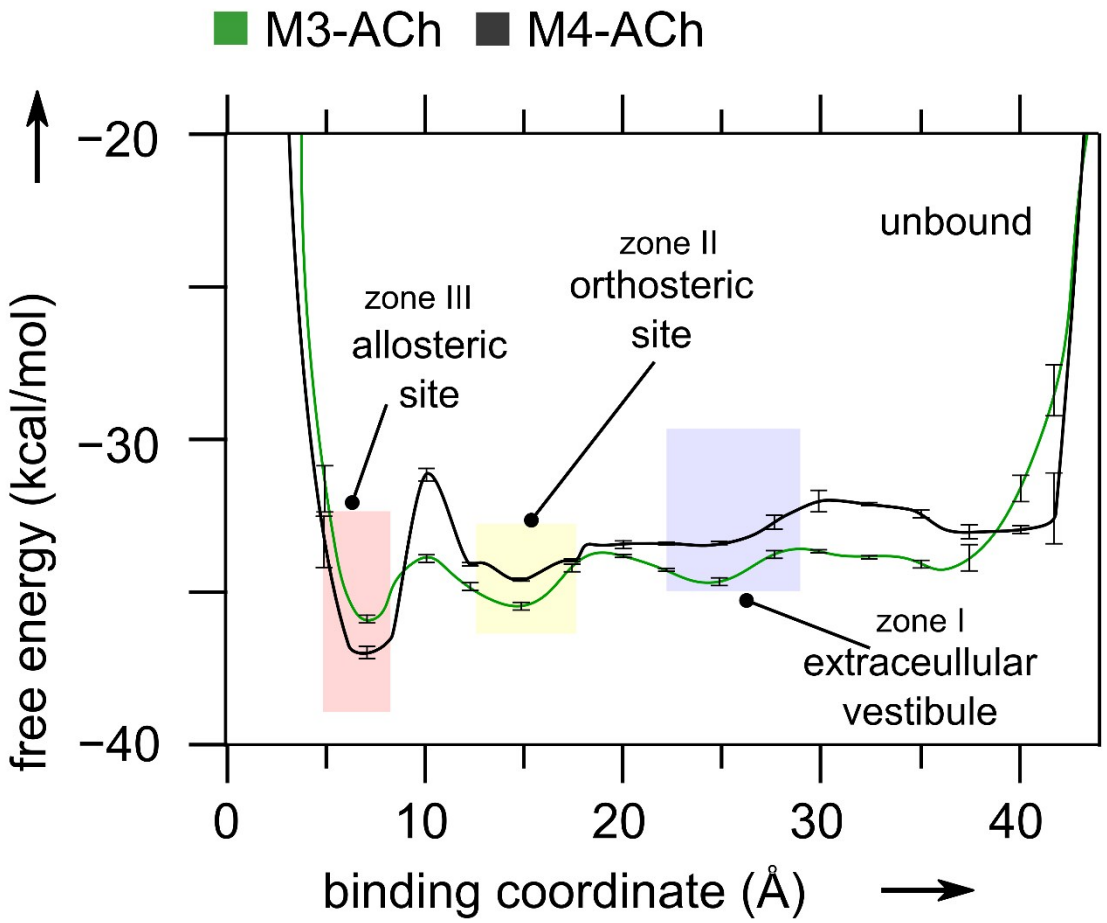


Fig. S7 Free energy profiles of the binding process of ACh to M3 (green), and M4 (black) receptors. The binding coordinate is defined as the distance between the quaternary nitrogen of ACh and the oxygen in the side chain of $D^{2.50}$.

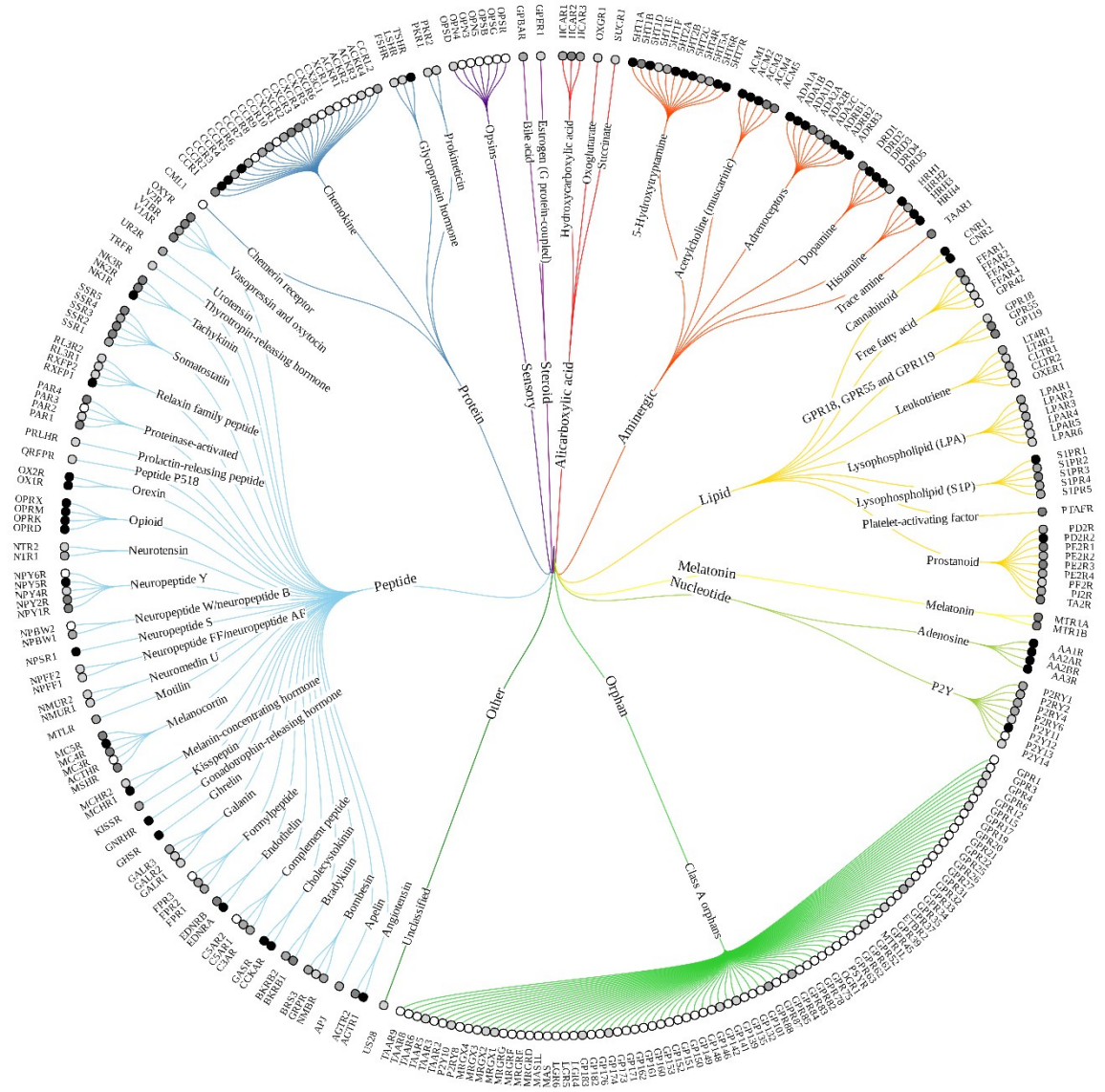
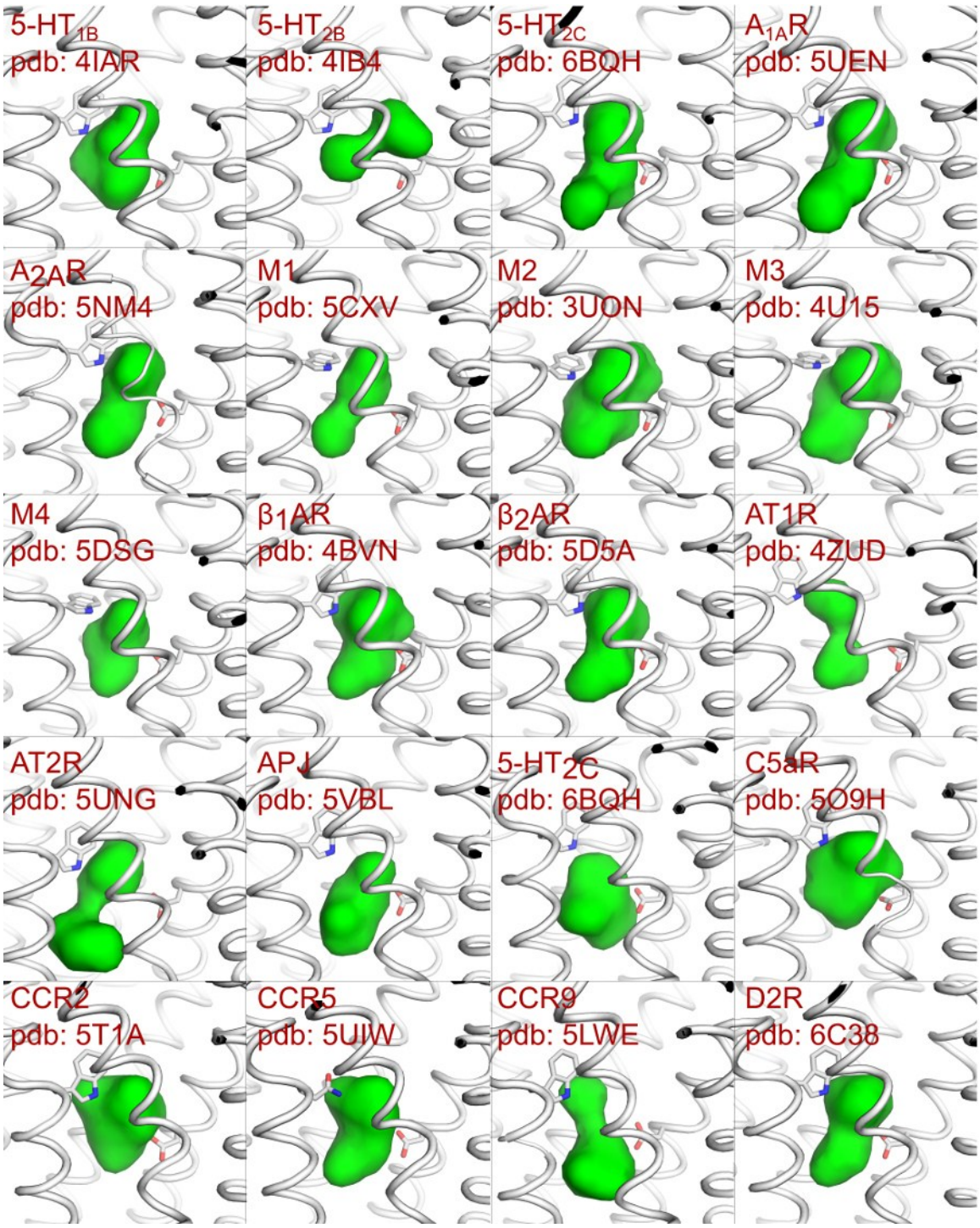


Fig. S8 The family A GPCRs. White circles: GPCRs without crystal structures. Gray and black circles: GPCRs with crystal structures. The number of known ligands is correlated to the color depth of the circles. More than 200 crystal structures of ligand-bound GPCRs were inspected in this work. The Fig. was generated from GPCRDB web service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5-HT2B
pdb: 4IB4
5-HT2C
pdb: 6BQH
A1AR
pdb: 5UEN
A2AR
pdb: 5NM4
M1
pdb: 5CXV
M2
pdb: 3UON
M3
pdb: 4U15
M4
pdb: 5DSG
β1AR
pdb: 4BVN
β2AR
pdb: 5D5A
AT1R
pdb: 4ZUD
AT2R
pdb: 5UNG
APJ
pdb: 5VBL
5-HT2C
pdb: 6BQH
C5aR
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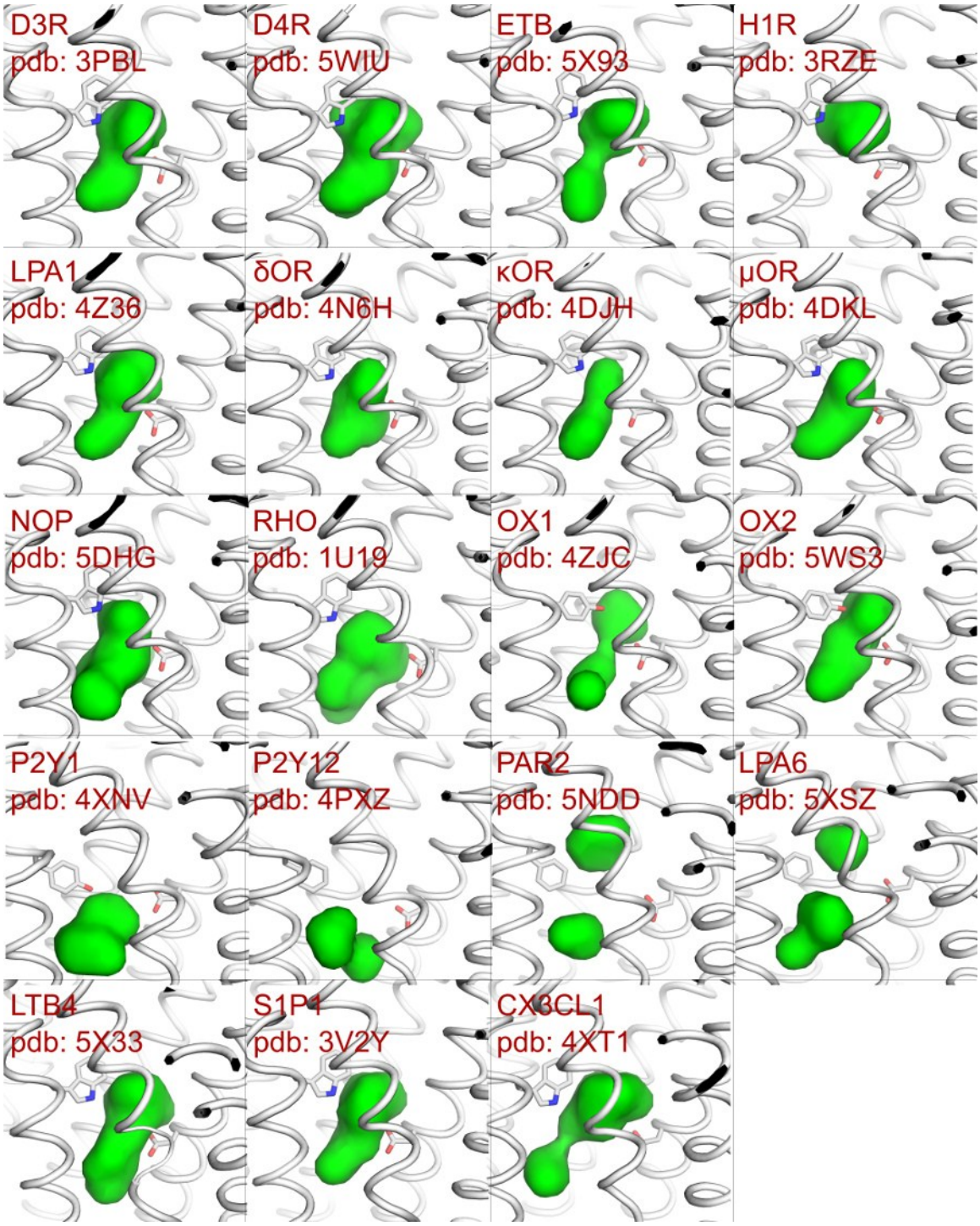

Fig. S9 The volume shapes of newly discovered ligand binding site for all currently known 39 unique crystal structures of family A GPCRs.

Movie S1. MD simulation of M3-ACh; ACh trajectory towards allosteric binding site of M3 receptor.

Movie S2. MD simulation of M4-ACh; ACh trajectory towards allosteric binding site of M4 receptor.