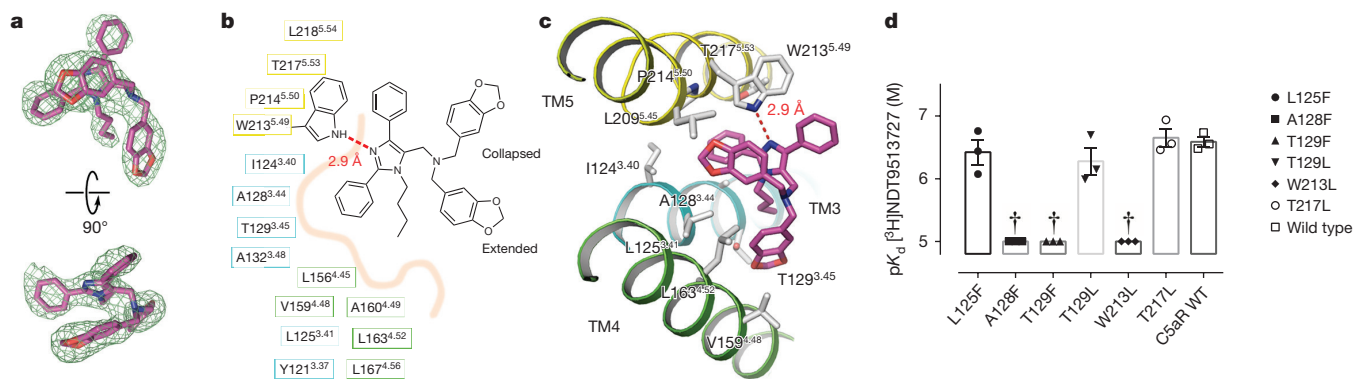


**Figure 1 | Ribbon and schematic representation of the C5aR1 crystal structure asymmetric unit.** **a**, Ribbon diagram of the C5aR1 structure, viewed parallel to the membrane. NDT9513727 in sphere representation with carbon, nitrogen and oxygen atoms coloured magenta, blue and red, respectively. Transmembrane helices, loops and approximate membrane boundaries are marked. **b**, Schematic cylinder representation of C5aR1

monomer in rainbow colouration (N terminus, blue; C terminus, red); approximate positions of other solved allosteric sites are labelled. **c**, As in **a**, rotated 90° to view from the cytoplasmic space. **d**, As in **c**, in surface representation to examine the shape complementarity of the ligand-binding site.

the mutation of Ala128<sup>3.44</sup> and Thr129<sup>3.45</sup> (two residues highly conserved across C5aR1 orthologues that sit at the hydrophobic core of the binding pocket) to phenylalanine severely affected NDT9513727 binding to C5aR1 (Fig. 2d and Extended Data Fig. 6). Notably, mutation

of Thr129<sup>3.45</sup> to leucine had no effect on NDT9513727 binding, suggesting that the bulky phenylalanine substitutions disrupt the shape complementarity of the hydrophobic pocket to the ligand, and that more conservative mutations can be tolerated across this region. Finally,



**Figure 2 | The NDT9513727 ligand-binding site and mutational analysis.** **a**,  $F_o - F_c$  OMIT density contoured at  $2.5\sigma$ , calculated before ligand inclusion. NDT9513727 shown as sticks and coloured as in Fig. 1. **b**, Schematic of ligand interactions in the extra-helical NDT9513727-binding site. Boxed colour scheme follows rainbow colouration in Fig. 1b. Hydrogen bonds depicted as dashed red lines

distances in Å. **c**, View down the side of the receptor showing specific interactions of NDT9513727. **d**, [ $^3$ H]NDT9513727 binding data for C5aR1 mutants across the allosteric site. Data are mean  $\pm$  s.e.m. and representative of three biologically independent experiments performed in duplicate. Dagger denotes ambiguous values due to near complete loss of specific binding.