Supplementary information, Fig. S7

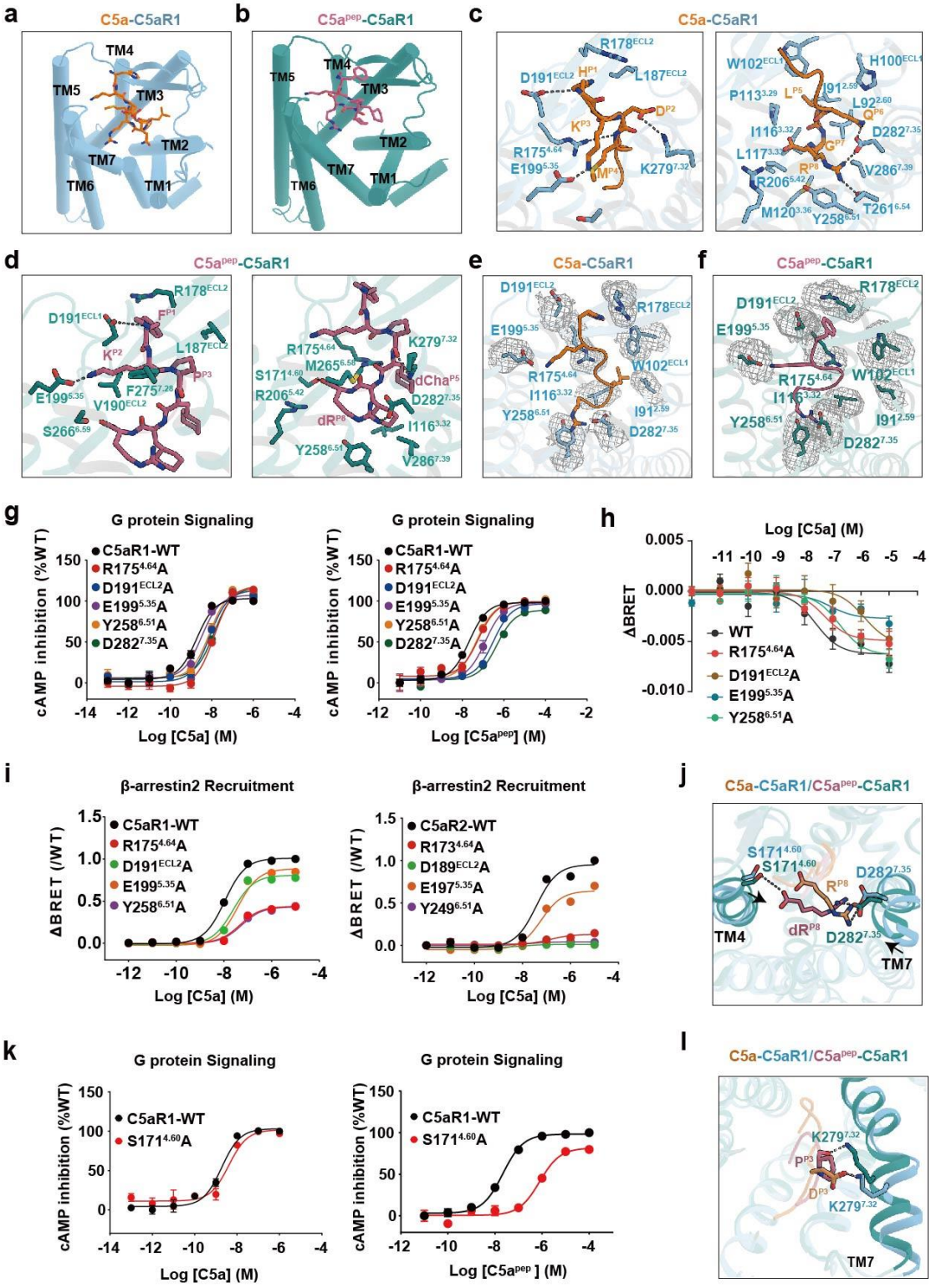

Fig. S7. Molecular details between C5aR1 and C5a or C5a^{pep}.

a-b, The C-terminus of C5a (**a**) and C5a^{pep} (**b**) penetrate deeply into the TM helical bundle of C5aR1 in a similar binding pose. The C-terminus of C5a and C5a^{pep} are shown as sticks, C5aR1 is shown as cartoon with cylindrical helices.

c-d, Detailed interaction of C5aR1 with C5a (**c**) or $C5a^{pep}$ (**d**). The interactions between P1-P4 of C5a or $C5a^{pep}$ and C5aR1 are shown in the left panel, that between P5-P8 of C5a or $C5a^{pep}$ and C5aR1 are shown in the right panel. Polar interactions are highlighted as black dashed lines.

e-f, High-quality electron density facilitated unambiguous assignment of key residues in the binding pocket of C5a-bound (**e**) and $C5a^{pep}$-bound (**f**) C5aR1.

g, Representative curve for effects of the $R175^{4.64}A$, $D191^{ECL2}A$, $E199^{5.35}A$, $Y258^{6.51}A$ and $D282^{7.35}A$ mutations in C5aR1 on C5a (left panel) and $C5a^{pep}$ (right panel) induced G protein signaling examined by cAMP inhibition assay. Data are presented as the mean ± SEM of three independent experiments performed in triplicate.

h, The representative dose response curves of the C5a-induced BRET ratio in HEK293 cells overexpressing FlAsH-BRET S2 wild type and corresponding mutants. Values are mean ± SEM from three independent experiments performed in triplicate.

i, Representative curve for effects of the $R175^{4.64}A$, $D191^{ECL2}A$, $E199^{5.35}A$ and $Y258^{6.51}A$ mutations in C5aR1 (left panel) and the $R173^{4.64}A$, $D189^{ECL2}A$, $E197^{5.35}A$ and $Y249^{6.51}A$ C5aR2 (right panel) on C5a induced β-arrestin2 recruitment examined by BRET assay. Data are presented as the mean ± SEM of three independent experiments performed in triplicate.

j, Structural alignment of C5a-bound and $C5a^{pep}$-bound C5aR1 showing that dR^{P8} in $C5a^{pep}$ forms polar interactions with both $S171^{4.60}$ in TM4 and $D282^{7.35}$ in TM7, leading to inward shift of TM4 and TM7, but the R^{P8} in C5a lost the polar interactions with $S171^{4.60}$. Polar interactions are highlighted as black dashed lines.

k, The $S171^{4.60}A$ mutation greatly impaired $C5a^{pep}$-induced G protein signaling (right panel) but had no effect on the potency and efficacy in response to C5a (left panel), the signaling was monitored by cAMP inhibition assay. Data are mean ± SEM from at least three independent experiments.

l, $K279^{7.32}$ of C5aR1 forms a salt-bride with the side chain of D^{P3} in C5a, instead a H-bond with the main chain of P^{P3} in $C5a^{pep}$. Polar interactions are highlighted as black dashed lines.