METHODS

No statistical methods were used to predetermine sample size. The experiments were not randomized and investigators were not blinded to allocation during experiments and outcome assessment.

StaR generation. Full-length human C5aR1 (1–350) was used as background for the generation of the conformationally thermostabilized receptor using a mutagenesis approach described previously. Mutants were analysed for thermostability in the presence of the radioligand [^{3}H]NDT9513727. The C5aR1 StaR is the full-length receptor with 11 thermostabilizing mutations.

Cell culture. HEK293T cells were purchased from the American Type Culture Collection and were cultured in DMEM supplemented with 10% (v/v) fetal bovine serum (FBS). Cells were transfected using GeneJuice (Merck Millipore) according to the manufacturer's instructions and collected after 48 h. Cell lines were not tested for mycoplasma contamination.

Thermostability measurement. Transiently transfected HEK293T cells were incubated in 50 mM HEPES–NaOH pH 7.5, 150 mM NaCl, supplemented with cOmplete Protease Inhibitor Cocktail tablets (Roche), with 1% (w/v) *n*-dodecyl-β-D-maltopyranoside (DDM) at 4 °C for 1 h. All subsequent steps were performed at 4 °C. Samples were incubated with 200 nM [^{3}H]NDT9513727 for 1 h and crude lysates cleared by centrifugation at 16,000*g* for 15 min. Thermostability of the receptor was determined as previously described. Thermal stability (T_m) is defined as the temperature at which 50% ligand binding is retained.

Radioligand binding. For saturation binding experiments HEK293T membranes transiently expressing C5aR1 or C5aR1(W213L) were incubated with varying concentrations of [^{3}H]NDT9513727 (final assay concentration of 0–1,000 nM, assay buffer: 50 mM HEPES–NaOH pH 7.5, 150 mM NaCl, 1% (w/v) DDM). For competition binding experiments, HEK293T membranes transiently expressing C5aR1 were incubated with 200 nM [^{3}H]NDT9513727 and varying concentrations of cold ligands NDT9513727, W-54011, PMX-53 or C5a agonist (final assay concentration of 0–10 μM, assay buffer: 50 mM HEPES–NaOH pH 7.5, 150 mM NaCl, 1% (w/v) DDM). Binding assays were incubated for 2 h at 4 °C and the reactions were terminated by ligand separation via immobilized metal ion affinity chromatography (IMAC). Specific binding was determined by subtracting mock-transfected controls. Saturation and competition binding data were globally fitted to one site-specific binding or one site heterologous competition.

Membrane preparation. cDNA encoding the human C5a receptor or C5a StaR construct was transfected into HEK293T cells using the transfection reagent Genejuice (Novagen). Forty-eight hours after transfection, cells were collected and washed twice with ice-cold PBS. The pellet was resuspended in ice-cold buffer containing 20 mM Tris-HCl, pH 7.4, 1 mM EDTA and homogenized with an Ultraturax for 30 s at maximum speed. After centrifugation at 48,000*g* for 30 min at 4 °C, the pellet was resuspended and spun again. The final pellet was resuspended and frozen at −80 °C before use. Protein was determined using the BCA protein assay method.

^{125}I-C5a radioligand-binding assay. After thawing, membrane homogenates were re-suspended in the binding buffer (50 mM HEPES, pH 7.4, 1 mM $CaCl_2$, 0.5% bovine serum albumin) to a final assay concentration of 5 μg (wild type) or 20 μg (StaR) protein per well. Competition experiments were carried out using 25–30 pM of ^{125}I-C5a (in a total reaction volume of 250 μl) for 120 min on ice. At the end of the incubation, membranes were filtered onto a unifilter, a 96-well white microplate with bonded GF/C filter pre-incubated with 0.5% polyethylenimine, with a Tomtec cell harvester and washed five times with PBS. Non-specific binding was measured in the presence of 10 μM C5a. Radioactivity on the filter was counted (1 min) on a microbeta counter after the addition of 50 μl of scintillation fluid. Half-maximal inhibitory concentration (IC_{50}) values were determined using Prism.

Truncation constructs. A panel of N- and C-terminal truncation variants of C5aR1 were designed and tested on the basis of secondary structure prediction. The most suitable construct emerging from this screen comprised residues 29–333 with the inclusion of a three-alanine spacer and a C-terminal deca-histidine tag.

Expression, membrane preparation and protein purification. The truncated C5aR1 StaR(29–333) construct was expressed with a C-terminal deca-histidine tag in *Spodoptera frugiperda* Sf21 cells (Oxford Expression Technologies) using ESF 921 medium (Expression Systems) supplemented with 10% (v/v) heat-inactivated FBS (Sigma-Aldrich) and 1% (v/v) penicillin/streptomycin (PAA Laboratories) with a Bac-to-Bac Expression System (Invitrogen). Cells were infected at a density of 2.5×10^6 cells ml^{-1} with baculovirus at an approximate multiplicity of infection of 2. Cultures were grown at 27 °C with constant shaking and collected by centrifugation 48 h after infection. All subsequent steps were performed at 4 °C unless otherwise stated. Membranes were prepared by resuspension of cells in buffer containing 50 mM HEPES pH 7.5, 150 mM NaCl, supplemented with cOmplete Protease Inhibitor Cocktail tablets (Roche), followed by disruption using a microfluidizer at 60,000 pounds per square inch (M-110L Pneumatic, Microfluidics). Membranes were collected by ultracentrifugation at 258,420*g*, resuspended in 50 mM HEPES–NaOH pH 7.5, 150 mM NaCl with cOmplete Protease Inhibitor Cocktail tablets (Roche), and stored at −80 °C until use. To purify the receptor, membranes were thawed at room temperature and incubated with 5 μM NDT9513727 for 30 min before solubilization with 1.2% (w/v) (DDM) and 0.12% (w/v) CHS (cholesteryl hemisuccinate; Anatrace, CH210) for 1 h. Insoluble material was removed by ultracentrifugation at 298,834*g* and the receptors were immobilized by batch binding to a 5 ml HiTrap TALON crude cartridge (GE Healthcare, 28-9537-67) connected to an ÄKTA FPLC system pre-equilibrated in buffer A: 50 mM HEPES pH 7.5, 150 mM NaCl, 0.03% (w/v) DDM, 0.003% (w/v) CHS and 2 μM NDT9513727. The bound material was eluted in buffer containing 300 mM imidazole. The protein was then concentrated using a 15 ml 100 kDa cut off Vivaspin Turbo Polyethersulfone (PES) concentrator (Sartorius, VS15T42) centrifuged at 932*g* for 3 min cycles at 4 °C in a Beckman Coulter Allegra X12-R centrifuge fitted with a swinging bucket SX4750A ARIES rotor and subjected to preparative SEC in 50 mM HEPES–NaOH pH 7.5, 150 mM NaCl, 0.12% (w/v) DDM, and 0.012% (w/v) CHS on a Superdex 200 10/300 Increase column (GE Healthcare). Receptor purity was analysed by SDS–PAGE. Fractions containing the pure, monomeric receptor were concentrated to 18–23 mg ml^{-1} in a 0.5 ml 100-kDa cut off Vivaspin Polyethersulfone (PES) concentrator (Sartorius, VS0142). Protein concentration was determined using the calculated extinction coefficient of the receptor at 280 nm (ε280, calc = 56,225 M^{-1} cm^{-1}) and confirmed by quantitative amino acid analysis.

Crystallization. Non-fusion C5aR1 StaR(29–333) was crystallized in LCP at 22.5 °C. The concentrated protein (~20 mg ml^{-1}) was mixed with monoolein (Nu-Check) supplemented with 10% (w/w) cholesterol (Sigma Aldrich) and 50 μM NDT9513727 using the twin-syringe method[29]. The final protein:lipid ratio was 40:60 (w/w). A 50 nl bolus was dispensed on 96-well glass bases and overlaid with 750 nl precipitant solution using a Mosquito LCP from TTPLabtech and sealed with Laminex Film Covers (Molecular Dimensions). 20–50 μm elongated plate shaped crystals of C5aR1 StaR were grown in 100 mM tri-sodium citrate across a pH range of 5.5–6.0, 200 mM Na/K tartrate, 35–45% (v/v) polyethylene glycol 400, and 0.2 μM NDT9513727.

Diffraction data collection and processing. Single crystals were mounted for data collection, flash-frozen and stored in liquid nitrogen without addition of cryoprotectant. Diffraction data from 11 crystals, collected at Diamond Light Source beamline I24, were merged to assemble a 99.3% complete dataset to a final resolution of 2.7 Å. X-ray diffraction data were measured on a Pilatus 6M detector at Diamond Light Source beamline I24 using a beam size of 10 μm × 10 μm. Crystals displayed diffraction initially out to approximately 2.5 Å following exposure to a non-attenuated beam for 0.07 s per 0.25 degree of oscillation. It was possible to collect ~25° of useful data from each crystal before radiation damage became severe. Data from individual crystals were integrated using XDS[30]. Data merging and scaling were carried out using the program AIMLESS[31] from the *CCP*4 suite of programs[32,33]. Data collection statistics are reported in Extended Data Table 1.

Structure solution and refinement. The structure was solved by molecular replacement using the program Phaser[34] using the μ-opioid receptor structure (PDB code 4DKL) as the input model searching for two copies in the asymmetric unit. Initial refinement was carried out with REFMAC5[35] using maximum-likelihood restrained refinement in combination with the jelly-body protocol. Manual model building was performed in Coot[36]. Further and final stages of refinement were performed with Phenix refine[37] with positional, individual isotropic *B*-factor refinement and TLS. The later stages of refinement were performed with release of all non-crystallographic symmetry (NCS) restraints. The final refinement statistics (R_{work}/R_{free} = 20.8/23.8%) are presented in Extended Data Table 1. Structure quality was assessed with MolProbity[38].

Molecular dynamics simulations. Each system was pre-processed with the Protein Preparation Wizard method in Maestro (Maestro v.11.1, Schrödinger, New York). Each system was solvated and enclosed in an orthorhombic simulation box after embedding the complex in a pre-equilibrated POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) bilayer by means of the System Builder method as implemented in Maestro. OPLS 3 force field was adopted[39]. Simulations were performed on GPU-equipped workstations using Desmond (D. E. Shaw Research, New York) and Maestro-Desmond Interoperability Tools. First, each system was minimized for 5,000 steps. Minimized systems were gradually thermalized up to 300 K within the NVT ensemble. Harmonic position restraints were applied to solute heavy atoms (50 kcal mol^{-1} Å^{-2}). Then, volume and density were equilibrated in the NPT ensemble for 200 ps at target temperature of 300 K and target pressure of 1 bar using a Nosé–Hoover chains thermostat[40] and a Martyna–Tobias–Klein barostat[41] with a 2.0 ps relaxation time, gradually removing residual restraints (10 kcal mol^{-1} Å^{-2}) set on the protein C_α carbon atoms. Production runs were performed in the NPT ensemble with semi-isotropic pressure coupling control on unconstrained systems. Short range van der Waals and Coulomb interactions