

Life Sciences Reporting Summary

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Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study. [For final submission](#): please carefully check your responses for accuracy; you will not be able to make changes later.

► Experimental design

1. Sample size

Describe how sample size was determined.

For calcium release assays and protein thermostability assays, the experiments were repeated as least three times. The sample size was chosen based on the data consistency.

2. Data exclusions

Describe any data exclusions.

Not applicable.

3. Replication

Describe the measures taken to verify the reproducibility of the experimental findings.

The cell-based signaling assays and protein thermostability assays were reliably repeated more than 3 times with the same results.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

Not applicable.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Not applicable.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a Confirmed

- ☐ ☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
- ☐ ☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☐ ☒ A statement indicating how many times each experiment was replicated
- ☐ ☒ The statistical test(s) used and whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- ☒ ☐ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
- ☒ ☐ Test values indicating whether an effect is present
*Provide confidence intervals or give results of significance tests (e.g. *P* values) as exact values whenever appropriate and with effect sizes noted.*
- ☐ ☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
- ☐ ☒ Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

See the web collection on [statistics for biologists](#) for further resources and guidance.

► Software

Policy information about [availability of computer code](#)

7. Software

Describe the software used to analyze the data in this study.

All the software used for the crystallographic study and data analysis is described in the Methods section, including HKL200, Phenix, Coot, Pymol and Prism.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* [guidance for providing algorithms and software for publication](#) provides further information on this topic.

► Materials and reagents

Policy information about [availability of materials](#)

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

There are no restrictions for the data and materials in our study. We will distribute the related materials upon request to outside research investigators under a Material Transfer Agreement as requested by our institute.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

The anti-FLAG M1 antibody was produced from hybridoma cells in our lab. The binding to FLAG peptide was confirmed by western blot and the successful use of the antibody resin in the affinity chromatography.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

The insect Sf9 cells were obtained from ExpressionSystems (<https://expressionsystems.com/>). The HEK293T cells were obtained from Dr. Kunhong Xiao at University of Pittsburgh.

b. Describe the method of cell line authentication used.

No.
The Sf9 cells were used to express the protein of interest. The normal signaling behaviors of C5aR in the HEK293T cells were confirmed. The replicates of each experiment were conducted with cells from different passages, and no significant difference was observed among these replicates.

c. Report whether the cell lines were tested for mycoplasma contamination.

No. However, we used Plasmocin in our cell media to prevent mycoplasma contamination.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by [ICLAC](#), provide a scientific rationale for their use.

Not applicable.

► Animals and human research participants

Policy information about [studies involving animals](#); when reporting animal research, follow the [ARRIVE guidelines](#)

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

Not applicable.

Policy information about [studies involving human research participants](#)

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

Not applicable.