# nature portfolio

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## **Reporting Summary**

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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FOI	an statistical arialyses, commit that the following items are present in the figure legend, table legend, main text, or inferious section.
n/a	Confirmed
	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
$\boxtimes$	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
	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.
$\boxtimes$	A description of all covariates tested
$\boxtimes$	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
	For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i> ) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
$\boxtimes$	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
$\boxtimes$	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
$\times$	Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated
	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.

#### Software and code

Policy information about availability of computer code

Data collection

X-ray diffraction data were collected at beamline BL18U1 of Shanghai Synchrotron Radiation Facility (SSRF) using a Pilatus 36M detector.

Data analysis

All diffraction data sets were automatically processed using autoPROC. Structure was solved by molecular replacement with CCP4i2 (version 1.1.0). Automated model building was performed by PHENIX (version 1.20-4487) and CCP4i2(version 1.1.0). Improvement of the initial model was carried out manually by COOT (version 0.9.8), and the refinement was conducted using PHENIX (version 1.20-4487) and REFMAC5 of CCP4 (version 8.0).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

#### Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The structures in the manuscript has been deposited at the Protein Data Bank (PDB) under the PDB accession code 7WJQ. The CTD of GSDMB (PDB ID:5TJ4) was used as a searching model for molecular replacement to solved the structure of IpaH7.8-GSDMB complex.

Field-spe	cific reporting			
Please select the o	ne below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.			
\times Life sciences	Behavioural & social sciences Ecological, evolutionary & environmental sciences			
For a reference copy of	he document with all sections, see <a href="mailto:nature.com/documents/nr-reporting-summary-flat.pdf">nature.com/documents/nr-reporting-summary-flat.pdf</a>			
Life scier	nces study design			
All studies must dis	close on these points even when the disclosure is negative.			
Sample size	All biological and cell biological experiments used in this study were repeated at least three times.			
Data exclusions	No data were excluded in this study.			
Replication	All the experiments in this work were repeated at least three times. We detailed the replication of all the experiments in figure legends and method session in the manuscript.			
Randomization	Our study focused on the function of proteins only through structural biology, biochemistry and cell biology approaches, and no animal or human subject was used in this work, so randomization is not applicable for the all experiments mentioned in the manuscript.			
Blinding	We performed all the experiments using automated methods, so blinding is not applicable for this study.			
Reportin	g for specific materials, systems and methods			
We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.				
Materials & ex	perimental systems Methods			
n/a Involved in th				
Antibodies	ChIP-seq			
☐ X Eukaryotic	cell lines Flow cytometry			
Palaeonto	ogy and archaeology MRI-based neuroimaging			
Animals and other organisms				
Human research participants				
Clinical data				
Dual use research of concern				
Antibodies				
Antibodies used	Anti HA (CST, 3724S, clone: C29F4) Dilution 1:2000 https://www.cellsignal.cn/products/primary-antibodies/ha-tag-c29f4-rabbit-mab/3724?site-search-type=Products&N=4294956287&Ntt=ha&fromPage=plp			
	Anti FLAG (CST, 14793S, clone: D6W5B) Dilution 1:2000 https://www.cellsignal.cn/products/primary-antibodies/dykddddk-tag-d6w5b-rabbit-mab-binds-to-same-epitope-as-sigma-s-anti-flag-m2-antibody/14793?_=1670414436110&Ntt=FLAG&tahead=true			
	Anti C7MA (About ab20020E dans) ERR20161\ Dilution 1:1000			

Anti GZMA (Abcam, ab209205, clone: EPR20161) Dilution 1:1000

https://www.abcam.cn/granzyme-a-antibody-epr 20161-ab 209205.html # lb

Anti β-actin (Proteintech, 20536-1-AP) Dilution 1:1000

https://www.ptgcn.com/products/ACTB-Antibody-20536-1-AP.htm

HRP linked Anti-Rabbit IgG antibody (CST, 7074S) Dilution 1:10000

https://www.cellsignal.cn/products/secondary-antibodies/anti-rabbit-igg-hrp-linked-antibody/7074?site-search-

type=Products&N=4294956287&Ntt=secondary+antibody&fromPage=plp

Validation

High quality commercial monoclonal antibodies were used for biological and cell biological experiments in this study. References for all the monoclonal antibodies can be obtained from the manufacturer's website listed above.

### Eukaryotic cell lines

Policy information about <u>cell lines</u>

Cell line source(s)

HEK293T cells (ATCC), NK92MI cells (ATCC).

Authentication HEK293T and NK92MI cell lines mentioned in the manuscript were from ATCC, so no additional authentication was

performed in this study.

Mycoplasma contamination Cell lines used in this work were routinely tested for mycoplasma every month, and all cell lines were tested negative for mycoplasma contamination.

Commonly misidentified lines No commonly misidentified cell lines were used in our study.

(See <u>ICLAC</u> register)