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Reporting Summary

Nature Research 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 Research policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	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.*
☐	☒	A description of all covariates tested
☐	☒	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. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	We used SerialEM v3.8.0 Beta for automated collection of cryo-EM data.
Data analysis	MotionCor2, CTTFFIND4.1.13, goCTF v1.2.0, crYOLO v1.3.6 and v1.5.3, RELION3.08, RELION3.1, ResMAP v1.1.4, Coot v0.8.9.1, Phenix v1.18.2-3874, pymol v2.4.0, chimera v1.14, chimeraX v1.0, Schrodinger prime 2020-1, PIXL search engine v1.0, ImageJ v2.0, GraphPad PRISM 7.0

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 Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

Extended protein purification protocols are available on Protocols.io (https://www.protocols.io/groups/hao-wu-lab). Raw cryo-EM data are available on EMPIAR under the accession numbers EMPIAR-10594 (NLRP1-DPP9) and EMPIAR-10595 (NLRP1-DPP9-VbP). The cryo-EM maps are available on the Electron Microscopy Data Bank under the accession numbers EMD-22074 (NLRP1-DPP9) and EMD-22075 (NLRP1-DPP9-VbP). The atomic coordinates are available on the Protein Data Bank under the accession numbers 6X6A (NLRP1-DPP9) and 6X6C (NLRP1-DPP9-VbP). Pymol session files and the image analysis macro are available on OSF [http://doi.org/10.17605/OSF.IO/X7DV8]. All other data can be obtained from the corresponding authors upon reasonable request.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No sample size calculations were performed. Experiments were performed with at least 3 biological replicates for each experiment presented. This is standard to the field, and this sample size was sufficient to observe the generally high effect size and binary outcomes of these experiments, i.e. active or inactive inflammasome processing by Western blot, high/low LDH release to measure inflammatory cell death, and ASC speck formation by confocal microscopy. All sample sizes are consistent with inflammasome literature and extent of experimental conclusions.
Data exclusions	No data were excluded.
Replication	All experiments were confirmed with multiple biological replicates as detailed in Methods or Figure Legends.
Randomization	No randomization was performed, as this does not apply to the in vitro and cellular systems used throughout this study.
Blinding	Researchers were not blinded to confocal microscopy experiments, which was not necessary given the binary nature of their effect and very large effect size. An ImageJ script was used for automated quantification of ASC specks with minimal user input.

Reporting 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 & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Primary antibodies used in this study include: DPP9 rabbit polyclonal Ab (1:1000, Abcam, Ab42080), FLAG® M2 mouse monoclonal Ab (1:1000, Sigma, F3165), GAPDH rabbit monoclonal Ab (1:1000, Cell Signaling Technology, 14C10), NLRP1-CT mouse monoclonal Ab (1:1000, R&D Systems, MAB6788), CASP1 rabbit polyclonal Ab (1:1000, Cell Signaling Technology, 2225), GSDMDC1 rabbit polyclonal Ab (1:1000, Novus, NBP2-33422), V5 rabbit polyclonal Ab (1:1000, Abcam, Ab9116), HA tag rabbit monoclonal Ab (1:1000, Cell Signaling Technology, 3724). Secondary antibodies used in this study include: IRDye 680 RD Streptavidin (1:1000, LI-COR, 926-68079), IRDye 800CW anti-rabbit (1:10000, LI-COR, 925-32211), IRDye 800CW anti-mouse (1:10000, LI-COR, 925-32210), IRDye 680CW anti-rabbit (1:10000, LI-COR, 925-68073), IRDye 680CW anti-mouse (1:10000, LI-COR, 925-68072).
Validation	All antibodies used in these studies have been evaluated by our groups in previously peer-reviewed publications, including in knockout cells. In particular, GSDMD (Novus, NBP2-33422), CASP1 (Cell Signaling Technology, 2225), and DPP9 (Abcam, Ab42080) antibodies were validated in knockout cells in Johnson et. al., 2018 Nat Med, whereas the NLRP1-CT antibody (R&D systems, MAB6788) was validated in Johnson et. al 2020 Cell Death Dis. as well as through transient expression in HEK cells in this paper. The Streptavidin antibody (LI-COR, 926-68079) was validated previously (Griswold et. al, Cell Chem Bio 2019). All other antibodies have been validated extensively in peer-reviewed publications as detailed on the manufacturers' websites.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HEK293T cells stably expressing GSDMD and caspase-1 were previously described (Johnson et. al., 2018 Nat Med). DPP8/9 knockout HEK cells were generated in this study using previously validated DPP9 KO cells (Griswold et. al, 2019 ACS Chem Bio). Wild-type HEK293T (ATCC), Expi293F (ThermoFisher), and Sf9 (ThermoFisher) cells were purchased from the manufacturer.
Authentication	Cell lines were verified by manufacturer's website and Identity of these cell lines were frequently checked by their morphological features. HEK293T (ATCC) https://www.atcc.org/en/Products/All/CRL-3216.aspx. Expi293F (ThermoFisher) https://www.thermofisher.com/order/catalog/product/A14527#/A14527. Sf9 (ThermoFisher) https://www.thermofisher.com/order/catalog/product/11496015.
Mycoplasma contamination	Expi293F and Sf9 cells for protein production were not tested regularly for mycoplasma contamination. All other cell lines regularly tested negative for mycoplasma using the MycoAlert Mycoplasma Detection Kit (Lonza).
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines are used in this study.