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 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	Isothermal Titration Calorimetry : MicroCal PEAQ-ITC family Analysis v.1.40 x-ray: MXCuBE v.Qt 2.3; SIMPLON v.1.6.0; Albula v.3.2.0 ; ADXV v.1.9.1 ; XDSME v.0.6.5.5. Mass spectrometry: Tune 3.4; Xcalibur 4.4 Immunofluorescence: AF6000
Data analysis	x-ray: XDS VERSION Mar 15, 2019 BUILT=20190315 ; STARANISIO server (https://staraniso.globalphasing.org/); PHASER v.2.8.3; PHENIX v. 1.20.1_4487; BUSTER v.2.10.4; COOT v.0.9.8.6 Mass spectrometry: Xi software suite v.1.7.6.4; xiFDR v.2.2beta5 Cross-linking data: DisVis webserver (https://wenmr.science.uu.nl/disvis/) Molecular modeling: HADDOCK 2.4 webserver (https://wenmr.science.uu.nl/haddock2.4/) SDS-PAGE protein band quantification: ImageJ v.1.52k Isothermal Titration Calorimetry : NITPIC v.2.1.0 ; SEDPHAT v.15.2b

immunofluorescence:
ImageJ v.2.3.0
Statistical analyses:
Microsoft excel v.16.78; Prism 9
Molecular graphics :
PyMol V.2.06 ; Chimera v.1.17.3
Sequence alignements :
Clustal Omega (https://www.ebi.ac.uk/jdispatcher/msa/clustalo)
Motif search :
SLIMsearch (http://slim.icr.ac.uk/tools/slimsearch/input)

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

Coordinates of the refined x-ray structure of IKKbeta and structure factor amplitudes generated in this study have been deposited in the PDB database under accession number 8OMV (https://www.rcsb.org/structure/8OMV). CLMS raw and processed data are available on JPOST and ProteomeXchange database with identifier PXD037534 (https://proteomecentral.proteomexchange.org/cgi/GetDataset?ID=PXD037534-1&test=no). All other data generated in this study are provided in the main text, Supplementary Information and Data files, and in the Source Data files. Source data are provided with this paper.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	not applicable
Reporting on race, ethnicity, or other socially relevant groupings	not applicable
Population characteristics	not applicable
Recruitment	not applicable
Ethics oversight	not applicable

Note that full information on the approval of the study protocol must also be provided in the manuscript.

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	Sample size calculations were not required for this study.
Data exclusions	No data were excluded for interaction, activity and functional analyses. The x-ray diffraction cut-off was determined based on the statistics shown in Supplementary Table 1.
Replication	Interaction, activity and functional data were reproduced in at least two independent experiments. The crystal structure of IKKbeta was determined form a single x-ray dataset. Mass-spetrometry data were acquired on one biological sample per condition.
Randomization	Randomization was not required for this study.
Blinding	Blinding was not required for this study.

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
[ ]	[x] Antibodies
[ ]	[x] Eukaryotic cell lines
[x]	[ ] Palaeontology and archaeology
[x]	[ ] Animals and other organisms
[x]	[ ] Clinical data
[x]	[ ] Dual use research of concern
[x]	[ ] Plants

Methods

n/a	Involved in the study
[x]	[ ] ChIP-seq
[x]	[ ] Flow cytometry
[x]	[ ] MRI-based neuroimaging

Antibodies

Antibodies used

Antibody used at the dilutions recommended by the manufacturer.
Anti-6xHis: Sigma, Cat# H1029, clone HIS-1, RRID: AB260015, Lot# 106M4768V and Lot#: 025M4780V
anti-HA: Santa Cruz biotechnology, Cat# sc-7392, clone F-7, RRID: AB_2894930 Lot# L1218
anti-HA beads: Santa Cruz biotechnology, sc-7392AC, Lot# E1217
anti-IKKbeta: Cell Signaling Technology, Cat# 2684, RRID:AB_2122298, Lot# 3
anti-IkappaBalpha: Santa Cruz biotechnology, Cat# sc-371, clone C-21, RRID: AB_2235952, Lot# F1412
anti-phospho-Ser32,Ser36-IkappaBalpha: Cell Signaling Technology, Cat# 9246, clone 5A5, RRID:AB_2267145, Lot# 23
anti-Gluc: New England Biolabs, Cat# E8023, product discontinued;
anti-Gluc: Invitrogen, Cat# PAI-181; RRID:AB_2539912
anti-phospho-tyrosine, Millipore, Cat# 05-321, clone 4G10, RRID:AB_309678, Lot# 2310354
anti-HSP90, Santa Cruz biotechnology, Cat# sc-13119, clone F-8, RRID: AB_675659 Lot# F2917
anti-NEMO, Santa Cruz biotechnology, cat# sc-166398, clone F-10, RRID: AB_2011719, Lot# F1020
mouse IgG: Santa Cruz biotechnology, Cat# sc-2025, RRID:AB_737182 Lot# A1923
anti-p65: Santa Cruz biotechnology, Cat# sc-8008, clone F-6, RRID:AB_628017 Lot# l1516
anti-mouse secondary antibody-AlexaFluor 488: Invitrogen, Cat# A-11001, RRID:AB_2534069 Lot# 727756

Validation

All antibodies were validated by the manufacturer for the specific uses reported in this study. For validation of anti-Gluc antibodies against the G1 and G2 fragments see Poirson et al. 2017, FEBS J., 284, 3171 and Fig. 6g of this study.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

Sf9 insect cells: purchased from Oxford Expression Technologies, Cat# 600104
Sf21 insect cells: purchased from Oxford Expression Technologies, Cat# 600105
*Phoenix-eco: purchased from ATCC
*HEK293T cells for immunoprecipitation: purchased from ATCC
MRC-5 cells: purchased from ATCC, Cat# CCL-171™
HEK293T cells for GPCA: kindly provided by Yves Jacob, Institut Pasteur, Paris
Mouse Embryonic Fibroblasts KO for IkBalpha, IkBbeta and IkBepsilon: kindly provided by Alexander Hoffmann, UCLA

Authentication

Cell lines marked by * were purchased in 2009. These cell lines were characterized by ATCC, using a comprehensive database of short tandem repeat (STR) DNA profiles.
The other cell lines were not authenticated.

For all cell lines frozen aliquots of freshly cultured cells were generated and experiments were done with resuscitated cells cultured for less than 20 passages.

Mycoplasma contamination

All cell lines were negative for mycoplasma contamination

Commonly misidentified lines (See ICLAC register)	none

Plants

Seed stocks	not applicable
Novel plant genotypes	not applicable
Authentication	not applicable