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	EMBL PETRA III beamline P14
Data analysis	Data fitting and visualization: Origin 2018, Qtiplot 09.8.09 SPR: Biacore S200 Evaluation Software 1.1. MS: Analyst TF software 1.7.1 (Sciex), Peak View softwareTM V2.2 (Sciex) Crystallography: XDS (BUILT=20190315), CCP4i 7.0.0.78, AIMLESS 0.7.4, COOT 0.9, PHASER, PHENIX 1.20.1, Jligand 1.0.40, Pymol 1.8 Quantitative western blot analysis: Odyssey CLx (LI-COR) Image Studio Lite 5.2 Statistical analysis and visualization: LibreOffice Calc 6.0.7.3 biochemical modeling: COPASI 4.37 (Build 264) steric volume calculations: Gaussian 16 (Revision A.03), SambVca 2,1

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

Crystal structures of JNK1–1aR-IN-8, –1aS-IN-8, and –1a'R-IN-8 are deposited in the Protein Data Bank with accession codes 8PTA [http://doi.org/10.2210/pdb8pta/pdb], 8PT9 [http://doi.org/10.2210/pdb8pt9/pdb], and 8PT8 [http://doi.org/10.2210/pdb8pt8/pdb]. The following X-ray structures are available from the PDB: 3V6S [http://doi.org/10.2210/pdb3v6s/pdb], 2ERK [http://doi.org/10.2210/pdb2erk/pdb] and 3S95 [http://doi.org/10.2210/pdb3s95/pdb]. 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 (N/A)
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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	No sample size was applied in this study to predetermine sample sizes for experiments using cell lines. A sample size of three was used to evaluate the spread of the data and was determined based upon other studies with similar methodologies (PMID: 26538579, PMID: 33188182, PMID: 38078976).
Data exclusions	No data were excluded from the analyses.
Replication	All experiments were replicated as stated in the figure legends.
Randomization	There was no need for the randomization of our samples.
Blinding	There was no blinding, as the same investigator performed most experiments and analyzed the data.

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

The primary antibodies were the following: anti-phospho JNK (Cell Signaling #9251; 1:1000), anti-phospho-c-Jun(Ser73) (Cell Signaling #9164; 1:1000), anti-phospho p38 (Cell Signaling #9215, 1:3000), anti-phospho MK2 (Cell Signaling #3007, 1:1000), anti-α-tubulin (Sigma #T6199; 1:10000), or anti-FLAG (Sigma #F1804; 1:10000).

The secondary antibodies were the following: IRDye 680RD (Goat anti-mouse IgG; Li-cor #926-68070; 1:0000) or IRDye 800CW (Goat anti-rabbit IgG, Li-Cor #926-32211; 1:5000).

Validation

The primary antibodies used in this study were all validated by the manufacturers and can be checked using their respective catalog number on the following websites:
Cell Signaling: https://www.cellsignal.com
Sigma: http://www.sigma.com

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

HEK293T (ATCC, CRL-3216), AP-1 Reporter - HEK293 (BPS Bioscence, #60405), HEK293T MKK6EE (created from HEK293T see PMID: 32649858), SH-SY5Y MKK7ACT (Created from SH-SY5Y, ATCC CRL-2266),

Authentication

The cell lines were not authenticated.

Mycoplasma contamination

The cell lines were not tested for mycoplasma contamination, apart from .SH-SY5Y MKK7ACT, which was tested negative by MycoAlert PLUS Mycoplama Detection Kit (Lonza #LT07-710).

Commonly misidentified lines
(See ICLAC register)

Commonly misidentified cell line was not used in this study.