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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 Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	ImageJ, Odyssey IR Imaging System, SymPhoTime 64
Data analysis	ImageJ, Prism 7.02, Microsoft Excel 2010, SymPhoTime64, Odyssey IR Imaging System

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/reviewers upon request. 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

Provide your data availability statement here.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

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

Sample size	No statistical method was used to predetermine sample size. The samples sizes were selected based on previous studies with similar methodologies.
Data exclusions	No data were excluded.
Replication	All attempts at replication were successful.
Randomization	Sample allocation was random.
Blinding	Strains were assigned numbers instead of descriptions during data acquisition and analysis.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☐	☒ Unique biological materials
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants

Methods

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

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	All strains and plasmids can be obtained upon request. The FLipTR probe will be available commercially in the future.

Antibodies

Antibodies used	goat anti-Ypk1 1:1000 (Cell Signaling, no longer produced); mouse anti-phospho-Ypk1-T662 1:500 (see ref 7); rabbit anti-phospho-Sch9-T737 1:250 (see ref 45); rabbit anti-Sch9 1:10000 (see ref 21); rabbit anti-phospho-p38 T180/T182 1:1000 (Cell Signaling 9211S); rabbit anti-Hog1 1:1000 (Santa Cruz Technology sc-9079); and the appropriate infrared dye-coupled secondary antibodies used at a 1:10000 dilution (alexa Fluor 680-conjugated secondary anti-mouse and anti-rabbit Ab from Li-Cor Biosciences 926-68072 and 926-68073 respectively, and IRDye® 800 conjugated secondary anti-goat Ab from Rockland Bioconcept 605-732-125). anti-GFP antibody (Abcam Ab290)
Validation	Cell Signaling anti phospho p38 (ref 9211S): Antibody detects endogenous levels of p38 MAPK only when activated by phosphorylation at threonine 180 and tyrosine 182 (tested by Western Blot). This antibody does not cross-react with the phosphorylated forms of either p42/44 MAPK or SAPK/JNK.Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues around Thr180/Tyr182 of human p38 MAPK. Antibodies are purified by protein A and peptide affinity chromatography. Species reactivity: Human, Mouse, Rat, Monkey, D. melanogaster, Pig, S. cerevisiae. Santa Cruz anti Hog1 sc-9079:Hog1 Antibody (y-215) is a rabbit polyclonal IgG; 200 µg/ml. epitope corresponding to amino acids 221-435 mapping at the carboxy terminus of Hog1 of Saccharomyces cerevisiae origin. validated by western blot.

Anti-GFP Ab290 from AbCam: polyclonal IgG. This antibody is provided as whole antiserum. It is not possible to determine the exact antibody concentration, since whole serum contains many other host serum proteins besides the antibody of interest. On Western blot the antibody detects the GFP fraction from cell extracts expressing recombinant GFP fusion proteins and has also been shown to be useful on mouse sections fixed with formalin. In Immunocytochemistry, the antibody gives a very good signal on recombinant YES-GFP chimeras expressed in COS cells (McCabe et al. 1999 and figure below). It is routinely used in Immunoprecipitation (IP) and IP-Western protocols and has been used successfully in HRP Immunohistochemistry at 1:200 on whole-mount mouse embryos.

The anti-Ypk1 ab is not produce by cell signaling anymore.

See ref 7, 45, and 21 for the validation of the custom-made ab.