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<br>*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<br>*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 | No software was used |
| Data analysis | Prism 9 was used for statistical analysis |

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

All data generated or analyzed during this study are included in this published article and its supplementary information files. The Supplementary Information file contains all supplementary figures and the original uncropped Western blots. The source data behind all graphs in the manuscript are in the Supplementary Data file

## 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 | NA |
| Reporting on race, ethnicity, or other socially relevant groupings | NA |
| Population characteristics | NA |
| Recruitment | NA |
| Ethics oversight | NA |

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 sizes are indicated in each figure legend. The sample size was determined according to the experiment type. For fluorescence recovery after photobleaching studies, a minimum number of 26 cells (with each measurement conducted on a different cell) was measured for each sample, since in our experience with this method (extending over 30 years), about 15 measurements are required to obtain accurate reproducible results. For immunoblots , we used n equal to or greater than 4. |
| Data exclusions | No data was excluded from the analysis. |
| Replication | Yes, attempts at replication were successful. |
| Randomization | Allocation of cells for the experiments was random, as they were split and plated at random while preparing all samples, including control samples. |
| Blinding | No blinding was used, as all samples were prepared simultaneously for the experiment from the same cell stock. |

# 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 |
| ☒ | ☐ Clinical data |
| ☒ | ☐ Dual use research of concern |
| ☒ | ☐ Plants |

### Methods

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

## Antibodies

| | |
|---|---|
| Antibodies used | 12CA5 murine monoclonal IgG anti-HA tag, Roche Diagnostics cat. #11-66-606-001 |

| | |
|---|---|
| Antibodies used | 9E10 murine monoclonal IgG anti-myc tag, BioLegend cat. #626802<br>HA.11 rabbit IgG anti-HA tag, BioLegend cat. #923502<br>Anti-pSmad2/3 rabbit IgG, Cell Signaling Technology cat. #8828<br>Anti-total (t) Smad2/3 murine IgG, Santa Cruz Biotechnology cat. #sc-133098<br>Anti-pAkt (phospho-Ser473) rabbit antibody, Cell Signaling Technology cat. #9271<br>Anti-pAkt (phospho-Thr308) rabbit antibody, Cell Signaling Technology cat. #9275<br>Anti-tAkt rabbit antibody, Cell Signaling Technology cat. #9272<br>Anti-pPI3K (phospho-PI3K p85 (Tyr458)/p55 (Tyr199) rabbit antibody, Cell Signaling Technology cat. #4228<br>Anti-tPI3K (total PI3K (p85)) rabbit antibody, Upstate Biotechnology cat. #06497<br>Anti-pErk1/2 (diphosphorylated Erk1/2) murine monoclonal antibody, Sigma-Aldrich, cat. #M8159<br>Anti-tErk1/2 rabbit antibody, Cell Signaling Technology cat. #4695S<br>Anti-beta actin mouse antibody, MP Biomedicals cat. #08691001<br>Normal goat gamma-globulin, Jackson ImmunoResearch Laboratories cat. #005-000-002<br>Alexa 488-goat IgG anti-rabbit IgG, Invitrogen-Molecular Probes cat. #R37116<br>Alexa 546-goat F(ab')2 anti-mouse IgG, Invitrogen-Molecular Probes cat. #A-11018<br>Alexa 488-goat F(ab')2 anti-rabbit F(ab')2, Invitrogen-Molecular Probes cat. #A-11070<br>Peroxidase-goat anti-mouse antibody, Jackson ImmunoResearch Laboratories cat. #115-035-062<br>Peroxidase-goat anti-rabbit antibody, Jackson ImmunoResearch Laboratories cat. #111-035-144 |
| Validation | All validations are on the web sites of the manufacturers and no new unvalidated antibodies were used. |

## Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

| | |
|---|---|
| Cell line source(s) | AML12 murine hepatocyte cell line, ATCC cat. #CRL-2254 |
| Authentication | STR profiling and interspecies contamination test for AML12 cells was performed by the cell line authentication service from IDEXX (Kornwestheim, Germany) using the CellCheckTM Mouse system that includes 19 species-specific STR markers |
| Mycoplasma contamination | Cells were routinely tested for mycoplasma contamination by RT-PCR every 2 months and found negative. |
| Commonly misidentified lines<br>(See ICLAC register) | No commonly misidentified cell lines were used. |

## Plants

| | |
|---|---|
| Seed stocks | NA |
| Novel plant genotypes | NA |
| Authentication | NA |