nature portfolio

Corresponding author(s): Silvie Foldynova-Trantirkova, Lukas Trantirek

Last updated by author(s): Jan 22, 2025

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	- Topspin 4.0.6 for NMR data acquisition; - BD FACSuite software V1.0.6 (BD Biosciences) for collecting flow cytometry data; - ZEN BLUE 2.6 for confocal microscopy imaging; - CELEROMICS software for fluorescence microscopy imaging; - Image Lab Touch Software 2.4.0.04 for SDS-PAGE gels scanning; - Image Studio 3.1.4 for Western blot membranes scanning;
Data analysis	- MestReNova v14.0.1-23559 software for NMR data analyses; - BD FACSuite software V1.0.6 (BD Biosciences) for flow cytometry data analyses; - ZEN BLUE 3.1 for confocal miscroscopy analyses; - Adobe Photoshop 2020 for SDS-PAGE gel and fluorescent microscopy images brightness / contrast adjustments; - Adobe Illustrator CS6 V16.0.0 for figure assembly; - Image Studio 3.1.4 for band intensity quantification;

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

Plasmids (and their maps) generated in this study are available in the Addgene plasmid repository under the ID numbers 232477, 232478, 232479, 232480, 23248, and 232781. All data generated in this study are available in the Article and Supplementary Information section. The NMR data generated in this study have been deposited in the public data repository (https://doi.org/10.6084/m9.figshare.28266740).

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	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	Approximately 5x10^7 cells were used for the preparation of the in-cell NMR samples. For FCM analysis, the total amount of 10,000 positively-gated cells was analyzed. Confocal microscopy images were acquired at least five times for each experimental condition with a 63x objective (containing approximately 20 cells) and 20x objective (approximately 200 cells in the scanned area). Fluorescence microscopy images were acquired at least three times for each experimental condition with 4x objective to scan area containing approximately 10 spheroids or with 10x objective to scan area containing approximately 500 cells.
Data exclusions	No data were excluded from the analysis.
Replication	All attempts to replicate data were successful, giving consistent results.
Randomization	Randomization is not applicable to this type of study. Here, the experimental design is not based on comparing two or more random experimental groups under different treatments, but on analysis of defined experimental groups under defined conditions.
Blinding	No blinding has been necessary, since the employed experimental methods do not allow subjective interpretation of their outcomes.

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

- Anti-Flag mouse monoclonal IgG1, clone M2, affinity purified, Cat. #F1804 (Sigma Aldrich: https://www.sigmaaldrich.com/CZ/en/product/sigma/f1804)
- Goat Anti-Mouse IgG, Alexa Fluor 594-conjugated, Cat. #115-585-146 (Jackson Immuno Research: https://www.jacksonimmuno.com/catalog/products/115-585-146)
- Goat Anti-Mouse, Horseradish Peroxidase conjugated, Cat. #115-035-003 (Jackson Immuno Research: https://www.jacksonimmuno.com/catalog/products/115-035-003)

Validation

- Anti-Flag mouse monoclonal IgG1, clone M2, affinity purified, Cat. #F1804: certificate of analysis can be found on manufacturer's website: https://www.sigmaaldrich.com/certificates/COFA/F1/F1804/F1804-BULK_____SLCD6338__.pdf

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

HEK293T cells were purchased from ATCC.
Flp-In T-REx 293 cells were purchased from TermoFisher.
MCF7 cells were obtained from ATCC.

Authentication

The cell lines were not further authenticated.

Mycoplasma contamination

The cell lines were tested negative for mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used.

Plants

Seed stocks

N/A

Novel plant genotypes

N/A

Authentication

N/A

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Sample preparation: Approx. 100,000 cells were taken to assess their viability based on propidium iodide staining and to determine the target protein expression levels using GFP reporter. Cells were resuspended in 200 µL of DPBS buffer, stained with 1 µL (1 mg/mL) of propidium iodide, and analyzed by a BD FACSVerse flow cytometer using BD FACSuite software. To evaluate the cell cycle synchronization, the cells were taken after the appropriate synchronization procedure, before the in-cell NMR sample preparation. Cells were first washed with 1 x DPBS and centrifuged at 1000 rpm for 5 min. The pellet was thoroughly resuspended in 300 µL 1 x DPBS, and finally, 2.5 mL of ice-cold 70 % ethanol (EtOH) was added. The sample was then vortexed and stored at -20 ºC for up to 1 week but at least 1 hour prior to staining. The sample of fixed cells was washed twice in ice-cold 1 x DPBS (5 mL and 1 mL), with the centrifugation steps at 15 000 rpm and 4 ºC for 5 min. After the second centrifugation, the sample was resuspended in 100 µL 1 x DPBS and incubated for 1 hour in the dark with ~ 3 µL of the MPM-2 antibody (anti-phospho-Ser/Thr-Pro, Cy5 conjugate) (Millipore, Sigma-Aldrich, USA) that stains cells containing mitosis-specific phophorylations. Next, the sample was washed twice in 1 mL of ice-cold 1 x DPBS, centrifuged at 15 000 rpm, 4 ºC for 5 min and resuspended in 200 µL of 1 x DPBS. Lastly, ~ 10 µL of propidium iodide (PI) was added to the sample to visualize the DNA content as well as 0.5 µL of RNAse (Thermo Fischer Scientific, USA) to get rid of possible RNA remnants from the sample. Thus, the sample was incubated on the shaker at 37 ºC in the dark for approximately 40 min and subsequently taken for FCM analysis.
Instrument	BD FACSVerse Flow Cytometer
Software	BD FASCSuite V1.0.6 was used to collect and analyze the data.
Cell population abundance	Not applicable, since we did not use FASC sorting.
Gating strategy	Samples for viability estimation were FSC-A, and SSC-A gated, followed by FSC-A/FSC-H gating to select singlet cells. Subsequently, the resulting gating was performed. In the case of cell cycle synchronization assays samples were PI-A/PI-W gating to select single cells, followed by the resulting gating.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.