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	No special software other than indicated was used to collect data.
Data analysis	Data analysis was performed using R or PRISM GraphPad. No custom code was used for analyzing the data.

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

Structural datasets: PDB ID 9GWJ, EMDB ID EMD-55042

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	We did not predetermine a sample size beforehand. The treatment involved prolonged exposure to inhibitors, given the robust nature of the expected outcomes, even a small sample size was presumed sufficient to detect significant effects.
Data exclusions	No replicates were excluded. From AP-MS experiments, baits showing poor or no enrichment of expected interactors were excluded. All attempts to repeat the experiment were successful.
Replication	We generally performed at least three independent biological replicates per condition. For some AP-MS experiments more replicates were performed to bolster robustness of the results.
Randomization	No randomization was performed since it was not relevant to the study design.
Blinding	No blinding was performed since it was not relevant to the study since no clinical samples were involved. All experiments were conducted in cell lines and blinding was not necessary as the data were generated by digital read-outs.

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 GTPBP4/NOG1 antibody (ab184124); Anti-RSL24D1, Sigma-Aldrich (HPA062724); Anit-MRTO4/Anti-C1orf33; Sigma-Aldrich (WH0051154M1); Anit-GFP antibody rabbit polyclonal [PABG1], Chromotek/Proteintech (PABG1-100); 5f8 α-Red FPs (RFP), Chromotek/Proteintech (5f8); ANTI-FLAG® M2 antibody, Merck (F3165-1MG), Anti-SPATA5, Chromotek/Proteintech (32104-1-AP), Anti-SPATA5L1, Chromotek/Proteintech (18414-1-AP), Anti-CINP, abcam (ab180955)

Validation

Anti GTPBP4/NOG1 antibody (ab184124); Anti-RSL24D1, Sigma-Aldrich (HPA062724); Anit-MRTO4/Anti-C1orf33, Sigma-Aldrich (WH0051154M1); Anit-GFP antibody rabbit polyclonal [PABG1], Chromotek/Proteintech (PABG1-100); 5f8 α-Red FPs (RFP), Chromotek/Proteintech (5f8); ANTI-FLAG® M2 antibody, Merck (F3165-1MG), Anti-SPATA5, Chromotek/Proteintech (32104-1-AP), Anti-SPATA5L1, Chromotek/Proteintech (18414-1-AP), Anti-CINP, abcam (ab180955) were validated by the manufacturer. No further validation was performed.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

HCT116 (CCL-247), American Type Culture Collection (ATCC); HEK293-EBNA1-6E (CVCL_HF20), National Research Council Canada (NRC)

Authentication

The cell line obtained from a commercial source, and was not authenticated further.

Mycoplasma contamination

Mycoplasma contamination was tested, and confirmed negative.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell line was used in this study.

Plants

Seed stocks

Not applicable

Novel plant genotypes

Not applicable

Authentication

Not applicable