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 custom software was used, all software used in this manuscript is publicly available and described in the methods. Software used: data collection for immunofluorescence was performed with Leica LAS X software, data collection for mass-spectrometry was performed with Thermo Scientific Xcalibur, data collection for immunoblotting was performed with Image Lab.
Data analysis	No custom software was used, all software used in this manuscript is publicly available and described in the methods. Software used: GraphPad Prism 9, ImageJ, Microsoft Excel, Image Lab version 6.0.1, RStudio version 1.4.1717, MaxQuant version 1.5.2.8

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

Mass spectrometry data have been deposited in the ProteomeXchange Consortium

(http://proteomecentral.proteomexchange.org) with the dataset identifier ProteomeXchange: PXD048274.
Project Name: Preservation of Ester-linked Modifications Reveals Glutamate/Aspartate mono-ADP-ribosylation by PARP1 and its Reversal by PARG
Project accession: PXD048274
Project DOI: Not applicable
Reviewer account details:
Username: reviewer_pxd048274@ebi.ac.uk
Password: hloYS5Mj
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	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 statistical methods were used to determine sample size. For immunofluorescence experiments, around 100 cells per sample were imaged to achieve representative sampling. Two to four independent replicates were performed for each experiment to confirm reproducibility according to common practices in the field (2-4 .biological replicates).
Data exclusions	No data were excluded.
Replication	Two to four independent replicates were performed for each experiment to confirm reproducibility, all observations were found to be reproducible.
Randomization	Samples were not divided into experimental groups, all replicates for all individual experiments were simultaneously prepared and processed.
Blinding	All samples related to the same experiment were handled simultaneously. During handling, samples were clearly labeled (and thus not blinded), but were however processed in random order to avoid bias. During MS data acquisition samples were clearly labeled and thus not blinded. All data analysis was performed with unbiased software in an unsupervised manner and all samples of the same experiment were analysed in exactly the same manner.

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 following primary antibodies were used for immunoblotting: Anti-H3 polyclonal antibody Cell Signaling Technology Cat# 9715S, Anti-PARP1 polyclonal antibody Abcam Cat# ab32138, Anti-GAPDH monoclonal antibody Millipore Cat# CB1001, anti-GFP antibody Takara Cat#632381, Anti-PARG monoclonal antibody Cell Signaling Technology Cat#66564,

Anti-Ubiquitin monoclonal antibody Cell Signaling Technology Cat# 3936S, Streptavidin (anti-biotin) Cell Signaling Technology Cat#3999, Anti-mono/poly-ADP-ribose antibody (E6F6A) Cell Signaling Technology Cat# 83732, Anti-Poly-ADP-ribose binding reagent (MABE1031) Millipore Cat# MABE1031, Anti-H3S10-ADP-ribose AbD33644 Bio-Rad Cat#TZA022, Anti-PARP1S499-ADP-ribose AbD34251 Bio-Rad Cat#TZA022, Anti-Mono-ADP-ribose AbD33205 Bio-Rad Cat#TZA021, Anti-Mono-ADP-ribose AbD43647 Bio-Rad Cat#TZA020.

The following secondary antibodies were used: Anti-mouse IgG HRP-conjugated secondary Amersham Cat# NA931V, Anti-rabbit IgG HRP-conjugated secondary Merck Cat# GENA934-1ML.

, Anti-Mouse Alexa-Fluor 594-conjugated goat secondary Invitrogen Cat# A11005

Validation

All commercial antibodies were validated by manufacturers for the use at least in immunoblotting and immunofluorescence. Anti-H3 polyclonal antibody was validated by the manufacturer for the use in western blotting on a variety of cell lines (https://www.cellsignal.com/products/primary-antibodies/histone-h3-antibody/9715). Anti-PARP1 polyclonal antibody was validated by the manufacturer for use in western blotting and reacts with human samples (https://www.abcam.com/en-de/products/primary-antibodies/parp1-antibody-e102-ab32138#), and additionally validated by us with PARP1KO cells (Bonfiglio et al., Cell, 2020). Anti-GAPDH is validated by the manufacturer for the use in western blotting in human samples (https://www.merckmillipore.com/DE/de/product/Anti-GAPDH-Mouse-mAb-6C5,EMD_BIO-CB1001?ReferrerURL=https%3A%2F%2Fwww.google.com%2F). Anti-GFP antibody is validated by Takara for the use in western blotting (https://www.takarabio.com/documents/Certificate%20of%20Analysis/632380/632380-632381-070313.pdf). Anti-PARG was validated by the manufacturer for the use in western blotting on a variety of human cell lines (https://www.cellsignal.com/products/primary-antibodies/parg-d4e6x-rabbit-mab/66564). Anti-ubiquitin was validated by the manufacturer for the use in western blotting in a variety of human cell lines. Ubiquitin (P4D1) Mouse mAb detects ubiquitin, polyubiquitin and ubiquitinated proteins. This antibody may cross-react with recombinant NEDD8 (https://www.cellsignal.com/products/primary-antibodies/ubiquitin-p4d1-mouse-mab/3936). Anti-biotin reagent is streptavidin (a protein with very high affinity for its natural ligand, biotin) conjugated with HRP and has been validated by the manufacturer for the use in western blotting (https://www.cellsignal.com/products/wb-ip-reagents/streptavidin-hrp/3999). Anti-mono/poly antibody E6F6A has been validated by the manufacturer for the use in western blotting (https://www.cellsignal.com/product/productDetail.jsp?productId=83732). Since our use of this antibody in this study, the antibody has been discontinued and replaced with D9P7Z, which we have not used. Anti poly-ADPr binding reagent (MABE1031) was validated for western blotting by the Kraus lab (Gibson et al., Biochemistry, 2017). The anti-mono-ADPr antibodies AbD33644, AbD34251, AbD43647 and AbD33205 were extensively validated in previous published papers for immunoblotting and immunofluorescence, among other applications (Bonfiglio et al., Cell, 2020; Longarini et al., Mol Cell, 2023).

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	U2OS WT cell lines were obtained from ATCC. HPF1KO U2OS cells were generously provided by Ivan Ahel (University of Oxford). PARGKO U2OS cells were generously provided by Roderick J. O'Sullivan (University of Pittsburgh).
Authentication	U2OS cell lines were obtained, authenticated by STR profiling and confirmed mycoplasma free by ATCC cell line authentication services.
Mycoplasma contamination	Cells were routinely tested for mycoplasma contamination and confirmed mycoplasma negative.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines were used.

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A