nature portfolio

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Last updated by author(s): Apr 20, 2026

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	*Provide a description of all commercial, open source and custom code used to collect the data in this study, specifying the version used OR state that no software was used.*
Data analysis	Image analysis: ImageJ Fiji x64 v1.5 Representation and analysis of AlphaFold stucture:UCSF ChimeraX v1.9 Statistical analysis: Graphpad prism v9.1, Excel (Microsoft 365) Vmax and Km:drc package in R Gene ontology analysis: Metascape V3.5 (http://https://metascape.org/) RNA-seq analysis: STAR v2.5.3a, HTSeq-count v0.6.1p1, DESeq2 v1.16.1 WGS analysis: FASTQC v0.11.7; BWA-MEM software v0.7.12; samtools v1.9; GATK Mutect2 v4.0.10.1; SigProfilerMatrixGenerator v1.0

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

RNA-seq and WGS datasets for this study have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under accession number PRJEB110854 (https://www.ebi.ac.uk/ena/browser/view/PRJEB110854). Cell lines and reagents used in this study are available upon request from the corresponding author (patricia.kannouche@gustaveroussy.fr).

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	Most of in-vitro cell biology (IP, WB, IF cell viability) experiments were performed with at least 2 biological replicates or more if indicated. Sample sizes were not determined by statistical power analysis but chosen according to standard practices in the field. For immunoblots or IF, one representative experiment was presented. For in-vivo experiments, the number of mice was calculated according to statistical power analysis and consideration of standard attrition rate, yielding N=6 mice per group (Kaplan-Meier survival).
Data exclusions	N/A
Replication	The number of replicates in each experiment is indicated in the figure legends and/or methods.
Randomization	N/A
Blinding	Researchers were not blinded during data collection and analysis as the study design included appropriate controls and did not require blinding. Data collection and analysis of different conditions were performed at the same time and using identical methods. Cell imaging was performed automatically without bias between different samples.

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

Flag-M2 (F1804 Sigma-Aldrich 1:2000);
GFP (ab290 Abcam 1:2000);
Rev3 Nter (GTX02533 GeneTex 1:500);
MAD2L2 (ab180579 Abcam 1:1000);
TASP1 (16739-1-AP Proteintech 1:500);
p-KAP1(S824) (A300-767A-M Bethyl 1:1000);
KAP1 (ab10483 Abcam 1:2000);
p-CHK1(S345) (2348 Cell Signaling 1:1000);
CHK1 (sc8408 Santa Cruz 1:1000);
p-RPA32(S33) (A300-264A Bethyl 1:5000);
RPA32 (A300-244A Bethyl 1:5000);
p125 (sc8797 Santa Cruz 1:2000);
α-actin (A5441 Sigma-Aldrich 1:10000);
b-catenin (610153 BD Transduct 1:10000).
53BP1 (ab21083 Abcam 1:500)
RAD51 (PC130 Millipore 1:1000)
γH2AX (05-636 Millipore 1:500)
MCM2 (ab4461 Abcam 1:5000)
H4 (93269 Active Motif 1:1000)
GAPDH (ab9484 Abcam 1:2000)

Secondary antibodies
Polyclonal Goat anti-mouse-HRP, DAKO P0447 1:5000
Polyclonal Goat anti-rabbit-HRP, DAKO P0448 1:5000
Polyclonal Donkey anti-goat-HRP; Promega V8051 1:5000
anti-rabbit IgG Alexa Fluor®488 (Invitrogen cat.no. A-21206), 1:1000
anti-mouse IgG Alexa Fluor®488 (Invitrogen cat.no. A-21202), 1:1000
anti-rabbit IgG Alexa Fluor®594 (Invitrogen cat.no. A-11005), 1:1000
anti-mouse IgG Alexa Fluor®594 (Invitrogen cat.no. A-11012), 1:1000

Validation

All antibodies used in this study are commercial and validated by the manufacturers. In addition, all antibodies produced the expected band size and/or subcellular localization and were used in several previous publications. Antibodies against damage-specific phosphorylations (e.g. phosphorylation of RPA, H2A.X, CHK1) were validated by the induction of expected bands/foci following specific types of stress.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

Most of experiments used SV40-transformed MEF Flag-REV3L as parental cell line which has been previously described (PMID: 34533226, 22319213)
We also used:
SV40 MEF Rev3l+/+ (PMID: 16397225) and MEF wt (PMID: 11701123)
human osteosarcoma cell line U2OS (BT-B-96) and HEK293T (CRL-3216) were obtained from ATCC.
Primary human fibroblasts (405-VI) and SV40-immortalized MRC5-VI were kindly provided by Alain Sarasin (GR, Villejuif).

Authentication

None of the cell lines used in this study were authenticated

Mycoplasma contamination

Cells were regularly tested (every 6-8 weeks) for Mycoplasma contamination using MycoStrip kit (Invivogen, cat. #rep-mysnc-100).

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used in this study

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	The study used C57BL/6J mice harboring mutation in Rev3l gene (Rev3lDG-AA/DG-AA). The mice were generated in LEAT platform (Imagine institute, Paris). Each littermate was genotyped. For genetic crosses, Rev3lflag/flag knock-in mice (PMID: 23386725)and heterozygous Rev3l+/– mice (PMID: 11050392), were bred with Rev3lDG-AA/DG-AA mice as required. All mice were housed in the Gustave Roussy PFEP facility in individually ventilated cages under specific pathogen-free conditions and under standard laboratory conditions with a 12 h light/12 h dark cycle, at a controlled temperature (22 ± 2 °C) and humidity (50 -60%). Routine pathogen screening was performed.
Wild animals	N/A
Reporting on sex	Sex was not considered in the study design and we did not collect sex-specific observations.
Field-collected samples	N/A
Ethics oversight	All experimental procedures were conducted in accordance with French government and institutional animal welfare regulations, and approved by the French Animal Experimentation Ethics Committee No. 26 (protocol #02005.02, APAFIS #33215-202109241556796 v3).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

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