

Reporting Summary

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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                                                                                                                                                                                                                                                                                      |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> The exact sample size ( <i>n</i> ) for each experimental group/condition, given as a discrete number and unit of measurement                                                                                                                               |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly                                                                                                                                    |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided<br><i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>                                                               |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A description of all covariates tested                                                                                                                                                                                                                     |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons                                                                                                                                        |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> 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) |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i> ) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted<br><i>Give P values as exact values whenever suitable.</i>                     |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings                                                                                                                                                           |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes                                                                                                                                     |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i> ), 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 | FCM: Cell Sorter Software ver2.1.6<br>Simple Western: Compass SW software v7.0.0                                                                                   |
| Data analysis   | Statistical analysis: Prism v10 (GraphPad Software)<br>Image processing: ImageJ v1.53a (NIH; <a href="https://imagej.nih.gov/ij/">https://imagej.nih.gov/ij/</a> ) |

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](#)

The raw deep-sequencing reads generated in this study have been deposited in the NCBI Sequence Read Archive (PRJNA1390995). All other data supporting the findings of this study have been deposited in Zenodo (DOI: 10.5281/zenodo.17982883). Uncropped PCR and blot images are provided as Supplementary

Information. The gating strategies for all flow cytometry plots are also provided in the Supplementary Information. Newly generated plasmids and all other materials are available from the corresponding author upon reasonable request. All data supporting the findings of this study, including uncropped PCR and blot images as well as numerical source data for graphs, are provided in Supplementary Data 1. Additional information is available from the corresponding author upon reasonable request.

## 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

Reporting on race, ethnicity, or other socially relevant groupings

Population characteristics

Recruitment

Ethics oversight

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](https://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

Data exclusions

Replication

Randomization

Blinding

## 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                                 | <input type="checkbox"/>            | Involved in the study         |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | Antibodies                    |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | Eukaryotic cell lines         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | Palaeontology and archaeology |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | Animals and other organisms   |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | Clinical data                 |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | Dual use research of concern  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | Plants                        |

### Methods

|                                     |                                     |                        |
|-------------------------------------|-------------------------------------|------------------------|
| n/a                                 | <input type="checkbox"/>            | Involved in the study  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | ChIP-seq               |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | Flow cytometry         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | MRI-based neuroimaging |

## Antibodies

### Antibodies used

ATR (E1S3S) Rabbit mAb Cell signaling 1394  
 Phospho-ATR (Ser428) Antibody Cell signaling 2853  
 ATM (D2E2) Rabbit mAb Cell signaling 2873  
 Anti-ATM (phospho S1987) Abcam ab315019  
 Chk1 (2G1D5) Mouse mAb Cell signaling 2360  
 Human/Mouse/Rat Phospho-Chk1 (S317) Antibody RnD systems AF2054  
 NRF2 (D1Z9C) XP® Rabbit mAb Cell signaling 12721  
 p53 (1C12) Mouse mAb Cell signaling 2524  
 Phospho-p53 (Ser15) Antibody Cell signaling 9284  
 Caspase-3 Antibody Cell signaling 9662  
 Cleaved Caspase-3 (Asp175) Antibody Cell signaling 9661  
 DNA-PKcs Polyclonal antibody Proteintech 28534-1-AP  
 Phospho-DNA-PK (Thr2609) Polyclonal Antibody Invitrogen PA5-105749  
 β-Tubulin Antibody Cell signaling 2146  
 GAPDH Antibody (6C5) Santa Cruz sc-32233  
 Peroxidase AffiniPure Donkey Anti-Mouse IgG (H+L) Jackson Immuno Research Laboratories, Inc. 715-035-150  
 Peroxidase AffiniPure Donkey Anti- Rabbit IgG (H+L) Jackson Immuno Research Laboratories, Inc. 715-035-152  
 Alexa Fluor® 647 Annexin V BioLegend 640912

### Validation

All primary antibodies were validated by the manufacturer for the indicated applications (Western blot, immunofluorescence).

## Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

### Cell line source(s)

Mouse embryonic stem cells (male; C57BL/6J background) were kindly provided by Professor Takeshi Yagi at Osaka University.  
 HEK293T cells (female) were obtained from ATCC.

### Authentication

None of the cell lines used were formally authenticated.

### Mycoplasma contamination

Cell lines were not tested for mycoplasma contamination.

### Commonly misidentified lines (See [ICLAC](#) register)

No commonly misidentified or cross-contaminated cell lines were used in this study.

## Plants

### Seed stocks

This study did not involve any plant materials.

### Novel plant genotypes

This study did not involve any plant materials.

### Authentication

This study did not involve any plant materials.

## 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

For reporter ES cells, cells were washed with PBS(−) and detached using 1× TrypLE™ Express solution. After incubation for 5 min at 37 °C, cells were collected in DMEM containing 10% FBS. The collected cells were centrifuged at 300 × g for 3 min. Cell pellets were washed once with PBS(−) and resuspended in PBS(−) containing 7-AAD for viability analysis. Cells were filtered

through a cell strainer and kept on ice prior to flow cytometric analysis.

For HEK293T, cells were washed with PBS(–) and detached using 1× TrypLE™ Express solution. After incubation for 5 min at 37 °C, cells were collected in DMEM containing 10% FBS. The collected cells were centrifuged at 300 × g for 3 min. Cell pellets were washed once with PBS(–) and resuspended in PBS(–) containing DAPI for viability analysis. Cells were filtered through a cell strainer and kept on ice prior to flow cytometric analysis.

For mouse MSCs, cells were washed with PBS(–) and detached using 1× TrypLE™ Express solution. After incubation for 5 min at 37 °C, cells were collected in DMEM containing 10% FBS. The collected cells were centrifuged at 300 × g for 3 min. Cell pellets were washed once with PBS(–) and resuspended in PBS(–) containing DAPI for viability analysis. Cells were filtered through a cell strainer and kept on ice prior to flow cytometric analysis.

For apoptosis assay, cells were washed with PBS(–) and detached using 1× TrypLE™ Express solution. After incubation for 5 min at 37 °C, cells were collected in DMEM containing 10% FBS. The collected cells were centrifuged at 300 × g for 3 min. Cell pellets were washed once with PBS(–) and stained with 200 µL of Zombie NIR for 15 min at room temperature, followed by incubation in 200 µL of Annexin V Binding Buffer containing Alexa Fluor 647–Annexin V 10 µL, for 15 min at room temperature. Cells were filtered through a cell strainer and kept on ice prior to flow cytometric analysis.

Instrument

SH800

Software

Cell Sorter Software

Cell population abundance

For each sample, between 10,000 and 30,000 7-AAD–negative (live) cell events were recorded and analyzed on the SH800 cell sorter.

Gating strategy

For reporter ES cells, cells were first gated on FSC-A and SSC-A to exclude debris and select the main cell population. Single cells were then identified by gating on FSC-W and FSC-H. Live cells were defined as 7-AAD<sup>–</sup> events. Within the live singlet population, cells were separated into TagBFP<sup>–</sup> and TagBFP<sup>+</sup> fractions; the TagBFP-negative fraction was defined as DSB cells. Reporter outcomes were then quantified within the live cell gate based on mEGFP and mCherry fluorescence: mEGFP<sup>+</sup>/mCherry<sup>+</sup> cells were classified as knock-in, whereas mEGFP<sup>+</sup>/mCherry<sup>–</sup> cells were classified as EJ-TI.

For HEK293T, cells were first gated on FSC-A and SSC-A to exclude debris and select the main cell population. Single cells were then identified using FSC-W and FSC-H. Live cells were defined as DAPI<sup>–</sup> events. Within the live singlet population, mEGFP<sup>+</sup> cells were quantified and classified as knock-in cells.

For mouse MSCs, cells were first gated on FSC-A and SSC-A to exclude debris and select the main cell population. Single cells were then identified using FSC-W and FSC-H to remove doublets. Live cells were defined as DAPI<sup>–</sup> events. Within the live singlet population, mEGFP<sup>+</sup> cells were quantified and classified as knock-in cells.

For apoptosis assay, cells were first gated on FSC-A and SSC-A to exclude debris and select the main cell population. Single cells were then identified using FSC-W and FSC-H. Live cells were defined as Zombie NIR<sup>–</sup> events. Apoptotic cells were then quantified within the live cell gate based on Annexin V-AF647 and mCherry fluorescence: Annexin V-AF647<sup>+</sup>/mCherry<sup>+</sup> cells were classified and quantified as apoptotic cells.

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