Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- 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.
- 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.

Software and code

Policy information about availability of computer code

- Data collection: Autoradiograms were imaged with the Typhoon FLA 7000 utilizing the FujiFilm FLA 7000 version 1.12 user interface. Immunoblots were imaged using the Amersham Imager 680.
- Data analysis: Autoradiograms and immunoblots were analyzed using Image J version 1.52.

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 list of figures that have associated raw data.
- A description of any restrictions on data availability.

No datasets were generated or analyzed for this study.

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
Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

**Sample size**
No sample size calculations were performed. All experiments were performed at least twice with a representative result shown. Quantified results were from the number of replicates indicated in the Figure legends.

**Data exclusions**
No data were excluded from the analysis.

**Replication**
All attempts at replication were successful. Experiments were performed at least twice and key results were replicated independently by two and in some cases three different people.

**Randomization**
Randomization was not relevant to this study. For molecular biology studies, common extracts were subject to the indicated treatments. For colony survival cells from a common pool were treated with increasing levels of genotoxic agent.

**Blinding**
For electron microscopy experiments, the scorer was blinded to experimental conditions before counting pre-incision and reversed fork structures.

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

<table>
<thead>
<tr>
<th>n/a</th>
<th>Involved in the study</th>
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<tbody>
<tr>
<td>☑</td>
<td>Antibodies</td>
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<tr>
<td>☑</td>
<td>Eukaryotic cell lines</td>
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<tr>
<td>☑</td>
<td>Palaeontology</td>
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<td>☑</td>
<td>Animals and other organisms</td>
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<tr>
<td>☑</td>
<td>Human research participants</td>
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<td>☑</td>
<td>Clinical data</td>
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</tbody>
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### Methods

<table>
<thead>
<tr>
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<tr>
<td>☑</td>
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<tr>
<td>☑</td>
<td>Flow cytometry</td>
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<tr>
<td>☑</td>
<td>MRI-based neuroimaging</td>
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</tbody>
</table>

### Antibodies

**Antibodies used**
All unique materials are available from commercial suppliers or are available upon request from the authors.

**Validation**
hFANCD2 antibody was validated by western blotting with NALM-6 cell lysate.
FLAG tag antibody manufacturer validated by western blot with mammalian crude cell lysates.
Histidine tag antibody manufacturer validated by western blot with mammalian and non-mammalian crude cell lysates.
Histone H3 antibody manufacturer validated by western blotting with K562, DAE, CTLL-2, BAEC, and C6 cell lysates.
hMCM3 antibody manufacturer validated by western blotting K-562, KNRK, and NIH/3T3 whole cell lysates.
hMCM4 and hMCM5 antibodies manufacturer validated by western blotting RIPA extract of HeLa cells.
hNEIL3 antibody manufacturer validated by positive WB detected in HEK-293 cells, A431 cells, human skin tissue, mouse skin tissue.
Phospho-hCHK1 (ser345) antibody manufacturer validated by Western blot analysis of extracts from COS cells treated with UV or MMS.
hTIMELESS antibody manufacturer validated by western blotting transfected 293T cell lysates.
hUbiquitin antibody manufacturer validated by western blotting with 293T whole cell lysates.
hVinculin antibody manufacturer validated by western blotting on RIPA lysates of human A431 cells, lysates from chicken embryonic fibroblasts (CEF), or mouse 3T3/A31 fibroblasts.

All other antibodies used in this study were validated by western blotting with Xenopus egg extracts.

### Eukaryotic cell lines

**Policy information about** [cell lines](#)

**Cell line source(s)**
HAP1 cells were originally obtained from Horizon Discovery. CH12-F3 murine B lymphoma cells were a gift from M. Neuberger (MRC Laboratory of Molecular Biology) and were originally obtained from T. Honjo (Kyoto University; see Nakamura et al., Int Immunol. 1996).

**Authentication**
All knockouts were confirmed by PCR. Additionally, cell lines were further validated by Western blot (NEIL3 KO) or FANCD2 ubiquitylation assay (FANCL and FANCB KO).
**Mycoplasma contamination**

All cell lines tested negative for mycoplasma contamination using the MycoAlert Mycoplasma Detection Kit (Lonza).

**Commonly misidentified lines**

(See [ICLAC register](#))

No commonly misidentified cell lines were used for this study.

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**Animals and other organisms**

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

<table>
<thead>
<tr>
<th>Laboratory animals</th>
<th>Xenopus laevis purchased from Nasco. Females used for egg collection were aged &gt; 2 years. Males used for sperm chromatin preparation were aged &gt; 1 year.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild animals</td>
<td>This study did not involve wild animals.</td>
</tr>
<tr>
<td>Field-collected samples</td>
<td>This study did not involve samples collected from the field.</td>
</tr>
<tr>
<td>Ethics oversight</td>
<td>All animal work was approved by the Harvard Medical Area Standing Committee on Animals (HMA IACUC Study ID IS00000051-3, approved 10/25/2017). The institution has an approved Animal Welfare Assurance (#A3431-01) from the Office of Laboratory Animal Welfare.</td>
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Note that full information on the approval of the study protocol must also be provided in the manuscript.