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.

- 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       |
|-----------------------|
| Western - LiCor Odyssey System |
| Flow Cytometry - Aurora Cytex |
| Microscopy - Leica SP8 |
| RT-PCR - PCR 7500 Fast Real-Time PCR System (Thermo Fisher) |
| Mass Spec - Orbitrap Fusion |
| BioRad PharosFX imager |

| Data analysis         |
|-----------------------|
| Western - ImageStudio 5.2 |
| Flow Cytometry - FlowJo 10.8.1 and SpectroFlo 2.2.0.4 |
| Microscopy - Lasm 3.5.2, 18963 |
| Statistical Analysis and Graphing - GraphPad Prism 9 |
| Mass Spec - Proteome Discoverer 2.0 |
| Image Lab Software version 5.1.2 |

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.
### Human research participants

Policy information about **studies involving human research participants and Sex and Gender in Research.**

| Reporting on sex and gender | 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.

- [x] 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-list.pdf](https://nature.com/documents/nr-reporting-summary-list.pdf)

### Life sciences study design

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

| Sample size | Sample sizes were chosen based on prior published studies [Daddacha, W. et al. SAMHD1 Promotes DNA End Resection to Facilitate DNA Repair by Homologous Recombination. Cell reports 20, 1921-1935, doi:10.1016/j.celrep.2017.08.008 2017] and in consultation with bio statistical support. |
|-------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Data exclusions | No data was excluded. |
| Replication | All experiments in the study were replicated at least twice (except mass spectrometry which was done once) with consistent results. In general, western analysis and microscopy studies were replicated 2-5 times, DRG assays were repeated 2-3 times, size exclusion chromatography (SEC) and dNTP quantification studies were replicated twice, and ChIP experiments were replicated twice and performed in biological duplicates. EMSA was replicated 5 times. |
| Randomization | Cells in culture were treated with specific conditions/treatments and thus was not random. Analysis was randomized and validated by multiple lab personnel. Since the study does not involve patient or animal work but involves specific treatments of cultured cells and their outcome, randomization is not fully relevant to the study. |
| Blinding | Cells in culture needed to be treated for specific conditions so this could not be blinded; however, analysis was blinded/random and validated by multiple lab personnel. |

### 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

| Method       | Involved in the study |
|--------------|-----------------------|
| Antibodies   |                       |
| Eukaryotic cell lines |                   |
| Palaeontology and archaeology |             |
| Animals and other organisms |             |
| Clinical data |                       |
| Dual use research of concern |               |

Methods

| Method       | Involved in the study |
|--------------|-----------------------|
| ChiP-seq     |                       |
| Flow cytometry |                     |
| MRI-based neuroimaging |          |

Antibodies

| Antibodies used | |
|-----------------|---------------------------------------------------|
| SAMHD1 (Origene # T502024; 1:1000 for Western, 1 µL per 1 mg lysate for IP; and Abcam # ab177462; 1:5000 for Western, 1:100 for IF); IgG (Invitrogen # 10500C and Sigma # N103); Pan acetylated lysine (Cell Signaling Technology # 9441S; 1:1000 for Western, 1 µL per 1 mg lysate for IP); GFP (Santa Cruz Tech # SC966; 1:1000 for Western; and Abcam # ab290; 1:5000 for Western, 1 µg per 2 mg lysate for IP); SirT1 (Abcam # 32441; 1:5000 for Western, 2 µL per 1 mg lysate for IP); SirT2 (ThermoFisher; 1:1000 for Western); SirT6 (Abcam # ab62768; 1:2000 for Western); SirT7 (Abcam # ab67448; 1:1000 for Western); FLAG (Cell Signaling Technology # 23685; 1:1000 for Western; and Santa Cruz Tech # sc-51590; 1:1000 for Western); a-Tubulin (Sigma # T6074; 1:10,000 for Western); GAPDH (Sigma, C6954; 1:2000 for Western); SAMHD1 K354Ac (Pierce, custom made; 1:1000 for Western); H2AX (Cell Signaling Technology # s139; 1:2000 for IF; and Millipore # 05-636; 1:4000 for IF, 1:1000 for Western); BrdU (BD Biosciences # 347580; 1:200 for IF); RPA32 (Santa Cruz Tech # sc-14692; 1:400 for Western); pRPA32 S4/8 (Bethyl # A700-009; 1:1000 for Western); ChIP (Millipore # MABE1060; 1:1000 for Western); RAD50 (Abcam # ab228935; 1:500 for Western); MRE11 (Abcam # ab30725; 1:1000 for Western); NBS1 (Abcam # ab23996; 1:2000 for Western); H2AX (Bethyl # A300-082a; 1:800 for Western); p-Chk2 (Cell Signaling Technology # 2661; 1:100 for IF); GST (Santa Cruz Tech # sc-138; 1:1000 for Western). Secondary antibodies used for Western (at 1:10,000) are: donkey anti-rabbit IR Dye 800 (Lico Biosciences #926-32213); donkey anti-rabbit IR Dye 680 (Lico Biosciences # 926-68023); donkey anti-mouse IR Dye 800 (Lico Biosciences #926-32213); donkey anti-mouse IR Dye 680 (Lico Biosciences # 926-68022); Streptavidin-conjugated IR Dye 800 (Lico Biosciences #926-32290). Secondary antibodies used for IF (at 1:1000) are: goat anti-mouse Alexa Fluor 555 (Invitrogen # A21424); goat anti-rabbit Alexa Fluor 647 (Invitrogen # A21244); goat anti-rabbit Alexa Fluor 488 (Invitrogen # A11034). |

Validation

All commercial antibodies have been validated by published studies [see below for PubMed PMID] or on the manufacturer’s site (link provided below, where required). Custom site-specific anti-acetyl SAMHD1 K354 antibody has been validated in Figure 2.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s) HEK293T, HCT116, HeLa and U20S mammalian cell lines were purchased from American Type Culture Collection (ATCC, Manassas, VA). U20S-235 mCherry-LacI-FokI cell line were provided by Dr. Roger Greenberg and U20S-DR-GFP cell line were obtained from Dr. Jeremy Stark. AsiSI ER-U20S (DNA DSB inducible via AsiSI) cells were provided by Dr. Gaelle Legube.

Authentication HEK293T, HCT116, HeLa and U20S mammalian cell lines were authenticated by ATCC via STR testing; Authenticated U20S
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

U2OS-DR-GFP cells expressing GFP (and RFP where applicable) were harvested, washed with PBS twice and resuspended in 200 µl PBS. Samples were subjected to flow cytometry.

Instrument

Aurora Cytek

Software

SpectroFlo software was used to conduct the flow cytometry experiments in Aurora Cytek and FlowJo software was subsequently used to analyze the data.

Cell population abundance

RFP population ranged between 40-60%, indicating transfection efficiency of the DR-GFP U2OS cells. Within the RFP population, GFP positive cells ranged from 1-8% (when there is homologous recombination present).

Gating strategy

Samples were first gated for % cells using FSC/SSC plots and then the cells were gated for RFP positive cells. Within the RFP positive cells, cells were gated for GFP population as an output for HR efficiency.

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