

## 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 | We used commercial software, available as a package with the respective instrument, for data collection. This includes Typhoon FLA 9000 capture software (GE Healthcare, Version 1.0), Zen capture software (Zeiss, Version 2.3 blue edition), Tanon MP software (Tanon, Version 1.0). XL-MS data were collected using Q Exactive Orbitrap mass spectrometer (Thermo Scientific). The data was demultiplexed using bcl2fastq v2.20 software from Illumina.                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| Data analysis   | Only commercial or publicly available software was used for data analysis. This includes image J (NIH, Version 1.53k) for the analysis of gel data; graphs and numerical data (including statistics/error bars) was analyzed and plotted by Prim (GraphPad, Version 10.0); Peptides containing the isopeptide bonds were identified using pLink (Version 2.0). END-seq data were analyzed bowtie (Version 1.2.1.1), samtools (Version 1.9), bedtools (v2.25.0). Sequence Alignment of SPO11 and TOP6BL were conducted using Clustal W within the JalView software (Version 2.11.4.1) and were manually refined based on structural alignment performed by Foldseek on the web ( <a href="https://search.foldseek.com/search">search.foldseek.com/search</a> ). The construct sequence of SPO11 and TOP6BL were exported from snapgene software (Version 8.0, <a href="http://www.snapgene.com">www.snapgene.com</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 cross-linking mass spectrometry raw data and peak search results generated in this study have been deposited to the ProteomeXchange Consortium (<https://proteomecentral.proteomexchange.org>) via the iProX partner repository with the dataset identifier PXD058354. The END-seq data generated in this study have been deposited to the Gene Expression Omnibus (<https://www.ncbi.nlm.nih.gov/geo>, GEO accession: GSE281557). The mouse reference genome was used for END-seq (GRCm38 - mm10).

## 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://www.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 &amp; experimental systems

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

## Antibodies

|                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|-----------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Antibodies used | <p>For immunofluorescence, primary antibodies used were mouse anti-SYCP3 (1:200 dilution; Santa Cruz, sc-74568), rabbit anti-SYCP1 (1:200, Abcam, ab15090), rabbit anti-RPA2 (1:200 dilution; gift from professor Mengcheng Luo, Wuhan University), mouse anti-γH2AX (1:500 dilution; Millipore, 5636) and rabbit anti-MVH (1:200 dilution; abcam, ab13840). Secondary antibodies used were Alexa Fluor® 594 AffiniPure™ Donkey Anti-Rabbit IgG (H+L) (1:500 dilution; Jackson ImmunoResearch, 711-585-152), Alexa Fluor® 594 AffiniPure™ Donkey Anti-Mouse IgG (H+L) (1:500 dilution; Jackson ImmunoResearch, 711-585-151) and Alexa Fluor® 488 AffiniPure™ Donkey Anti-Mouse IgG (H+L) (1:500 dilution; Jackson ImmunoResearch, 711-545-151).</p> <p>For western blot, primary antibodies used were His Tag Mouse Monoclonal Antibody (1:2000, HRP Conjugated, AF2879-50μL, clone name: BC11 Beyotime), Strep II Tag Mouse Monoclonal Antibody (1:2000 dilution; HRP Conjugated, AF2927, clone name: 11D6, Beyotime), Flag Tag Mouse Monoclonal Antibody (1:2000 dilution; HRP Conjugated, AF2855-50μL, clone name: 2E6, Beyotime), MBP Tag Mouse Monoclonal Antibody (1:2000 dilution; HRP Conjugated, AF2915, clone name 1A9, Beyotime).</p>                                                                                                                                                                                                                                                                                                                                                                |
| Validation      | <p>All commercial antibody have been frequently used in previous studies and their specificity for use in mouse is well established. The anti-RPA2 antibody was previously validated in study of Luo laboratory (Wu et al., 2021, DOI: 10.3389/fcell.2021.715733. eCollection 2021.). Validation for anti-SYCP3 antibody is available at <a href="https://www.scbt.com/zh/p/scp-3-antibody-g-3">https://www.scbt.com/zh/p/scp-3-antibody-g-3</a>. Validation for anti-SYCP1 is available at <a href="https://www.abcam.cn/products/primary-antibodies/scp1-antibody-ab15090.html">https://www.abcam.cn/products/primary-antibodies/scp1-antibody-ab15090.html</a>. Validation for anti-MVH is available at <a href="https://www.abcam.cn/products/primary-antibodies/ddx4--mvh-antibody-ab13840.html">https://www.abcam.cn/products/primary-antibodies/ddx4--mvh-antibody-ab13840.html</a>. Validation for His Tag Mouse Monoclonal Antibody is available at <a href="https://www.beyotime.com/product/AF2879-50%CE%BCI.htm">https://www.beyotime.com/product/AF2879-50%CE%BCI.htm</a>. Validation for Strep II Tag Mouse Monoclonal Antibody is available at <a href="https://www.beyotime.com/product/AF2927.htm">https://www.beyotime.com/product/AF2927.htm</a>. Flag Tag Mouse Monoclonal Antibody is available at <a href="https://www.beyotime.com/product/AF2855-50%CE%BCI.htm">https://www.beyotime.com/product/AF2855-50%CE%BCI.htm</a>. MBP Tag Mouse Monoclonal Antibody is available at <a href="https://www.beyotime.com/product/AF2915.htm">https://www.beyotime.com/product/AF2915.htm</a>.</p> |

## Eukaryotic cell lines

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

|                                                                   |                                                                                |
|-------------------------------------------------------------------|--------------------------------------------------------------------------------|
| Cell line source(s)                                               | Expi293F cells were kindly provided by Cell Bank, Chinese Academy of Sciences. |
| Authentication                                                    | Cell lines were not authenticated.                                             |
| Mycoplasma contamination                                          | Cell lines were not verified to be free of mycoplasma contamination.           |
| Commonly misidentified lines (See <a href="#">ICLAC</a> register) | No commonly misidentified species used.                                        |

## 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      | <p>Mice were fed regular rodent chow with ad libitum access water and food in 12-hour light-dark cycle in a temperature and humidity-controlled environment (20-26°C, 30-70% humidity). Euthanasia was by CO2 asphyxiation. The Spo11 D277N mouse line was generated by Shanghai Biomodel Organism Co., Ltd using the CRISPR/Cas9 technology. The donor oligos were designed to change the D277 codon GAT (D) to AAT (N). The resulting point-mutation founders were backcrossed with adult C57BL/6J mice (WT; from Shanghai Biomodel Organism Co., Ltd) to obtain heterozygous mutant animals. Homozygous mutant mice were produced by self fertilization of heterozygous. 12 dpp female mice and 19 dpp male mice were used for phenotypic analysis in this study.</p> |
| Wild animals            | This study did not involve wild animals.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| Reporting on sex        | We separately studied the phenotypes of Spo11 D277N mutations in males and females using the common methods in the field.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| Field-collected samples | The study did not involve samples collected from the field.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| Ethics oversight        | The experiments performed in this study were approved by the Ethics Committee of Center for Excellence in Molecular Cell Science, Chinese Academy of Sciences (2022-088)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |

## Plants

|                       |               |
|-----------------------|---------------|
| Seed stocks           | no applicable |
| Novel plant genotypes | no applicable |
| Authentication        | no applicable |