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Reporting Summary

Nature Research 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 Research policies, see Authors & Referees 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	Image and movie capture: SharpCap: https://docs.sharpcap.co.uk/2.9/ Leica Application Suite X (LAS X): https://www.leica-microsystems.com/ Sperm motility data collection (data analysis see below): Hamilton Thorne IVOS II: https://www.hamiltonthorne.com/ High-throughput sequencing: Illumina NextSeq 500 System: https://www.illumina.com/
Data analysis	Image and movie data analysis: FIJI (ImageJ) v2.0.0-rc.69/1.52p: https://fiji.sc/ Statistics and graph generation: RStudio v1.2.5042: https://www.rstudio.com/ ggplot2 v3.1.0: https://ggplot2.tidyverse.org/ Igor Pro v7.08: https://www.wavemetrics.com/products/igorpro Sperm motility data analysis: CASAnova: https://uncnri.org/CASAnova/ High-throughput sequencing data analysis: umitools: https://github.com/weng-lab/umitools piPipes: https://github.com/bowhan/piPipes BEDtools v2.18: https://github.com/arq5x/bedtools2

SAMtools-1.10: http://www.htslib.org/
Bowtie 2 v2.4.1: http://bowtie-bio.sourceforge.net/bowtie2/index.shtml
DESeq2 v3.11: https://bioconductor.org/packages/release/bioc/html/DESeq2.html
HTSeq v0.11.2: https://htseq.readthedocs.io/en/release_0.11.1/
STAR v2.7: https://github.com/alexdobin/STAR
BWA v0.7.17: https://github.com/lh3/bwa

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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

All sequencing data are available through the NCBI Sequence Read Archive using accession number PRJNA480354.

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	The investigators reached and confirmed all the conclusions in this manuscript using data collected from 3–11 individual, age-matched (2–4 month old mice and two independently generated mutant lines for all major experiments. The sample size was determined by the standard in the field that enables statistical meaningful comparison for the given experiment. We aimed to exceed the standard sample size unless the availability of data was restricted by the mouse phenotype defects (e.g. male fertility defects in pi6 mutants rendered fewer data points than the controls in Fig 2a.)
Data exclusions	No data are excluded.
Replication	We validated the reproducibility of our data using (1) 3–11 individual animals of identical genetic background (C57BL/6) and a similar age (2–4 month) and (2) two mutant mouse lines independently generated and validated by different CRISPR guides. All observations in this study, including the mutant phenotype and molecular changes, are reproducible in both mutant mouse lines.
Randomization	Animals used in this study were grouped based entirely on their genotypes, involving no subjective animal allocation.
Blinding	The investigators are blinded in experiments for which the order of data collection does not affect the outcome, such as measuring sperm motility and fertilization capability (i.e. in vitro fertilization and intracytoplasmic injection). For sequencing experiments, blinding is not ideal or relevant. Libraries from wild-type and mutant animals are sequenced in the same run when possible to minimize artificial differences created by separate sequencing runs, which requires prior knowledge of the sample identities.

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
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	For meiotic chromosome immunofluorescence: Rabbit polyclonal anti-SCP1 antibody: Abcam Cat# ab15087; Lot# gr215384-18; dilution 1:1000 Mouse monoclonal anti-SCP3 antibody: Abcam Cat# ab97672; Lot# gr255063-1; dilution 1:1000
Validation	Anti-SCP1 antibodies for mouse SYCP1 proteins (ab15087): Data for validation of the antibodies specificity for immunofluorescence was reported on the manufacturer's (Abcam) website: https://www.abcam.com/scp1-antibody-ab15087.html. In our hands, the immunostaining pattern using the antibodies (Supplementary Fig. 2d) is consistent with that from the manufacturer and 20 other publications. Anti-SCP3 antibodies for mouse SYCP3 proteins (ab97672): Data for validation of the antibodies specificity for immunofluorescence was reported on the manufacturer's (Abcam) website: https://www.abcam.com/scp3-antibody-cor-10g117-ab97672.html. In our hands, the immunostaining pattern using the antibodies (Supplementary Fig. 2d) is consistent with that from the manufacturer and 60 other publications.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	NIH/3T3 was purchased from ATCC: https://www.atcc.org/Products/All/CRL-1658.aspx
Authentication	The cell line was tested and authenticated by ATCC using morphology, karyotyping, and PCR.
Mycoplasma contamination	The cell line was tested negative for mycoplasma by ATCC using cultures, DNA staining, and PCR.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell line was used in this study.

Animals and other organisms

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

Laboratory animals	C57BL/6J mice: JAX# 000664; wild-type control for all experiments; 2–4 month old males pi6em1 mice: generated in the C57BL/6J background in this study; 2–4 month old males pi6em2 mice: generated in the C57BL/6J background in this study; 2–4 month old males pi17–/– mice: generated in the C57BL/6J background in this study; 2–4 month old males B6D2F1/J mice: JAX# 100006; oocyte donors for intracytoplasmic injection experiments; 2–4 month old females B6SJLF1/J mice: JAX# 100012; oocyte donors for in vitro fertilization experiments; 2–4 month old females Swiss Webster mice: Taconic; surrogate mothers for embryo transfer; 2–4 month old females
Wild animals	No wild animals were used in this study.
Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	The Umass Medical School (UMMS) Institutional Animal Care and Use Committe (IACUC) approved the animal procedures under protocol D16-00196 on 16 February 2017.

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