nature portfolio

Corresponding author(s): Mingxi Liu,Siyu Liu,Xiaoyu Yang,Jianping Wu,Yuchuan Zhou

Last updated by author(s): May 1, 2025

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	Imaging data were collected using Leica LAS X (v3.7.5) and Zeiss Zen software (for FRAP). Flow cytometry was performed using BD FACSuite (v1.0.6).
Data analysis	Data analysis was performed using GraphPad Prism (v8.3.0) for statistical analysis and graphical representation, FlowJo (v10) for flow cytometry analysis. For metabolomics data analysis, ProteoWizard MSConvert was used to convert raw MS data into MzXML format, and the data was processed with XCMS. Proteome Discoverer (version 2.4) was employed for proteomic analysis.

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

All data supporting the findings of this study are available within the article and its Supplementary Information files or from the corresponding author upon reasonable request. Source data are provided with this paper. The mass spectrometry proteomics data have been deposited in the ProteomeXchange Consortium

via the iProX partner repository under the dataset identifier PXD060989. Additionally, the metabolomics data have been uploaded to MetaboLights and can be accessed under the dataset identifiers MTBLS12397 and MTBLS12402.

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	Only male participants were included in this study. Sex was determined based on self-report and confirmed by clinical examination. No gender-based analysis was performed.
Reporting on race, ethnicity, or other socially relevant groupings	All participants were Han Chinese. Race/ethnicity was self-reported and confirmed through clinical records. No additional socially relevant groupings were assessed.
Population characteristics	The study population consisted of adult males diagnosed with idiopathic asthenozoospermia, as defined by the 5th Edition WHO Laboratory Manual for Human Semen Examination. Exclusion criteria included Klinefelter syndrome, AZF deletions, testicular cancer, varicocele, cryptorchidism, and prior chemotherapy or radiotherapy. Healthy controls were age-matched males with normal semen parameters and a history of fathering at least one child.
Recruitment	Participants were recruited from three hospitals in China: Shanghai General Hospital, Reproductive and Genetic Hospital of CITIC-Xiangya, and the First Affiliated Hospital of Nanjing Medical University. All participants provided written informed consent. Recruitment bias was minimized by applying consistent diagnostic and exclusion criteria.
Ethics oversight	All patients provided written informed consent for participation, and the study was approved by the ethics committees of Shanghai General Hospital, Shanghai Jiao Tong University (Approval No. 2021-SQ-112); the Reproductive and Genetic Hospital of CITIC Xiangya (Approval Nos. LL-SC-2017-025 and LL-SC-2019-034); and the First Affiliated Hospital of Nanjing Medical University (Approval No. 2019-SR-472).

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

Life sciences study design

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

Sample size	For the mouse experiments, the sample size was determined based on previous experience and reports for each experiment to ensure sufficient power to detect specific effects. For the human study, we included 800 men with idiopathic infertility and 159 fertile controls. This sample size was determined based on patient availability during the recruitment period and to ensure adequate statistical power for detecting genetic variants associated with male infertility.
Data exclusions	No exclusion of data was made.
Replication	All experimental findings were reproduced in multiple independent experiments.The number of independent experiments or biological replicates is indicated in the figure legends.
Randomization	For animal studies, mice were grouped based on their genotype rather than being randomly assigned to experimental groups. For human studies, randomization was not applied due to study design and ethical considerations. Participants were grouped based on their fertility status and genetic variants.
Blinding	For mice, blinding was not applicable to this study as mice were grouped based on genotype. For genetic and clinical data analysis of human subjects, blinding was not possible due to the nature of the data, but all analyses followed pre-established protocols to minimize bias.

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 and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☐	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used

1. Anti-TEX44 (prepared in-house, 1:2000 for WB, 1:200 for IF, 1:100 for IP);
2. Anti-FLAG (MBL, PM020, 1:200 for IP);
3. Anti-FLAG (Sigma-Aldrich, F3165, 1:5000 for WB, 1:1000 for IF);
4. Anti-HA (MBL, m180-3, 1:200 for IP);
5. Anti-HA (Sigma-Aldrich, H6908, 1:5000 for WB, 1:1000 for IF);
6. Anti-EGFP (abcam, ab13970, 1:1000 for IF);
7. Anti-TOMM20 (abcam, ab283317, 1:500 for IF);
8. Anti-CPT1B (abcam, ab134988, 1:2000 for WB);
9. Anti-CPT1B (Proteintech, 22170-1-AP, 1:200 for IF);
10. Anti-GPX4 (Proteintech, 67763-1-Ig, 1:200 for IF);
11. Anti-CDH2 (Proteintech, 66219-1-Ig, 1:200 for IF);
12. Anti-SEPT4 (Proteintech, 12476-1-AP, 1:400 for IF);
13. Anti-β-tubulin (ABclonal, AC021, 1:5000 for WB);
14. Anti-β-Actin (Santa, sc-47778, 1:1000 for WB).

Validation

Anti-TEX44 antibody was validated by Western Blot in Tex44 knockout mouse testes, showing complete absence of the expected band. All other commercial antibodies used in this study were validated by the manufacturers for their respective applications. Information regarding antibody validation can be found on the supplier's website.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	HEK293T, HELA cells were obtained from the American Type Culture Collection. Expi293F cells were obtained from the National Collection of Authenticated Cell Cultures.
Authentication	None of the cell lines used were authenticated in the laboratory.
Mycoplasma contamination	All cell lines were tested for mycoplasma contamination and confirmed to be free of contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

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	The study involved laboratory C57BL/6 male mice aged 8-12 weeks.
Wild animals	The study did not involve wild animals.
Reporting on sex	The study included only male mice. Sex was determined based on physical examination for the mice.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	Animal experiments were approved by the Ethics Committee of Nanjing Medical University (Approval No. IACUC-1810020) and conducted in accordance with institutional guidelines for the care and use of laboratory animals.

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

Plants

Seed stocks	No plants were used in this study.
Novel plant genotypes	No plants were used in this study.
Authentication	No plants were used in this study.

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	Caudal epididymides were dissected from euthanized C57BL/6 mice and immersed in 1 mL of modified human tubal fluid. The tissue was finely minced using scissors and incubated at 37°C in a 5% CO_2 incubator for 5 minutes to facilitate spermatozoa release. Spermatozoa in HTF medium were treated with various concentrations of palmitoyl-CoA (0, 10 nM, 100 nM, and 1 µM), while the final concentration of L-carnitine was maintained at 1 mM across all samples. After treatment, sperm were stained with 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) to assess intracellular ROS levels. The staining was carried out at 37°C for 20 minutes in a light-protected environment, and during the last 5 minutes, Hoechst was added to counterstain the nuclei.
Instrument	Flow cytometry analysis was performed using a BD FACSVerse flow cytometer (BD Biosciences).
Software	Data analysis were conducted with FlowJo V10 software.
Cell population abundance	Each sample consisted of an analysis of 10000 spermatozoa.
Gating strategy	For ROS detection, spermatozoa were first gated by FSC/SSC to exclude debris. The Hoechst-positive spermatozoa population was then analyzed for DCFH fluorescence.

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