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Corresponding author(s): Maria Agustina Battistone

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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	All data were collected using previously published methods or commercially available kits. The Methods section provides descriptions of each technique and references. Confocal images using a Nikon Yokagawa CSU-W spinning disc/ SoRa super-resolution system for imaging, with Nikon Elements software (5.4). The H&E-stained slides were digitally scanned using a NanoZoomer 2.0RS digital scanner (Hamamatsu, Japan). Flow cytometry data were collected using the BD FACSDiva software and SpectroFlo. Luminescence detection was performed using the G:BOX MINI-9 imaging system and GeneTools Image Analysis Software. Proteomics samples were run on a Velos Orbitrap Elite (Thermo Scientific). Optical density readings were measured using a microplate reader (Promega GloMax Discover, Madison, WI) at 450 nm. All experimental details and observations were systematically recorded in LabArchives for secure documentation, and Microsoft Excel was employed for data compilation.
Data analysis	Statistical analysis: Prism 10.4.0, Flow cytometry data: Flowjo 10.10.0, Sperm parameters: Hamilton Thorne's CASA version 14 (Hamilton Thorne Inc., Beverly, MA), Confocal and fluorescence images: Nikon Elements software (5.4) and Fiji software (2.1.0), Proteomics data: Peptide sequences (and hence protein identity) were determined by matching protein databases with the acquired fragmentation pattern using SEQUEST (Thermo Fisher Scientific), Proteomic datasets of each group were compared using VENNY 2.1.

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 mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD058247: Reviewer access details
Log in to the PRIDE website using the following details:
Project accession: PXD058247
Token: iRvdgJ9jwt2o
Alternatively, reviewers can access the dataset by logging in to the PRIDE website using the following account details:
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Project accession: PXD062944
Project DOI: 10.6019/PXD062944
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Log in to the PRIDE website using the following details:
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Alternatively, reviewer can access the dataset by logging in to the PRIDE website using the following account details:
Username: reviewer_pxd062944@ebi.ac.uk
Password: ealLxAsgJAjn

All data supporting the findings of this study are available within the paper and its Supplementary Information.

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	Semen samples are from men. No data on gender.
Reporting on race, ethnicity, or other socially relevant groupings	Human semen samples from patients undergoing routine semen analysis at the assisted reproduction unit of Cruces and Galdakao University Hospitals of Bizkaia (Spain) were utilized independently of their race, ethnicity or social status.
Population characteristics	A total of 36 human semen samples from patients aged 25-50 years were utilized in this study. All samples were classified as normozoospermic (n=26) or asthenozoospermic (n=12) according to the World Health Organization guidelines. In normozoospermic group only patients with female factor infertility were included, whereas asthenozoospermic group comprised patients without known cause for their infertility condition.
Recruitment	Human semen samples were obtained between January 2022 and July 2023. Ejaculates were collected by masturbation after 3-5 days of sexual abstinence. After routine semen analysis, sperm fraction and seminal plasma were frozen separately until usage.
Ethics oversight	Human semen samples were obtained from patients undergoing routine semen analysis at the assisted reproduction unit of the Cruces and Galdakao University Hospital of Bizkaia (Spain). All patients gave signed informed consent to the Declaration of Helsinki.

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 all experiments requiring statistical analysis, we aimed to have a minimum of n = 4 biological replicates in order to derive p-values. We noted exact sizes either within the methods section or the figure legends. Sample size selection was based on similar types of experiments in

	literature.
Data exclusions	Data excluded was based on supplementary readouts that were inconsistent with the model, for example, low antibody levels, no pups obtained in the controls (fertility assesment), low sperm count/motility in the controls.
Replication	All measures were at least replicated 3 times. Replications were successful.
Randomization	Allocation was randomly made.
Blinding	Immunofluorescence image quantification was conducted in a blindly manner by 2 investigators using generic labeling.

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

Immunofluorescence: rat anti-mouse-F4/80 (5), chicken anti-V-ATPase B1 subunit(5), rabbit anti-AQP9(5), goat anti-mouse-IgA (1mg/mL, 62-6720, Invitrogen), goat anti-mouse-IgG1 (1mg/mL, A10551, Invitrogen), goat anti-mouse-IgG2b (1mg/mL, M32407, Invitrogen), goat anti-mouse-IgG2c (0.8mg/mL, 115-035-208, Jackson ImmunoResearch), goat anti-mouse-IgG3 (M32707, Invitrogen), donkey anti-mouse-IgM (0.8mg/mL, 715-035-020, Jackson ImmunoResearch), rabbit anti-mouse-CD19 (0.2mg/mL: sc-8500-R, Santa Cruz), rat anti-mouse-CD21/CD35 (0.5mg/mL, 14-0211-81, Invitrogen), Armenian Hamster anti-mouse-CD3 (0.5mg/mL, 100303, Bioegend), rat anti-human/mouse-AID (0.5mg/mL, 14-5959-80, Invitrogen), rabbit anti-alpha smooth muscle actin (SMA) (0.2mg/mL, ab5694, Abcam), rabbit anti-mouse-CD31 (ab28364, Abcam), rabbit anti-Ki67 (0.031 mg/mL, MA5-14520, Thermofisher Scientific), rat anti-mouse-BLC6 (25ug/mL, 358512, BioLegend), rat anti-mouse-IgD (0.5mg/mL, 405702, Biolegend), goat anti-mouse-CD20 (0.5mg/mL, sc-7735, Santa Cruz), rat anti-mouse/human PNAD (0.5mg/mL, 120801, BioLegend), Syrian hamster anti-mouse PDPN (0.5mg/mL, ab11936, Abcam, Waltham, MA) and donkey anti-human-IgG (1.5mg/mL, 709-545-149, Jackson ImmunoResearch). The secondary antibodies used were Alexa Fluor 488 goat anti-mouse IgG (20 µg/ml; A-11001, Invitrogen), Alexa Fluor 647 donkey anti-rat IgG (3 µg/ml; 712-606-150, Jackson ImmunoResearch, West Grove, PA), Alexa Fluor 488 donkey anti-mouse IgG (20 µg/ml; A21202, Invitrogen), Alexa Fluor 488 donkey anti-chicken IgY (15 µg/ml; 703-545-155, Jackson ImmunoResearch), Alexa Fluor 647 donkey anti-rabbit IgG (1.5 µg/ml; 711-606-152, Jackson ImmunoResearch), Alexa Fluor 488 goat anti-rabbit IgG (1:800 dilution; 111-545-144, Jackson ImmunoResearch), DyLight 649 goat anti-hamster (STAR104D649, AbD Serotec), FITC donkey anti-human IgG (15 µg/ml; 709-545-149, Jackson ImmunoResearch), Cy3 donkey anti-rabbit IgG (7.5 µg/ml; 711-165-152, Jackson ImmunoResearch),Alexa Fluor 488 donkey anti-goat IgG (1:100 dilution; 705-545-147, Jackson ImmunoResearch), Cy3 donkey anti-rat IgG (1:800 dilution; 712-166-153, Jackson ImmunoResearch), Cy3 donkey anti-goat IgG (1:800 dilution; 705-165-1470, Jackson ImmunoResearch) DyLight 405 Donkey anti-rat IgG (1.4mg/ml; 712-475-150, Jackson ImmunoResearch).

Flow cytometry: anti-mouse antibodies (1:500 dilution) against CD45 Brilliant Violet 711 (0.2 mg/ml; Clone 30-F11, 563709, BD Biosciences, San Jose, CA), CD45 PE-Firetm700 (0.2 mg/ml; Clone 30-F11, 103177, BioLegend), CD45 PE (0.5 mg/ml; Clone 30-F11, 103105, BioLegend), CD45 BUV395 (0.2 mg/ml; Clone 30-F11; 565967, BD Biosciences), Ly6G PE (0.2 mg/ml; Clone 1A8, 551461, BD Biosciences), F4/80 PE/Cyanine7 (0.2 mg/ml; Clone BM8, 123113, BioLegend, San Diego, CA), Ly6C FITC (0.5 mg/ml; Clone AL-21; 553104, BD Biosciences), MHC Class II (I-A/I-E) Alexa Fluor 700 (0.2 mg/ml; Clone M5/114.15.2, 56-5321-82, Thermo Fisher Scientific, Waltham, MA), CD4 PE (0.2 mg/ml; Clone RM4-5, 100511, BioLegend), CD4 BV480 (0.2 mg/ml; Clone RM4-5; 565634, BD Biosciences), CD44 BUV737 (0.2 mg/ml; Clone IM7; 612799, BD Biosciences), CD11b APC/Cyanine7 (0.2 mg/ml; Clone M1/70, 101225, BioLegend), CD1d BUV805 (0.2 mg/ml; Clone IB1; 741965, BD Biosciences), CD19 BV750 (0.2 mg/ml; Clone 1D3; 747332, BD Biosciences), IgD BUV615 (0.2 mg/ml; Clone 217-170, 751474, BD Biosciences), CD138 BV510 (0.2 mg/ml; Clone 281-2, 563192, BD Biosciences), CD3 BV421 (0.2 mg/ml; Clone 17A2, 100227, BioLegend), CD20 PE (0.2 mg/ml; Clone SA275A11, 150409, BioLegend), B220 Pacific Blue (0.5 mg/ml; Clone RA3-6B2, 103230, BioLegend), FAS APC/FireTM810 (0.2 mg/ml; Clone SA367H8, 152623, BioLegend), CD5 Alexa Fluor®594 (0.5 mg/ml; Clone 53-7.3, 100632, BioLegend), GL7 PerCP/Cyanine5.5 (0.2 mg/ml; Clone GL7, 144609, BioLegend), CD8 FITC (0.5 mg/ml; Clone 53-6.7, 100706, BioLegend), CXCR5 BV605 (0.1 mg/ml; Clone L138D7, 145513, BioLegend), CD62L PE/Cyanine5 (0.2 mg/ml; Clone MEL-14, 104410, BioLegend), CD21/CD35 Alexa fluor®647 (0.5 mg/ml; Clone 7E9, 123423, BioLegend), CD38 Alexa Fluor® 700 (0.5 mg/ml; Clone 90, 102741, BioLegend), PD-1 APC/Cyanine7 (0.2 mg/ml; Clone 29F.1A12, 135223, BioLegend), CD23 PerCP (0.1 mg/ml; Clone #011, 50695-R011, SinoBiological Inc.), CD27 PerCP-eFluorTM710 (0.2 mg/ml; Clone LG.7F9, 46-0271-80, Invitrogen). For intranuclear staining of the Foxp3 and intracellular staining of IgM and IgG: (1:100 dilution) anti-Foxp3-APC (0.2 mg/ml; Clone FJK-16s, 17-5773-82, ThermoFisher Scientific), IgM BV786 (0.2 mg/ml; Clone R6-60.2, 564028, BD Biosciences) IgG PE/Cyanine7 (0.2 mg/ml; Clone Poly4053, 405315, BioLegend).

ELISA: horseradish peroxidase (HRP)-anti-mouse IgG (1:5000; 115-035-205, Jackson ImmunoResearch), IgA (1:3000; 62-6720, Invitrogen), IgG1 (1:2000; A10551, Invitrogen), IgG2b (1:5000; M32407, Invitrogen), IgG2c (1:5000; 115-035-208, Jackson

	ImmunoResearch), IgG3 (1:2000; M32707, Invitrogen), IgM (1:5000; 715-035-020, Jackson ImmunoResearch) and human totIgG (1:10000, 709-035-149, Jackson ImmunoResearch) and human IgA (1:10000; 109-035-011, Jackson ImmunoResearch).
Validation	Data provided in the manuscript. All used antibodies are quality control tested by manufacturer.

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	Female C57BL/6-Tg(Foxp3 DTR/EGFP)23.2Spar/Mmjax (Foxp3-DTR mice) and male C57BL/6 WT mice were acquired from Jackson Laboratory and bred at the Massachusetts General Hospital (MGH) animal facility to produce Foxp3-DTR or WT (control) male littermates. Foxp3-DTR transgenic mice express the diphtheria toxin receptor (DTR) fusion protein controlled by the endogenous forkhead box P3 (Foxp3) promoter/enhancer regions. Male mice aged between six to forty weeks were used for all experiments.
Wild animals	N/A
Reporting on sex	This study exclusively utilized male subjects due to their direct relevance to investigating male reproductive health.
Field-collected samples	N/A
Ethics oversight	IACUC protocol 2003N000216

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

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	The human study was approved by The Medicine Research Ethics Committee of the Basque Country, Spain (protocol number: PI2019184).
Study protocol	The study protocol is not publicly available but was made available to all patients prior to study enrollment.
Data collection	All samples and clinical data were collected at the assisted reproduction unit of the Cruces and Galdakao University Hospitals of Bizkaia (Spain) between January 2022 and July 2023.
Outcomes	Human semen samples were obtained from patients undergoing routine semen analysis at the assisted reproduction unit of the Cruces and Galdakao University Hospital of Bizkaia (Spain). Ejaculates were collected by masturbation after 3-5 days of sexual abstinence. All patients gave signed informed consent to the Declaration of Helsinki. Semen parameters and the presence of round cells (RC) were evaluated using the Sperm Class Analyzer® CASA System (Microptics®). After semen analysis, all samples were classified as normozoospermic or asthenozoospermic according to the World Health Organization guidelines .

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

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	The proximal and distal segments of the epididymis and testis were mechanically minced and enzymatically digested for 30 min at 37ºC in RPMI 1640 medium containing collagenase type I (0.5 mg/mL) and collagenase type II (0.5 mg/mL), following established protocols(5). The resulting suspensions were filtered through a 70 µm nylon mesh strainer, rinsed with 2% fetal bovine serum (FBS) in PBS with 2 mM EDTA, and centrifuged for 5 min at 400 g. The pellet was treated with ACK lysing buffer (#A10492-01, Gibco, Grand Island, NY) for 1 min, followed by centrifugation for 5 min at 400 g. Cell suspensions were then incubated for 30 min with the superficial antibodies diluted in 2 % FBS in PBS with BD Horizon Brilliant Stain buffer (563794, BD Biosciences) and TruStain FcX anti mouse CD16/32 (101320, Biolegend). DAPI (62248, ThermoScientific), LIVE/DEAD™ Fixable Blue Dead Cell Stain Kit, for UV excitation (L23105, Invitrogen) or LIVE/DEAD™ Fixable Yellow Dead Cell Stain Kit, for 405 nm excitation (L34967, Invitrogen) were used as viability markers. For intranuclear staining of the Foxp3 and intracellular staining of IgM and IgG, suspensions were incubated fixed for 20 min with 1x Fixation/Permeabilization working solution and washed with 1x Permeabilization Buffer, following the manufacturer's instructions (#00-5523-00, Thermo Fisher Scientific). Fixed cells were then incubated overnight with the antibodies diluted in 1x Permeabilization Buffer (00-5523-00, Thermo Fisher Scientific) containing BD Horizon Brilliant Stain buffer (563794, BD Biosciences). After incubation, cells were washed with 2% FBS in PBS and filtered through a 40 µm cell strainer.
Instrument	Flow cytometry data were collected using the BD FACSAria II flow cytometer (BD Biosciences) or AURORA flow cytometer (Cytek® Aurora System)
Software	Flowjo 10.10.0
Cell population abundance	No sorting was performed
Gating strategy	Events were first gated based on FSC/SSC leaving "small, non-complex" events (debris) and quantitative beads outside. Singlets were gated twice using FSC-A/FSC-H and SSC-A/SSC-H. LIVE and stained cells were gated using negative unstained, FMO controls to set the boundaries.

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