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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	N/A
Data analysis	DESeq2 library (v 1.38.3); R language (v 4.2); bioMart library (v 3.16); (CLC-GW, ver.10.1.1, Qiagen); WGCNA; MIENTURNET; GraphPad Prism 9 (v.9.5.1); Proteome Discoverer (ThermoScientific; v2.5) (PD 2.4); Cytoscape StringApp; MetaboAnalyst 5.0; Whole metaGenome Sequence Assembly pipeline, version 2 (WGSA2); MetaScape; Scalable Precision Medicine Open Knowledge Engine (SPOKE); QuPath (v0.4.3); . Ilastik (v1.4.0); Syglass (v.1.7.2-79); Imaris (v10.0)

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

Data Availability Statement

Fig 1. A,B) Aggregated from NASA OSDR (https://osdr.nasa.gov/bio/)

Fig 2. A; Extended Data Fig 1. A,B) only available per comms from NASA (contact Scott Smith) under specific usage conditions and deidentified in order to preserve astronaut anonymity.

Fig 2. B; Suppl Info 1,2) underlying data from NASA OSDR (https://osdr.nasa.gov/bio/): OSD-462

Fig 2. C; Fig 3. A,B; Fig 4; Extended Data Fig 2,3,5,6,8; Suppl Info 3,4,8,9) underlying datasets from NASA OSDR (https://osdr.nasa.gov/bio/): OSD-102, 163, 253, 336, 342, 462, 513, 457, 530, 532, // Overbey et al., Nature. In Review. Nature ID: 2022-12-20632: The Space Omics and Medical Atlas (SOMA): A comprehensive data resource and biobank for astronauts // Hourebi et al., Nature Metabolomics. Submitted. NATMETAB-A23069187.: Secretome profiling captures acute changes in oxidative stress, brain homeostasis and coagulation from spaceflight

Fig 2. D; Extended Data Fig 8; Suppl Info 5,6) underlying datasets from NASA OSDR (https://osdr.nasa.gov/bio/): OSD-72, 212, 249, 250, 465, 466

Fig 2. E; Extended Data Fig 8; Suppl Info 7) Rat data at request of CNSA authors (https://doi.org/10.3389/fphys.2020.00939) // MHU-3 data at request of JAXA authors (https://ibsls.megabank.tohoku.ac.jp/metabolite-list) // Hourebi et al., Nature Metabolomics. Submitted. NATMETAB-A23069187.: Secretome profiling captures acute changes in oxidative stress, brain homeostasis and coagulation from spaceflight.

Fig 3. C) data available from Peptide Atlas (https://peptideatlas.org/): PASS00239

Fig 3. D; Fig 5. B,C; Suppl Info 10,11,12,15) imaging data is available on request to k.siew@ucl.ac.uk. Currently this is not available in a repository due to the extremely large size of the raw image files, and will be made available as soon as these repositories have storage capabilities.

Fig 5. A; Extended Data Fig. 8,9,) urinary biochemistry data are available on request to k.siew@ucl.ac.uk. Once OSDR is upgraded to support these type of data they will be made publicly available on this repository.

Fig 5. D; Extended Data Fig. 10; Suppl Info 17) raw 2D spatial transcriptomic data is available on request to k.siew@ucl.ac.uk. Once OSDR is upgraded to support these type of data they will be made publicly available on this repository.

Extended Fig. 1 C; Extended Data Fig. 8) Data available per comms with Mason group // Kim et al. Nature. In Review. Nature ID: 2023-02-01822: Single-cell multi-ome and immune profiles of the Inspiration4 crew reveal variegated, cell-type, sex-specific differences to spaceflight and recovery.

Extended Data Fig. 4) kidney and bodyweight data are available on request to abehesht@broadinstitute.org for BNL-1/2/3 mice and to valery.boyko@nasa.gov and NASA OSDR (https://osdr.nasa.gov/bio/): OSD-513

Extended Data Fig. 7) qPCR data are available are available on request to schisler@unc.edu.

Suppl Info 13, 14, 16) underlying datasets from NASA OSDR (https://osdr.nasa.gov/bio/): OSD-336

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	As indicated in the text (See table 1), the findings apply mostly male humans; due to the crew make up of the human crewed space flight missions. We are not at liberty to disaggregate the sex/gender data due to the onerous conditions of anonymity for the small number of astronauts and ethical concerns around identification.
Reporting on race, ethnicity, or other socially relevant groupings	We have not subclassified or studied different racial or ethnic groups in these studies, due to the onerous conditions of anonymity for the small number of astronauts and the ethical concerns around identification.
Population characteristics	We are unable to provide population characteristics due to the onerous conditions of anonymity for the small number of astronauts and the ethical concerns around identification.
Recruitment	Participants were recruited as part of the selection process for the relevant space missions. The details of which are not accessible to the general public in all instances.
Ethics oversight	All crews from the Inspiration4, NASA, JAXA spaceflights provided informed written consent prior to their participation. More specifics are below: Inspiration4 subjects were consented at an informed consent briefing (ICB) at SpaceX (Hawthorne, CA), and samples were collected and processed under the approval of the Institutional Review Board (IRB) at Weill Cornell Medicine, under Protocol 21-05023569. All crew members have consented for data and sample sharing. Tissue samples were provided by SpaceX Inspiration4 crew members after consent for research use of the biopsies, swabs, and biological materials. The procedure followed guidelines set by the Health Insurance Portability and Accountability Act (HIPAA) and operated under Institutional Review Board (IRB) approved protocols. Experiments were conducted in accordance with local regulations and with the approval of the IRB at Weill Cornell Medicine (IRB #21-05023569). NASA IRB (CSA defers to NASA's IRB), ESA IRB, JAXA IRB for their respective crewmembers from Biochemical Profile (NASA IRB Pro0797) and Nutritional Status Assessment: SMO 016E (pro0326) projects. All crews provided informed written consent

prior to participation.

The JAXA human spaceflight study was proposed to and supported by the 2014 International Life Sciences Research Announcements, JAXA, and NASA. Ethics committee approvals were obtained at the University of Tsukuba (No. 251, Nov. 27, 2015), JAXA (JX-IRBA-20-071, Aug. 30, 2016), NASA (Pro1995, Feb. 28, 2017), ESA (2017_04_09, Apr. 20, 2017). Informed consent was obtained by the personal information manager of the study, and de-identified samples were made available to researchers who performed sample processing and data analysis.

Cosmonaut data were obtained from previously published data [https://link.springer.com/article/10.1007/s10517-013-2310-2]

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	Due to the nature of our study we used samples from animals that had been in spaceflight (usually as part of the 'Rodent Research' missions to the International Space Station) or had been taken from costly experiments using the Galactic Cosmic Radiation Simulator at Brookhaven national laboratories. We also used samples and/or data from humans who had undergone spaceflight (in historical NASA missions, Roscosmos missions, and JAXA missions to the ISS or modern commercial missions with SpaceX such as inspiration4). Our sample numbers were therefore limited to the number of samples made available to us in the original experimental designs or mission parameter limitations (restricted by practical considerations of weight to lauch, available space and resource consumption). In all cases, we endeavoured to use the maximum number of biological replicates available to us to maximise experimental power.
Data exclusions	Data were only excluded on technical grounds due to poor sample preservation quality where positive control measures or QC standards failed, or where the identity of samples matching to animals/participant ID number could not be validated and mislabelling was suspected.
Replication	All -omics studies were performed using a minimum of three technical & three biological replicates. All low-throughput studies (e.g. imaging, qPCR, morphometry) were reproduced in a minimum of 3 biological replicates.
Randomization	Where feasible to do so, all samples were analysed using a randomised block design for each assay, with a new randomisation applied to each new assay.
Blinding	Where feasible to do so, all sample collections were done in a blinded fashion. Data were assigned coded identifiers for analysis, with decoding of original experimental groups only done at the end of statistical analysis to reduce the risk of 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	Sheep polyclonal IgG Anti-human NCC pT46, pT50, pT55 (which targets the conserved mouse NCC pT44, pT48, pT53) [S908B; MRC-PPU Reagents]

	Rabbit polyclonal IgG Anti-Rat total NCC (which cross-reacts with mouse and human total NCC) [AB95302; Abcam but also sold under SPC-402D; StressMarq Biosciences and AB3553; Chemicon(Merck)]
Validation	Both these antibodies have been extensively used in the research communities and validated in both knockdown, overexpression and physiologically stimulated in vitro cellular systems [https://mrcppureagents.dundee.ac.uk/product/71984]. They have also been validated orthogonally by IHC and Western blot in mouse tissues, from models which have inhibition (Gitelman syndrome) or stimulation (Gordon syndrome) of NCC abundance and phosphorylation due to alterations in upstream regulators. As well as by colocalisation with other anti-NCC antibodies targeting different protein regions and also in NCC knockout mouse kidney tissues. [DOI 10.1002/emmm.200900058] [DOI: 10.1093/hmg/ddv185] [DOI: 10.15252/emmm.201505444]

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	As indicated in the text (See table 1), the strains and species of all animals are reported where these were known. Exact ages were not always available and were obtained from NASA Genelab Open Science Data Repository (see detailed citations for all misisons in the online methods section)
Wild animals	N/A
Reporting on sex	As indicated in the text (See table 1), the findings apply mostly female animals (roughly two thirds of animal missions were female). As we were collecting data from previously conducted experiments we did not have the ability to alter this sex disparity. Only one study had both males and females, which enabled us to perform sex difference analyses for parameters such as blood and urine electrolytes.
Field-collected samples	N/A
Ethics oversight	For all animal data coming from from NASA Genelab Open Science Data Repository (see detailed citations for all misisons in the online methods section) the ethical oversight information and protocol number can be found in the Protocol section (e.g. https://osdr.nasa.gov/bio/repo/data/studies/OSD-102). For BNL-1/2/3 - Brookhaven National Laboratory IACUC Protocol 506 “miRNA Signature Detection and Countermeasures Against HZE Radiation Exposure for Tissue Degeneration”. For NSRL-22A - All care and procedures were approved by the Institutional Animal Care and Use Committees (IACUC) at BNL and CHOP and were in accordance with the AAALAC and National Institute of Health (NIH) guidelines for the care and use of laboratory animals. For RR-10 - NASA John F. Kennedy Space Center Institutional Animal Care and Use Committee (IACUC) Research Protocol Review IACUC Protocol #: FLT-20-133 "The Role of CDKN1a/p21 Pathway in Microgravity-Induced Bone Tissue Regenerative Arrest - A Spaceflight Study of Transgenic CDKN1a/p21-Null Mice in Microgravity (SpaceX-21)"

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