

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  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The exact sample size ( <i>n</i> ) for each experimental group/condition, given as a discrete number and unit of measurement   |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided<br><i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>   |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A description of all covariates tested   |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> 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) |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i> ) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted<br><i>Give P values as exact values whenever suitable.</i>                                |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i> ), 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	<div>R (v4.1.2) limma (v3.52) pheatmap (v1.0) FGSEA (v1.22) FlowJo™ v10.8 Software (BD Biosciences) WebGestalt 2019 MassHunter Qualitative analysis 10.0 MassHunter PCDL Manager 8.0 Seurat (v4.2.0) Cell Ranger (v6.1.1) GSVA (v1.42.0) Proteograph Analysis Suite (PAS) DIA-NN search engine (v1.8) DEP (version 1.18) Proteome Discoverer 1.4.1.14 Mascot 2.5 clusterprofiler(v4.4) enrichplot(v1.16) Percolator</div>

XCalibur  
Guppy version 6.2.1  
eventalign (v1.1)  
minimap2 (v2.24)  
subread (v2.0.1)  
DESeq2 (v1.36.0)  
featureCounts (v2.0.1)  
SARTools (v1.8.1)  
m6Anet  
methylKit (v3.18)  
pipeline-transcriptome-de  
Cytoscape (v3.8.2)  
Maplet (v1.1.2)  
RCy3 (v2.14.2)  
MassProfiler 8.0

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 are in the following NASA Open Science Data Repositories. Both the release link (not yet active; to be released upon publication) and the private preview link (active):

OSD-569

Release Link: <https://osdr.nasa.gov/bio/repo/data/studies/OSD-569/>

Preview Link: <https://osdr.nasa.gov/bio/repo/data/studies/OSD-569/preview/SQ3QgEzNMH5q1X19jkWJxVLF5Lx5RQtp>

OSD-571

Release Link: <https://osdr.nasa.gov/bio/repo/data/studies/OSD-571/>

Preview Link: <https://osdr.nasa.gov/bio/repo/data/studies/OSD-571/preview/Wr-IXUj2sAQ4dOp4UcAuajlGfMcZAX0E>

## 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

Sex information is collected and annotated in the NASA OSDR repository for each sample collected.

Reporting on race, ethnicity, or other socially relevant groupings

No race/ethnicity data is reported.

Population characteristics

The crew member composition was of two races and ages ranged from 29-51.

Recruitment

Participants were recruited by SpaceX and mission commander Jared Isaacman.

Ethics oversight

All 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.

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	The entire Inspiration4 crew was profiled, which was limited by the size of the Dragon capsule (n=4).
Data exclusions	No data has been excluded.
Replication	Replication tests are difficult as mission parameters cannot be repeated. Where possible, data validation was performed via western blots to validate proteomic findings.
Randomization	This is not relevant to the study as we were profiling the entire crew longitudinally (pre-flight and post-flight).
Blinding	Blinding was not possible because all subjects were astronauts in the same crew.

## 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
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input 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	DyLight® 488 Lycopersicon Esculentum (Tomato) Lectin (1:100, Vector Laboratories) anti-PECAM-1 (1:100, Novus Biologicals, Centennial, CO) anti-rabbit IgG Alexa Fluor® 568 (1:1000, Life Technologies) anti-LGALS3BP mouse monoclonal IgG (1:1000 dilution, sc-374541, Santa Cruz Biotechnology) anti-FCN3 rabbit polyclonal IgG (1:500 dilution, 11867-AP, Proteintech) anti-mouse HRP-labeled IgG goat (1:5000, Jackson Laboratory) anti-rabbit HRP-labeled IgG goat (1:5000, Jackson Laboratory)
Validation	All antibodies are commercial antibodies validated by manufacturer and used according to the manufacturer's instructions.

## 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	For spaceflight mouse groups (RR-18), ten-week-old C57BL/6 male mice were used. For proteomic profiling of EVP cargo, tissues were isolated from 6-8 week-old naive female C57BL/6 mice and processed as previously described.
Wild animals	The study did not involve wild animals.
Reporting on sex	For spaceflight mouse groups, only male mice were used. For proteomic profiling of EVP cargo, female mice were used.
Field-collected samples	The study did not involve field-collected samples.
Ethics oversight	For spaceflight mouse groups (RR-18), animal experiments were approved by the National Aeronautics and Space Administration (NASA) Animal Care and Use Committee (IACUC) on October 14, 2021 (Protocol Number: RR-18), Roskamp Institute IACUC on October 7, 2021 (Protocol Number RR-18). For proteomic profiling of EVP cargo, mouse studies were performed in accordance with institutional, IACUC and AAALAS guidelines,

and according to Weill Cornell Medicine animal protocol #0709-666A

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

## Plants

Seed stocks

N/A

Novel plant genotypes

N/A

Authentication

N/A