

Reporting Summary

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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

Cell Ranger arc (v2.0.0)
 Cell Ranger (v6.1.1)
 Azimuth (v0.3.2)
 Seurat (v4.2.0)
 chromVAR (v1.16.0)
 VGenes (vPre-release3)
 DESeq2 (cRNA: v1.34.0. Mouse GeneLab: v.1.38.3)
 ComplexHeatmap (v.2.15.1)
 EnhancedVolcano (v.1.16.0)
 fgsea (v1.22)
 GSVA (v1.42.0)
 msigdbr (v7.4.1)
 gprofiler (v.e111_eg58_p18_30541362)
 GeneOverlap (v.1.34.0)
 pheatmap (v1.0)
 bbtools (v38.92)
 XTree (v0.92i)
 Kraken2 (v2.1.2)
 bracken (v2.6.2)

vegan (v2.6.2)
 MetaSPAdes (v3.14.3)
 MetaQUAST (v5.0.2)
 Bowtie2 (v2.2.3)
 samtools (v1.0, 1.9)
 MMseqs2 (v13.4511)
 Diamond (v2.0.14)
 Snakemake workflow management system (v7.7.0)
 STAR (v2.7.0f)
 UMI tools (v1.1.2)
 featureCount (v2.0.0)
 BayesPrism (v2.0)

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](#)

Datasets have been uploaded to two data repositories: the NASA Open Science Data Repositories (OSDR; osdr.nasa.gov; comprised of GeneLab9 and the Ames Life Sciences Data Archive [ALSDA]), and the TrialX database. Identifiers for publicly downloadable datasets in the OSDR are documented below. Select data can be visualized online through the SOMA Data Explorer: <https://soma.weill.cornell.edu/#main>. Also, the single-cell data can be visualized online through Inspiration4 Multiome Data Explorer: https://epigenetics.weill.cornell.edu/apps/l4_Multiome/. All code used to generate Figures and analyses from this project is available at <https://github.com/eliah-o/inspiration4-omics>.

Human research participants

Policy information about [studies involving human research participants and Sex and Gender in Research](#).

Reporting on sex and gender	Yes, sex information was collected and is stated in Figure 3. However, all analyses are performed with the crew as a single cohort.
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

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 qPCR to validate transcriptomic findings. Experiments involving human material (including the qPCR), were only performed once due to limited immune cell availability.

Randomization	This is not relevant to the study as we were profiling the entire crew longitudinally (at different timepoints pre-flight and post-flight) and therefore it was not possible to randomize the collection.
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 checked="" type="checkbox"/> | <input 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 |

Methods

- | | |
|-------------------------------------|--|
| n/a | Involved in the study |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> Flow cytometry |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging |

Antibodies

Antibodies used

- CD3 (T cells) (BD, cat no.555342)
- CD19 (B cells) (Biolegend, cat no.302207)
- CD14 (Monocyte) (BD, cat no.563420)
- CD56 (for NK cells) (BD, cat no.564058)

Validation

The cells were resuspended and incubated with monocyte blocking solution (Biolegend, cat no.426102) and Fc Receptor Blocking Solution (Biolegend, cat no.422301) for 20 mins.

Information from the manufacturer:

CD56 (5 ul per million cells in 100 ul): This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1×10^6 cells in a 100- μ l experimental sample (a test).

CD3 (20 ul per million cells in 100 ul): This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1×10^6 cells in a 100- μ l experimental sample (a test).

CD14 (5 ul per million cells in 100 ul): This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1×10^6 cells in a 100- μ l experimental sample (a test).

CD19 (5 ul per million in 100 ul): For flow cytometric staining using the μ g size, the suggested use of this reagent is ≤ 0.25 μ g per million cells in 100 μ l volume. It is recommended that the reagent be titrated for optimal performance for each application. For flow cytometric staining using the test sizes, the suggested use of this reagent is 5 μ l per million cells in 100 μ l staining volume or 5 μ l per 100 μ l of whole blood.

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 granulocyte-depleted peripheral blood samples were subjected to fluorescence-activated cell sorting to sort out different subsets of immune cells. Briefly, the frozen samples were thawed, and washed in cell staining buffer (Biolegend, cat no.420201). The cells were resuspended and incubated with monocyte blocking solution (Biolegend, cat no.426102) and Fc Receptor Blocking Solution (Biolegend, cat no.422301) for 20 mins, followed by incubation with CD3 (T cells) (BD, cat no.555342), CD19 (B cells) (Biolegend, cat no.302207), CD14 (Monocyte) (BD, cat no.563420), and CD56 (for NK cells) (BD, cat no.564058) for 30 minutes, washed twice in the cell staining buffer and resuspended in the same buffer. Right before sorting DAPI was added to eliminate dead cells during sorting.

Instrument

BD FACSAris Fusion. Serial #: 127896. Model #: 25172

Software

BD FACSDiva™ software

Cell population abundance

	B	T	NK	Mono
C001_L-44	13,811	202,316	75,105	28,333
C001_R+1	7,868	67,863	18,412	7,680
C001_R+82	24,615	184,790	42,614	20,720
C002_L-44	47,067	806,810	48,108	58,376
C002_R+1	6,508	53,583	7,527	12,484
C002_R+82	54,712	442,954	46,448	35,244
C003_L-44	66,392	360,694	66,942	53,040
C003_R+1	3,153	55,281	5,034	134
C003_R+82	101,015	765,104	68,155	85,385
C004_L-44	60,718	394,584	66,840	67,139
C004_R+1	55,157	117,920	19,661	20,398
C004_R+82	85,358	757,223	101,530	141,895

Gating strategy

The gating strategy was shown in the Extended Data Fig. 3d.

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