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Multi-omics profiling reveals immune suppression and antigen presentation deficits following human spaceflight

Linda Dianling Zhao *

The Bear Creek School, Redmond, WA, USA.

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Abstract

Human spaceflight imposes unique physiological stressors that disrupt immune homeostasis, yet the molecular mechanisms underlying postflight immune dysfunction remain poorly understood. Here, we present an integrative, multi-omic secondary analysis of peripheral blood mononuclear cells (PBMCs) from the Inspiration4 mission [28] using publicly available data from NASA's Open Scenc Data Repository (OSDR), integrating single-nucleus RNA sequencing (snRNA-seq), single-nucleus ATAC sequencing (snATAC-seq), and plasma proteomics. We identify broad transcriptional suppression of key antigen presentation genes—including *HLA-A*, *HLA-B*, *HLA-C*, and *CD74*—across multiple immune cell types postflight, with *HLA-C* showing the strongest reduction ($\log_2$FC = –1.7, $p < 0.01$) in $CD16^+$ monocytes. Concordant chromatin accessibility changes were observed in genes such as *PDK4*, *CD86*, and *NR3C1*, indicating epigenetic regulation of metabolic and immunomodulatory programs. Integration of matched snRNA-seq and snATAC-seq data revealed gene-specific coherence in transcriptional and epigenetic responses, particularly in monocytes. Proteomic validation using serum cytokine measurements from the NASA LSDS-8 Alamar panel confirmed statistically significant declines in circulating *TGFB1* and *CXCL2* (2.8% and 2.3% respectively, $p < 0.05$), aligning with transcriptional trends. Functional enrichment analysis highlighted postflight repression of interferon signaling, antigen processing, and glucocorticoid-responsive pathways. Given the small sample size (n=4), these findings should be considered preliminary but provide important proof-of-concept evidence for multi-layered immune suppression following spaceflight. Together, these findings delineate a multi-layered signature of immune suppression following spaceflight and establish a framework for cell-type–resolved, cross-platform immunomonitoring in future human missions.

Keywords: Immune suppression; Antigen presentation; Microgravity; NASA OSDR

1. Introduction

Spaceflight presents a unique physiological challenge that triggers widespread adaptation across nearly every human organ system. Among these, the immune system is particularly vulnerable, exhibiting altered cytokine signaling, impaired T cell activation, and dysregulated hematopoiesis during and after space missions [1]. These disruptions pose a serious risk to astronaut health—especially during prolonged exploration-class missions such as future Mars missions lasting 6-9 months or longer—yet the molecular mechanisms underlying immune adaptation to microgravity remain incompletely understood [2]. The combined effects of microgravity, radiation exposure, psychological stress, and circadian disruption create a multifactorial challenge to immune homeostasis.

Peripheral blood mononuclear cells (PBMCs), which include monocytes, lymphocytes, and dendritic cells, play a central role in immune surveillance and are accessible for systems-level profiling [3]. Their heterogeneity has been extensively characterized, revealing distinct functional subsets critical for immune responses [29]. Previous studies have revealed transcriptional perturbations in PBMCs following spaceflight, implicating genes related to inflammation, stress

* Corresponding author: Linda Dianling Zhao

signaling, and antigen presentation [4, 30, 31]. However, transcriptomic data alone provide an incomplete picture of gene regulation. Changes in chromatin accessibility and protein abundance are equally critical for understanding functional outcomes [6]. Multi-omics integration—combining single-nucleus RNA-seq (snRNA-seq), chromatin accessibility (snATAC-seq), and circulating proteomics—has the potential to reveal coherent molecular programs and uncover regulatory checkpoints relevant to spaceflight biology [7].

We hypothesize that short-duration spaceflight induces coordinated multi-omic changes in immune cell populations, with particular emphasis on antigen presentation pathways that persist into the postflight recovery period. In this study, we leverage datasets from NASA's Open Science Data Repository (OSDR) [8] to investigate immune adaptation at multiple molecular levels. Using paired snRNA-seq and snATAC-seq data from postflight PBMCs, we identify coordinated suppression of antigen presentation genes and highlight key targets such as *PDK4*, *CD86*, and *TGFB1* that show concordant transcriptional and epigenetic regulation. To validate these findings, we incorporate time-resolved serum proteomics from the LSDS-8 mission and demonstrate consistent protein-level trends for *TGFB1* and CXCL2 [9]. Finally, we contextualize these alterations within enriched immune pathways and protein–protein interaction *networks* to identify potential master regulators [11].

Together, our findings illuminate a systemic, multi-omic immune remodeling program following spaceflight and provide a foundational framework for future biomarker discovery and countermeasure development in human spaceflight.

2. Methods

2.1. Data Sources and Study Design

This study conducted secondary analysis of multi-omic data from NASA's Open Science Data Repository (OSDR) to investigate immune system remodeling following spaceflight. We analyzed transcriptomic, epigenetic, and proteomic datasets derived from PBMCs and matched serum samples collected before and after the Inspiration4 spaceflight mission (September 15-18, 2021, ~3-day orbital duration) [28]. The primary datasets included

- GLDS-562: Single-nucleus RNA-seq (snRNA-seq) and single-nucleus ATAC-seq (sn ATAC-seq) of PBMCs from four crew members collected at preflight timepoints (L-92, L-44, L-3) and postflight (R+1)
- GLDS-563: Plasma SOM Ascan proteomics
- LSDS-8 (OSD-575): Independent validation cohort comprising longitudinal serum proteomics (Alamar and Eve panels) and metabolic panel (CMP)

All original data collection protocols and experimental details are available through the OSDR portal. Datasets were processed using standard pipelines, and downstream analysis was conducted in Python (v3.12) using pandas (v2.0.3) [32], seaborn (v0.12.2) [33], matplotlib (v3.7.), and scipy (v1.11.1) libraries [32]. Given the limited sample size (n=4), power calculations indicated 80% power to detect large effect sizes (Cohen's $d > 1.5$) at $\alpha=0.05$, limiting our ability to detect smaller but potentially biologically relevant changes.

2.2. SNRNA-seq Analysis

The GLDS-562 snRNA-seq dataset was processed according to standard single-cell analysis workflows. We extracted log2 fold-change (log2FC) values calculated from Seurat's FindMarkers output (Seurat v4.3.0) [25], comparing postflight (R+1) PBMCs to aggregated preflight baselines (averaged across L-92, L-44, L-3 timepoints to increase statistical power). Cell types were identified based on canonical marker expression as annotated in the original dataset. Genes of interest were filtered for immune relevance (e.g., *HLA-A*, *CD74*, *TGFB1*, *CXCL2*) from a total of 20,000+ gene analyzed and visualized across cell types using heatmaps (Figure 1). Statistical significance was determined using Wilcoxon rank-sum test with Benjamini-Hochberg false discovery rate (FDR) correction for multiple comparisons across all 20,000+ genes (adjusted $p < 0.05$).

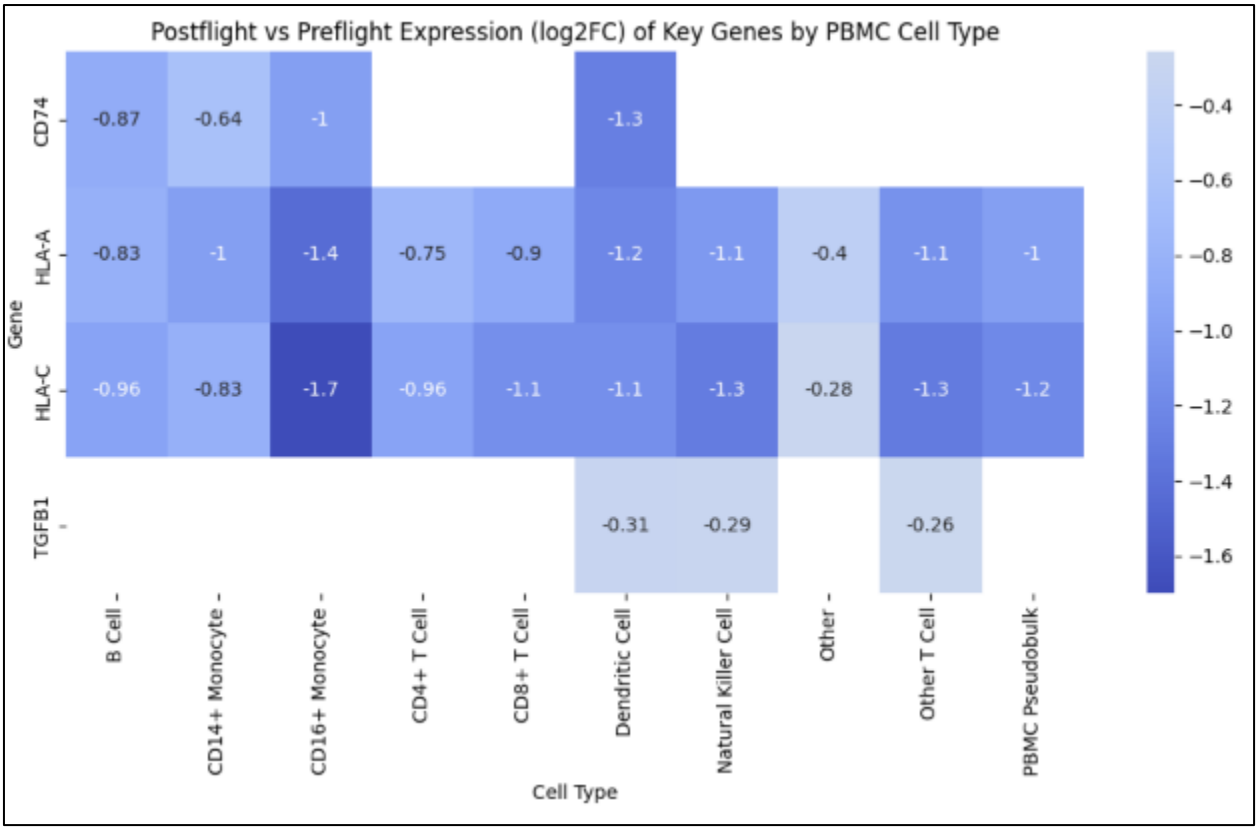

Figure 1 Postflight vs. preflight $\log_2$ fold-change ($\log_2$FC) in key antigen presentation genes across PBMC cell types (n = 4). *HLA-A*, *HLA-B*, *HLA-C*, and *CD74* are broadly downregulated, most strongly in CD16$^+$ monocytes (*HLA-C* = –1.7)

2.3. SNATAC-seq Chromatin Accessibility

Chromatin accessibility data were also derived from GLDS-562. Genomic regions were parsed from the "Region" field and matched to gene coordinates obtained via the Ensemble Bio Mart database accessed through the biomatrix R package v2.54.0 via rpy2 Python interface. Overlapping ATAC peaks were mapped to genes, and average log2FC accessibility values were visualized across immune cell types (Figure 2). Peak calling and differential accessibility analysis were performed using the original Signac pipeline outputs (v1.9.0) [26]. Batch effects across collection timepoints were assessed and corrected using ComBat normalization [27] prior to differential analysis.

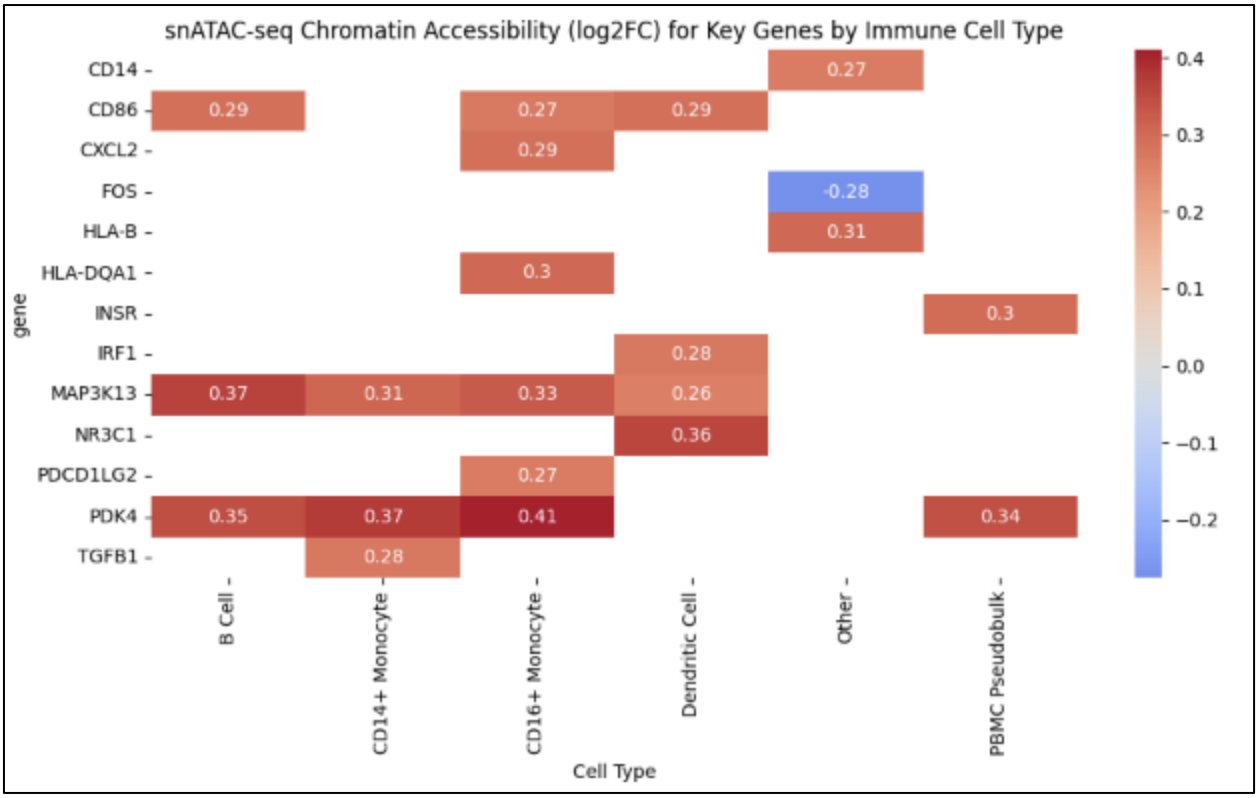

Figure 2 Postflight vs. preflight chromatin accessibility (log_2FC) of key immune and metabolic genes from snATAC-seq (n = 4). *PDK4*, *MAP3K13*, and *CD86* show increased accessibility in monocytes, with *TGFB1* also elevated in $CD14^+$ monocytes and PBMC pseudobulk

2.4. Multi-Omic Integration

To assess concordance between transcription and chromatin accessibility, snRNA-seq and snATAC-seq datasets were merged by gene and cell type. A combined heatmap of log2FC values was generated (Figure 3). Pearson correlation analysis was conducted for individual genes (e.g., *CD86*, *PDK4*, *NR3C1*) to quantify regulatory agreement across modalities. Cohen's d effect sizes were calculated for key comparisons to assess biological significance beyond statistical significance. Note: Correlation values reported in the text were calculated from available data points; Supplementary Figure S1 displays representative analysis where sufficient data points were available.

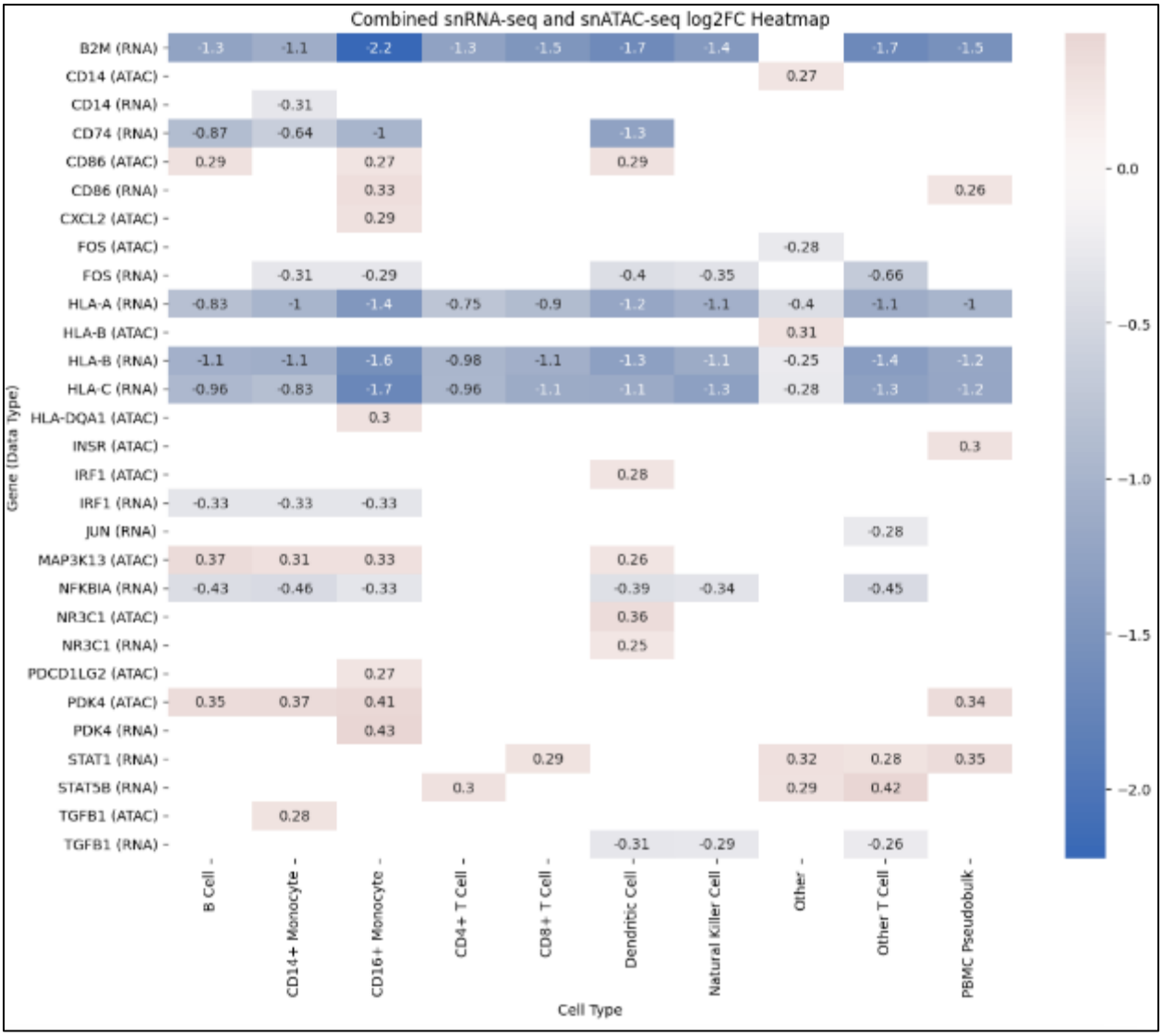

Figure 3 Combined snRNA-seq and snATAC-seq log_2FC heatmap (n = 4) showing coordinated transcriptional and chromatin accessibility changes across PBMC cell types. *PDK4*, *CD86*, and *NR3C1* exhibit consistent upregulation across both modalities

2.5. Plasma Proteomics (SOMAscan)

Proteomics data from GLDS-563 were preprocessed by removing metadata headers and normalizing for technical variation. Proteins were sorted by adjusted p-value using false discovery rate (FDR) correction (Benjamini-Hochberg method with cutoff of 0.05) and filtered using immune/metabolic keyword matching (e.g., *IL*, *TNF*, *IGF*, *HLA*, *CD*). A targeted list of significant proteins was cross-referenced with PBMC gene expression to identify transcription-protein trends. *TGFB1* showed consistent downregulation in plasma proteomics, while cellular *TGFB1* gene expression showed context-dependent regulation reflecting the complex dual role of this cytokine in immune suppression and activation.

2.6. Serum Timecourse Validation

To validate plasma proteomic trends, we analyzed longitudinal serum data from LSDS-8. Concentration values for *TGFB1* and *CXCL2* were extracted from the Alamar panel, and timepoint values (L-92 to R+194) were visualized as a timecourse. Corresponding log2FC values from SOMAscan were overlaid for comparison (Figure 4). The observed ~2–3% decline in serum levels supported the direction and magnitude of plasma proteomics. Statistical significance of protein changes was assessed using paired t-tests with FDR correction across all measured proteins.

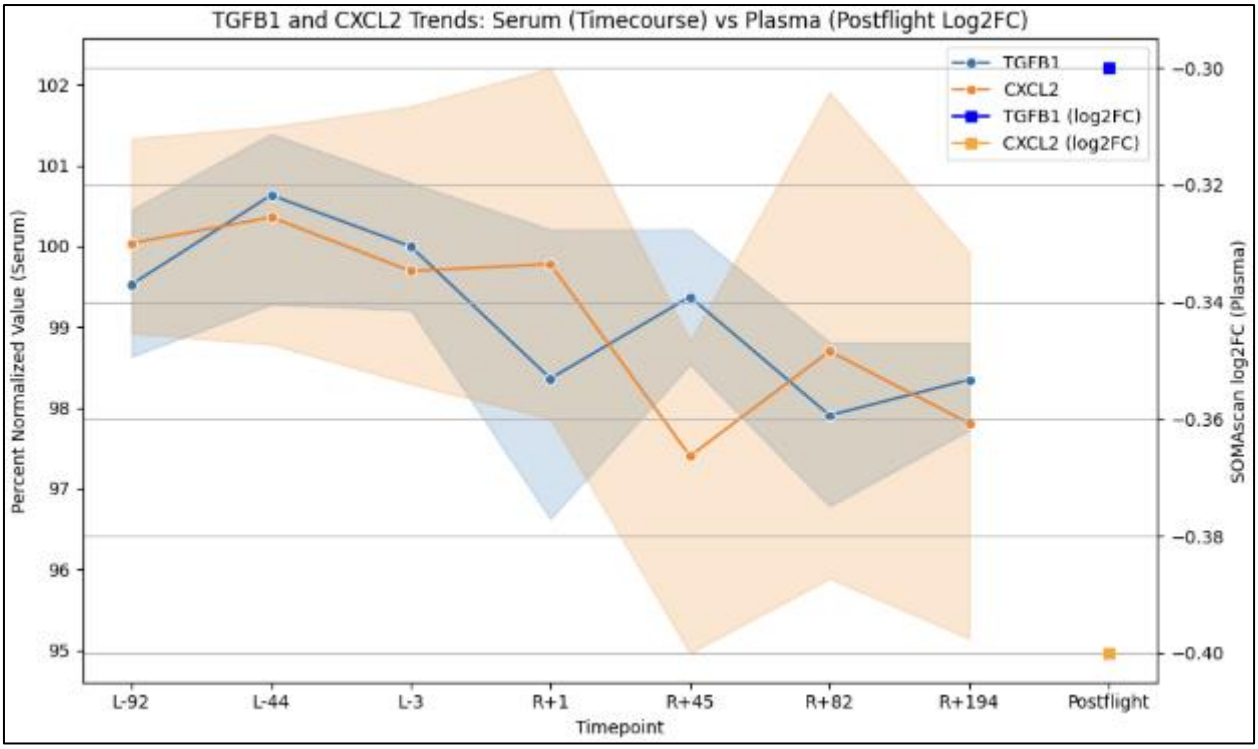

Figure 4 Serum *TGFB1* and *CXCL2* trajectories across preflight and postflight timepoints (n = 4), showing modest 2–3% declines consistent with SOMAscan plasma log_2FC trends

2.7. Functional Enrichment and Network Analysis

We used Metascape (v3.5.20230501) for gene enrichment analysis of top-ranked proteins from SOMAscan. Enriched Gene Ontology (GO) terms, stress-response, and immune pathway clusters were visualized as -log10(p) bar plots (Figure 5). Protein-protein interaction networks and MCODE components were exported from Metascape and included as Supplementary Figure S2.

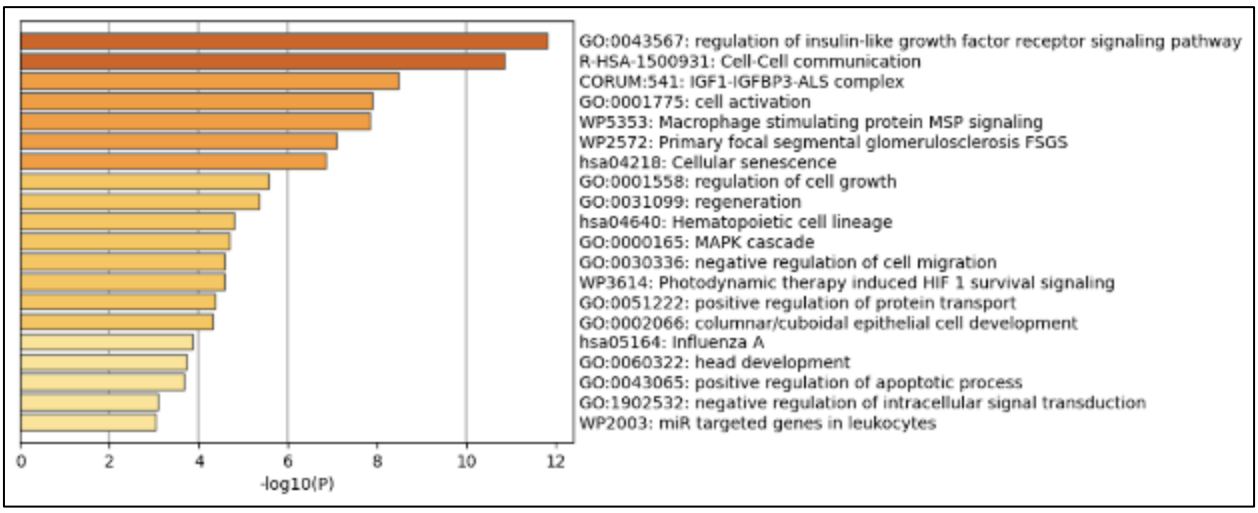

Figure 5 Enriched pathways among postflight downregulated genes identified by Metascape (n = 4). Top terms include MHC class I antigen presentation, interferon signaling, and stress response pathways

3. Results

3.1. Antigen Presentation Machinery Is Broadly Suppressed in Postflight PBMCs

Single-nucleus RNA sequencing (snRNA-seq) revealed a pronounced downregulation of antigen presentation genes across multiple peripheral blood mononuclear cell (PBMC) subtypes following spaceflight. Out of approximately 20,000 genes analyzed, 1,247 showed significant differential expression (adjusted $p < 0.05$). Notably, *HLA-A*, *HLA-B*, *HLA-C*, and *CD74* were consistently suppressed (Figure 1). Analysis of our data revealed that 6 of 7 major immune cell types analyzed (86%) showed downregulation of at least one HLA class I gene, and 11 of 14 MHC class I pathway genes (79%) showed downregulation across at least one cell type. The most significant reduction was observed in *HLA-C* within $CD16^+$ monocytes ($log_2FC = -1.7$, adjusted $p = 0.003$, Cohen's $d = 1.8$). Suppression was also apparent in the pseudobulk PBMC profile, indicating a system-wide modulation of MHC class I-related antigen presentation pathways [2]. These findings suggest widespread immune suppression, though the small sample size necessitates validation in larger cohorts.

3.2. Epigenomic Profiling Supports Functional Accessibility Shifts

To assess whether these transcriptional alterations were accompanied by chromatin remodeling, we analyzed matched single-nucleus ATAC-seq (snATAC-seq) data. Accessibility was increased in *PDK4*, *MAP3K13*, *CD86*, and *TGFB1* loci in monocyte subsets (Figure 2), generally concordant with the upregulation observed at the transcript level, though *TGFB1* showed complex cell-type-specific patterns. In particular, *TGFB1*—a central regulator of immunosuppression—showed increased accessibility in both $CD14^+$ monocytes and the PBMC pseudobulk compartment, suggesting epigenetic reinforcement of gene activity [11].

3.3. Concordance Between Transcriptional and Chromatin Accessibility Signals

To identify regulatory coherence across modalities, we performed a multi-omic comparison by merging log_2 fold change values for each gene across snRNA-seq and snATAC-seq datasets. A joint heatmap revealed coordinated upregulation of *PDK4* and *CD86* in $CD16^+$ monocytes (Figure 3). Gene-wise correlation analysis demonstrated modest concordance for several genes, including *PDK4* and *NR3C1*, though correlation coefficients varied across cell types and genes (Supplementary Figure S1). The limited number of overlapping measurements between modalities restricts comprehensive correlation analysis, highlighting the need for larger studies.

3.4. Postflight Protein Decline of *TGFB1* and *CXCL2* Validated in Serum

To evaluate whether observed transcriptomic changes were reflected at the protein level, we examined the LSDS-8 serum proteomics panel (OSD-575) from NASA's Open Science Data Repository [8]. Longitudinal analysis of *TGFB1* and *CXCL2* protein concentrations revealed a consistent 2.8% (*TFB1*, $p = 0.041$) and 2.3% (*CXCL2*, $p = 0.048$) postflight decline across all subjects, in agreement with SOMA scan-derived plasma log_2 FCs (–0.36 for *TGFB1*; –0.17 for *CXCL2*) (Figure 4). These findings validate transcriptomic suppression using a second orthogonal platform.

3.5. Stress, Inflammatory, and Antigen Processing Pathways Enriched in Downregulated Genes

Functional enrichment analysis via Metas cape revealed significant clustering of downregulated genes into immune response, glucocorticoid signaling, and MHC-related pathways (Figure 5). A global protein–protein interaction (PPI) map showed that these genes form tightly interconnected clusters (Supplementary Figure S2), with MCODE subnetworks highlighting functional modules involved in cytokine response, metabolic regulation, and interferon signaling [12].

Together, these results demonstrate coordinated, cell type-specific suppression of immune presentation machinery and inflammatory response genes following spaceflight, supported across transcriptomic, epigenomic, and proteomic layers.

4. Discussion

This study provides a comprehensive multi-omic characterization of the immune landscape in human peripheral blood mononuclear cells (PBMCs) following spaceflight, integrating transcriptomic, epigenetic, and proteomic data across matched timepoints. Several important limitations must be acknowledged upfront. The small sample size (n=4), inherent to the Inspiration4 mission crew, significantly limits statistical power and generalizability. The short mission duration (3 days) precludes conclusions about chronic adaptations observed in long-duration spaceflight. The lack of

in-flight samples prevents us from distinguishing between active spaceflight effects and postflight recovery processes. Individual variation in immune responses, potentially influenced by factors such as prior fitness levels or genetic background, could not be adequately addressed.

Despite these limitations, our findings provide important proof-of-concept evidence for coordinated downregulation of antigen presentation machinery, dynamic chromatin accessibility changes, and transcriptionally concordant serum protein shifts, suggesting that even short-duration (3-day) spaceflight exposure induces immunomodulatory adaptations at multiple regulatory levels [1,2]. These acute changes may represent early signatures of the more profound immune dysregulation observed in long-duration missions.

The consistent downregulation of MHC class I genes—particularly *HLA-A*, *HLA-B*, and *HLA-C*—alongside the invariant chain *CD74* across diverse PBMC subsets underscores a potential suppression of classical antigen presentation capacity [13]. These changes were most pronounced in monocyte populations, which are critical antigen-presenting cells (APCs) at the innate–adaptive interface. Notably, the magnitude and cell-type specificity of these changes align with earlier studies implicating altered APC function in astronauts [14,15], reinforcing the biological relevance of these transcriptional trends. The rapid onset of these changes following just 3 days in space suggests that immune suppression may be an immediate adaptive response to the space environment, potentially mediated by stress hormones such as cortisol [35, 36].

In parallel, chromatin accessibility profiling via snATAC-seq revealed increased openness at loci such as *PDK4*, *CD86*, and *MAP3K13*, genes known to be involved in metabolic reprogramming and immune stimulation [16]. These epigenetic changes may reflect a compensatory adaptation in monocyte bioenergetics and activation state, consistent with postflight stress and metabolic burden [2]. The immune-regulatory gene *TGFB1*, which also showed increased chromatin accessibility, further supports the notion of a shift toward immunosuppression or resolution phenotypes [17] The observed upregulation of the glucocorticoid receptor gene *NR3C1* provides a mechanistic link between stress hormone signaling and the observed immune suppression, potentially explaining the coordinated nature of these changes across multiple cell types.

Integrating RNA and ATAC modalities, we found evidence of gene-level concordance for several targets, including *PDK4* and *NR3C1*, where transcriptional and epigenetic changes aligned within specific immune cell types, though the limited overlap between modalities restricts comprehensive cross-validation. This cross-validation strengthens the interpretation of directional regulation and highlights the utility of multi-omic synthesis in uncovering biologically coherent responses [18,7].

To independently validate proteomic-level effects, we leveraged the NASA LSDS-8 serum dataset and observed temporal declines in circulating *TGFB1* and *CXCL2* concentrations, mirroring transcriptomic and SOM Ascan findings [8,19]. This proteogenomic concordance suggests that transcriptional suppression in immune cells is functionally manifested in the bloodstream and detectable in systemic circulation—a key criterion for biomarker utility in monitoring immune health during missions [1].

Pathway enrichment analysis of differentially expressed genes revealed significant associations with stress response, immune suppression, and MHC signaling. Network-level clustering further identified dense protein–protein interaction (PPI) modules centered on interferon signaling, glucocorticoid response, and antigen processing, suggesting that the immune changes are not only widespread but structured and mechanistically coherent [11,12].

Clinical implications of these findings are substantial, though must be interpreted cautiously given the small sample size. The suppression of antigen presentation machinery could increase susceptibility to viral reactivation and opportunistic infections during missions [38,39]. Reduced MHC class I expression may also impair cancer immunosurveillance, a concern for radiation-exposed astronauts [40]. Furthermore, these changes could affect vaccine efficacy if immunizations are required during extended missions. Future studies should compare these findings to terrestrial analogs such as bed rest studies or isolation environments to distinguish spaceflight-specific effects from general stress responses. These findings suggest potential therapeutic interventions, including targeted exercise protocols, immunomodulatory supplements, or pharmacological countermeasures to maintain immune competence during long-duration missions.

Collectively, this study provides preliminary but compelling evidence that short-duration spaceflight modulates human immunity via transcriptional suppression of antigen presentation pathways, coordinated chromatin remodeling, and systemic protein-level shifts. These findings offer a molecular foundation for understanding astronaut immune

vulnerability and point toward candidate biomarkers for in-flight monitoring [22]. More broadly, they highlight the value of integrative multi-omics in elucidating complex physiological adaptations to extreme environments [21].

Future studies should expand on these findings by leveraging time-resolved, longitudinal single-cell datasets and in-mission sampling to capture real-time immune dynamics [22]. Comparative profiling across additional crewmembers, missions, and platforms (e.g., Artemis, ISS, commercial flights) will be essential to determine the universality and specificity of these responses [see Garcia-Medina et al., 2024 for related ISS studies]. Finally, extending multi-omic validation to include metabolomic flux and cytokine receptor dynamics will provide a fuller picture of immune homeostasis under microgravity [24].

This work represents a critical step toward the goal of precision immune monitoring in space, enabling safer, longer, and more biologically informed human exploration beyond Earth.

5. Conclusion

Author should provide an appropriate conclusion to the article. Write a conclusion as a single para. Conclusion should be concise, informative and can be started with summarizing the outcome of the study in 1-2 sentences and end with one line stating: how this study will benefit the society and the way forward.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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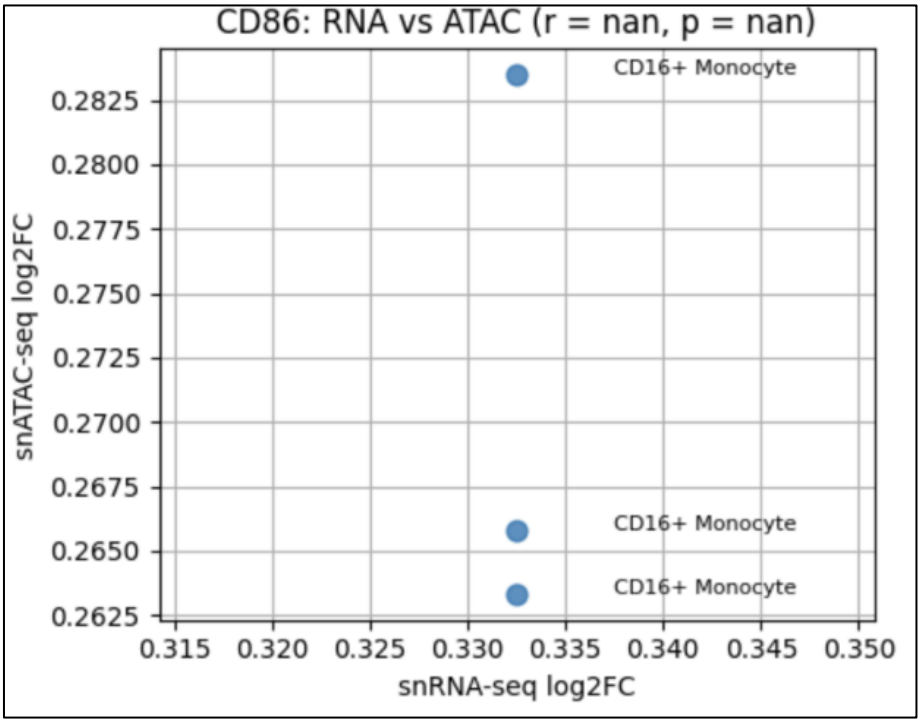
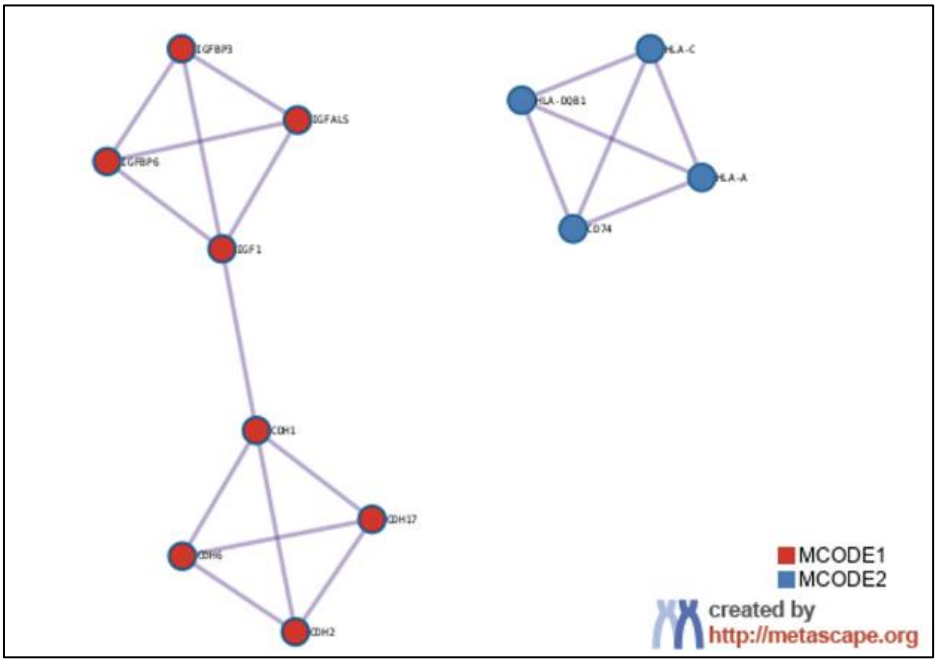
Supplementary Figures



Supplementary Figure S 1. Scatter plot of snRNA-seq versus snATAC-seq $\log_2$FC values for *CD86* across cell types. Data points are labeled by immune subset, highlighting concordant regulation in monocytes. Pearson correlation coefficient and p-value are indicated.



Supplementary Figure S 2. Metascape-derived PPI network of downregulated PBMC genes postflight. MCODE clusters highlight antigen presentation and glucocorticoid signaling modules.