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Research Paper

# Exploratory longitudinal analysis of a PIN1-associated gene panel in PBMCs during orbital spaceflight

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**Abstract.** Spaceflight exposes astronauts to a combination of microgravity, radiation, circadian disruption, isolation, and operational stress that may influence immune and transcriptional regulation. In this exploratory study, we examined peripheral blood mononuclear cell RNA-sequencing data from three Axiom-3 astronauts sampled before launch (L−7) and on International Space Station Days 4, 7, and 10. A separate Galactic-07 participant sampled before launch and 3 h after suborbital flight was included for descriptive context only. The analysis focused on a preselected panel comprising PIN1 and ten biologically associated genes involved in cell-cycle regulation, stress responses, transcriptional control, innate immunity, and oxidative responses. Participant-adjusted longitudinal analysis identified significant overall time-associated variation in PIN1, TP53, PML, MYC, NCF1, NCF2, IRF3, POLR2A, and SFN after Benjamini–Hochberg correction. Several additional genes exhibited descriptive downward expression patterns without meeting the adjusted significance threshold. Because the cohort was small and the measurements were limited to bulk-PBMC mRNA abundance, the findings do not establish altered PIN1 protein activity, a mission-specific regulatory network, or biomarker performance. Instead, they provide hypothesis-generating evidence supporting further evaluation of PIN1-associated transcriptional responses in larger astronaut cohorts using protein-level, functional, and cell-type-resolved analyses.

**Keywords:** PIN1-associated gene panel, orbital spaceflight, PBMC, RNA sequencing, exploratory longitudinal analysis.

Araştırma Makalesi

# Yörüngesel uzay uçuşu sırasında PBMC'lerde PIN1 ile ilişkili bir gen panelinin keşifsel boylamsal analizi

**Öz.** Uzay uçuşu, mikrogravite, radyasyon, sirkadiyen düzensizlik, izolasyon ve operasyonel stresin birlikte etkili olduğu çok faktörlü fizyolojik bir ortam oluşturmaktadır. Bu keşifsel çalışmada, Axiom-3 görevine katılan üç astronottan fırlatma öncesinde (L−7) ve Uluslararası Uzay İstasyonu'ndaki 4., 7. ve 10. günlerde alınan periferik kan mononükleer hücrelerine ait RNA dizileme verileri incelenmiştir. Fırlatma öncesinde ve suborbital uçuştan 3 saat sonra örneklenen ayrı bir Galactic-07 katılımcısı yalnızca tanımlayıcı bağlamda değerlendirilmiştir. Analiz, PIN1 ve hücre döngüsü, stres yanıtı, transkripsiyonel kontrol, doğuştan bağışıklık ve oksidatif yanıtlarla biyolojik olarak ilişkili diğer 10 gen olmak üzere toplam 11 genden oluşan önceden belirlenmiş bir panele odaklanmıştır. Katılımcı etkisini dikkate alan boylamsal analiz ve Benjamini–Hochberg düzeltmesi sonrasında PIN1, TP53, PML, MYC, NCF1, NCF2, IRF3, POLR2A ve SFN için zamanla ilişkili anlamlı değişiklikler belirlenmiştir. Bazı diğer genlerde aşağı yönlü tanımlayıcı ekspresyon örüntüleri gözlenmekle birlikte, bu değişiklikler çoklu test düzeltmesi sonrasında anlamlılık sınırına ulaşmamıştır. Küçük örneklem büyüklüğü ve ölçümlerin toplu PBMC mRNA düzeyiyle sınırlı olması nedeniyle bulgular PIN1 protein aktivitesindeki değişimi, göreve özgü düzenleyici bir ağı veya biyobelirteç performansını göstermemektedir. Sonuçlar, daha büyük astronot kohortlarında protein düzeyi, fonksiyonel ve hücre tipi çözünürlüklü analizlerle değerlendirilmesi gereken hipotez üretici transkripsiyonel örüntüler sunmaktadır.

**Anahtar Kelimeler:** PIN1 ile ilişkili gen paneli, yörüngesel uzay uçuşu, PBMC, RNA dizileme, keşifsel boylamsal analiz.

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## 1. INTRODUCTION

The human body undergoes significant physiological changes during spaceflight due to the combined effects of microgravity, cosmic radiation, circadian rhythm disturbance, isolation, and operational stress (Garrett-Bakelman et al., 2019; da Silveira et al., 2020). A thorough examination of a one-year ISS expedition found significant changes in telomere dynamics, gene expression, DNA methylation, and immune-related pathways in Peripheral Blood Mononuclear Cells (PBMCs) (Garrett-Bakelman et al., 2019). Spaceflight biology is characterized by mitochondrial stress and systemic molecular reconfiguration across immunological and metabolic networks, as revealed by multi-omics studies (da Silveira et al., 2020). This paradigm emphasizes the significance of identifying crucial regulatory nodes that change during spaceflight.

One such regulatory node is PIN1 (NIMA-interacting 1), a special peptidyl-prolyl isomerase that catalyzes cis-trans isomerization at phosphorylated Ser/Thr-Pro patterns. (Zhou & Lu, 2007). PIN1 has a wide range of impacts on cell-cycle regulation, stress responses, protein turnover, and signal transduction networks by influencing substrate shape, stability, subcellular localization, and function following phosphorylation (Lu & Zhou, 2007). PIN1 has been specifically connected to pathways related to ubiquitination and to the fate of certain phosphoproteins involved in proteostatic regulation and signaling fidelity (Jeong et al., 2024). In the context of tau biology, PIN1's neurobiological relevance is especially clear. Because it reduces tau's capacity to bind microtubules and to preserve microtubule integrity, tau hyperphosphorylation is a major pathogenic event in Alzheimer's disease. (Liu and others, 2024). While PIN1 deficiency encourages age-related tau pathology and neurodegeneration, PIN1 activity can restore the correct structure of phosphorylated tau (Liou et al., 2003). According to recent research, PIN1 may reduce harmful tau forms, offering protection against several tau-related illnesses (Qiu et al., 2024). PIN1's biological significance extends beyond neurodegeneration. PIN1 has been demonstrated to affect p53 activity following genotoxic stress in the context of cell-cycle regulation and DNA damage response. (Zacchi and others, 2002). PIN1 helps control mitotic entry by interacting with CDC25C and Wee1 (Lin et al., 2015). In the immune system, PIN1 influences antiviral responses by changing IRF3 through phosphorylation-dependent isomerization (Saitoh et al., 2006). It also regulates Toll-like receptor signaling by managing IRAK1-dependent production of type I interferon (Tun-Kyi et al., 2011). Additionally, NCF1 and NCF2 serve as regulatory components of the NOX2 complex and are closely linked to oxidative burst capacity and innate immune responses (Holmdahl et al., 2016). Research specifically focused on PIN1 or the PIN1-associated gene panel discussed here remains limited. Large-scale omics studies in human spaceflight have shown effects on immunity, transcriptional regulation, and systemic stress responses, but have not included analyses focused on PIN1 or its network (Garrett-Bakelman et al., 2019; Stratis et al., 2023; Wu et al., 2024). However, an early cDNA microarray study in the mouse brain, using the hindlimb-unloading model as a microgravity analog, found broad transcriptomic changes after 2 weeks of unloading, especially in pathways related to immune function, memory and learning, and cell connectivity (Frigeri et al., 2008). Pin1 was among the differentially expressed transcripts, indicating that the PIN1 axis may respond to microgravity-like conditions. Still, these results came from a ground-based mouse model and do not directly reflect the temporal regulation of the PIN1-centered network in PBMCs during human spaceflight (Frigeri et al., 2008). Research specifically examining PIN1 expression during human spaceflight remains limited. Large-scale human spaceflight studies have identified changes in immune, metabolic, and transcriptional pathways but have not systematically evaluated a preselected PIN1-associated transcript panel. In a mouse hindlimb-unloading model, Pin1 was reported among the differentially expressed transcripts, providing a biological rationale for exploratory assessment under altered-gravity conditions. However, findings from a ground-based animal model cannot establish the temporal regulation of PIN1 or functionally associated genes in human PBMCs. Accordingly, the present study evaluates time-associated expression patterns in a hypothesis-driven gene panel and does not assume the existence of a PIN1-centered regulatory network.

The MESSAGE (Microgravity Associated Genetics) Science Mission conducted during Axiom-3 provides a human space-biology framework for examining longitudinal gene-expression changes in astronaut peripheral blood samples (Tastan et al., 2025). The present exploratory study focused on PIN1, and ten biologically associated genes involved in cell-cycle regulation, stress responses, antiviral signaling, oxidative biology, and transcriptional control. The primary objective was to characterize time-associated changes in this preselected gene panel within the Axiom-3 orbital cohort. Data from the Galactic-07 participant were evaluated separately and descriptively. The study was designed to generate hypotheses for subsequent validation rather than to establish a PIN1-centered regulatory network, functional inhibition of PIN1, or a clinically applicable biomarker.

## 2. MATERIALS AND METHODS

### 2.1. Ethical Approval and Consent to Participate

The Microgravity Associated Genetics MESSAGE proposal (Study eIRB Number: STUDY00000620) was approved by the NASA Institutional Review Board (IRB) in accordance with ethical standards and the Code of Federal Regulations for the Protection of Human Subjects (NASA 14CFR1230 and FDA 21CFR50 and 56, if applicable) (Approval Date: September 21, 2023). The MESSAGE Science Mission proposal (No: 61351342/020-275) received approval from the Ethics Commission of Üsküdar University (Approval Date: July 03, 2023) (Tastan et al., 2025). All participants provided written informed consent before participation, as required by the approved institutional review board protocols.

### 2.2. Sample Collection

The orbital and suborbital datasets were treated as separate cohorts. The orbital cohort comprised three Axiom-3 astronauts, each sampled before launch (L−7) and on ISS Days 4, 7, and 10, resulting in 12 longitudinal samples. The suborbital cohort comprised one Galactic-07 participant sampled before launch (L−7) and 3 h after flight, resulting in two samples. Because the suborbital cohort included a single participant, these observations were used for descriptive comparison only and were not incorporated into the primary longitudinal hypothesis tests. Blood samples from age- and sex-matched healthy donors were processed in parallel as technical or biological reference samples. Still, they were not included in the primary longitudinal analysis of the Axiom-3 cohort. PBMCs were isolated, cryopreserved, and stored at −80 °C. In-flight samples were stored in the Minus Eighty Degree Laboratory Freezer for ISS. PBMCs were not cultured, expanded, or stimulated ex vivo before RNA isolation (Tastan et al., 2025).

### 2.3. Transcriptome Analysis

Transcriptome analysis was performed as previously described in our earlier studies (Tastan et al., 2025; Demir et al., 2025; Cam et al., 2025). RNA-seq analysis was performed using RNA isolated directly from uncultured peripheral blood/PBMC-derived samples collected during the MESSAGE Science Mission. PBMCs were not cultured, expanded, or ex vivo stimulated before RNA isolation. RNA isolation was performed by SZA OMICS using automated QIAsymphony-based extraction with manual extraction when required, depending on sample type and compatibility. RNA concentration and purity were evaluated using NanoDrop and Qubit or Victor Nivo measurements, and RNA integrity was assessed using RNA Integrity Number values. Library preparation was performed using the Illumina Stranded Total RNA Prep with Ribo-Zero Plus Kit. Libraries were sequenced on the Illumina NovaSeq 6000 platform using 2 × 100-bp paired-end reads, with approximately 20–25 million reads generated per sample. Raw reads were evaluated using FastQC, and adapter and low-quality sequences were removed using Trim Galore. Reads were aligned to the GRCh38 human reference genome using HISAT2. Gene-level counts were generated using HTSeq. Raw counts were used for statistical modeling in DESeq2, whereas variance-stabilized values were used for heatmaps and other descriptive visualizations. RNA quantity and purity were assessed spectrophotometrically using NanoDrop measurements, including A260/A280 and A260/A230 ratios, and RNA concentration was further evaluated fluorometrically using Qubit or Victor Nivo-based assays. RNA integrity was assessed using RNA Integrity Number (RIN) values before library preparation. No sample was reported as failed at the pre-library QC step. Library preparation was performed using the Illumina Stranded Total RNA Prep, Ligation with Ribo-Zero Plus Kit on the Hamilton NGS STAR platform. Paired-end sequencing was performed using a 2 × 100 bp configuration. Sequencing quality was evaluated using total read number and Q30 metrics, and low-quality bases and adapter sequences were removed before alignment. RNA samples that passed the sequencing provider's pre-library QC and library preparation criteria were included in the transcriptomic analysis, following previously established workflows for the MESSAGE project transcriptomics (Tastan et al., 2025; Demir et al., 2025; Cam et al., 2025).

### 2.4. Statistical Analysis

Statistical analyses were performed on gene expression data obtained across the available mission phases: Pre-Launch (L−7 day), Post-Launch Suborbital (L+3 hr), ISS Day 4, ISS Day 7, and ISS Day 10. Data distribution was assessed before comparison, and the Kruskal–Wallis test was applied to non-normally distributed datasets, including Pre-Launch (L−7 day), ISS Day 4, ISS Day 7, and ISS Day 10 (Kruskal & Wallis, 1952). The primary inferential

analysis was restricted to the 12 longitudinal samples from the three Axiom-3 astronauts. Gene-level raw counts were modeled using DESeq2 with participant and mission time included in the design formula (~ participant + time). An overall time effect was evaluated using a likelihood-ratio test comparing the full model with a reduced participant-only model. Planned contrasts compared ISS Days 4, 7, and 10 individually with the pre-launch L−7 baseline. Multiple-testing correction was performed using the Benjamini–Hochberg procedure, and adjusted $p < 0.05$ was considered statistically significant. Effect sizes are reported as $\log_2$ fold changes together with standard errors or 95% confidence intervals. The Galactic-07 participant was evaluated descriptively and was not included in inferential testing. Owing to the small orbital cohort, all statistical findings were interpreted as exploratory. Heatmaps were produced using row-wise z-score normalization to emphasize relative expression changes within each gene across mission phases. Hierarchical clustering was applied to both genes and time points using Ward.D2 linkage with Euclidean distance. Genes were reordered according to clustering results and annotated by functional class where applicable. To improve figure readability, heatmap contrast was increased, font sizes were enlarged, and dense gene sets were reduced or organized into biologically grouped panels. Biological Process enrichment was used as the primary ontology, and significantly enriched terms were defined using adjusted $p < 0.05$. Enriched terms were visualized as dot plots using ggplot2. Co-expression networks were generated from the same expression matrices by calculating pairwise Pearson correlations between genes across mission phases. Network edges were defined using an absolute correlation threshold of $|r| > 0.8$. Networks were visualized using igraph, ggraph, and tidygraph, with nodes annotated by functional class and edges representing strong positive or negative correlations. PIN1, TP53, PML, PLK1, HIPK2, MYC, NCF1, NCF2, IRF3, POLR2A, and SFN were selected a priori based on documented biological relationships with phosphorylation-dependent signaling, cell-cycle and stress regulation, innate immune signaling, oxidative responses, and transcriptional control. The panel was hypothesis-driven and was not intended to represent a comprehensive PIN1 regulatory network. Known and predicted protein associations were examined using STRING for Homo sapiens. The analysis used a minimum interaction score for network edges and no additional interactors beyond the eleven submitted proteins. The STRING network was used solely to provide external database-based biological context and was not inferred from the astronaut expression data.

## 3. RESULTS

### 3.1. Experimental Overview of the MESSAGE Space Biology Mission

Transcriptome profiling was used in the MESSAGE (Microgravity Associated Genetics Research) project, Turkey's first human space biology mission, to examine how microgravity affects the expression of genes related to the immune system and aging. Peripheral blood samples were taken over the course of five mission phases: the International Space Station (ISS) mission days 4, 7, and 10; the suborbital phase (immediate pre- and post-flight); and the pre-launch baseline (L–7). All samples were cryopreserved at -80 °C; to protect RNA integrity, in-flight samples were kept in the Minus Eighty Degree Laboratory Freezer for ISS (MELFI). High-throughput RNA sequencing (RNA-seq) was then used to evaluate the samples (Tastan et al., 2025; Demir et al., 2025; Cam et al., 2025). We summarized the experimental setup and sampling schedule for the MESSAGE space biology mission (Tastan et al., 2025; Demir et al., 2025; Cam et al., 2025). This method enabled a time-resolved assessment of transcriptomic responses to acute and prolonged microgravity exposure (Tastan et al., 2025; Demir et al., 2025; Cam et al., 2025). The MESSAGE Science Mission integrated orbital and suborbital human spaceflight sampling with PBMC transcriptomic analysis to investigate the behavior of a PIN1-associated gene panel (**Figure 1**). Figure 1 summarizes the MESSAGE sampling design and transcriptomic workflow. The primary longitudinal cohort comprised three Axiom-3 astronauts sampled at L−7 and on ISS Days 4, 7, and 10. A separate Galactic-07 participant was sampled at L−7 and 3 h after suborbital flight and was evaluated descriptively. Following RNA sequencing and quality control, expression of a preselected panel comprising PIN1 and ten biologically associated genes was examined. Participant-adjusted longitudinal modeling was performed only for the Axiom-3 cohort, while the Galactic-07 observations were not incorporated into inferential testing.

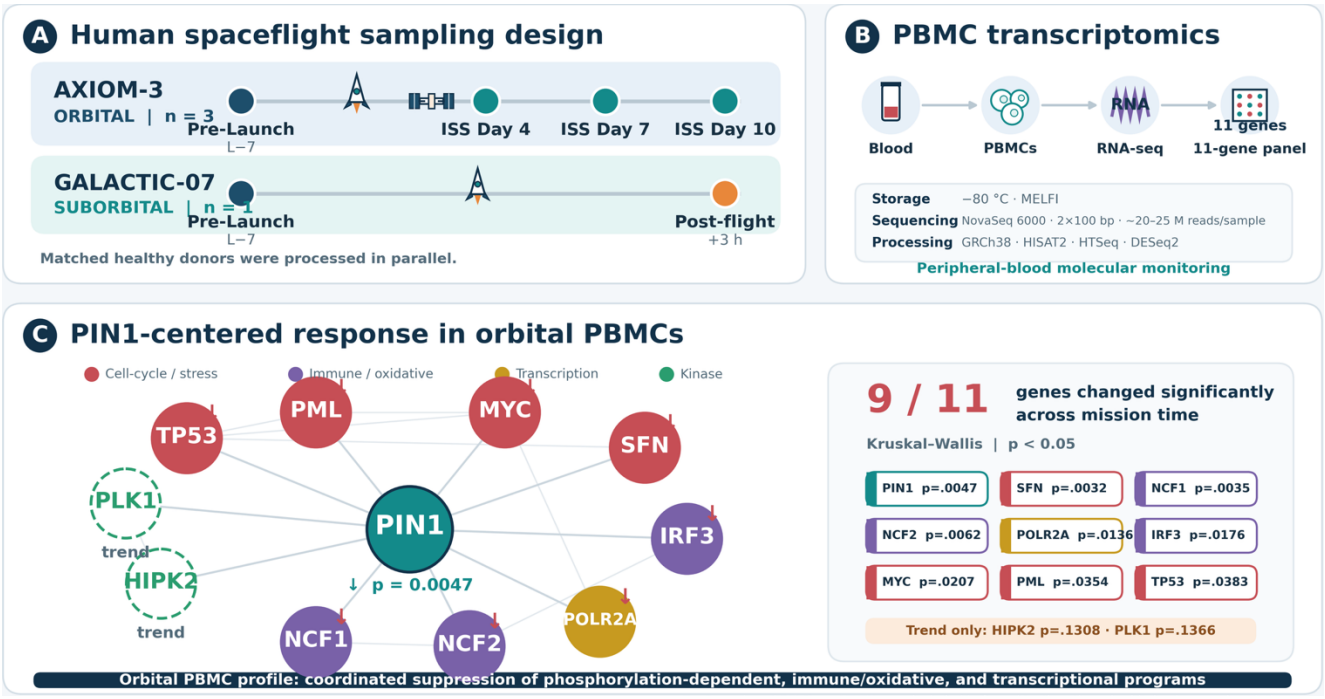


**Figure 1.** MESSAGE Science Mission Sampling Design, PBMC Transcriptomics Workflow and Exploratory Analysis of a PIN1-Associated Gene Panel

(A) The Axiom-3 orbital cohort comprised three astronauts sampled at L−7 and on ISS Days 4, 7 and 10. The Galactic-07 suborbital dataset comprised one participant sampled at L−7 and 3 h after flight and was evaluated descriptively. (B) RNA sequencing and bioinformatic processing included quality assessment, alignment to GRCh38, gene-level read counting, and participant-adjusted DESeq2 analysis. (C) Summary of the preselected eleven-gene panel. Statistical findings shown in this panel are derived from the longitudinal Axiom-3 model and are reported using Benjamini–Hochberg-adjusted p-values. Diagrammatic grouping of genes represents broad biological annotations and does not demonstrate direct molecular regulation or a mission-derived interaction network.

### 3.2. Exploratory Longitudinal Expression Changes in the PIN1-Associated Gene Panel

Normalized RNA-seq read counts for PIN1 and its 10 related genes were visualized at five sampling time points during the mission. The primary longitudinal analysis included three Axiom-3 astronauts sampled at L−7 and on ISS Days 4, 7, and 10. In contrast, the Post-launch Suborbital time point was included only for visual presentation. Of the 11 genes examined, nine showed statistically significant time-dependent changes, while PLK1 and HIPK2 exhibited similar directional trends that did not reach statistical significance. Overall, the observed pattern indicates that most components of the PIN1-centered network showed lower expression levels at orbital time points than at pre-launch levels (Figure 2). PIN1 ($p = 0.0047$) and TP53 ($p = 0.0383$) showed comparatively high expression during the pre-launch phase when the expression profiles of PIN1 and its ten associated genes were examined. On the other hand, on days 4, 7, and 10, ISS showed reduced expression levels. Similar to baseline, PML exhibited decreased expression at orbital time points ($p = 0.0354$). MYC was one of the cell cycle-related genes that changed significantly over the course of the mission ($p = 0.0207$). Compared to pre-launch levels, MYC expression was much lower at ISS time points. On the other hand, although these changes did not achieve statistical significance, PLK1 ($p = 0.1366$) and HIPK2 ($p = 0.1308$) showed a trend toward reduced expression at orbital time points. Within the subnetwork associated with immune response and oxidative stress, the most prominent changes were observed in NCF1 and NCF2. Both NCF1 ($p = 0.0035$) and NCF2 ($p = 0.0082$) showed marked suppression at orbital time points relative to pre-launch levels. IRF3 likewise exhibited a significant decrease ($p = 0.0176$). The shared pattern across these three genes suggests a consistent reduction in transcriptional activity related to immune and oxidative response pathways, particularly during the orbital phase. Among genes associated with transcriptional regulation and stress response, POLR2A showed a significant time-dependent change ($p = 0.0136$). POLR2A expression was higher during the pre-launch phase and was markedly reduced at orbital time points. SFN was also among the genes showing significant change ($p = 0.0032$), exhibiting a distinctly suppressed expression profile during the orbital phase. The single Galactic-

07 participant was displayed separately and was not included in inferential analysis. These findings represent exploratory expression changes within a preselected transcript panel and do not demonstrate functional regulation of a PIN1-centered molecular network.

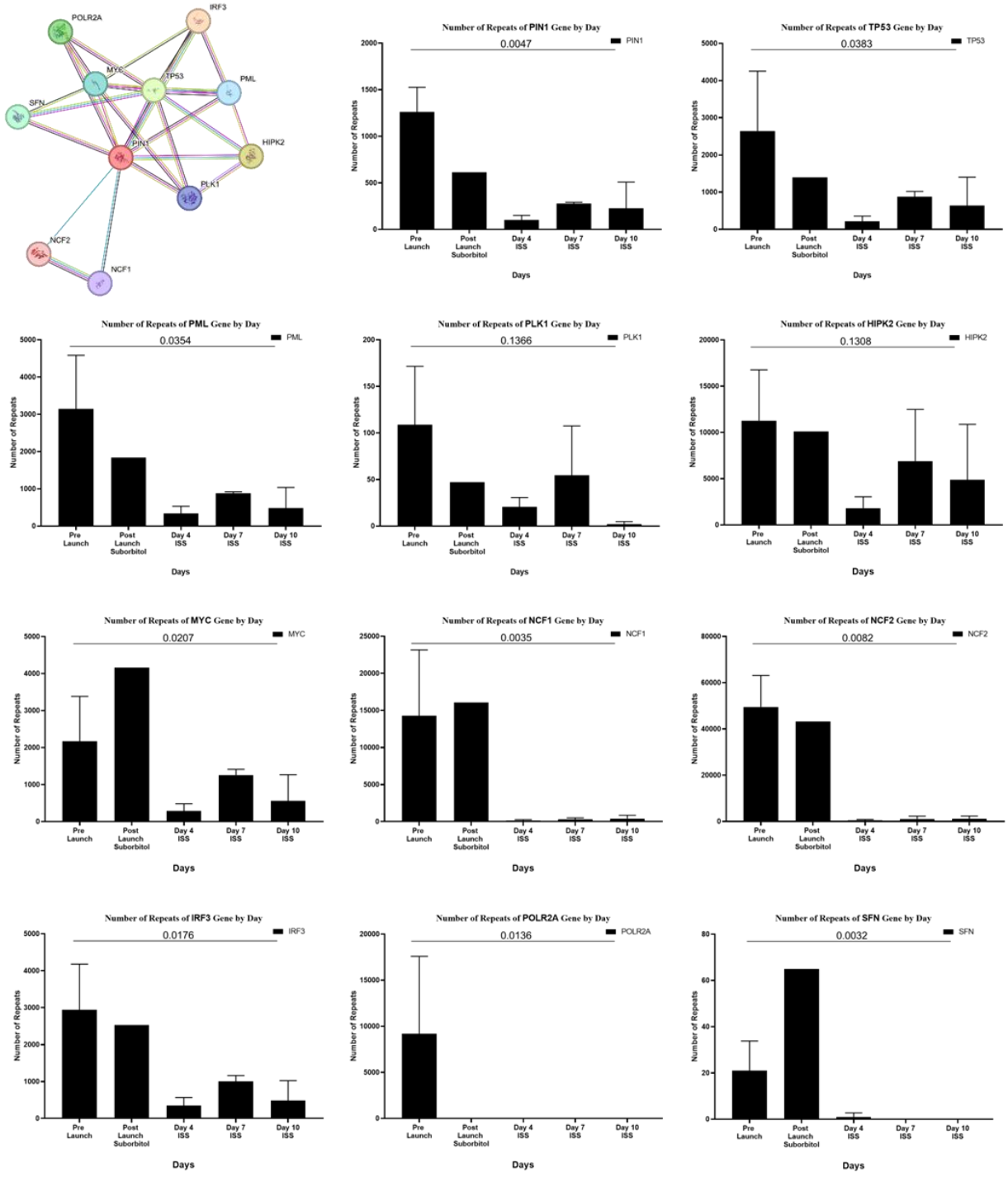


**Figure 2.** Database-Derived Protein-Association Context and Longitudinal Expression Profiles of the PIN1-Associated Gene Panel

(A) Known and predicted protein associations among the eleven preselected proteins were obtained from STRING, using a minimum interaction score. The network represents external database-derived information and was not inferred from the astronaut expression data. (B) Individual variance-stabilized expression values for three Axiom-3 astronauts sampled at L−7 and on ISS Days 4, 7, and 10. Measurements from the same participant are connected. Statistical annotations show Benjamini–Hochberg-adjusted results from participant-adjusted longitudinal models.

### 3.3. Descriptive Heatmap and Expression-Profile Analysis

A heatmap of variance-stabilized expression values illustrated gene-specific and participant-level expression patterns within the Axiom-3 orbital cohort (Figure 3A). Several genes showed relatively higher expression at L−7 and lower expression at one or more orbital time points, whereas PLK1 and HIPK2 exhibited more heterogeneous profiles. The descriptive standardized expression summaries similarly illustrated gene-specific temporal patterns across ISS Days 4, 7 and 10 (Figure 3B). These visualizations were used to summarize relative expression patterns and were not treated as independent evidence of gene co-expression, pathway activation or regulatory-network structure. Because these trajectories connect condition-level mean values, they represent ordered expression profiles rather than within-astronaut longitudinal measurements. Exploratory co-expression analysis identified a densely connected network dominated by strong positive correlations among several components of the PIN1-centered panel (Figure 3C). This coordinated structure was consistent with the broadly parallel expression changes observed across mission conditions. However, because the correlations were calculated from five condition-level averages, the network should be interpreted as hypothesis-generating rather than as definitive evidence of regulatory or protein–protein interactions. Gene set enrichment analysis indicated positive enrichment of the p53 pathway (NES = 2.45, FDR = 0.010) and apoptosis (NES = 1.82, FDR = 0.020), while MYC_TARGETS_V1 (NES = −2.10, FDR = 0.015) and MYC_TARGETS_V2 (NES = −1.75, FDR = 0.040) were negatively enriched (Figure 3D). DNA repair showed positive enrichment at the FDR boundary (NES = 1.45, FDR = 0.050), whereas reactive oxygen species and oxidative phosphorylation pathways did not meet the predefined FDR < 0.05 threshold. Collectively, these findings suggest a spaceflight-associated shift toward stress- and damage-response programs accompanied by suppression of MYC-related proliferative activity.

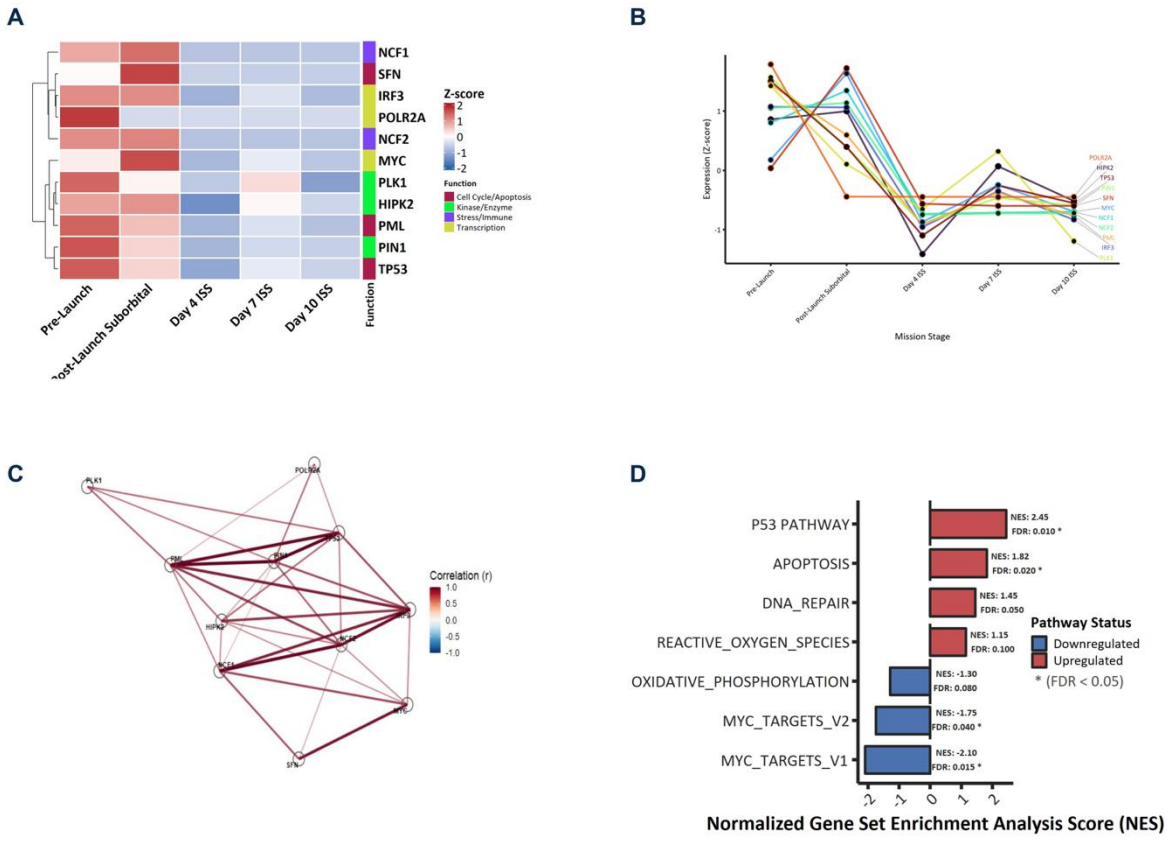


**Figure 3.** Descriptive Expression Patterns of the PIN1-Associated Gene Panel During Orbital Spaceflight

(A) Heatmap of variance-stabilized expression values from individual Axiom-3 samples after row-wise z-score standardization. Columns represent coded participant-level samples collected at L−7 and on ISS Days 4, 7 and 10. Genes were clustered using Euclidean distance and Ward.D2 linkage, whereas samples were retained in their predefined longitudinal order. Red and blue indicate expression above and below each gene's row mean, respectively.

(B) Descriptive standardized expression profiles across the four Axiom-3 sampling points. Statistical inference was based on participant-level DESeq2 models rather than the standardized visualization. (C) Exploratory co-expression network based on Pearson correlations calculated across the five condition-level profiles. Nodes represent genes, and edges indicate strong correlations ($|r|>0.80$); edge color indicates the direction and strength of the correlation, from negative (blue) to positive (red). (D) Gene set enrichment analysis summary showing Normalized Enrichment Scores (NES). Red bars denote positive enrichment, and blue bars denote negative enrichment; the corresponding False Discovery Rate (FDR) is shown beside each pathway, with asterisks indicating FDR < 0.05.

## 4. DISCUSSION

PIN1 is a special enzyme that isomerizes phosphorylated Ser/Thr-Pro motifs to control the structure and function of many phosphoproteins (Lu & Zhou, 2007; Jeong et al., 2024). Redox imbalance, large-scale molecular remodeling, and mitochondrial stress are key elements of space biology, according to multi-omics studies of spaceflight (da Silveira et al., 2020; Garrett-Bakelman et al., 2019). Additionally, PIN1 has been shown to exhibit reduced expression and undergo oxidative modification in the hippocampal regions of patients with Alzheimer's disease (Sultana et al., 2006). Therefore, the influence of microgravity-linked stress responses on a redox-sensitive regulatory node may be reflected in the lower PIN1 transcript abundance observed in this work. However, this conclusion needs to be supported by functional and protein-level investigations, as the available data show only transcript levels.

The well-established role of PIN1 in tau metabolism also makes these findings noteworthy from a neurobiological perspective. PIN1 has been shown to direct phosphorylated tau toward more functional conformations, and loss of PIN1 has been associated with age-dependent tau pathology (Liou et al., 2003; Qiu et al., 2024). Consequently, the decrease of PIN1 shown in peripheral blood could provide a basis for indirectly examining pathways linked to neurodegeneration during extended space missions. However, this interpretation should be viewed as a framework for generating hypotheses rather than as concrete proof, because transcriptomic alterations in PBMCs cannot be taken to reflect tau biology in the central nervous system directly. The marked decreases observed in NCF1 and NCF2 constitute one of the strongest signals in this study. NCF1 and NCF2 are regulatory components required for activation of the NADPH oxidase 2 (NOX2) complex and are closely associated with oxidative burst capacity (Holmdahl et al., 2016). Their shared downward expression pattern suggests that effector programs associated with innate immunity may be attenuated in PBMCs under microgravity. This finding is consistent with a review showing that microgravity can disrupt immune cell function in multiple ways (Lv et al., 2023). In addition, single-cell studies have identified conserved disruptions in interferon signaling and inflammatory pathways under both simulated and real microgravity conditions (Wu et al., 2024). The significant decrease in IRF3 is also notable in this context. PIN1 has been shown to target activated IRF3 in a phosphorylation-dependent manner, thereby suppressing antiviral responses (Saitoh et al., 2006). Nevertheless, the concurrent reduction of both PIN1 and IRF3 transcripts in the present study may indicate a more complex network-level remodeling rather than a simple linear PIN1→IRF3 relationship. NCF1 and NCF2 encode regulatory components of the NOX2 complex and are associated with oxidative-burst functions. Their lower transcript abundance at selected orbital time points, together with the expression pattern observed for IRF3, is compatible with altered transcription of genes involved in innate immune and oxidative-response pathways. However, the present transcriptomic data do not demonstrate reduced oxidative-burst capacity, impaired antiviral function, or attenuation of an immune-effector program. Moreover, bulk-PBMC profiles cannot distinguish within-cell transcriptional regulation from changes in PBMC subpopulation composition. These interpretations should therefore be evaluated using cell-type-resolved measurements and functional immune assays.

PIN1 has documented biological relationships with proteins involved in genotoxic-stress responses, cell-cycle control and transcriptional regulation (Zacchi et al., 2002; Lin et al., 2015). In the present study, the time-associated expression patterns of TP53, MYC and POLR2A provide exploratory evidence that selected transcripts linked to these processes may change during orbital flight. Nevertheless, lower transcript abundance cannot be interpreted as a globally suppressed proliferative state, reduced checkpoint activity, or impaired transcriptional capacity. Similarly, the absence of adjusted significance for PLK1 or HIPK2 should not be used as evidence of network-level reorganization. Previous studies have shown that pathways involved in the DNA damage response and transcriptional stress are altered during spaceflight (Garrett-Bakelman et al., 2019). DNA damage has also been reported to trigger

genome-wide degradation of promoter-bound RNA polymerase II (Steurer et al., 2022). The STRING associations provide literature- and database-derived context for the selected proteins but do not establish a regulatory network generated by spaceflight. Although the genes can be measured in peripheral blood, the present study does not evaluate biomarker sensitivity, specificity, reproducibility, or predictive performance. Consequently, the panel should be regarded as a hypothesis-generating set of candidate transcripts rather than a validated biomarker or multisystem-monitoring platform.

In this context, the decrease in POLR2A may be consistent with transcriptional stress associated with spaceflight; however, the present dataset does not provide sufficient mechanistic evidence to directly attribute this reduction to the cosmic radiation load or DNA damage. This PIN1-centered expression pattern may represent a candidate biomarker framework for space medicine. The fact that all genes in the panel are measurable in peripheral blood provides an important advantage for noninvasive monitoring strategies. Moreover, the simultaneous tracking of axes related to immunity, cell-cycle regulation, stress response, and neurodegeneration suggests that this network may form a candidate transcript panel requiring validation that is sensitive to mission progression. In this respect, the findings provide a conceptual basis for multisystem physiological monitoring, particularly during long-duration missions. Nevertheless, this study has several important limitations. First, the inherently limited sample size of space mission studies may have reduced statistical power for some genes. Second, the data are limited to mRNA levels and therefore do not provide direct information regarding protein abundance, enzymatic activity, post-translational modifications, or actual functional outputs. Third, it remains unclear to what extent the changes observed in PBMCs reflect biological processes occurring in the brain or other tissues. Finally, the biological interpretations proposed here are correlational and require validation through independent cohorts, protein-based analyses, and functional experiments. Accordingly, the present findings should be regarded not as definitive mechanistic conclusions, but rather as biological hypotheses and candidate biomarkers requiring further validation.

Several limitations should be considered. First, the orbital cohort comprised only three astronauts, limiting statistical power and the generalizability of the findings. Second, the Galactic-07 dataset contained a single participant and therefore permitted descriptive interpretation only. Third, bulk-PBMC RNA sequencing cannot distinguish transcriptional regulation within a cell type from changes in PBMC subpopulation composition. Cell-type proportions were not incorporated into the present analysis. Fourth, the study assessed mRNA abundance and did not measure PIN1 protein abundance, enzymatic activity, phosphorylation-dependent substrate interactions, subcellular localization, oxidative burst capacity, or antiviral function. Fifth, mission-related variables including circadian timing, diet, medication, radiation exposure, operational stress, and sample-processing conditions may have contributed to the observed expression patterns. Finally, the targeted panel and exploratory sample size require independent validation in larger cohorts with prespecified analytical criteria.

In conclusion, participant-adjusted exploratory analysis identified time-associated expression patterns in a preselected PIN1-associated gene panel in PBMCs from three Axiom-3 astronauts. PIN1, TP53, PML, MYC, NCF1, NCF2, IRF3, POLR2A, and SFN remained significant after correction for multiple testing, while other genes showed descriptive expression differences across orbital sampling points. The findings do not establish functional inhibition of PIN1, a mission-specific regulatory network, or a validated biomarker. Instead, they provide a hypothesis-generating basis for future studies incorporating independent astronaut cohorts, cell-type-resolved transcriptomics, proteomics, PIN1 activity measurements, and functional immune assays.

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**ETHICAL STATEMENT & GENERAL STATEMENTS**

This article meets the standards of research and publication ethics.

## ARTIFICIAL INTELLIGENCE USE DISCLOSURE

During the preparation of this manuscript ChatGPT (OpenAI) was used to translate; to improve grammar, spelling, punctuation, and/or linguistic fluency; to search literature, discover sources, and/or summarize sources; and to adjust the visualizations. AI was not used to generate the scientific content of the study. All AI-assisted suggestions were reviewed and verified by the author(s). All responsibilities for the content of this paper belong to the author(s).

## AUTHORS' CONTRIBUTIONS

Idea/Concept: C. Tastan; Design: C. Tastan and B. Aydin; Data Collection/Processing: Z.B. Uzun; Analysis/Interpretation: Z.B. Uzun, C. Yildirim and B. Aydin; Supervision: C. Tastan; Manuscript Writing: C. Tastan, Z.B. Uzun and B. Aydin. All authors have read and approved the final manuscript.

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## AVAILABILITY OF DATA AND MATERIALS

TÜBİTAK UZAY (Space Technologies Research Institute) securely archives the raw and processed data generated during the MESSAGE Science Mission, including participant-level RNA-sequencing and associated molecular datasets, under the governance of the Turkish Space Agency (TUA). Because of the small, mission-specific astronaut cohort and the risk of participant re-identification, these data are not made openly available without prior review. Qualified researchers may be granted access to the underlying participant-level data upon submission of a formal scientific request and approval by TÜBİTAK UZAY and TUA, subject to compliance with the applicable NASA Institutional Review Board approval (STUDY00000620), institutional ethics requirements, participant-consent provisions, confidentiality safeguards, and any required data-use agreements. Access requests should be directed to the corresponding author, who will coordinate the institutional review process with TÜBİTAK UZAY and TUA. Aggregated and non-identifying data supporting the principal findings of this study are provided within the article and its Supplementary Information.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

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