Mathematical Biosciences and Engineering

MBE, 20(5): 8531–8545.
DOI: 10.3934/mbe.2023374
Received: 10 December 2022
Revised: 31 January 2023
Accepted: 12 February 2023
Published: 03 March 2023

http://www.aimspress.com/journal/MBE

Research article

Effects of spaceflight on the spleen and thymus of mice: Gene pathway analysis and immune infiltration analysis

Yuru Han[1,2,3], Shuo Shi[4], Shuang Liu[1,2,3] and Xuefeng Gu[2,3,*]

[1] School of Health Science and Engineering, University of Shanghai for Science and Technology, Shanghai, China
[2] School of Pharmacy, Shanghai University of Medicine & Health Sciences, Shanghai, China
[3] Shanghai Key Laboratory of Molecular Imaging, Zhoupu Hospital, Shanghai University of Medicine and Health Sciences, Shanghai, China
[4] China COMAC Shanghai Aircraft Design and Research Institute, Shanghai, China

* **Correspondence:** Email: guxf@sumhs.edu.cn; Tel: +8602165883903; Fax: +862165883218.

Abstract: During space flight, the immune system function of the body is disrupted due to continuous weightlessness, radiation and other factors, resulting in an increased incidence of infectious diseases in astronauts. However, the effect of space flight on the immune system at the molecular level is unknown. The aim of this study was to identify key genes and pathways of spatial environmental effects on the spleen and thymus using bioinformatics analysis of the GEO dataset. Differentially expressed genes (DEGs) in the spleen and thymus of mice preflight and postflight were screened by comprehensive analysis of gene expression profile data. Then, GO enrichment analysis of DEGs was performed to determine the biological role of DEGs. A protein–protein interaction network was used to identify hub genes. In addition, transcription factors in DEGs were screened, and a TF-target regulatory network was constructed. Finally, immune infiltration analysis was performed on spleen and thymus samples from mice. The results showed that DEGs in the spleen and thymus are mainly involved in immune responses and in biological processes related to platelets. Six hub genes were identified in the spleen and 13 in the thymus, of which Ttr, Aldob, Gc and Fabp1 were common to both tissues. In addition, 5 transcription factors were present in the DEGs of the spleen, and 9 transcription factors were present in the DEGs of the thymus. The spatial environment can influence the degree of immune cell infiltration in the spleen and thymus. Our study bioinformatically analyzed the GEO dataset of spacefaring mice to identify the effects of the space environment on the immune system and the genes that play key roles, providing insights for the treatment of spaceflight-induced immune system disorders.

Keywords: spaceflight; spleen; thymus; immune system; immune cell filtration

1. Introduction

As technology advances, humans enter a new era of space exploration. In the future, astronauts will need to explore space further, and the cumulative time spent in space will increase. Radiation and changes in gravity can affect the thymus and spleen of astronauts and, thus, their immune system [1–3]. In a previous study, researchers comparing C57BL/6 mice in the flight group with those in the ground group after a 13-day mission of the Space Shuttle Atlantis found increased thymic DNA fragmentation, altered expression of 15/84 cancer-related genes and T-cell-related genes in the flight group [4]. Similarly, space radiation also affects the metabolome of the spleen. Again, using C57BL/6 mice, a total of 40 metabolites were identified using three different low doses of radiation exposure; the pathways that metabolites are involved in were identified, and the results showed that radiation exposure may affect mitochondria and DNA repair [5]. Furthermore, it has been shown that a week after landing, the weight of the thymus gland drops, which has a strong immunosuppressive effect on the immune system and can, in turn, lead to chronic immunodeficiency [6].

The effects of weightlessness on the thymus and spleen are more pronounced than the effects of radiation. Comparing mice exposed to a 1 g environment via centrifuge in the International Space Station with ground controls, Horie et al. found that erythroid-related genes regulated by the transcription factor GATA1 were significantly downregulated in spleen whole-transcript cDNA sequencing data from the space group of mice [7]. Gravity affects the expression and regulation of the immune response by significantly increasing the cytokine IL-1β and significantly decreasing IL-2 in the spleen [8]. In addition, the thymus is significantly affected by changes in gravity during spaceflight, and alterations in the organ environment can impair lymphocyte production, thus indirectly affecting acquired immunity [9]. Similarly, using embryonic thymus organ culture, gravity was found to significantly affect T-cell development. Mice that had undergone a 16-day flight had significantly fewer precursor cells, further suggesting that gravity plays a decisive role in T-cell development [10]. Certainly, a lot of effects of spaceflight are combinatorial effects of radiation and gravity, and the researchers' subjected mice to a chronic cumulative dose of 0.5 Gy of gamma radiation for a month in combination with simulated microgravity, and the study found that radiation and gravity may interact to alter immune cell phenotypes in the thymus and spleen in an organ-specific manner [11]. Therefore, it is crucial for the health of flight crew members to study the impact of the space environment on the immune system at the molecular level.

In this study, we aimed to elucidate the effects of spaceflight on the thymus and spleen at the molecular level. We downloaded the gene expression dataset GSE152382 from the GEO database and extracted gene expression data from the thymus and spleen. Differential expression analysis and enrichment analysis were used to determine which pathways in the thymus and spleen were affected by the space environment. Then, PPI networks were constructed to identify hub genes using the STRING database and Cytoscape software. Using the TRRUST database, we screened for transcription factors in differentially expressed genes in the thymus and spleen, and constructed TF regulatory networks. Finally, the immune cell infiltration status in spleen and thymus tissues was obtained using ImmuneCellAI-mouse tools.

2. Materials and methods

2.1. Gene expression profiles

The gene expression dataset GSE152382 was downloaded from the Illumina HiSeq 2500 platform of the Gene Expression Omnibus (GEO) database. In total, RNA sequencing data from thymus and spleen tissue from 6 wild-type mice on the ground and from a 31-day stay on the International Space Station were included in the study.

2.2. Identification of DEGs

The R package DESeq2 was used to identify the differentially expressed genes (DEGs) between the flight group and ground group. Only the genes with logFC (fold change) ≥ 1 and P value < 0.05 were identified as DEGs. PCA of transcriptome data was performed using the R package gmodels. The online Venn diagram tool (http://bioinformatics.psb.ugent.be/webtools/Venn/) was used to obtain the common DEGs.

2.3. Enrichment analyses of DEGs

Gene ontology (GO) analysis of DEGs was performed using the clusterProfiler package in R software. A p value < 0.05 was considered statistically significant. Enrichment analysis of pathways for the DEGs was performed using KOBAS (version 3.0). The pathways were considered from four databases, including KEGG, Reactome, BioCyc and Panther [12]. The threshold was set as p value < 0.05.

2.4. PPI network construction and identification of hub genes

Protein–protein interaction (PPI) network analysis was carried out on the DEGs using the STRING database. A combined score > 0.4 was used as the threshold to screen the interactions between genes. Cytoscape (http://www.cytoscape.org) (version 3.8.2) was used to visualize this PPI network. Five common algorithms (MCC, EPC, DMNC, Degree and BottleNeck) from the cytoHubba plug-in of Cytoscape were used to evaluate and select hub genes.

2.5. Mining transcription factors

The transcription factor database (TRRUST, https://www.grnpedia.org/trrust/) contains 6552 pieces of reported regulatory information on TF targets in humans and mice, and was used to identify transcription factors in the DEGs.

2.6. Identification of DEGs

We used the analytical tool ImmuneCellAI-mouse (http://bioinfo.life.hust.edu.cn/ImmuCellAI-mouse/#!/) to obtain the immune cell composition in thymus and spleen samples. ImmuCellAI-mouse is a tool that can estimate the abundance of 36 immune cell subpopulations in mouse tissues based on

gene expression profiles. Next, the immune cell abundance of the space mice and ground mice was visualized using the ggplot2 package, and the difference in immune cell abundance between the two groups was tested for significance using the Wilcoxon test.

3. Results

3.1. Identification of DEGs in the spleen and thymus

Figure 1 shows the flow diagram for study enrollment. The results of the PCA clustering analysis of the samples are shown in Supplementary Figure 1, with outliers being removed in subsequent studies. Significant changes in gene expression in spleen and thymus tissues of mice traveling for one month on the space station compared to those on the ground were identified. In total, 104 upregulated genes and 188 downregulated genes were identified in spleen tissue, and 261 upregulated genes and 50 downregulated genes were identified in thymus tissue (Figure 2).

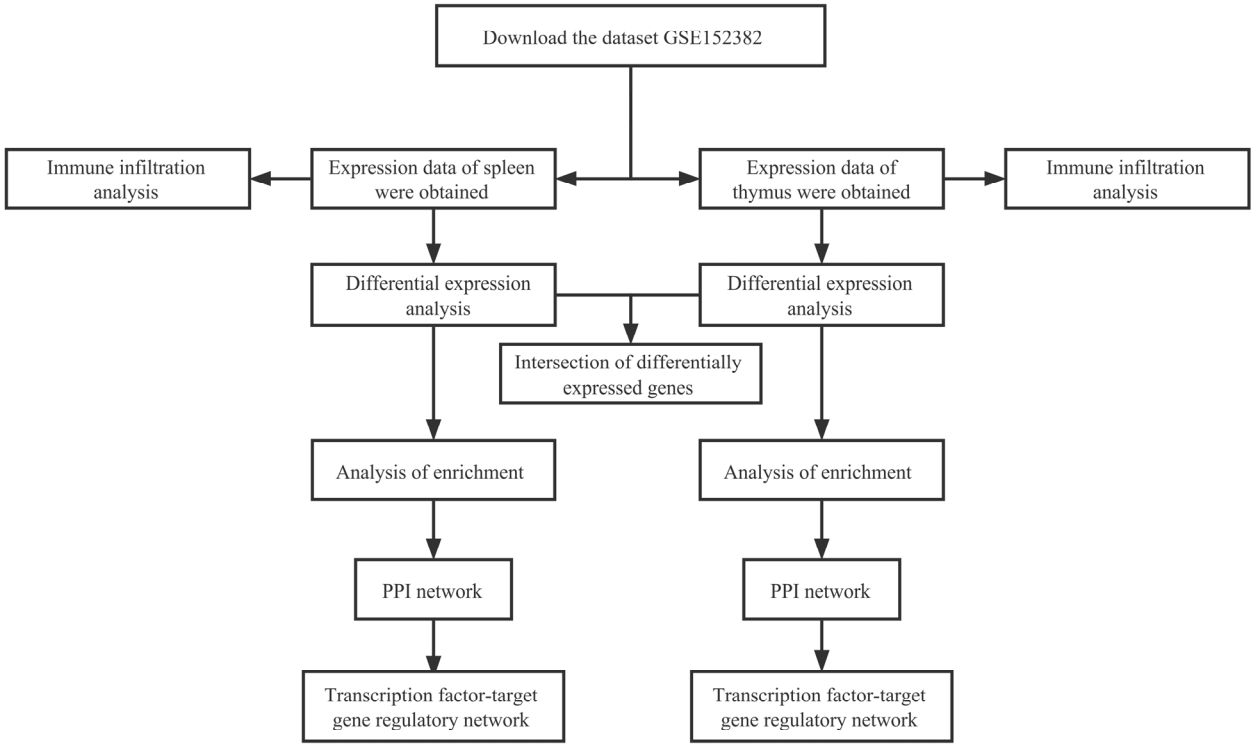

Figure 1. Flow chart of this study.

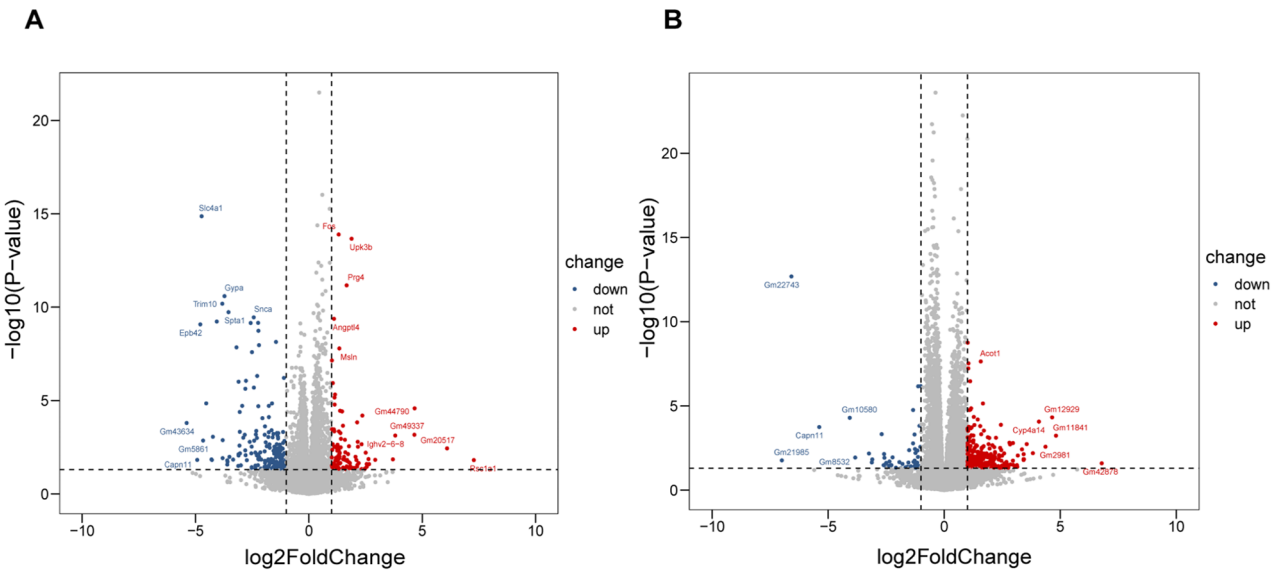

Figure 2. Volcano maps of DEGs. (A) Volcano map of DEGs in the spleen. (B) Volcano map of DEGs in the thymus.

3.2. Functional and pathway enrichment analysis of DEGs

To further understand the function of the DEGs, GO analysis and pathway enrichment analysis were performed using the clusterProfiler package and KOBAS. The enrichment results of DEGs in thymus and spleen tissues are shown in Figure 3A–D. GO analysis showed that DEGs in the spleen and thymus activated many immune-related biological processes, such as the humoral immune response, B-cell-mediated immunity and complement activation. Moreover, the results of the pathway enrichment analysis of DEGs in thymus and spleen tissues showed a high degree of similarity, and Table 1 displays the overlapping pathways. The Venn diagram shows the intersection of DEGs in the thymus and spleen (Figure 3E).

3.3. PPI network construction and identification of hub genes

The PPI network of DEGs in the spleen consisted of 149 nodes and 1102 edges (Figure 4A,B). Six genes were selected as hub genes, including Gc, Hpx, Fabp1, Ttr, Aldob and Uox. The PPI network of DEGs in the thymus consisted of 99 nodes and 457 edges (Figure 4C,D). Thirteen genes were selected as hub genes, including Apoh, Apob and Fga. Interestingly, Gc, Ttr, Fabp1 and Aldob are hub genes common to the spleen and thymus, suggesting that they have an important role in space travel.

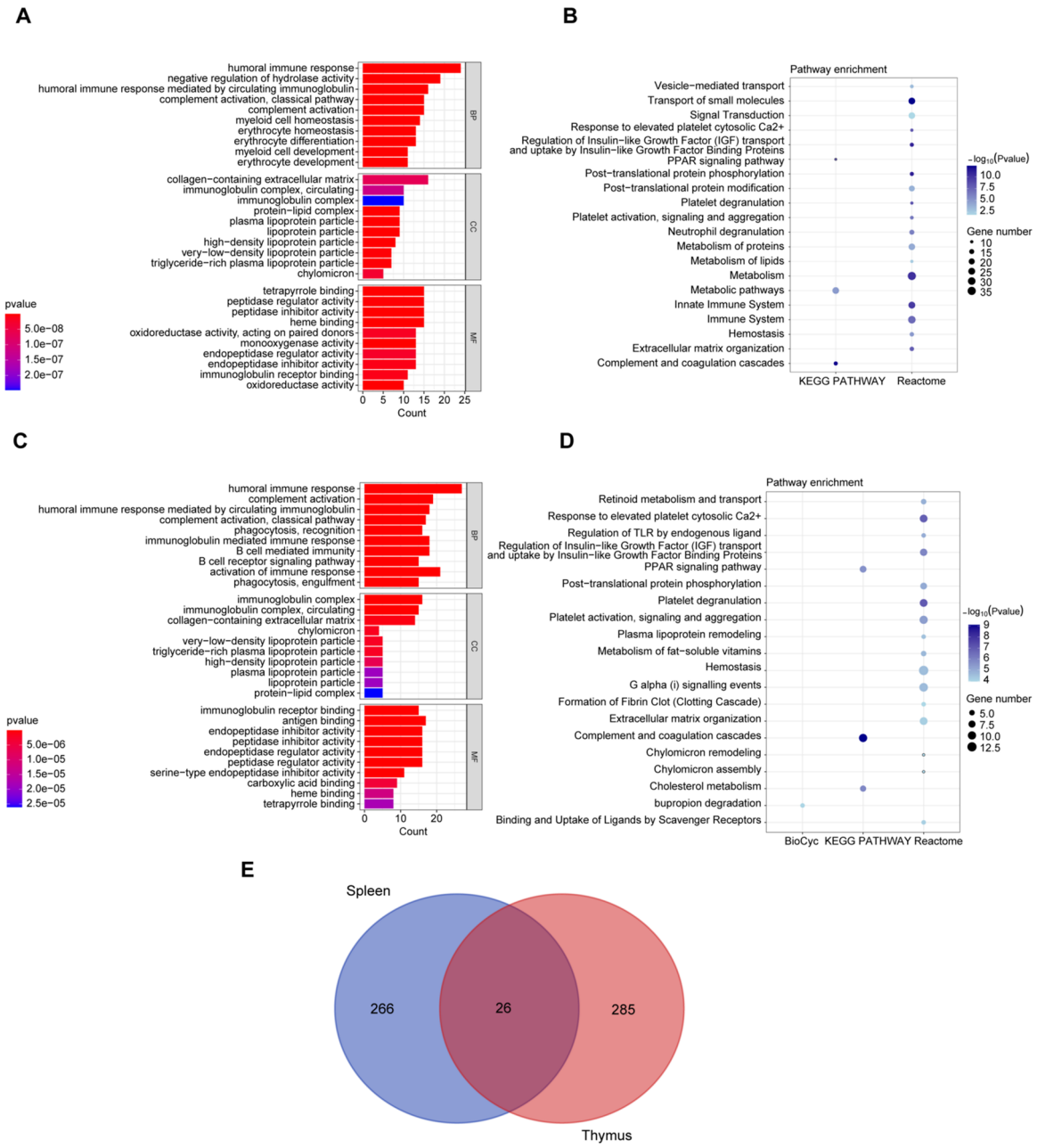

Figure 3. (A) Results of GO enrichment analysis of DEGs from the spleen. The top 10 BP, CC, and MF terms are shown separately. BP: Biological Process, CC: Cellular Component, MF: Molecular Function. (B) Top 20 items of KEGG enrichment analysis of DEGs in the spleen. (C) Results of GO enrichment analysis of DEGs in the thymus. The top 10 BP, CC, and MF terms are shown separately. (D) Top 20 items of KEGG enrichment analysis of DEGs in the thymus. (E) Venn diagram of the intersection of differentially expressed genes in the spleen and thymus.

Table 1. Intersection of the results of the top 20 KEGG analyses of the thymus and spleen.

ID	Term	Genes in thymus	Genes in spleen
mmu03320	PPAR signaling pathway	Cyp4a14, Cyp4a12a, Rxrg, Fabp1, Plin4, Apoa1, Apoa2	Cyp4a14, Cyp4a10, Angptl4, Fabp1, Apoa5, Plin4, Apoa1, Apoa2, Slc27a2
mmu04610	Complement and coagulation cascades	Kng1, Kng2, C4bp, Serpina1b, Serpina1d, F12, Hc, Fgg, Fga, Fgb	C4bp, C8a, Cfi, Kng1, Vtn, Plg, Serpina1c, C9, Serpina1d, Serpina1e, Fga, Fgb
R-MMU-109582	Hemostasis	Fga, Apob, Itih4, Lefty2, Kif26b, Apoa1, Vegfd, Apoh, F12, Itih3, Fgg, Gnat3, Fgb	Fga, Hpx, Apob, Orm1, Alb, Apoh, Adra2c, Plg, Serpina1c, Ahsg, Gata5, Apoa1, Fgb
R-MMU-114608	Platelet degranulation	Fga, Lefty2, Apoh, Itih4, Vegfd, Itih3, Fgg, Apoa1, Fgb	Hpx, Orm1, Alb, Apoh, Fga, Plg, Serpina1c, Ahsg, Apoa1, Fgb
R-MMU-1474244	Extracellular matrix organization	Klk1b16, Ttr, Optc, Spp1, Capn11, Fgg, Col2a1, Fga, Fgb	Elane, Matn4, Ttr, Tmprss6, Ctsg, Plg, Col4a6, Icam4, Capn11, Col4a4, Vtn, Fga, Fgb
R-MMU-381426	Regulation of Insulin-like Growth Factor (IGF) transport and uptake by Insulin-like Growth Factor Binding Proteins (IGFBPs)	Apob, Pappa2, Fga, Apol7b, Spp1, Fgg, Apoa1, Apoa2	Hpx, Apob, Alb, Fga, Serpina1c, Gpc3, Ahsg, Apol8, Apoa5, Msln, Apoa1, Apoa2
R-MMU-76002	Platelet activation, signaling and aggregation	Fga, Itih4, Lefty2, Apoh, Apoa1, Vegfd, Itih3, Fgg, Gnat3, Fgb	Fga, Hpx, Orm1, Alb, Apoh, Adra2c, Plg, Serpina1c, Ahsg, Apoa1, Fgb
R-MMU-76005	Response to elevated platelet cytosolic Ca2+	Fga, Lefty2, Apoh, Itih4, Vegfd, Itih3, Fgg, Apoa1, Fgb	Hpx, Orm1, Alb, Apoh, Fga, Plg, Serpina1c, Ahsg, Apoa1, Fgb
R-MMU-8957275	Post-translational protein phosphorylation	Apob, Fga, Apol7b, Spp1, Fgg, Apoa1, Apoa2	Hpx, Apob, Alb, Fga, Serpina1c, Gpc3, Ahsg, Apol8, Apoa5, Msln, Apoa1, Apoa2

3.4. Transcription factor regulatory network analysis of the DEGs

Transcription factors are important molecules that control gene expression. In this study, 5 transcription factors were identified among the differentially expressed genes in the spleen, including Msx2, Klf1, Sox6, Six3 and Fos. Similarly, 9 transcription factors were identified in the thymus, including Dtx1, Hnf4a, Irx3, Myog, Prox1, Pth, Runx1t1, Sox7 and Tfap2a. Figure 5 shows the regulatory network of differentially expressed transcription factors and target genes.

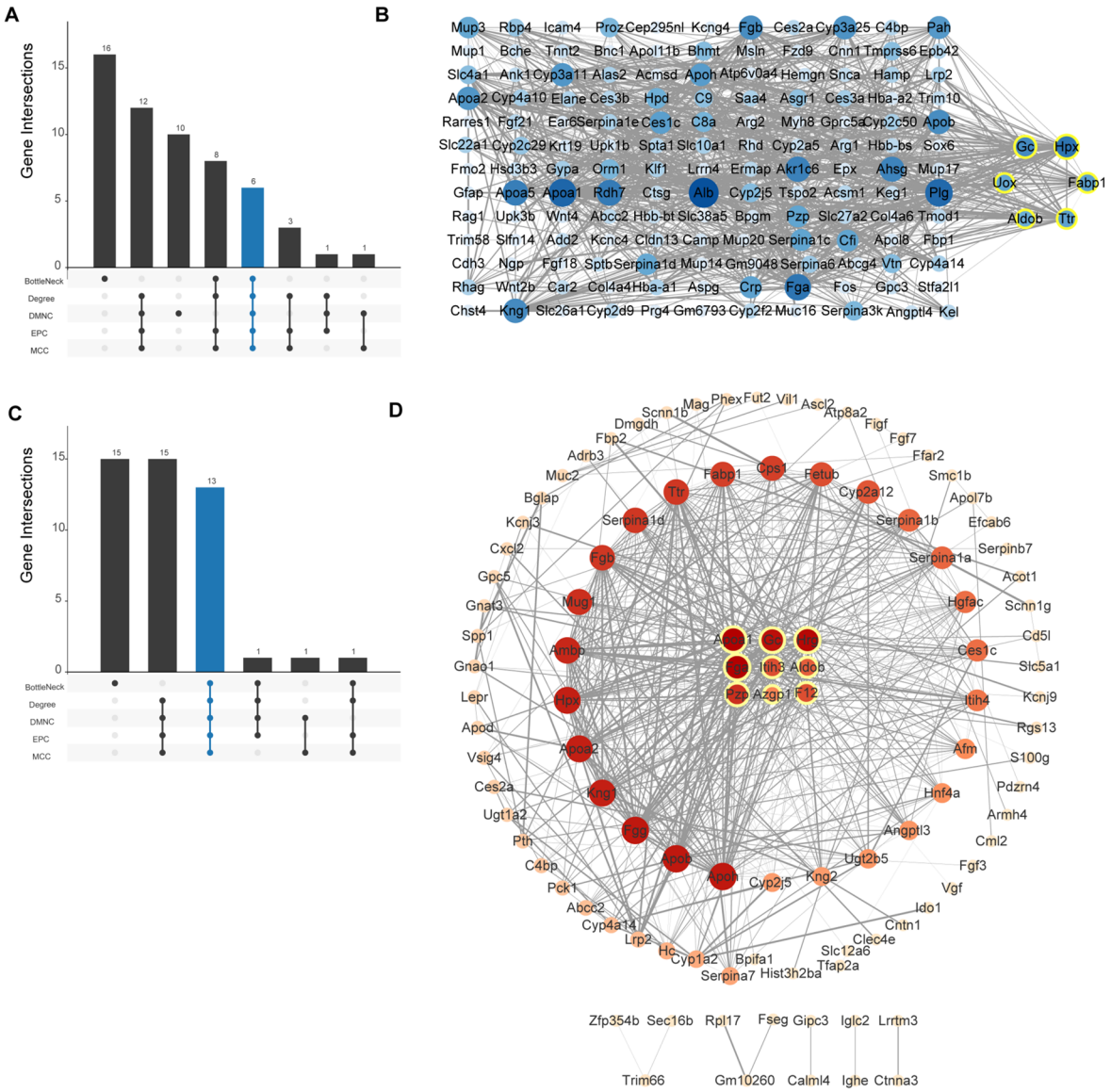

Figure 4. Protein–protein interaction network. (A) UpSet plots of hub genes in spleen identified by 5 algorithms of Cytoscape. Each black dot represents the hub genes identified by one algorithm. The dots connected by black lines represent the common hub genes identified by multiple algorithms. The numbers on the bars represent the number of genes. (B) PPI network plot of the spleen, where the genes in the yellow inner ring are the hub genes. (C) UpSet plots of hub genes in thymus identified by 5 algorithms of Cytoscape. Each black dot represents the hub genes identified by one algorithm. The dots connected by black lines represent the common hub genes identified by multiple algorithms. The numbers on the bars represent the number of genes. (D) PPI network plot of the thymus, where the genes in the yellow inner ring are the hub genes. The darker the color is, the larger the node, indicating the greater the degree of the node. The lines between the nodes represent the combined score.

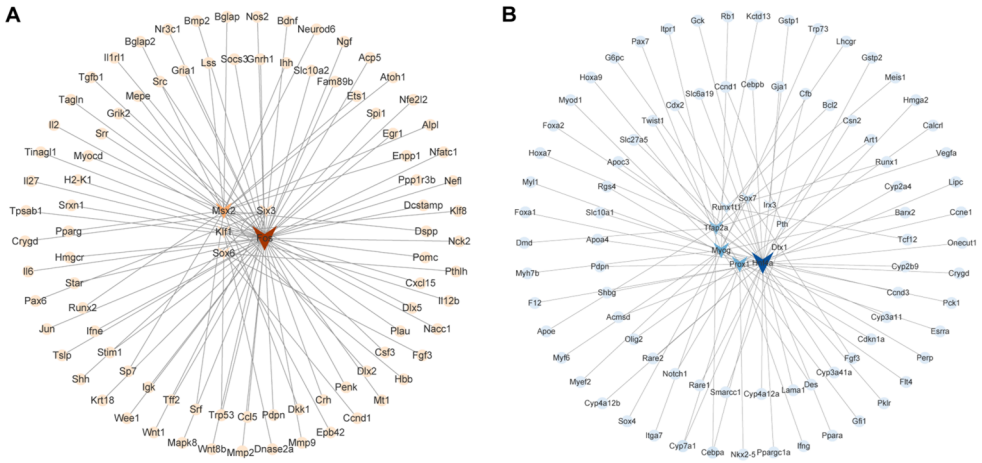

Figure 5. Transcription factor regulatory network. (A) The transcription factor regulatory network in the spleen. (B) The transcription factor regulatory network in the thymus. Triangles indicate transcription factors differentially expressed in the spleen, and circles are corresponding target genes.

3.5. Immune cell infiltration analysis

Thirty-six immune cell subpopulations were identified by ImmuCellAI-mouse. In the spleen, the proportions of Tgd cells and germinal center B cells were both significantly decreased compared to those in the control group. The proportion of M2 macrophages increased significantly (Figure 6A). In the thymus, the proportions of T cells, naive CD4 T cells and Treg cells decreased significantly, while the proportions of macrophages, marginal zone B cells and T helper cells increased significantly (Figure 6B). These results suggest that the space environment can influence the degree of immune cell infiltration and that immune cells in the thymus may be more sensitive to changes in the environment.

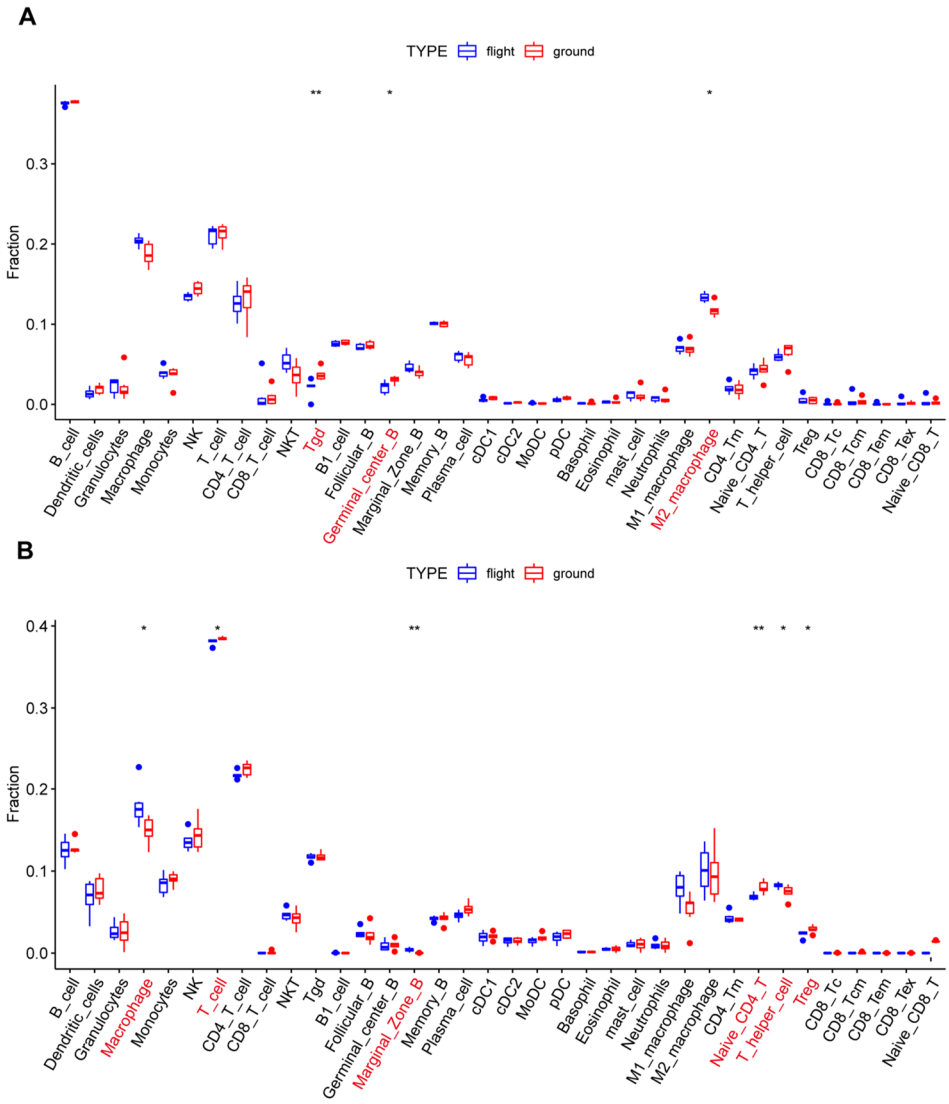

Figure 6. Results of immune infiltration analysis of the flight mice and ground mice samples. (A) Comparison of the boxplots of 36 immune cell subpopulations in spleen samples from flight mice and normal controls. (B) Comparison of the boxplots of 36 immune cell subpopulations in thymus samples from flight mice and normal controls. *p value < 0.05, **p value < 0.01, ***p value < 0.001.

4. Discussion

In this study, differential gene expression analysis identified 311 differentially expressed genes in the thymus, and 292 differentially expressed genes in the spleen of mice. Enrichment analysis revealed that differentially expressed genes in the thymus and spleen were primarily involved in immune responses, which is also consistent with previous studies. Interestingly, we intersected the differentially expressed genes in the thymus and spleen, and only 26 genes were found to be common. This suggests that the space environment affects immune organs by regulating the expression of different genes.

Subsequently, the results of KEGG pathway enrichment analysis of the thymus and spleen were

examined for pathways that were altered in both tissues. Nine of the first 20 items were identical, and 4 pathways were associated with platelets, including platelet degranulation, platelet activation, hemostasis and response to elevated platelet cytosolic Ca2+. This indicates that the space environment is capable of influencing platelets to some extent. In addition to their hemostatic effects, platelets are also the main inflammatory effector cells that can influence both innate and adaptive immune responses. Activated platelets have a thrombotic inflammatory function [13,14]. Dai et al. found that platelet function was inhibited in microgravity and activated in high gravity conditions, possibly as a novel mechanism for gravity change-related bleeding and thrombotic disorders [15]. Bednarska et al. studied the effect of UV-B radiation on platelet function in pigs, and found that UV-B radiation could partially activate platelets by stimulating platelet secretion processes and arachidonic acid metabolism [16]. This suggests that both gravity and radiation can activate platelets. Since the beginning of the space age, the majority of attention has been focused on the effects of environmental change on the cardiovascular system [17,18]. However, the effect of the spatial environment on platelet function may be a contributing factor to cardiovascular disease. Therefore, the results of our study suggest that the impact of gravity and radiation on platelets should be given special attention.

We performed protein-protein interaction analysis of differentially expressed genes in the thymus and spleen, and obtained key genes by Cytoscape. Gc and Aldob were hub genes shared by the spleen and thymus, which may play an important role in the immune system. Gc encodes the protein vitamin D binding protein (DBP). DBP performs multiple functions as a transporter of many ligands, and is a regulator of immune and inflammatory processes [19]. Among the hub genes identified in the spleen, Fabp1 encodes the fatty acid binding protein. Fabp1 is associated with the infiltration of immune cells, and researchers have found that Fapb1 is positively associated with the development of gastric cancer by regulating the immune microenvironment of the tumor [20]. In the thymus, Apoa1 encodes apolipoprotein A-1 (apoA-1), which is a major protein component of high-density lipoprotein (HDL). The apoA-1 plays an anti-inflammatory role by reducing integrin expression to inhibit immune cell transendothelial migration, inhibiting T cell contact-induced monocyte activation and cytokine production, inhibiting lipid peroxidation and interfering with the ability of innate immune receptors [21]. Hrg encodes histidine-rich glycoprotein, which binds a variety of ligands and is involved in the regulation of many processes, such as immune complex and pathogen clearance, cell chemotaxis and platelet activation [22].

TF-target information was downloaded from the TRRUST database to identify transcription factors in DEGs of the spleen and thymus. Five differentially expressed transcription factors were screened in the spleen, including Fos, Klf1, Msx2, Six3 and Sox6. Nine differentially expressed transcription factors were screened in the thymus, including Hnf4a, Prox1, Myog, Tfap2a, Runx11, Sox7, Irx3, Pth and Dtx1. KLF1 is a major regulator of erythropoiesis. Erythrocytes have the function of recognizing and storing antigens. A study found that exposure to hypoxic environments caused stress erythropoiesis. During stress erythropoiesis, the expression of Klf1 is significantly changed, and the expression of genes involved in the immune response was suppressed [23]. Sox6 promotes the ability of erythropoietin signaling to promote erythropoietic cell survival [24]. Therefore, the Klf1 and Sox6 may regulate the immune system by affecting erythropoiesis. Furthermore, the genes Fos, Msx2 and Sox6 are able to regulate the development of skeletal cells. Space flight adversely affects the bone marrow cell population and its function, leading to an imbalance in the skeletal reconstruction process [25]. Bone marrow tissues are home to a variety of immune cells, and is an important haematopoietic organ, along with the spleen and thymus. Platelet-associated pathways were

identified in functional enrichment analysis of DEGs. Therefore, these results suggested the space environment may affect the immune system by influencing haematopoiesis. Among the nine transcription factors identified in the thymus, we found that Prox1 and Dtx1 regulate the development of the lymphatic system [26,27]. The transcription factor Prox1 is a key regulator of lymphatic vessel production [28]. Middelhoff [29] found that, during inflammation-induced lymphangiogenesis, inflammatory stimulus-dependent activation of NF-κB signaling and Prox1 expression, which in turn activated the VEGFR-3 promoter, leading to increased receptor expression in lymphatic endothelial cells. Myog is a marker gene for myoid cells in the thymus [30]. Myoid cells could present exogenous antigens to T cells and induce proliferation of antigen-specific T cells [31].

Finally, we calculated the percentage of 36 immune cell subpopulations in the spleen and thymus samples. The space environment affected the ratio of immune cell subpopulations in both the spleen and thymus, and the thymus was more responsive. In the thymus, the ratio of T cells, naive CD4 T cells, T helper cells and Treg cells was significantly changed. Woods et al. studied the effects of space flight and weightlessness on T-cell development using fetal thymus organ cultures. They found that T-cell development was significantly weakened, and that impaired T-cell development appeared to occur at the pre-T-cell and CD4+ CD8+ T-cell stages [10]. This is consistent with our findings, suggesting that gravity plays a decisive role in the development of T cells. In addition, the proportion of Treg cells in the thymus decreased after entering space. Treg cells have a very important role in maintaining autoimmune tolerance and homeostasis. Absence or abnormal function of Treg cells can lead to the development of autoimmune diseases, such as amyotrophic lateral sclerosis [32] and rheumatoid arthritis [33]. Moreover, an imbalanced ratio of Th17 cells to Treg cells is associated with the development of many chronic inflammatory diseases [34,35].

5. Conclusions

In summary, we performed bioinformatics analysis of thymus and spleen tissues from mice after 31 days of stay on the ISS versus controls (thymus and spleen tissues from preflight mice). DEGs in both tissues were mainly associated with immunity and platelet activation. It is worth noting that, although both the thymus and spleen are immune organs and DEGs in both tissues are involved in the same biological processes, their molecular mechanisms may not be the same. Furthermore, the ratio of immune cells in the spleen and thymus was altered in mice, and the space environment may cause disorders of the immune system and consequent disease.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (81830052), the Special Program for Collaborative Innovation, the Construction project of Shanghai Key Laboratory of Molecular Imaging (18DZ2260400) and “Top-100 Talent Cultivation Plan” of Shanghai University of Medicine and Health Sciences, and Innovative Team of Intelligent Inspection and Active Health (ITIH).

Conflict of interest

We declare that there are no conflicts of interest.

References

1. J. J. Bong, Y. M. Kang, S. C. Shin, S. J. Choi, K. M. Lee, H. S. Kim, Identification of radiation-sensitive expressed genes in the ICR and AKR/J mouse thymus, *Cell Biol. Int.*, **37** (2013), 485–494. https://doi.org/10.1002/cbin.10065
2. K. Horie, T. Kato, T. Kudo, H. Sasanuma, M. Miyauchi, N. Akiyama, et al., Impact of spaceflight on the murine thymus and mitigation by exposure to artificial gravity during spaceflight, *Sci. Rep.*, **9** (2019), 19866. https://doi.org/10.1038/s41598-019-56432-9
3. R. Ito, L. P. Hale, S. M. Geyer, J. Li, A. Sornborger, J. Kajimura, et al., Late effects of exposure to ionizing radiation and age on human thymus morphology and function, *Radiat. Res.*, **187** (2017), 589–598. https://doi.org/10.1667/rr4554.1
4. D. S. Gridley, X. W. Mao, L. S. Stodieck, V. L. Ferguson, T. A. Bateman, M. Moldovan, et al., Changes in mouse thymus and spleen after return from the STS-135 mission in space, *PLoS One*, **8** (2013), e75097. https://doi.org/10.1371/journal.pone.0075097
5. E. C. Laiakis, I. Shuryak, A. Deziel, Y. W. Wang, B. L. Barnette, Y. Yu, et al., Effects of low dose space radiation exposures on the splenic metabolome, *Int. J. Mol. Sci.*, **22** (2021). https://doi.org/10.3390/ijms22063070
6. E. G. Novoselova, S. M. Lunin, M. O. Khrenov, S. B. Parfenyuk, T. V. Novoselova, B. S. Shenkman, et al., Changes in immune cell signalling, apoptosis and stress response functions in mice returned from the BION-M1 mission in space, *Immunobiology*, **220** (2015), 500–509. https://doi.org/10.1016/j.imbio.2014.10.021
7. K. Horie, H. Sasanuma, T. Kudo, S. I. Fujita, M. Miyauchi, T. Miyao, et al., Down-regulation of GATA1-dependent erythrocyte-related genes in the spleens of mice exposed to a space travel, *Sci. Rep.*, **9** (2019), 7654. https://doi.org/10.1038/s41598-019-44067-9
8. K. Felix, K. Wise, S. Manna, K. Yamauchi, B. L. Wilson, R. L. Thomas, et al., Altered cytokine expression in tissues of mice subjected to simulated microgravity, *Mol. Cell. Biochem.*, **266** (2004), 79–85. https://doi.org/10.1023/b:mcbi.0000049136.55611.dd
9. T. Akiyama, K. Horie, E. Hinoi, M. Hiraiwa, A. Kato, Y. Maekawa, et al., How does spaceflight affect the acquired immune system, *NPJ Microgravity*, **6** (2020), 14. https://doi.org/10.1038/s41526-020-0104-1
10. C. C. Woods, K. E. Banks, R. Gruener, D. DeLuca, Loss of T cell precursors after spaceflight and exposure to vector-averaged gravity, *Faseb J.*, **17** (2003), 1526–1528. https://doi.org/10.1096/fj.02-0749fje
11. R. Sadhukhan, D. Majumdar, S. Garg, R. D. Landes, V. McHargue, S. A. Pawar, et al., Simultaneous exposure to chronic irradiation and simulated microgravity differentially alters immune cell phenotype in mouse thymus and spleen, *Life Sci. Space Res.*, **28** (2021), 66–73. https://doi.org/10.1016/j.lssr.2020.09.004
12. K. Chen, S. Liu, C. Lu, X. Gu, A prognostic and therapeutic hallmark developed by the integrated profile of basement membrane and immune infiltrative landscape in lung adenocarcinoma, *Front. Immunol.*, **13** (2022), 1058493. https://doi.org/10.3389/fimmu.2022.1058493
13. P. R. B. Dib, A. C. Quirino-Teixeira, L. B. Merij, M. B. M. Pinheiro, S. V. Rozini, F. B. Andrade, et al., Innate immune receptors in platelets and platelet-leukocyte interactions, *J. Leukocyte Biol.*, **108** (2020), 1157–1182. https://doi.org/10.1002/jlb.4mr0620-701r

14. D. Lai, L. Tan, X. Zuo, D. Liu, D. Jiao, G. Wan, et al., Prognostic ferroptosis-related lncRNA signatures associated with immunotherapy and chemotherapy responses in patients with stomach cancer, *Front. Genet.*, **12** (2021), 798612. https://doi.org/10.3389/fgene.2021.798612
15. K. Dai, Y. Wang, R. Yan, Q. Shi, Z. Wang, Y. Yuan, et al., Effects of microgravity and hypergravity on platelet functions, *Thromb. Haemostasis*, **101** (2009), 902–910. https://doi.org/10.1160/TH08-11-0750
16. K. Bednarska, B. Wachowicz, Changes in blood platelets exposed to UV-B radiation, *J. Photochem. Photobiol. B Biol.*, **49** (1999), 187–191. https://doi.org/10.1016/s1011-1344(99)00057-3
17. M. Shen, W. H. Frishman, Effects of spaceflight on cardiovascular physiology and health, *Cardiol. Rev.*, **27** (2019), 122–126. https://doi.org/10.1097/crd.0000000000000236
18. N. A. Vernice, C. Meydan, E. Afshinnekoo, C. E. Mason, Long-term spaceflight and the cardiovascular system, *Precis. Clin. Med.*, **3** (2020), 284–291. https://doi.org/10.1093/pcmedi/pbaa022
19. B. Lisowska-Myjak, A. Jóźwiak-Kisielewska, J. Łukaszkiewicz, E. Skarżyńska, Vitamin D-binding protein as a biomarker to confirm specific clinical diagnoses, *Expert Rev. Mol. Diagn.*, **20** (2020), 49–56. https://doi.org/10.1080/14737159.2020.1699064
20. S. Liu, C. Ni, Y. Li, H. Yin, C. Xing, Y. Yuan, et al., The involvement of TRIB3 and FABP1 and their potential functions in the dynamic process of gastric cancer, *Front. Mol. Biosci.*, **8** (2021), 790433. https://doi.org/10.3389/fmolb.2021.790433
21. N. Vuilleumier, J. M. Dayer, A. von Eckardstein, P. Roux-Lombard, Pro- or anti-inflammatory role of apolipoprotein A-1 in high-density lipoproteins, *Swiss Med. Wkly*, **143** (2013), w13781. https://doi.org/10.4414/smw.2013.13781
22. M. Blank, Y. Shoenfeld, Histidine-rich glycoprotein modulation of immune/autoimmune, vascular, and coagulation systems, *Clin. Rev. Allergy Immunol.*, **34** (2008), 307–312. https://doi.org/10.1007/s12016-007-8058-6
23. H. Wang, D. Liu, P. Song, F. Jiang, X. Chi, T. Zhang, Exposure to hypoxia causes stress erythropoiesis and downregulates immune response genes in spleen of mice, *BMC Genomics*, **22** (2021), 413. https://doi.org/10.1186/s12864-021-07731-x
24. B. Dumitriu, M. R. Patrick, J. P. Petschek, S. Cherukuri, U. Klingmuller, P. L. Fox, et al., Sox6 cell-autonomously stimulates erythroid cell survival, proliferation, and terminal maturation and is thereby an important enhancer of definitive erythropoiesis during mouse development, *Blood*, **108** (2006), 1198–1207. https://doi.org/10.1182/blood-2006-02-004184
25. E. Ozçivici, Effects of spaceflight on cells of bone marrow origin, *Turk. J. Hematol.*, **30** (2013), 1–7. https://doi.org/10.4274/tjh.2012.0127
26. T. Elsir, A. Smits, M. S. Lindström, M. Nistér, Transcription factor PROX1: its role in development and cancer, *Cancer Metastasis Rev.*, **31** (2012), 793–805. https://doi.org/10.1007/s10555-012-9390-8
27. W. H. Liu, M. Z. Lai, Deltex regulates T-cell activation by targeted degradation of active MEKK1, *Mol. Cell. Biol.*, **25** (2005), 1367–1378. https://doi.org/10.1128/mcb.25.4.1367-1378.2005
28. P. Yu, J. K. Tung, M. Simons, Lymphatic fate specification: an ERK-controlled transcriptional program, *Microvasc. Res.*, **96** (2014), 10–15. https://doi.org/10.1016/j.mvr.2014.07.016

29. M. Middelhoff, H. Nienhüser, G. Valenti, H. C. Maurer, Y. Hayakawa, R. Takahashi, et al., Prox1-positive cells monitor and sustain the murine intestinal epithelial cholinergic niche, *Nat. Commun.*, **11** (2020), 111. https://doi.org/10.1038/s41467-019-13850-7
30. J. E. Park, R. A. Botting, C. Conde, D. M. Popescu, M. Lavaert, D. J. Kunz, et al., A cell atlas of human thymic development defines T cell repertoire formation, *Science*, **367** (2020). https://doi.org/10.1126/science.aay3224
31. M. Y. Matsumoto, H. Matsuo, T. Oka, T. Fukudome, K. Hayashi, H. Shiraishi, et al., Thymic myoid cells as a myasthenogenic antigen and antigen-presenting cells, *J. Neuroimmunol.*, **150** (2004), 80–87. https://doi.org/10.1016/j.jneuroim.2004.01.022
32. M. Rajabinejad, S. Ranjbar, L. Afshar Hezarkhani, F. Salari, A. Gorgin Karaji, A. Rezaiemanesh, Regulatory T cells for amyotrophic lateral sclerosis/motor neuron disease: A clinical and preclinical systematic review, *J. Cell. Physiol.*, **235** (2020), 5030–5040. https://doi.org/10.1002/jcp.29401
33. A. Avdeeva, Y. Rubtsov, D. Dyikanov, T. Popkova, E. Nasonov, Regulatory T cells in patients with early untreated rheumatoid arthritis: Phenotypic changes in the course of methotrexate treatment, *Biochimie*, **174** (2020), 9–17. https://doi.org/10.1016/j.biochi.2020.03.014
34. A. O. Naufel, M. C. F. Aguiar, F. M. Madeira, L. G. Abreu, Treg and Th17 cells in inflammatory periapical disease: a systematic review, *Braz. Oral. Res.*, **31** (2017). https://doi.org/10.1590/1807-3107bor-2017.vol31.0103
35. J. B. Yan, M. M. Luo, Z. Y. Chen, B. H. He, The function and role of the Th17/Treg cell balance in inflammatory bowel disease, *J. Immunol. Res.*, **2020** (2020), 8813558. https://doi.org/10.1155/2020/8813558

Supplementary

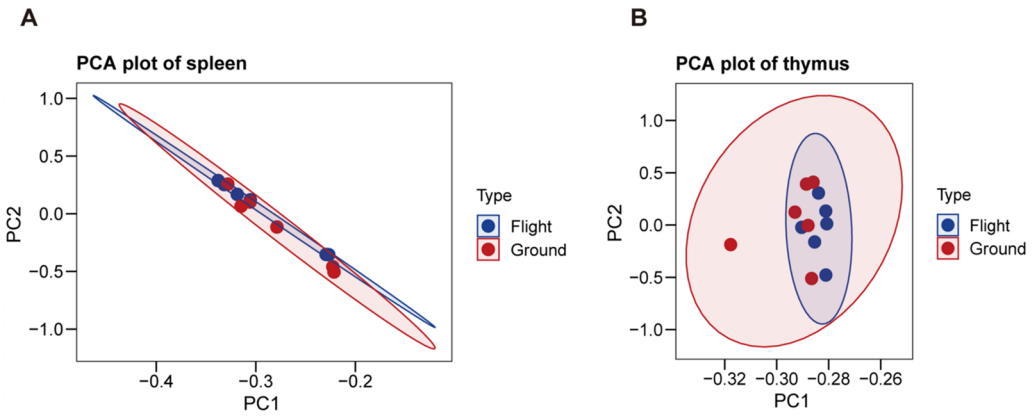

Figure S1. PCA of samples. (A) PCA plot of the spleen. (B) PCA plot of the thymus.