

## Neutrophil to lymphocyte ratio: A prognostic indicator for astronaut health

Amber M. Paul<sup>1,2</sup>, Siddhita D. Mhatre<sup>3</sup>, Egle Cekanaviciute<sup>1,2</sup>, Ann-Sofie Schreurs<sup>1,2</sup>, Candice G.T. Tahimic<sup>1,3</sup>, Ruth K. Globus<sup>1</sup>, Brian Crucian<sup>4</sup>, and Sharmila Bhattacharya<sup>1</sup>

<sup>1</sup>Space Biosciences Division, NASA Ames Research Center, Moffett Field, CA, 94035

<sup>2</sup>Universities Space Research Association, Columbia, MD, 21046

<sup>3</sup>KBRWyle, Moffett Field, CA, 94035

<sup>4</sup>Biomedical Research and Environmental Sciences Division, NASA Johnson Science Center, Houston, TX, 77058

### Abstract

Short-term and long-term spaceflight missions can cause immune system dysfunction in astronauts. Recent studies indicate elevated white blood cells (WBC) and polymorphonuclear neutrophils (PMN) in astronaut blood, along with unchanged or reduced lymphocyte counts, and reduced T cell function, during short-(days) and long-(months) term spaceflight. A high PMN to lymphocyte ratio (NLR) can act as a strong predictor of poor prognosis in cancer, and as a biomarker for subclinical inflammation in humans and chronic stress in mouse models, however, the NLR has not yet been identified as a predictor of astronaut health during spaceflight. For this, complete blood cell count data collected from astronauts and rodents that have flown for short- and long-term missions on board the International Space Station (ISS) was repurposed to determine the NLR pre-, in-, and post-flight. The results displayed that the NLR progressively increased during spaceflight in both human and mice, while a spike in the NLR was observed at post-flight landing, suggesting stress-induced factors may be involved. In addition, the ground-based chronic microgravity analog, hindlimb unloading in mice, indicated an increased NLR, along with induced myeloperoxidase expression, as measured by quantitative (q)PCR. The mechanism for increased NLR was further assessed *in vitro* using the NASA-developed rotating wall vessel (RWV) cell culture suspension system with human WBCs. The results indicated that simulated microgravity led to increased mature PMN counts, NLR profiles, and production of reactive oxygen species (ROS). Collectively, these studies show that an increased NLR is observed in spaceflight missions, and in chronic microgravity-analog simulation in mice, and that this effect may be potentiated by the oxidative stress response in blood cells under microgravity conditions. Furthermore, these results suggest that a disrupted NLR profile in spaceflight may further disrupt immune homeostasis, potentially causing chronic immune-mediated inflammatory diseases. Thus, we propose that the health status of astronauts during short- and long-term space missions can be monitored by their NLR profile, in addition to utilizing this measurement as a tool for interventions and countermeasure development to restore homeostatic immunity.

Support by Universities Space Research Association (USRA) and NASA Space Biology Program.



# Neutrophil to lymphocyte ratio: A prognostic indicator for astronaut health

Amber M. Paul<sup>2</sup>, Siddhita D. Mhatre<sup>3</sup>, Egle Cekanaviciute<sup>2</sup>, Ann-Sofie Schreurs<sup>2</sup>, Candice G.T. Tahimic<sup>3</sup>, Ruth K. Globus<sup>1</sup>, Brian Crucian<sup>4</sup>, and Sharmila Bhattacharya<sup>1</sup>

<sup>1</sup>Space Biosciences Division, NASA Ames Research Center, Moffett Field, CA, 94035, <sup>2</sup>Universities Space Research Association, Columbia, MD, 21046, <sup>3</sup>KBRWyle, Moffett Field, CA, 94035, and <sup>4</sup>Biomedical Research and Environmental Sciences Division, NASA Johnson Science Center, Houston, TX, 77058.

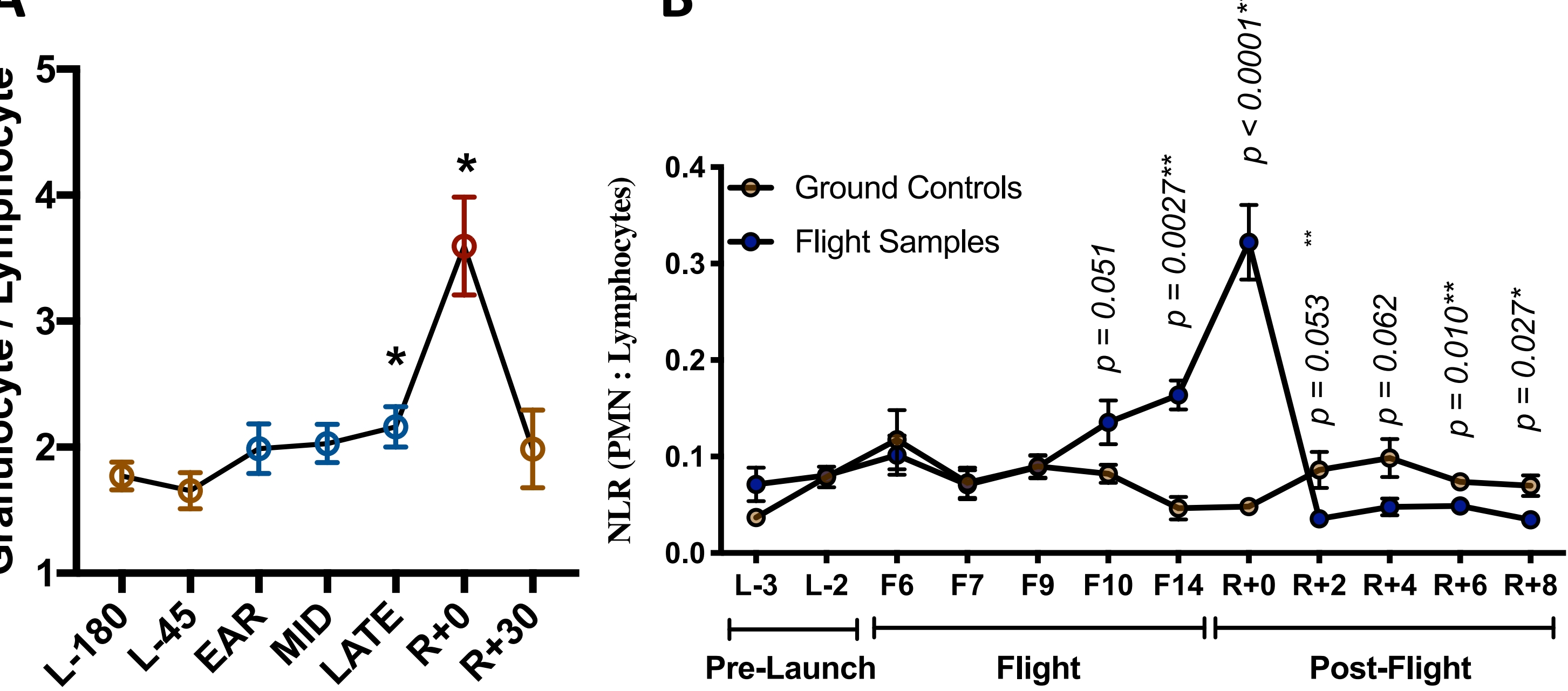
## Introduction

Short- and long-term spaceflight missions can cause immune system dysfunction in astronauts. Studies indicate elevated white blood cells (WBC) and polymorphonuclear neutrophils (PMN) in astronaut blood, along with unchanged or reduced lymphocyte counts, and reduced T cell function, during short-(days) and long-(months) term spaceflight. A high PMN to lymphocyte ratio (NLR) can act as a strong predictor of poor prognosis in cancer, and as a biomarker for subclinical inflammation in humans and chronic stress in mouse models, however, the NLR has not yet been identified as a predictor of astronaut health during spaceflight. For this, complete blood cell count data collected from astronauts and rodents that have flown for short- and long-term missions on board the International Space Station (ISS) was repurposed to determine the NLR pre-, in-, and post-flight. Collectively, these results suggest a disrupted NLR profile in spaceflight, which may further disrupt immune homeostasis. Under the hypothesis that spaceflight induces a higher NLR due to aberrant oxidative stress response, this study aimed to; **1: determine the NLR and GLR of astronauts and rodents during short- and long-term missions, and 2: determine the contributing role of reactive oxygen species (ROS) in this immune profile shifting.** For this, the ground-based microgravity analog, hindlimb unloading (HU) mouse model and the high-aspect rotating wall vessel cell culture system (HARV-RWV) were utilized to determine the effects of microgravity on the generation of ROS and resulting effects on the maturation and function of PMNs in human blood samples.

## Methods

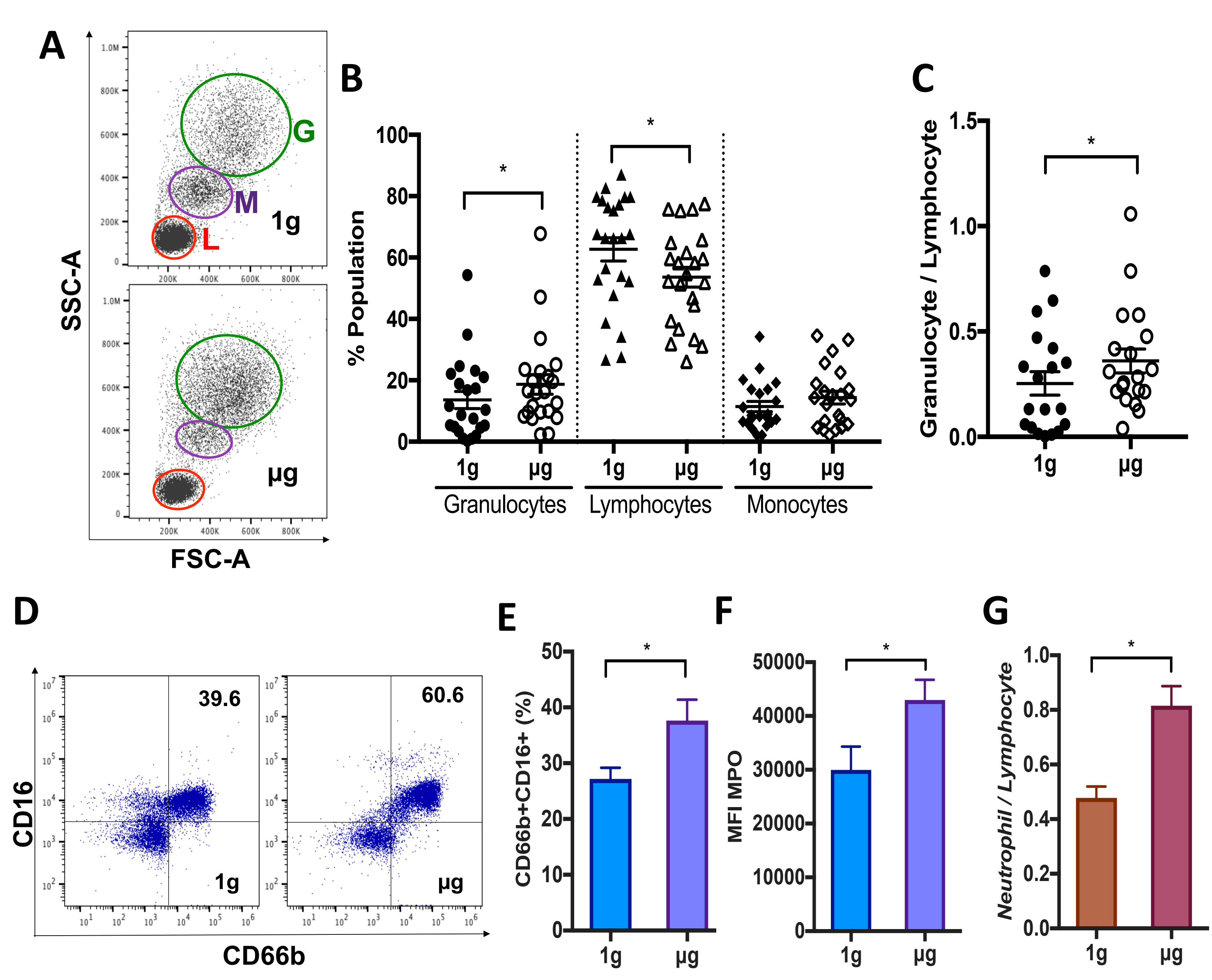
- Cell Culture:** Human whole blood was separated into cell types using ficoll- and histo- paque. Similarly, terminal mouse blood was collected and separated. RBCs were lysed and WBCs were grown in complete RPMI media.
- Phagocytosis assay:** Isolated blood cells were cultured with pHrodo red/green bioparticles. Cells were fixed, washed and analyzed using flow cytometry.
- Simulated microgravity:** 3D high-aspect rotating wall vessels (HARV-RWV, Synthecon) were used to simulate microgravity. Cells were suspended at  $5 \times 10^5$  cells/ml in 10 ml and rotated at 20 RPM ( $\mu g$ ) for 24 hr. Upright flasks were used as 1g controls. Following incubation cells were collected for appropriate assays.
- Hindlimb unloaded mice:** Females, 4-month old were subjected to tail restrained hindlimb unloading for 30 days.
- qPCR:** Total RNA was extracted from cells. qRT-PCR was performed using iQ SYBR green supermix.  $\beta$ -Actin, normalizing gene was used for calculating relative fold change (RFC).

## Spaceflight

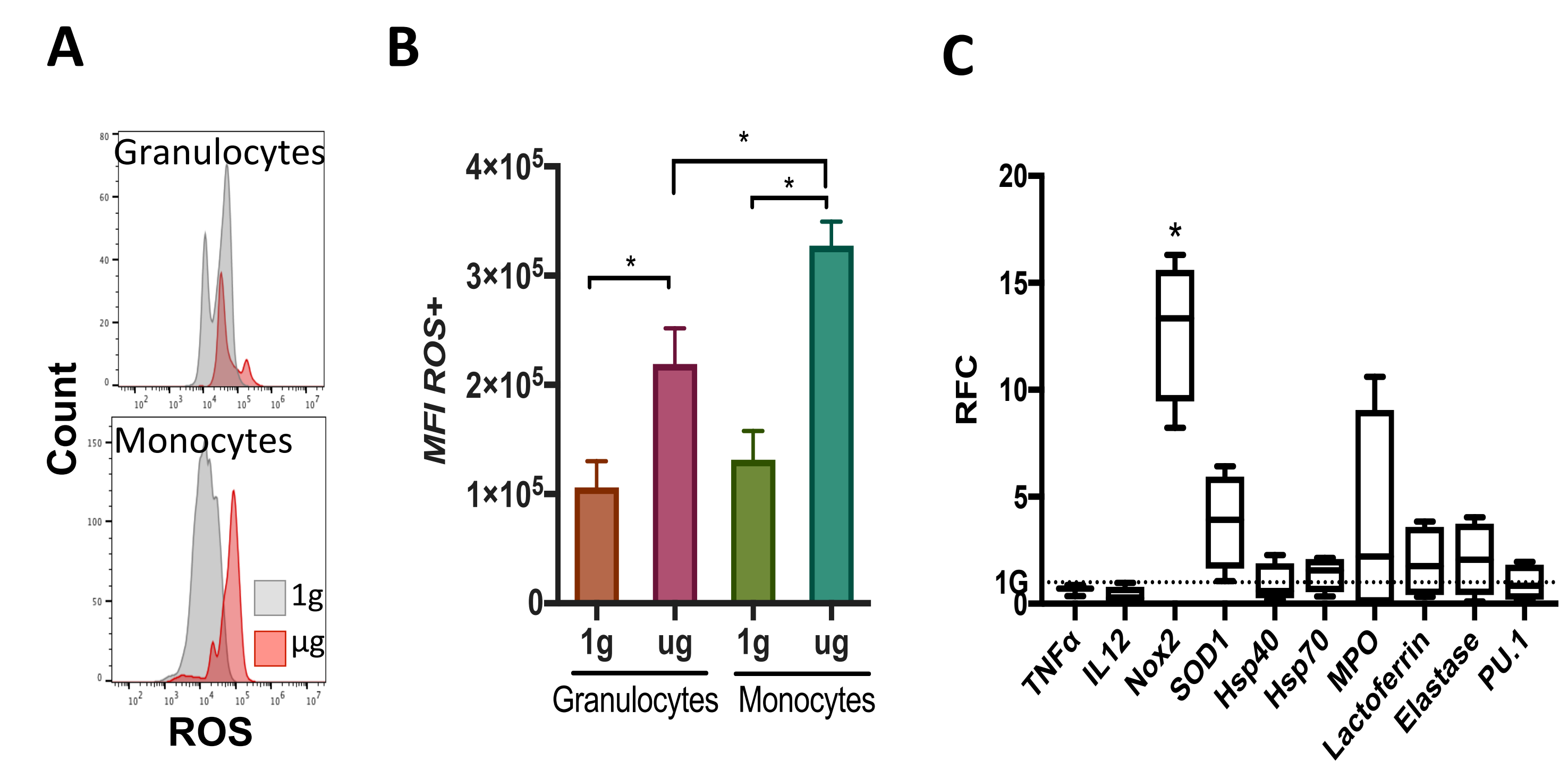


**Figure 1: Spaceflight increased GLR and NLR.** (A) Repurposed astronaut blood data shows progressive increase in GLR inflight and at post-return (B) Repurposed rodent data from STS-58 mission show progressive increase in NLR inflight, while a significantly increased NLR was displayed post-return.\* indicates  $p < 0.05$

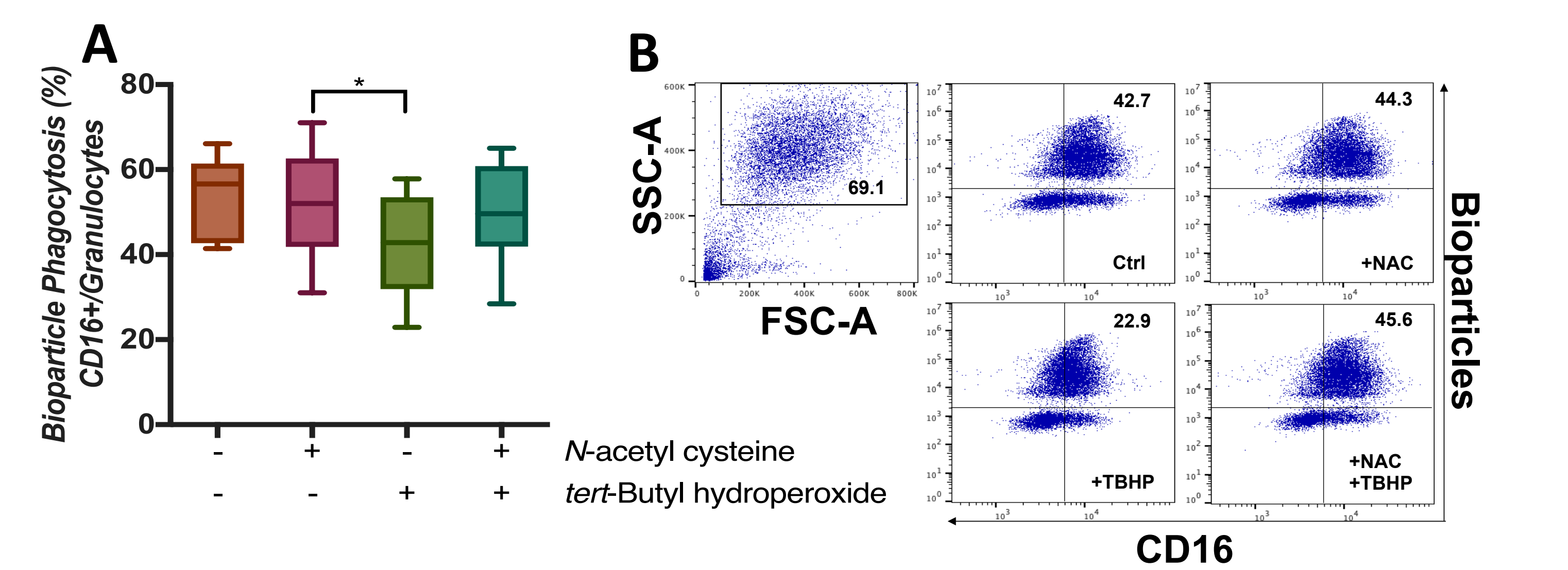
## Simulated microgravity models



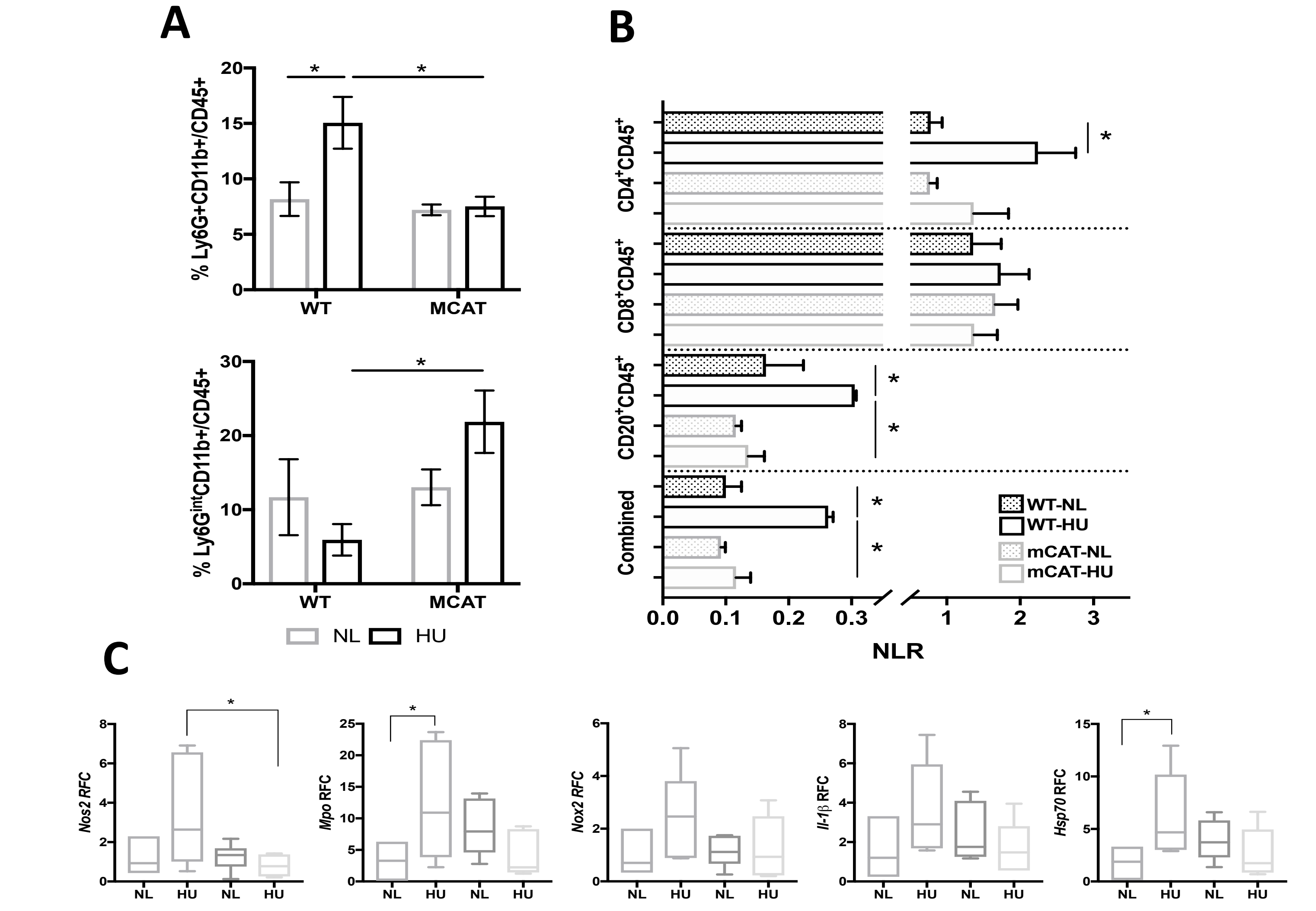
**Figure 2: Simulated microgravity resulted in increased granulocyte and PMN maturation, myeloperoxidase expression, and increased NLR.** (A) Flow scatter plot with different cell types marked, G, Granulocyte (green); M, Monocyte (purple); L, Lymphocytes (red) (B) Dot plot representing the % population of each cell type (C) Dot plot representing the granulocyte to lymphocyte ratio (D) Scatter plot marking mature neutrophils ( $CD66b^{high}$  and  $CD16^{high}$ ) (E) Graph depicting increased mature neutrophils in simulated microgravity (F) Graph shows increased levels of myeloperoxidase, marker for PMN degranulation a process that occurs in mature neutrophils (G) Graph shows increased mature neutrophil to total lymphocyte ratio in simulated microgravity conditions. \* indicates  $p < 0.05$



**Figure 3: Simulated microgravity induced oxidative stress.** (A) Histogram shows a shift in the intensity of CellROX suggesting increase in total ROS fluorescence in microgravity condition as compared to 1g controls (B) Quantification of mean fluorescence intensity of CellROX stain suggest increased ROS in granulocytes and monocyte population (C) qPCR analysis suggest increased neutrophil-specific and oxidative stress responsive genes represented as relative fold change (RFC). \* indicates  $p < 0.05$



**Figure 4: Simulated microgravity resulted in decreased phagocytosis** (A) Phagocytic ability of cells under oxidative stress was determined using *S.aureus*-bioparticles in cells stimulated with *tert*-Butyl hydroperoxide (ROS inducer) and *N*-acetyl cysteine (antioxidant). We observe reduced phagocytic ability of myeloid cells under ROS inducer treatment (B) Scatter plot showing myeloid bioparticle positive cells. \* indicates  $p < 0.05$ .



**Figure 5: Hindlimb unloaded mice (30days) displays increased NLR and MPO, while human mitochondrial catalase (mCAT) mitigates the phenotype** (A) Graph displays decreased neutrophils ( $Ly6G^{+}CD11b^{+}/CD45^{+}$ ) and increased immature neutrophils ( $Ly6G^{int}CD11b^{+}/CD45^{+}$ ) in mice blood (B) Histogram indicating increased helper T-cells ( $CD4^{+}CD45^{+}$ ) and B cell ( $CD20^{+}CD45^{+}$ ) lymphocyte count and an overall increase in the NLR count in HU mice (C) qPCR analysis show increased *Mpo*, *Nos2* and *Hsp70* expression in HU mice, which is mitigated with *mCAT* transgenic mice. \* indicates  $p < 0.05$

## Conclusion

- Progressive increased in GLR (humans) and NLR (rodent) in spaceflight and at post-flight return.
- Increased GLR, NLR and ROS in human blood under simulated microgravity, resulting in dysfunctional phagocytosis and robust MPO expression.
- Increased NLR in hindlimb unloaded mice, along with increased *Mpo* expression in blood.

## Acknowledgements

Would like to thank NASA Ames Space Biology Research program and Universities Space Research Association for funding this research, and the Life Science Data Archive (LSDA) for providing retrospective data from spaceflight missions.