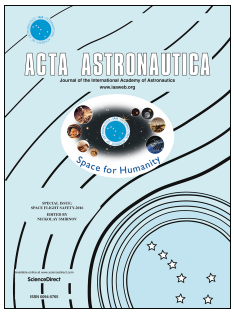

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Effects of rapid gravity load changes on immunophenotyping and leukocyte function of human peripheral blood after parabolic flight

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Abstract

One of the biological systems that suffers a physiological de-conditioning in space is the immune system. It is in charge of defending the body against pathogens and other aggressions. The aim of this work is to assess if there are any relevant changes in the aggregation of erythrocytes, cell count, immunophenotyping and functionality after parabolic flight. This effect has been assessed *ex vivo* using human peripheral blood, which was drawn from the radial vein (n=6 healthy volunteers) and anticoagulated with heparin and EDTA. Blood samples were split into two aliquots and maintained in two identical thermally isolated boxes; one stayed on the ground whereas the other one was subjected to parabolic flight. The parabolic flight consisted of 15 parabolas performed with a Mudry CAP-10B acrobatic aircraft. Each parabola consists of 8 seconds of hypogravity preceded and followed by 2 seconds of hypergravity. Any of the biological parameters measured showed no statistically significant differences. Altered gravity could increase aggregation of red blood cells, as demonstrated by a decrease in the number of single cells after parabolic flight exposure. No counting changes in haemoglobin concentration were observed when comparing the two different groups. Furthermore, potential functional alterations of monocytes and neutrophils cannot be rejected. Although these possible changes could be associated with hypogravity, other factors such as hypergravity and acceleration or deceleration cannot be ruled out. Our findings indicate that, under this specific experimental setup, there was no significant alteration in leukocyte

immunophenotyping and functional capacity when using *ex vivo* blood samples and short exposure to altered gravity.

Keywords: immune system, parabolic flight, altered gravity, cell aggregation, phagocytic capacity, oxidative burst

1. Introduction

Long-time exposure to altered gravity provokes physiologic changes in most of human tissues: heart and blood vessels, which can result in arrhythmias, muscle atrophy, lower bone mineral density and fracture risk, nauseas and disorientation, and an increased susceptibility to infections [1–5].

In addition to altered gravity, ionizing radiation, confinement, isolation and altered circadian rhythms, extreme temperatures, and constant noise are other stressing factors that face astronauts in space, which is called the space exposome [6,7].

1.1 Gravity, hypogravity and hypergravity

The ideal scenarios to study the effects of altered gravity along with the space exposome are spaceflights and the International Space Station (ISS) in which altered gravity and the space exposome converge [8]. These scenarios present two main disadvantages for scientific research: these opportunities are both rare and costly. Alternatively, real hypogravity conditions can be achieved on Earth under several experimental setups, even though the period and values of hypogravity may vary among platforms [8,9].

Between 2.2 and 9.3 seconds of hypogravity are achieved in Drop Towers with values close to 10^{-6} *g*, while sounding rockets achieve between 5 and 10 minutes of hypogravity with values of 10^{-4} *g*. During a parabolic flight, values from 10^{-2} and 10^{-3} *g* for 15-22 seconds are obtained [8]. Through these platforms along with the ISS and satellites, real hypogravity values can be achieved. There are other platforms such as clinostats, random positioning machines (RPM) and rotating wall vessel (RWV) that can achieve simulated gravity [9].

1.1.1 Parabolic flight

In this study, the platform used to assess the effect of hypogravity on human blood samples is the parabolic flight (PF). PF consists of a specific number of parabolas performed with an aircraft. Each parabola has a period of hypogravity preceded and followed by a period of hypergravity [10].

A PF campaign took place in Sabadell Airport (Barcelona, Spain) in March 2022 with 15 parabolas. In the last decade this platform has successfully performed optimal 5-8.5 seconds parabolas with an average of 0.01 *g* residual acceleration for both education and research purposes [11].

1.2 Human blood cells populations

The most abundant formed cell elements in blood are erythrocytes which, among other functions, transport oxygen from lungs to the cells [12]. Proportionately rare cells called leukocytes, defend the organism from pathogens. Leukocytes include neutrophils, basophils, eosinophils, lymphocytes, and monocytes. These cells along with bone narrow, thymus, lymph nodes, spleen, and mucosa-associated lymph tissues are part of the immune system [13].

1.3 Physiology and altered gravity

“Space anaemia” is one of the physiological changes firstly described after long term exposure to microgravity [14]. When human subjects are orbiting the Earth, there is a reduction in the plasma volume due to dehydration along with a corporal fluid redistribution [15]. Evidence suggests that this reduction would increase the erythrocyte concentration, that would in turn decrease erythropoietin release, and, consequently, lead to lower erythrocyte production. This physiological adjustment would also be boosted by the lower muscular workload and oxygen delivery requirements and the consequent skeletal muscle mass loss [6,14].

Regarding the immune system, in the first space missions, more than half of the crew members suffered a viral or bacterial infection [16]. Long periods of exposure to altered gravity provoke changes in the immune system [5]. An increased number of granulocytes and reduction in lymphocytes and monocytes has been described, probably in response to the stressful situation of returning to Earth conditions [17]. Macrophages are one of the most studied immune cells in altered gravity conditions. Their dysfunctionality could be due to the suppression of tumour necrosis factor alfa (TNF-α), a molecule which mediates the immune response, and a change in the expression of the Intercellular Adhesion Molecule 1 (ICAM-1) protein, an adhesion molecule required for initiating the immune response [18,19].

The hypothesis of this study is that short exposure to altered gravity *per se* may alter the immunophenotype and function of different blood cell subpopulations, whereas changes in blood cell count should not be expected under *ex vivo* conditions when peripheral blood samples are analysed. What is new in this study is the use of flow cytometry to understand the effects of a parabolic flight in *ex vivo* conditions.

2. Material and methods

2.1 Experimental design

Six healthy young people (three males and three females) volunteered to participate in this study. All the volunteers were summoned on the same day, at the same time in the morning at the Pedralbes Campus Medical Services of the *University of Barcelona* for blood drawing. A qualified person drew the participant blood samples. Peripheral blood of each participant was split in five aliquots. In three of them, the anticoagulant was ethylenediaminetetraacetic acid (EDTA) whereas in the two remaining it was lithium heparin. Two different anticoagulants were needed based on the analytical requirements. Heparin was used for functional assays, including phagocytosis detection and oxidative burst assessment. EDTA was used for cell counting and immunophenotyping. The whole blood extraction procedure started at 8:15 am and ended at 9:10 am (Table 1). In order to avoid vigorous shaking with potential impact in sample haemolysis, blood tubes were almost filled. The remaining air bubble allowed anticoagulant gently mixing.

One of the EDTA anticoagulated samples from each participant was used for quantitative haematological analysis (Department of Cell Biology, Physiology, and Immunology, University of Barcelona). The remaining samples were then divided into two identical boxes of expanded polystyrene, each of which contained two tubes from each participant, one anticoagulated with EDTA and one with heparin. The tubes were kept at room temperature and fixed inside the box by tight racks. Both boxes were transferred to the Sabadell Airport (QYS/LELL). The airport is a 30-minute drive from where the blood samples were drawn. One of the twin boxes was attached to the co-pilot's seat and exposed to changes of gravity load after parabolic flight (A condition) while the other box remained on the ground (B condition).

After the parabolic flight, both boxes were carried to the Germans Trias i Pujol Research Institute where the phenotypic and functional analyses were performed. Flow cytometric measurements included cell counting; identification of major lymphocyte populations (total T cells, helper T cells, cytotoxic T cells, B cells, and NK cells), also known as TBNK immunophenotyping; oxyhaemoglobin absorption; oxidative burst and phagocytosis assessment. The experimental design is shown in Figure 1.

2.1.1 Parabolic flight

The parabolic flight was carried out with a Mudry CAP-10B aerobatic aircraft operated by one of the pilots at the Barcelona-Sabadell Aviation Club. The aircraft has two seats, one for the pilot and the other was occupied by the box carrying blood samples. The box was properly attached to the seat to study the potential effects of hypogravity periods (see Fig. 2).

The parabolic flight lasted 20 minutes. Median temperature was 12ºC, ranging from 10 to 15ºC. The flight operation was carried out following the Visual Flight Rules (VFR). An accelerometer model BWT901CL (WitMotion, Shenzhen Co, China) was attached to the carrying blood box to measure the hypogravity periods. The accelerometer is a multisensory device used to assess the residual acceleration [20]. Samples were loosely attached to the cockpit avoiding vibrations or g-jitter.

The accelerometer was connected using Bluetooth to a mobile cell phone to collect all the information about the flight. Gravity values obtained during the parabolic flight are shown in Figure 3 and these values are expressed in acceleration. The parabolic flight consisted of 15 parabolas and the height ranged between 600 and 1200 metres. The hypogravity period lasted 8.5 seconds and reached a value of 0.02 ± 0.01 *g*. Each hypogravity period was preceded and followed by a hypergravity period that lasted 2 seconds and reached the value of 3.2 *g*. This aircraft has been used for other space-biological related experiments such as heart rate variability [21], sperm [22] and brain activity [23].

2.1.2 Human subjects

A total of six volunteers were recruited to participate in this study. Three of them were females while the others were males, (age 25, 22 – 31 years old; body weight 70.67, 54 – 90 kg; height 166.17, 152 – 175 centimetres; mean, range). None of them suffered from a diagnosed illness or was undergoing any medical treatment.

Volunteers signed the informed consent, and they did not receive any compensation for participating in the study. The study was developed in accordance with the World Medical Association Declaration of Helsinki concerning the ethical principles of human experimentation.

2.2 Sample processing

Samples were prepared within 4h after finishing parabolic flight for the assessment of haematologic profile, red blood cells scattering, phagocytosis, oxidative burst, and for the analysis and quantification of T/B/NK-cells, according to the following protocols.

2.2.1 Flow cytometry

Both phenotypic and functional analysis were conducted in the Germans Trias i Pujol Research Institute. Samples were acquired on the Attune NxT Acoustic Focusing Cytometer (Thermo Fisher

Scientific) equipped with 4 lasers (violet, blue, yellow-green and red; operating at 405, 488 561 and 637 nm, respectively) and 14 fluorescence detectors [24].

When a cell passes through the laser beam, the light is scattered in all directions. Side scatter (SSC) and forward scatter (FSC) provide information about the complexity and size of the cell, respectively. The combination of both light scatter parameters allows easy identification of lymphocytes, monocytes, and granulocytes. Fluorescence is also collected simultaneously, providing multiparametric information at a real time and single cell level [25]. The cytometric approach used in this work included erythrocytic and leucocytic counting, TBNK immunophenotyping, and functional measurements (oxidative burst and phagocytic activity).

2.2.1.1 Acoustophoretic orientation of erythrocytes

Briefly, 2 µl of blood were diluted in 1 ml of Hanks' Balanced Salt Solution (HBSS, Biowest). Diluted blood was acquired immediately on a flow cytometer after a 15 minute-incubation at room temperature and 37ºC, following a protocol described by Rico et al. [26]. The erythrocytic count was performed taking advantage of the different light absorption of erythrocytes, by collecting the blue and violet SSC simultaneously. Violet SSC was also used to analyse the oxyhaemoglobin absorption at 413 nm to evaluate potential variations over time.

2.2.1.2 Leucocyte counting

A volume of 100 µl of blood was diluted into 900 µl of HBSS and incubated in the presence 10 µl of Hoechst 33342 (1 mg/ml, stock concentration), a fluorescent dye used for specifically staining the nuclei of living or fixed cells. After 20 min incubation, 100 µl of the dilution were added to 900 µl of HBSS and were acquired in the flow cytometer. The Hoechst 33342 signal was collected in logarithmic scale and allowed the discrimination of nucleated and non-nucleated cells. The FSC and SSC were collected in linear scale.

2.2.1.3 TBNK immunophenotyping

The following monoclonal antibodies were used for the identification of TBNK subpopulations: FITC-CD3, PE-CD56, PB-CD8, APC-CD4, AF750-CD19 and PO-CD16 (Sysmex). A volume of 5 µl of each antibody was added to 50 µl of blood and incubated for 20 min at room temperature and light protected. After incubation, a fixative and lysing solution intended for lysing red blood cells and fixing white blood cells (CyLyse, Sysmex) was used following manufacturer instructions.

2.2.2 Functional analysis

2.2.2.1 Phagocytosis and oxidative burst

Samples were prepared following a minimal sample perturbation protocol [27]. Briefly, 50 µl of blood were diluted in 1 ml HBSS and incubated with 10 µl Hoechst 33342 (1 mg/ml stock concentration), 2 µl Dihydrorhodamine 123 (DHR 123) (0.01 mg/ml stock concentration) and 10 µl pHrodo™ Red BioParticles (1 mg/ml stock concentration) (ThermoFisher). pH-sensitive pHrodo™ dye conjugated to *E. coli* particles is non-fluorescent at neutral pH and exhibit and increasing fluorescent signal as pH decreases. This increase relates with phagocytic activity since cell cytosol has an acid pH. Dihydrorhodamine 123 is a non-fluorescent soluble dye used as indicator of Reactive Oxigen Species (ROS). When diffuses passively through the cell membrane, can be oxidated by ROS and transformed in fluorescent Rhodamine 123, allowing thus, the detection of ROS in the cells. For optimal stimulation and staining conditions, 1 µl Phorbol 12-myristate 13-acetate (PMA) (1 mg/ml stock concentration) was added. Non-stimulated control samples were prepared with 1 µl Dimethyl sulfoxide (DMSO). Samples were incubated for 30 min light-protected at 37ºC in a dedicated water bath prior to flow cytometry acquisition. Phagocytosis was determined in phagocytes in terms of pHrodo™ red mean fluorescent intensity (MFI), whereas measurement of oxidative burst in neutrophils was performed in terms of Rhodamine 123 MFI.

2.2.3 Data analysis

Datasets obtained from primary population description and haematological profiling were analysed using Excel software (Microsoft Corporation, v.2205 2018). Data from accelerometer was analysed using Matlab 2018a (Natick, Massachusetts: The MathWorks Inc, 2010). Flow cytometric data was analysed using FlowJo™ v.10.4. For statistical analysis, GraphPad™ Prism v.9 was used. Cell counting and functional activity between Group A and Group B were compared by using the non-parametric Wilcoxon Test. A p-value<0.05 was considered significant.

4. Results

The haematological profile of all the volunteers was within the range of normality, according to their sex and age (data not shown).

4.1 Phenotypic analysis

4.1.1 Red blood cell counting, aggregation and haemoglobin absorption

Single cell analysis of erythrocytes was performed using flow cytometry. Single cell counts were as follows: median = 108,951 cells/µL, ranging from 83,355 to 124,822 cells/µL (Group A) and median = 120,506 cells/µL ranging from 96,252 to 140,555 cells/µL (Group B) (see Fig. 4). No significant differences were obtained between groups (p-value = 0.3125; 95% CI =-104,000 – 370,000).

Oxyhaemoglobin absorption analysis was also performed using flow cytometry. Oxyhaemoglobin absorption values were as follows: median = 3,071/µL (Group A) and median = 3,116 /µL (Group B) (see Fig. 5). No significant differences were obtained between groups (p-value = 0.5625; 95% CI = -104.00 – 194.00).

4.1.2 Leucocyte counting

Single cell analysis of leucocytes was performed using cytometry according to staining with Ho342 and excluding aggregates and coincident events (erythrocytes). Single cell counts were as follows: median = 34.67 cells/µL, ranging from 28.46 to 68.97 cells/µL (Group A) and median = 35.55 cells/µL ranging from 28.81 to 69.07 cells/µL (Group B) (p-value = 0.6875, 95% CI = -1.8 – 4.7) (see Fig. 6A). Lymphocytes, monocytes and neutrophils were also determined with the following values: median lymphocytes Group A = 3.86 cells/µL, ranging from 3.01 cells/µL to 5.55 cells/µL and median lymphocytes Group B = 3.46 cells/µL, ranging from 3.17 cells/µL to 6.03 cells/µL (p-value = >0.99; 95%CI = -0.5 – 0.4) (see Fig. 6B); median monocytes Group A = 2.27 cells/µL, ranging from 1.28 cells/µL to 3.08 cells/µL and median monocytes Group B = 2.48 cells/µL, ranging from 1.54 cells/µL to 3.33 cells/µL (p-value = 0.03; 95%CI = 0.03 – 0.38) (see Fig. 6C); median neutrophils Group A = 27.07 cells/µL, ranging from 22.17 cells/µL to 60.80 cells/µL and median neutrophils Group B = 30.22 cells/µL, ranging from 22.22 cells/µL to 60.63 cells/µL (p-value = 0.84; 95% CI = -1.56 – 3.93) (see Fig. 6D). Neutrophil to lymphocyte ratios were also calculated with a median = 7.33, ranging from 5.10 to 14.41 (Group A) and median = 7.79, ranging from 5.97 to 13.74 (Group B) (p-value = 0.3125, 95% CI = -0.29 – 0.93) (see Fig 6E).

4.1.3 TBNK subsets

Peripheral blood obtained from 6 healthy donors was used for TBNK immunophenotyping. CD3, CD4, CD8, CD19, CD56 and CD16 marker expression were studied in both groups. FCS files were concatenated and subjected to dimensionality reduction using tSNE. $CD3^{-}CD19^{+}$ B cells, $CD3^{+}CD4^{+}$ T cells and $CD3^{+}CD8^{+}$ T cells, $CD3^{-}CD56^{+}CD16^{dim}$ NKs, and $CD3^{-}CD56^{dim}CD16^{+}$ NKs subsets were identified. After dimensionality reduction, no differences were observed when comparing the two different conditions (see Fig. 7).

4.2 Functional analysis

4.2.1 Phagocytosis

Single cell analysis of phagocytes provided the following pHrodo median fluorescence intensity values: MFI = 19,902 arbitrary units (a.u.), ranging from 14,850 to 25,167 a.u. (Group A) and MFI = 22,816 a.u. ranging from 18,642 to 28,794 a.u. (Group B) (see Fig. 8). No significant differences were obtained between groups (p-value = 0.1662; 95% CI = -415.1 – 6813).

4.2.2 Oxidative burst

Single cell analysis of neutrophils provided the following rhodamine 123 median fluorescence intensity values: MFI = 36,348 a.u., ranging from 23,841 to 50,336 a.u (Group A) and MFI = 32,520 a.u. ranging from 22,005 to 47,900 a.u. (Group B) (see Fig. 9). No significant differences were obtained between groups (p-value = 0.4375; 95% CI = -13,961 – 5,300).

5. Discussion

The objective of this study was to explore the potential effect of a short exposure to altered gravity *per se* on peripheral blood samples. We hypothesized potential alterations in the immunophenotype and function of different leucocyte subpopulations, whereas changes in blood cells count should not be expected under *ex vivo* conditions.

Red blood cell counting, aggregation and haemoglobin absorption

The first variable studied was the erythrocyte count, through single cells, along with the oxyhaemoglobin absorption. Both parameters are complementary and provide a closer approach of the effect of altered gravity to erythrocytes. There were no significant differences neither in red blood cells counting nor haemoglobin absorption. An increased aggregation of erythrocytes after exposition to simulated hypergravity for 30 minutes has been reported, whereas intermediate exposition did not show any differences *in vivo* [28]. In another study on twenty healthy men, after 15 minutes under 3 *g* hypergravity on a long-arm centrifuge, an increased aggregation of red blood cells was also found [29]. Interestingly, cells exposed to hypogravity did not show changes in cell aggregation [30]. Therefore, understanding the effect of altered gravity on erythrocyte aggregability and its underlying mechanisms needs to be clarified through additional experimental approaches.

Leucocyte counting and TBNK subsets

No differences were observed in total leucocyte counts when comparing the two different conditions. However, when enumerating lymphocytes, monocytes and neutrophils, single-cell monocytes were significatively reduced in the experimental group, suggesting changes in the normal aggregation capability when exposing to short periods of altered gravity.

Recently the ratio neutrophil to lymphocyte has been proposed to assess the astronaut's immune health [31,32]. No differences were observed in this ratio among our two experimental conditions.

Regarding TBNK subsets, no differences were found after clustering analysis. Our results could be explained by the fact that the experiment was *ex vivo* and the number of cells as well as the immunophenotype is unlikely to change after short-term altered gravity exposure followed by immediate sample processing. Moreover, the absence of effects on B cells after altered gravity were reported in two recent studies by Spielmann et al. and Bonnedoy et al. suggesting that real and simulated microgravity exposure may not affect B-cell homeostasis [33,34].

Functional analysis

Phagocytosis is the process by which a cell uses its plasma membrane to engulf a large particle, and is a major mechanism used to remove pathogens and apoptotic bodies by immune cells. This functional capacity is typical of several subtypes of circulating leukocytes, such as neutrophils, monocytes and macrophages, and can be elicited by oxygen-dependent mechanisms, such as reactive oxygen species (ROS) production (including hydrogen peroxide (H_2O_2), nitric oxide, etc.) and independent oxygen mechanisms through acidic environment. In our study, we studied the phagocytic capacity using pH-dependent pHrodo dye conjugated to *E. coli* particles and the oxidative burst by means of rhodamine 123, with and without PMA stimulation.

No statistical differences were observed between the two experimental conditions regarding functional measurements. In an experimental design similar to our approach, H_2O_2 production and phagocytosis were examined, showing non-significant differences in *ex vivo* samples. In the *in vivo* study, non-significant differences were detected when analysing phagocytosis, whereas H_2O_2 production was significantly increased after parabolic flight [35].

In another study, the functionality of macrophages was assessed under real and simulated hypogravity and hypergravity conditions, and a different effect on ROS production was found. Hypogravity reduced ROS production while hypergravity increased it, suggesting that ROS production is affected by gravity [36].

In summary, our results are not fully conclusive about an impaired functionality of the studied cells due to a lower phagocytic capacity and a higher ROS production, both in agreement with the published literature.

Study limitations and advantages

The main limitation of our study is the small sample size (n=6). However, this is a limiting factor in most of the space physiology related studies, due to the limited number of crew members and resources during spaceflights [6]. By contrast, in this study we applied cutting-edge novel methodology (acoustic focusing cytometry) to assess leukocyte immunophenotyping and functionality on human peripheral blood after a very short period of time once the parabolic flight was performed.

6. Conclusions

Peripheral blood samples exposed to short periods of hypogravity preceded and followed by seconds of hypergravity during parabolic flight did not show statistical differences in comparison with the control condition regarding leukocyte, lymphocyte and neutrophil counting, and TBNK immunophenotyping. Only monocyte counts showed significant differences, suggesting altered cell-to-cell interactions with gravity changes.

A higher aggregation of erythrocytes in the experimental condition can not be ruled out, as seen by an apparently reduced number of single cells although similar oxyhaemoglobin absorption. Also, a possible reduced phagocytic capacity and an increased ROS release after stimulation could indicate a possible effect of gravity on the cellular metabolism.

Anyway, our data point to a minor impact of altered gravity *per se* on *ex-vivo* blood components after short time exposure. Further research is required to delve deeper in the effect of gravitational alterations on human immune competence. An *in vivo* experiment in which volunteers would board the aircraft and their blood samples could be compared before and after the parabolic flight would help clarify this point.

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Legends for figures and tables

Figure 1. Experimental design. The experiment started at 8:15 in the morning with the blood extraction which lasted 55 minutes. After, the samples arrived at the Sabadell airport at 9:42 and the parabolic flight started at 10:20 until 10:43. Functional and phenotypic analysis started at 12:30 and lasted 240 and 1320 minutes respectively. Finally, the haematological profile started at 13:41 until 14:25.

Figure 2. Mudry CAP-10B aerobatic aircraft (A) and aircraft seats, one with the box containing samples and the accelerometer pointed (B).

Figure 3. Data obtained from the parabolic flight in which samples were exposed. On the Y left axis, the height was measured (meters); on the X axis, time (milliseconds); and on the Y right axis, acceleration values (g=9,81 m/s^2).

Figure 4. RBCs counting. Red blood cell counting in condition A (experimental group), and B (control group). The results are represented as event/µL. Analysis of erythrocytes was performed using flow cytometry. Medians are represented, dots indicate individual values of each participant and bars show minimum and maximum values. Significant differences were determined using the non-parametric Wilcoxon test. A p-value <0.05 was considered significant (*).

Figure 5. Oxyhaemoglobin absorption. Oxyhaemoglobin concentration in condition A (experimental group) and B (control group). The results are shown as total event/µL. Medians are represented, dots indicate individual values of each participant and bars show minimum and maximum values. Significant differences were determined using the non-parametric Wilcoxon test. A p-value <0.05 was considered significant (*).

Figure 6. Leukocyte populations count. Leukocytes (A), Lymhocytes (B), Monocytes (C), Neutrophils (D) and Neutrophil to Lymphocyte ratio are assessed in condition A (experimental group) and in condition B (control group). Results are shown in event/µL. Medians are represented, dots indicate individual values of each participant and bars show minimum and maximum values. Significant differences were determined using the non-parametric Wilcoxon test. A p-value <0.05 was considered significant (*).

Figure 7. tSNE displays of CD3, CD4, CD8, CD19, CD56 and CD16 markers expression showing merged conditions (A+B), experimental condition (A) and control group (B).

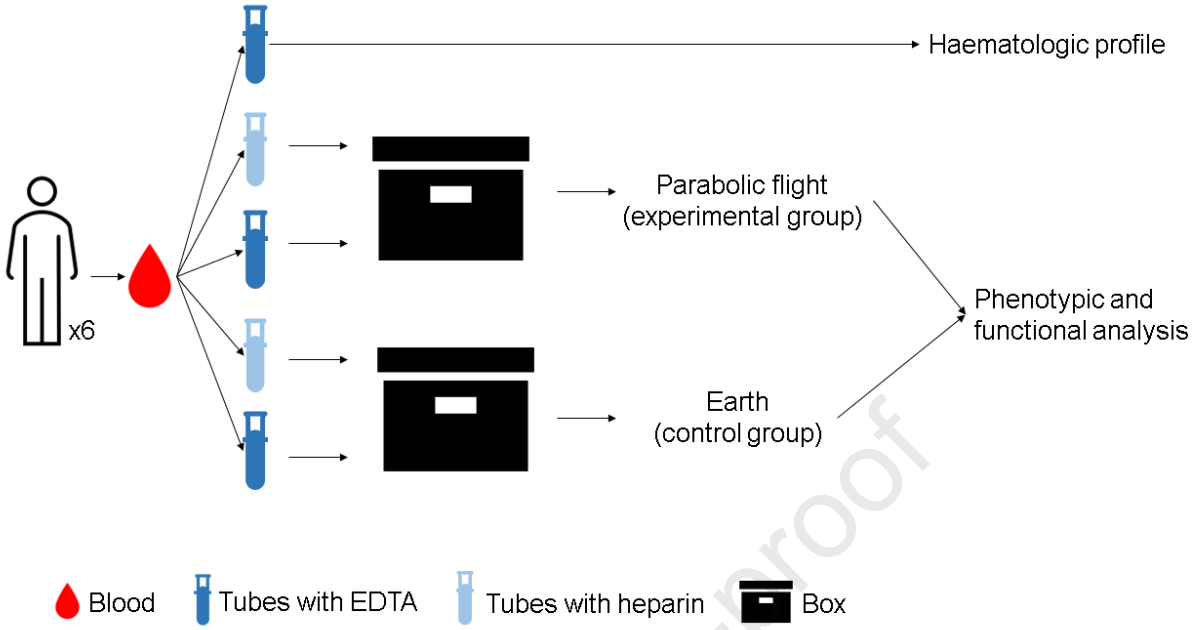
Figure 8. Single cell analysis of phagocyte activity. The results of pHrodo median fluorescence intensity values are expressed in arbitrary units for condition A and condition B. Medians are represented, dots indicate individual values of each participant and bars show minimum and maximum values. Significant differences were determined using the non-parametric Wilcoxon test. A p-value <0.05 was considered significant (*).

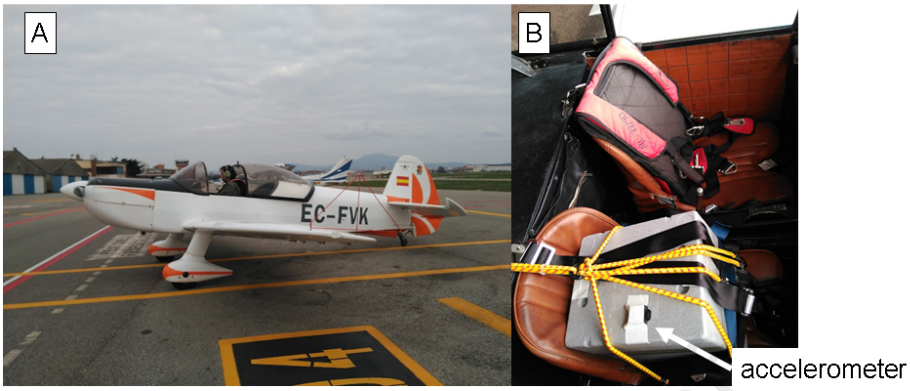
Figure 9. Single cells analysis of neutrophil oxidative burst. The results of rhodamine 123 median fluorescence intensity values are expressed in arbitrary units. Medians are represented, dots indicate individual values of each participant and bars show minimum and maximum values. Significant differences were determined using the non-parametric Wilcoxon test. A p-value <0.05 was considered significant (*).

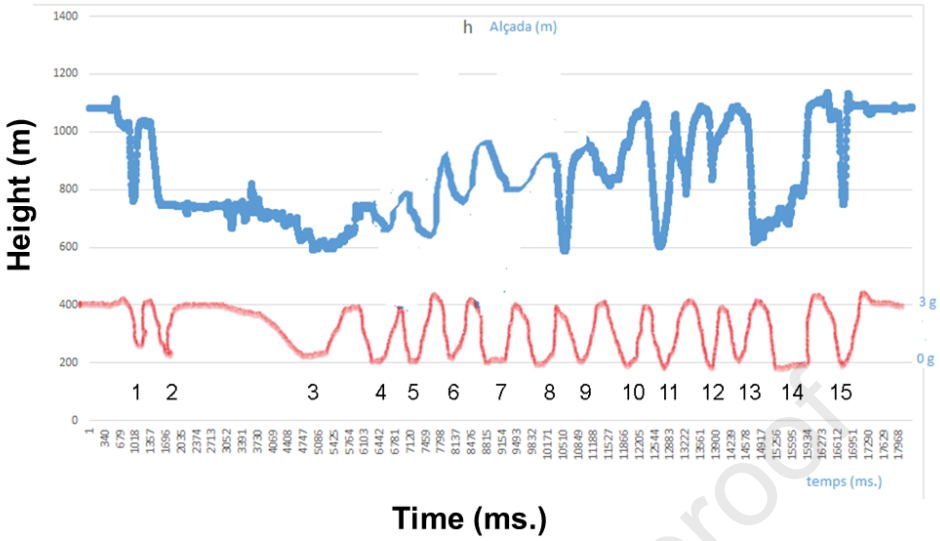
Table 1. Time of the different parts of the experimental procedures

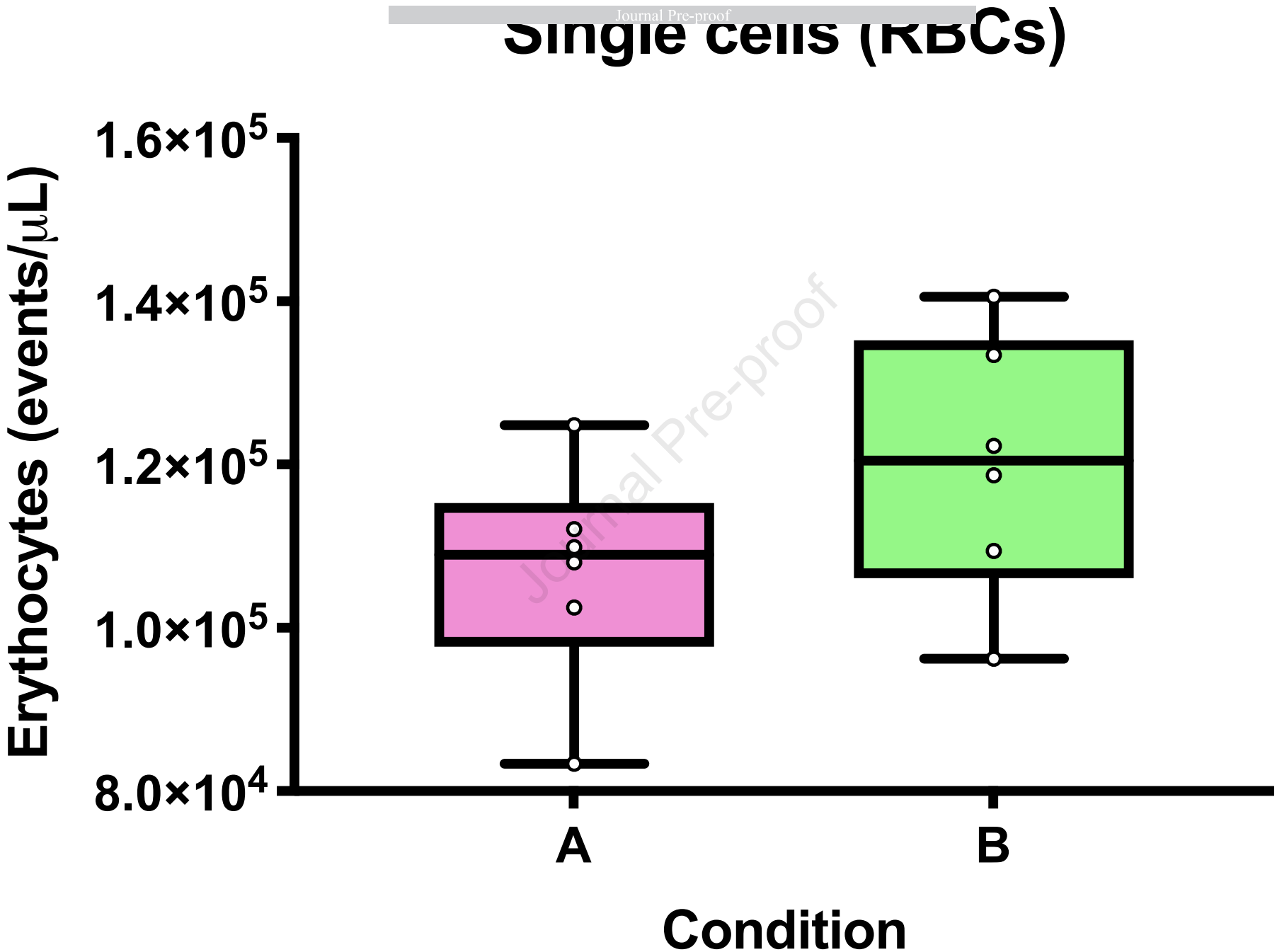
Table 1

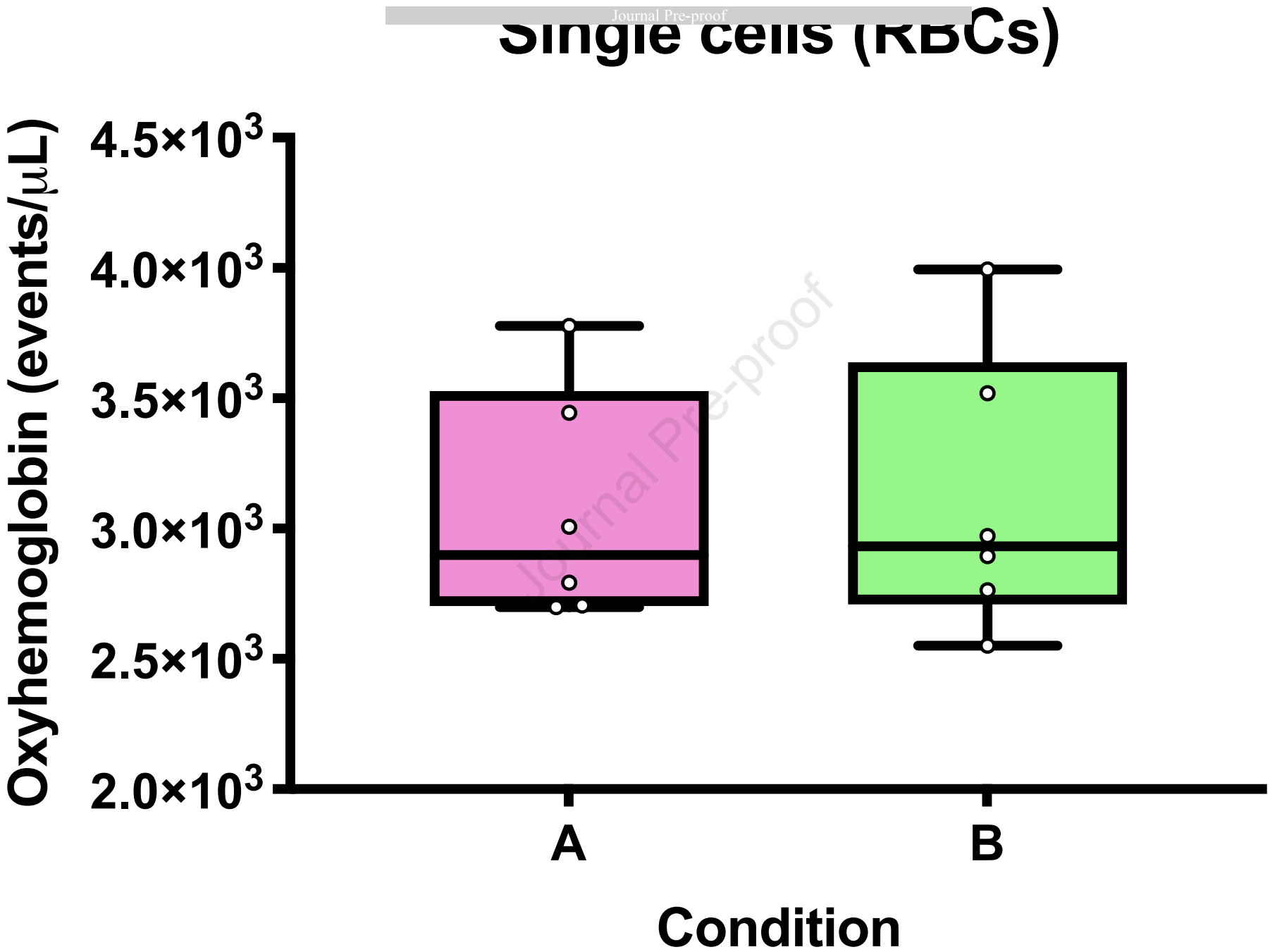
Activity	Starting time (duration in minutes)
Blood extraction	8:15 (55 minutes)
Arrival to the airport	9:42
Parabolic flight	10:20 (23 minutes)
Functional analysis	12:30 (240 minutes)
Phenotypic analysis	12:30 (1320 minutes)
Haematologic RBC profile	13:41 (44 minutes)

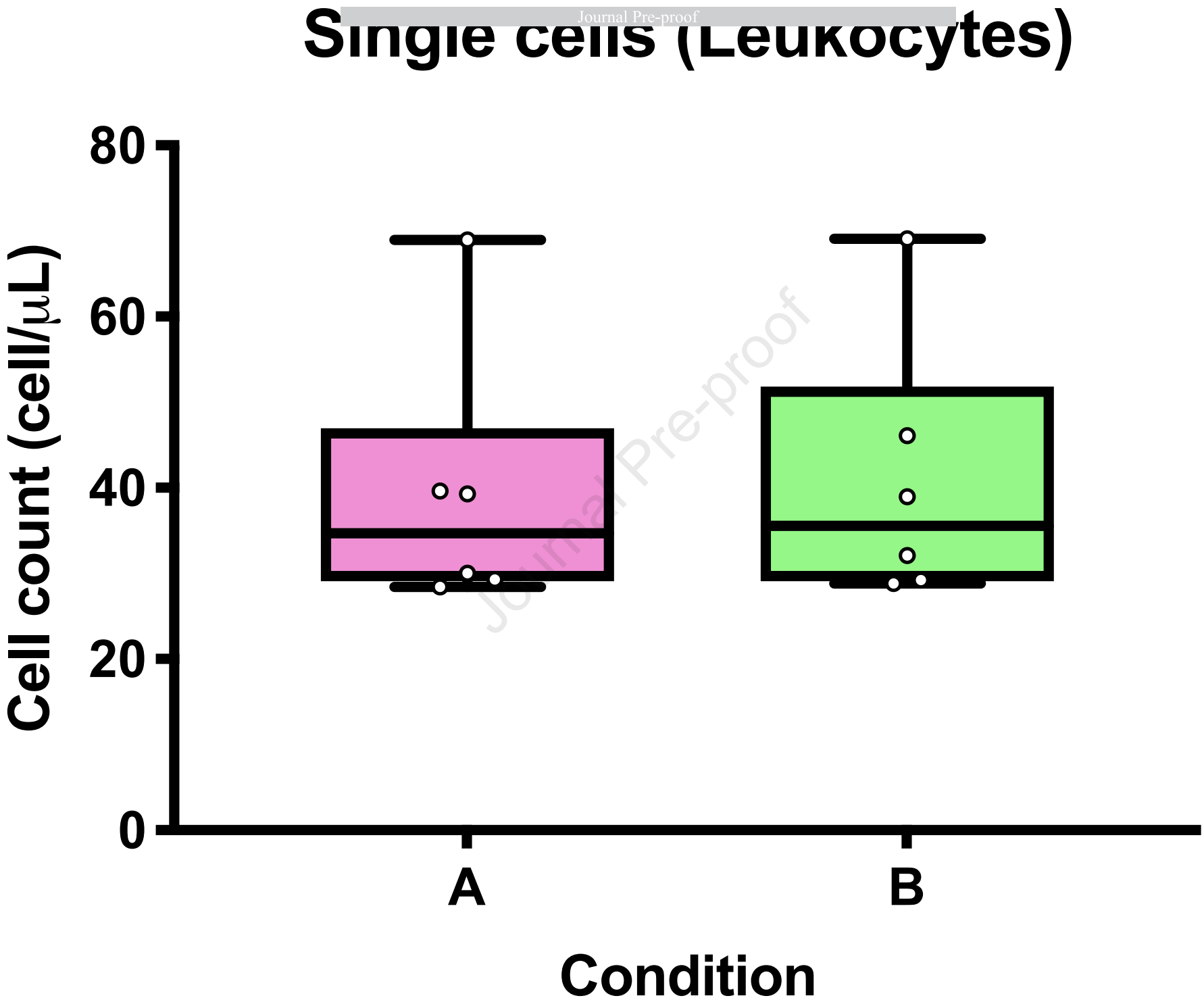




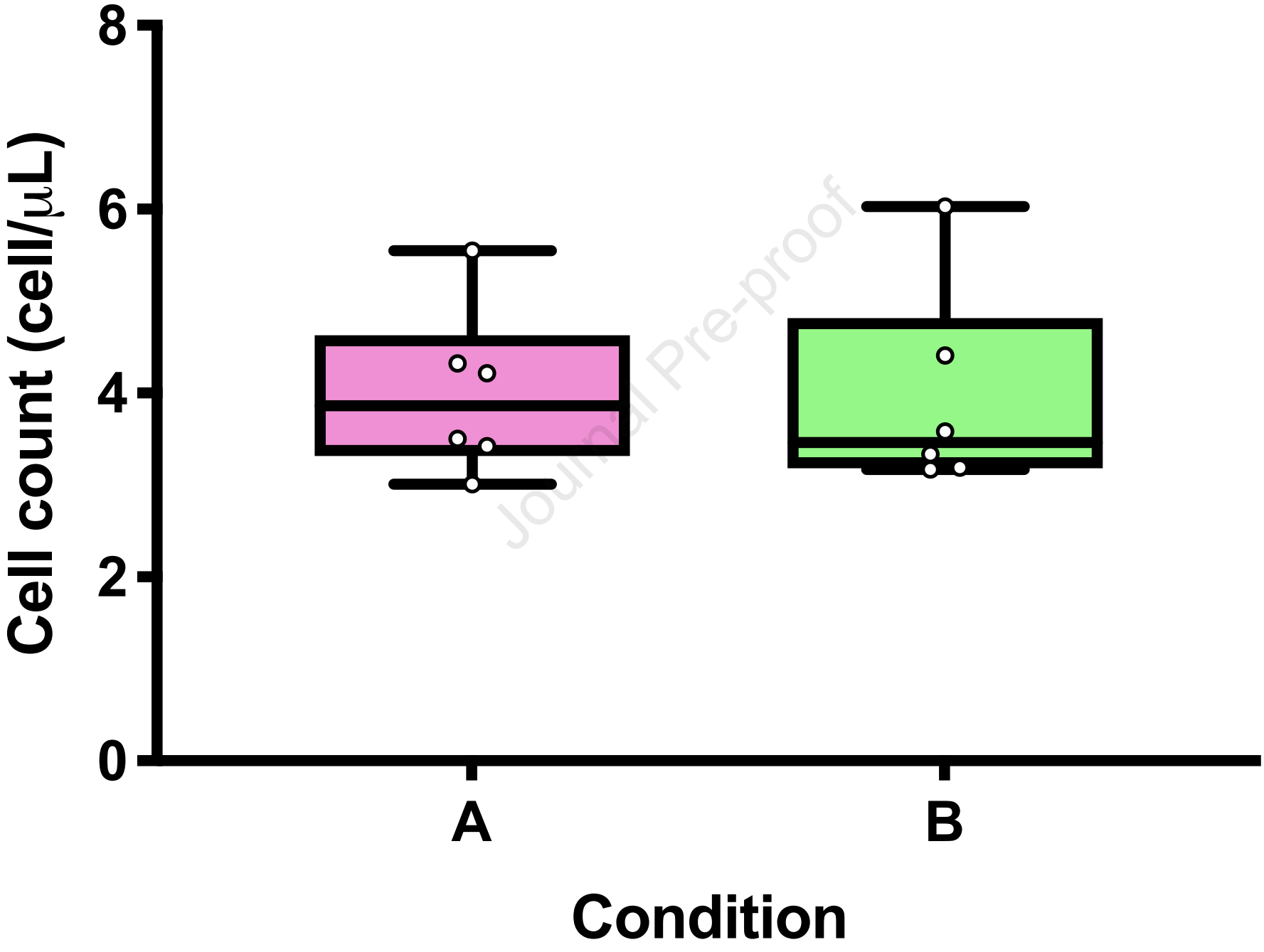


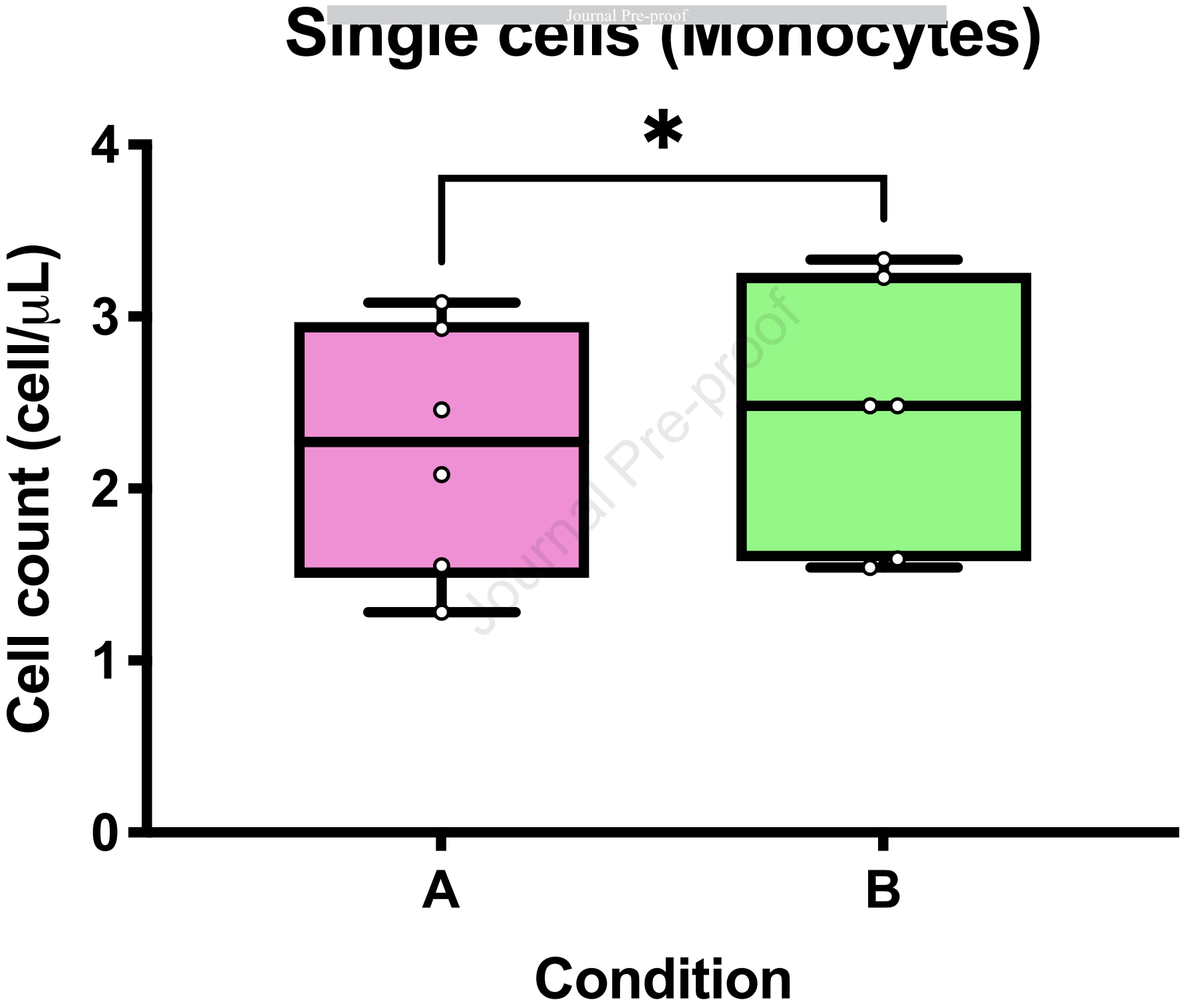


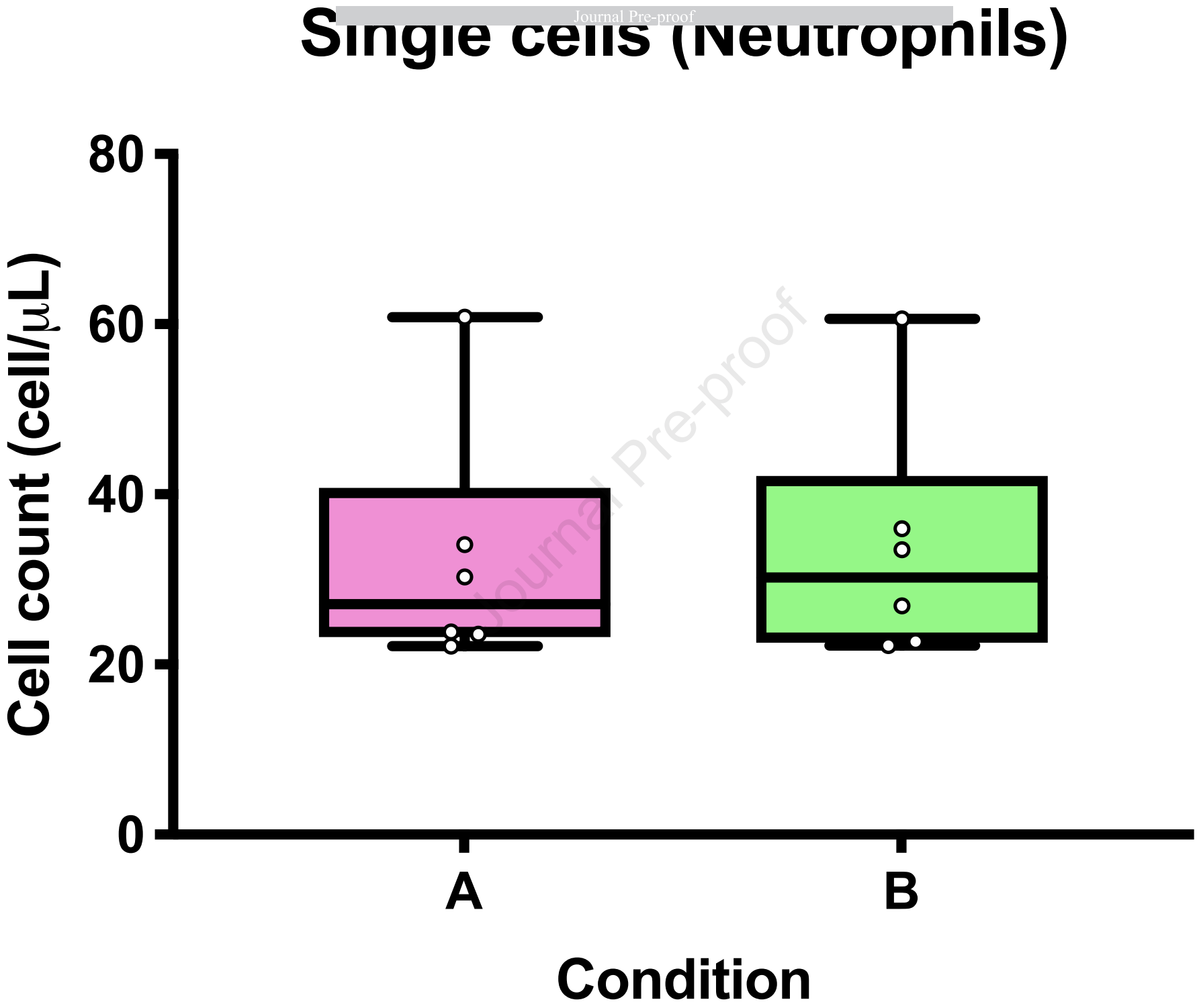


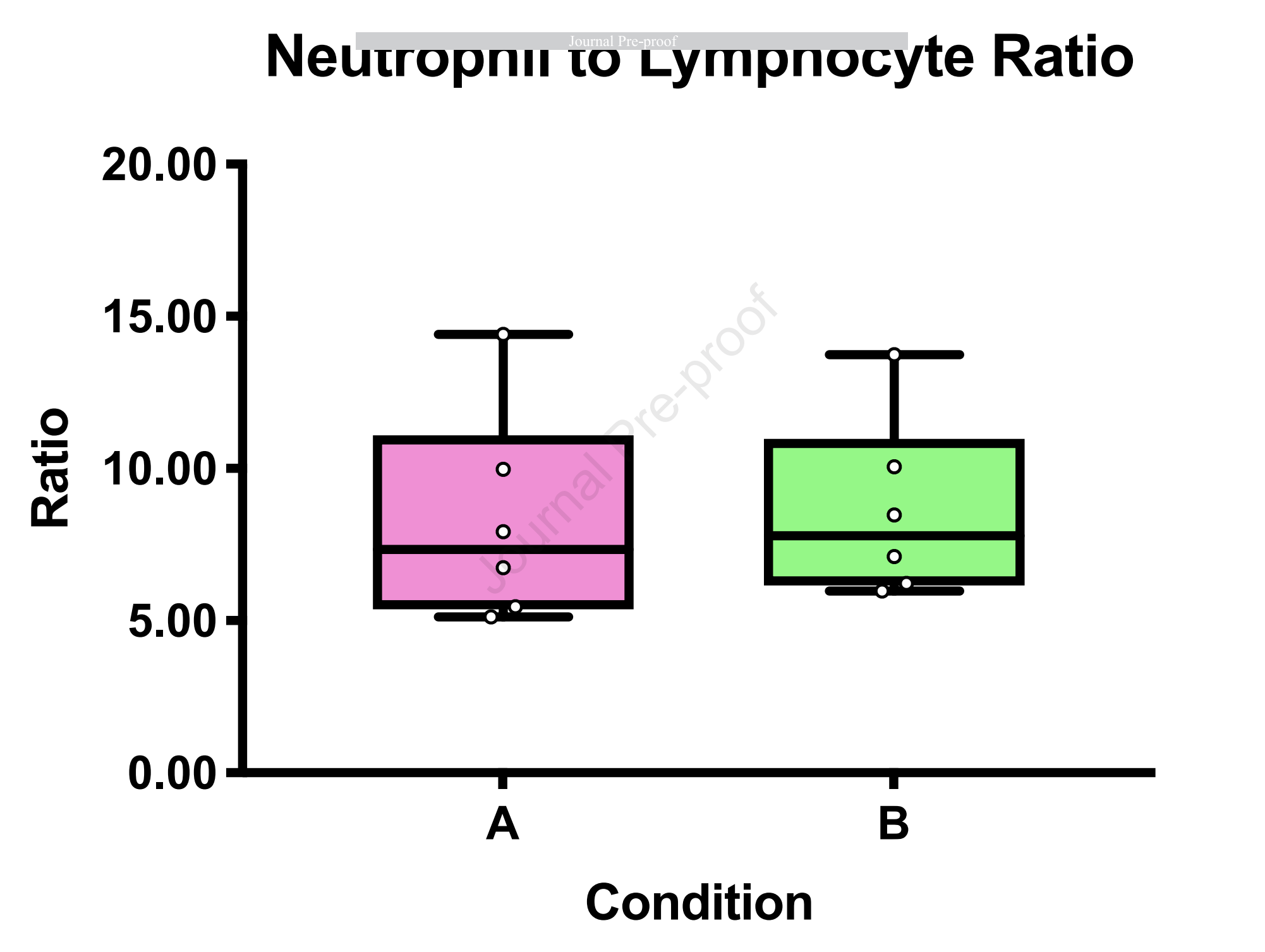


Single cells (Lymphocytes)

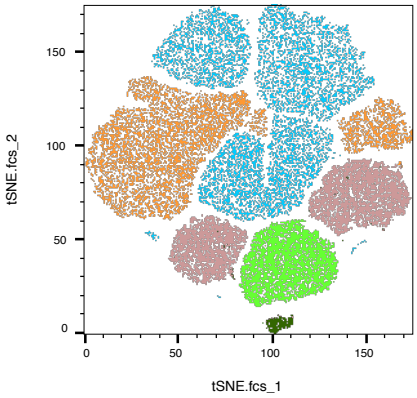








Group A



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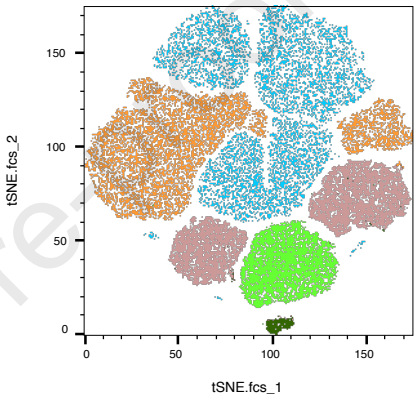
	concat_1.fcs	CD56dimCD16+	12187
	concat_1.fcs	CD56+CD16dim	641
	concat_1.fcs	CD19+	8776
	concat_1.fcs	CD8 T cells	25117
	concat_1.fcs	CD4 T cells	36992

Group B



	Sample Name	Subset Name	Count
	concat_1.fcs	CD56dimCD16+	15319
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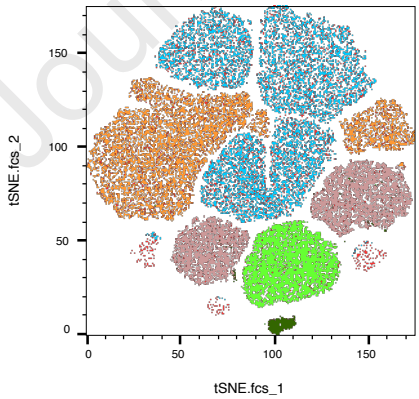
	concat_1.fcs	CD19+	10500
	concat_1.fcs	CD8 T cells	28029
	concat_1.fcs	CD4 T cells	40201

Global



	Sample Name	Subset Name	Count
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	concat_1.fcs	CD56+CD16dim	1535
	concat_1.fcs	CD19+	19277
	concat_1.fcs	CD8 T cells	53142
	concat_1.fcs	CD4 T cells	77193
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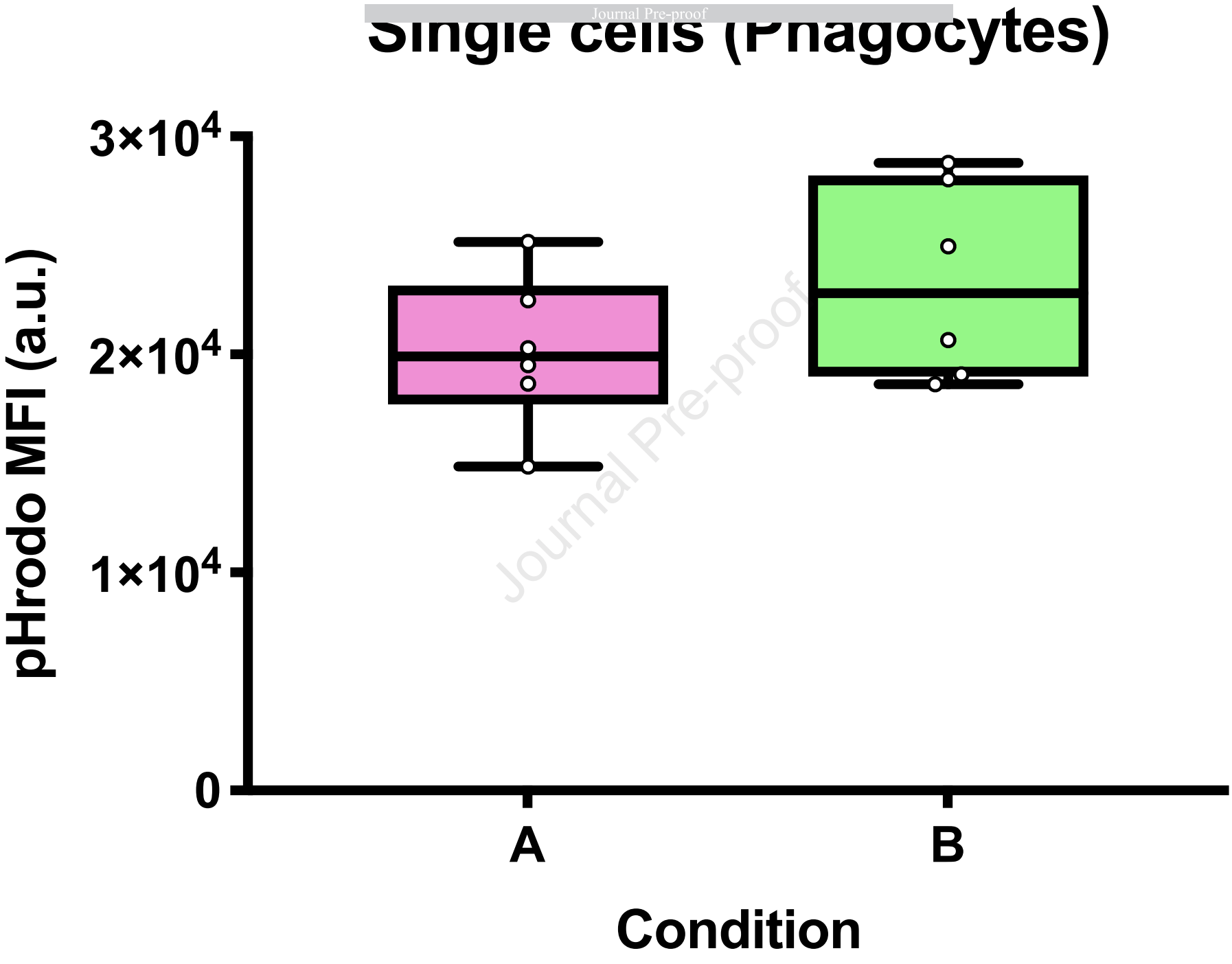

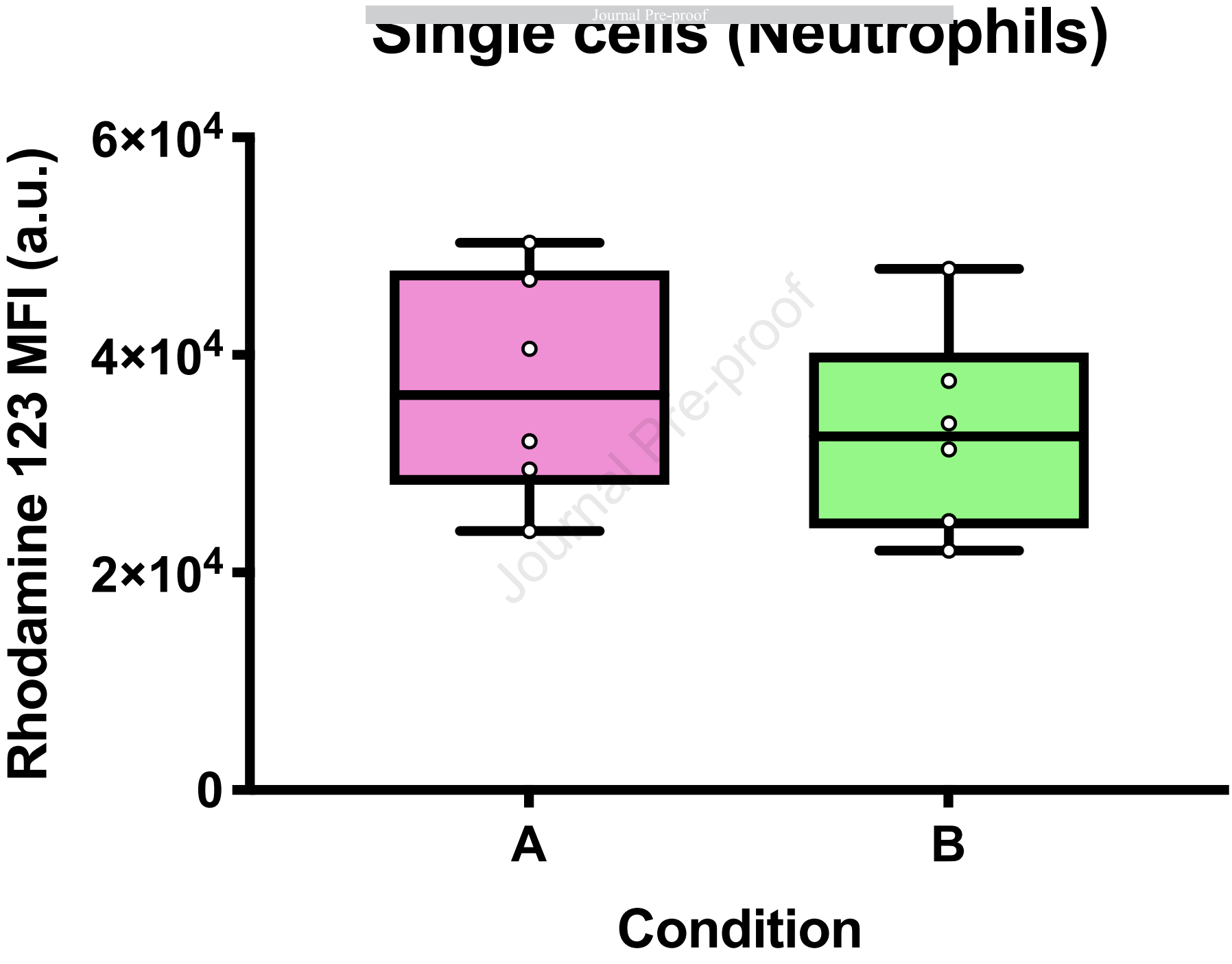

Highlights

- Parabolic flight did not cause substantial changes in peripheral blood cells counting.
- An erythrocyte aggregation response cannot be ruled out after *ex-vivo* parabolic flight.
- No significant alteration in leukocyte immunophenotyping and functional capacity were detected when using *ex vivo* blood samples and short exposure to altered gravity.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Michael D. Ward and Jolene A. Bradford are employees of ThermoFisher Scientific, Eugene, Oregon, USA, which is in the business of selling flow cytometers and flow cytometry reagents. The rest of the authors declare no potential conflicts of interest.