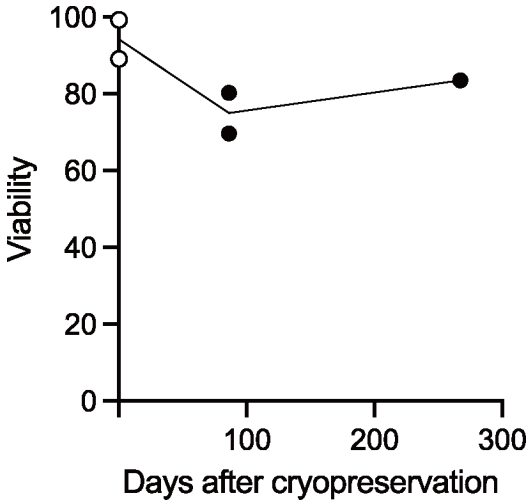
**Supplementary information**

**Title: Establishing a method for the cryopreservation of viable peripheral blood mononuclear cells in the International Space Station**

Hiroto Ishii[1, 2, 5], Rin Endo[1, 2, 5], Sanae Hamanaka[3, 5, #], Nobuyuki Hidaka[3, 5], Maki Miyauchi[1, 2], Naho Hagiwara[1], Takahisa Miyao[1], Tohru Yamamori[4], Tatsuya Aiba[3], Nobuko Akiyama[1,2] and Taishin Akiyama[1,2, *]

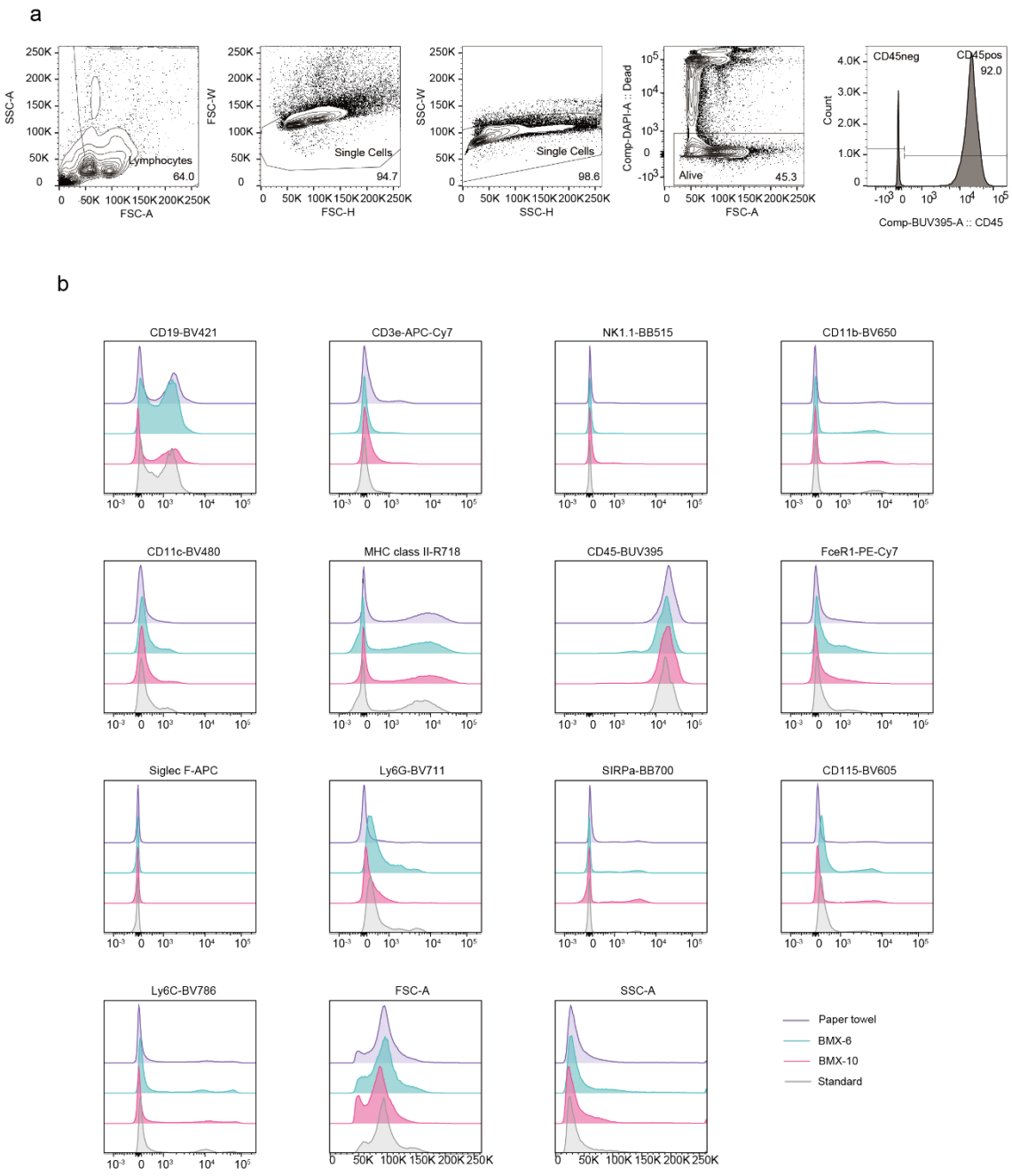
1. Laboratory for Immune Homeostasis, RIKEN Center of Integrative Medical Sciences, Yokohama 230-0045, Japan
2. Immunobiology, Graduate School of Medical Life Science, Yokohama City University, Yokohama 230-0045, Japan
3. Space Biomedical Research Group, Human Spaceflight Technology Directorate, JAXA, Tsukuba 305-8505, Japan
4. Japan Space Forum, Tokyo 101-0062, Japan
5. These authors contributed equally to this study

# Contact information for BMX: Sanae Hamanaka, Ph D.
E-mail: hamanaka.sanae@jaxa.jp
*Corresponding author: Taishin Akiyama, Ph.D
Postal address: 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama 230-0045, Japan
E-mail: taishin.akiyama@riken.jp




**Supplementary Figure 1**.

Cell viability after the long-term cryopreservation. Time 0 (open circles) indicates the sample without freezing. After wrapping with paper towels, cryopreserved samples in the CP-1 (1.5 ml tube) were stored at -80℃ for 86 days (N = 2) and 267 days (N =1).




**Supplementary Figure 2**. Gating strategy for flow cytometric analysis

**a**. Gating strategy for $CD45^+$ cells.

**b**. Flow cytometric profile of each cell surface marker. Typical data of each sample was exhibited. Purple histograms for wrapping by paper towel, blue histograms for BMX-6, red histograms for BMX-10, and gray histograms for the standard method involving the cryopreservation after the removal of red blood cells by hemolysis.