

## **Supplementary information for**

### **Lunar and Martian gravity alter immune cell interactions with endothelia in parabolic flight**

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This file includes:

Supplementary Figure 1

Supplementary Figure 2

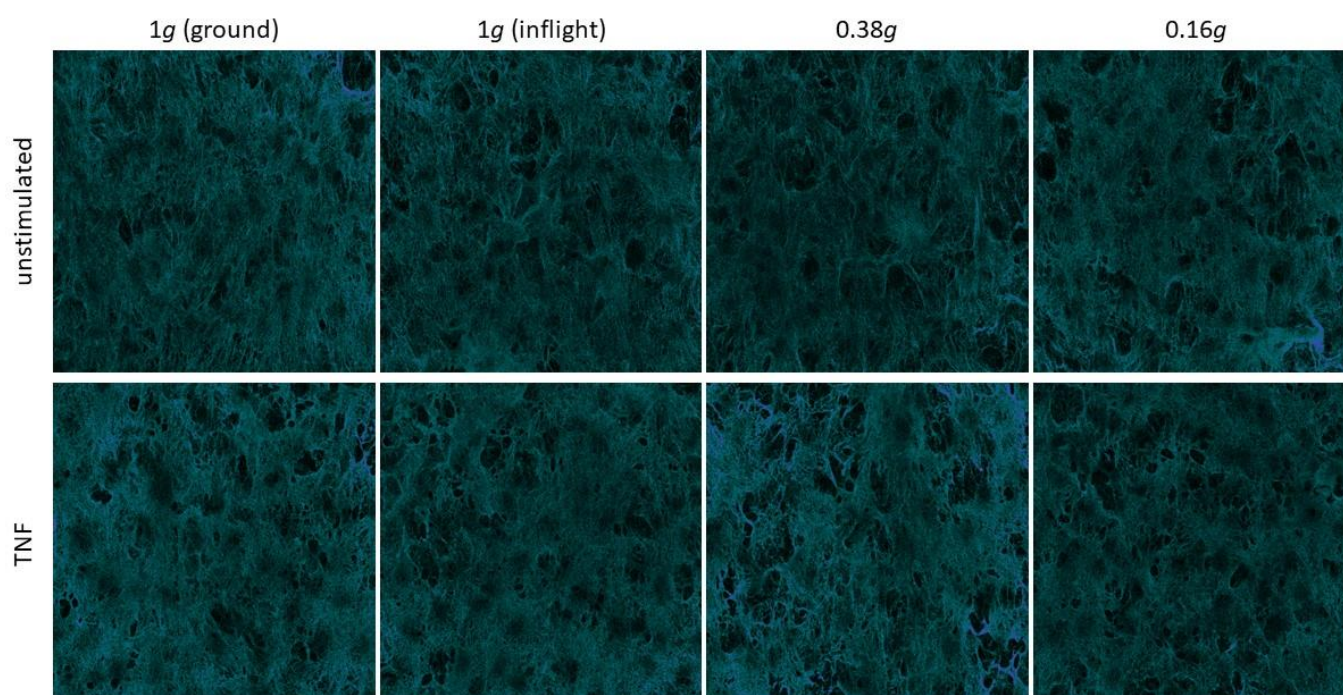
Supplementary Table 1

Separate video files:

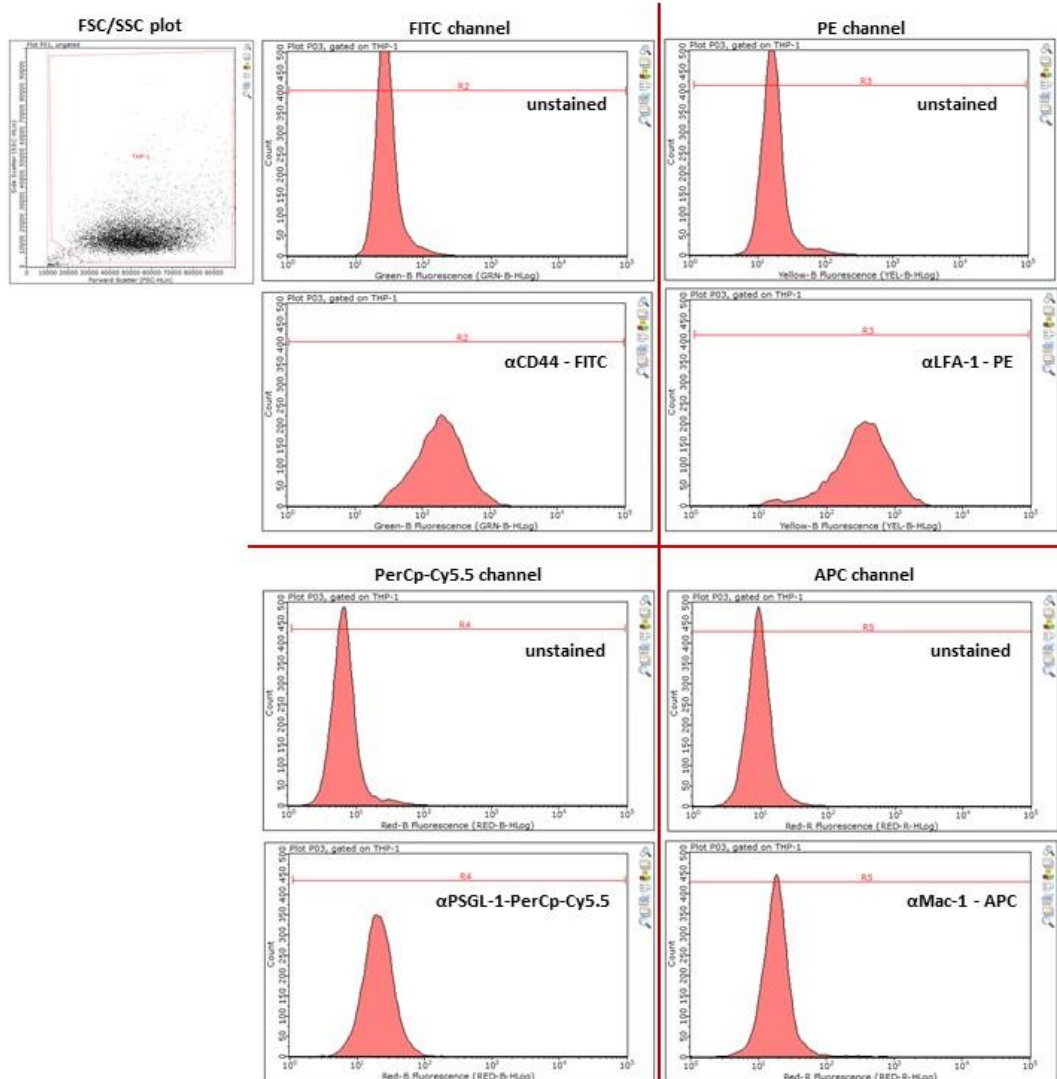
Supplementary video 1

Supplementary video 2

Supplementary video 3



**Supplementary Figure 1 Expression of glyocalyx on HUVECs.** Fixation of cells was performed either on ground at the time of start of parabolas (1g (ground)), in the plane before start of parabolas (1g (inflight)), or after eight parabolas in Martian (0.38g) or lunar (0.16g) gravity. Representative images of the glyocalyx component heparan sulfate by confocal microscopy.



**Supplementary Figure 2 Representative flow cytometry histograms.** FSC/SSC plot of THP-1 cells and histograms showing intensity of antibody binding in comparison to their respective negative control. Upper left: αCD44-FITC, upper right: αLFA-1-PE, lower left: αPSGL-1-PerCp-Cy5.5, lower right: αMac-1-APC.

**Supplementary Table 1 Surface adhesion marker expression on THP-1 cells.** Expression (MFI) of Mac-1, PSGL-1, CD44 and LFA-1 on THP-1 cells inflight before start of parabolas (1g) and after completion of P8 in lunar gravity (0.16g). Data show mean  $\pm$  STD, n=2, \*\*significant difference between expression on cells in 0.16g and different 1g control conditions (\*\*  $P<0.01$ ).

|               | Ground control                |              | Inflight - pre-parabola       |              | Inflight - after P8           |              | Inflight - 0.16g (flow chamber) |              |
|---------------|-------------------------------|--------------|-------------------------------|--------------|-------------------------------|--------------|---------------------------------|--------------|
|               | unst.                         | TNF          | unst.                         | TNF          | unst.                         | TNF          | unst.                           | TNF          |
| <b>Mac-1</b>  | <b>17.26 <math>\pm</math></b> | 21.71 $\pm$  | <b>20.41 <math>\pm</math></b> | 19.14 $\pm$  | <b>16.56 <math>\pm</math></b> | 19.25 $\pm$  | 28.62 $\pm$                     | 30.80 $\pm$  |
|               | <b>2.46**</b>                 | 7.12         | <b>3.79**</b>                 | 0.33         | <b>1.10**</b>                 | 1.68         | 4.18                            | 3.42         |
| <b>PSGL-1</b> | 23.30 $\pm$                   | 20.32 $\pm$  | 22.30 $\pm$                   | 21.16 $\pm$  | 23.41 $\pm$                   | 21.85 $\pm$  | 26.26 $\pm$                     | 23.14 $\pm$  |
|               | 0.64                          | 0.23         | 0.09                          | 0.67         | 1.24                          | 3.39         | 2.10                            | 1.57         |
| <b>CD44</b>   | 166.37 $\pm$                  | 185.22 $\pm$ | 162.08 $\pm$                  | 176.41 $\pm$ | 160.71 $\pm$                  | 184.36 $\pm$ | 151.05 $\pm$                    | 142.36 $\pm$ |
|               | 7.11                          | 34.83        | 8.02                          | 8.41         | 14.95                         | 69.33        | 38.37                           | 25.27        |
| <b>LFA-1</b>  | 370.66 $\pm$                  | 370.27 $\pm$ | 323.18 $\pm$                  | 333.60 $\pm$ | 352.64 $\pm$                  | 381.54 $\pm$ | 322.56 $\pm$                    | 254.97 $\pm$ |
|               | 29.82                         | 14.81        | 46.46                         | 30.60        | 67.39                         | 118.52       | 144.69                          | 59.09        |

#### Figure legends Supplementary Videos

**Supplementary Video 1 Computational fluid dynamics in lunar gravity** Modeling of cell movement in 0.16g with Discrete Phase Model of Ansys Fluent.

**Supplementary Video 2 Computational fluid dynamics in Martian gravity** Modeling of cell movement in 0.38g with Discrete Phase Model of Ansys Fluent.

**Supplementary Video 3 Computational fluid dynamics in normogravity** Modeling of cell movement in 1g with Discrete Phase Model of Ansys Fluent.