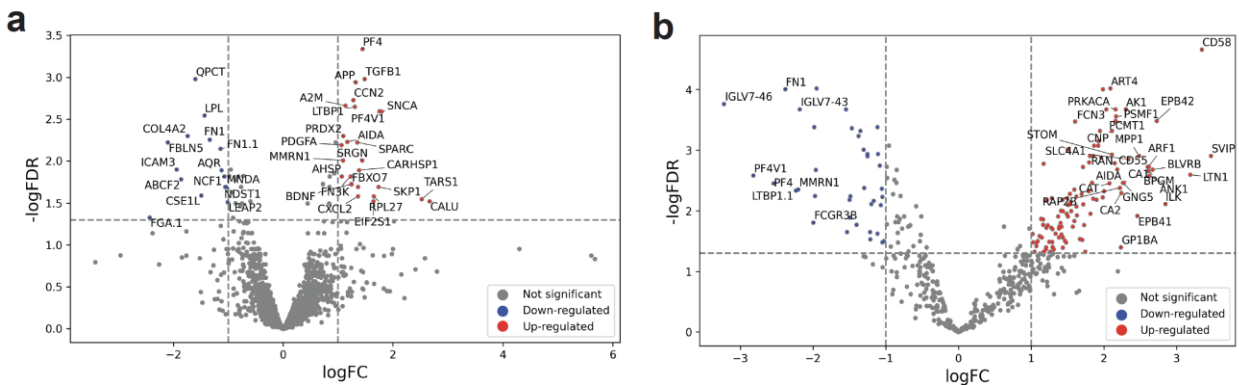
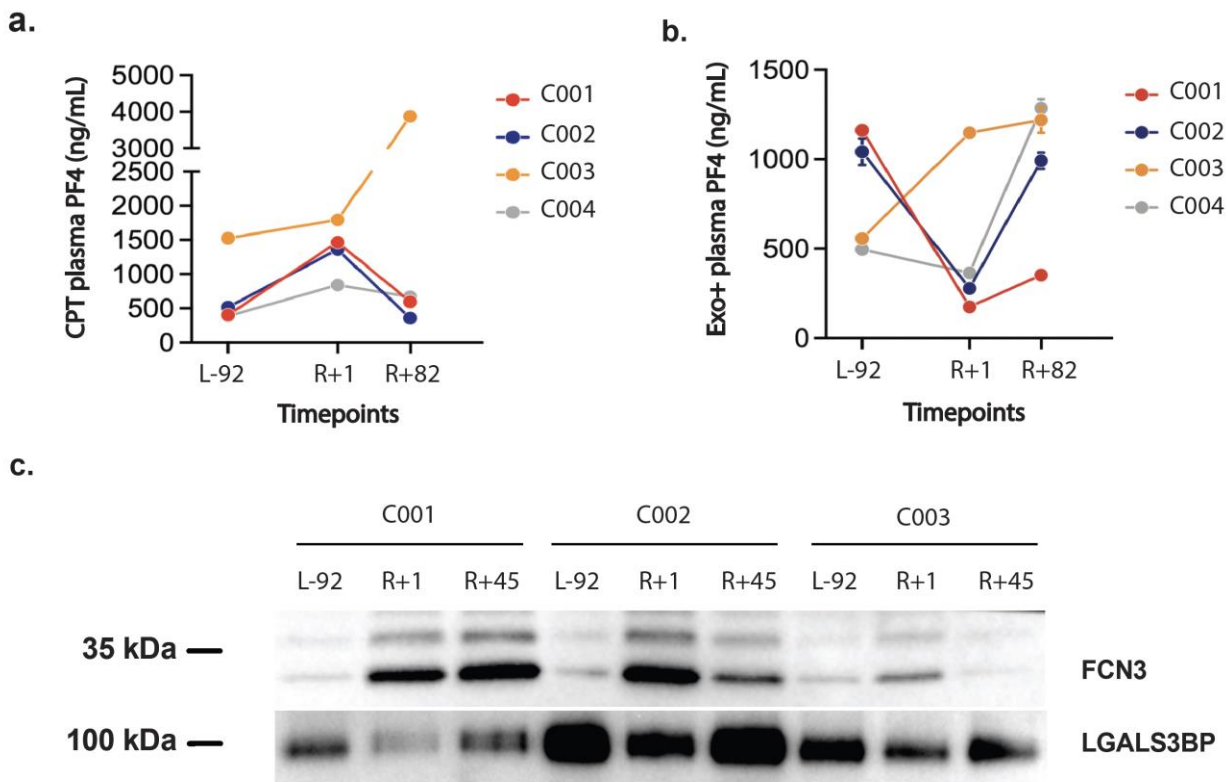


Supplementary Figure
Supplementary Figure 1. Volcano plots of acute changes after 3-day spaceflight.
a. Volcano plot of DAPs in plasma at R+1 vs preflight (p-adjusted < 0.05, |logFC|>1). **b.** Volcano plot of DAPs in EVPs at R+1 vs preflight (p-adjusted < 0.05, |logFC|>1).



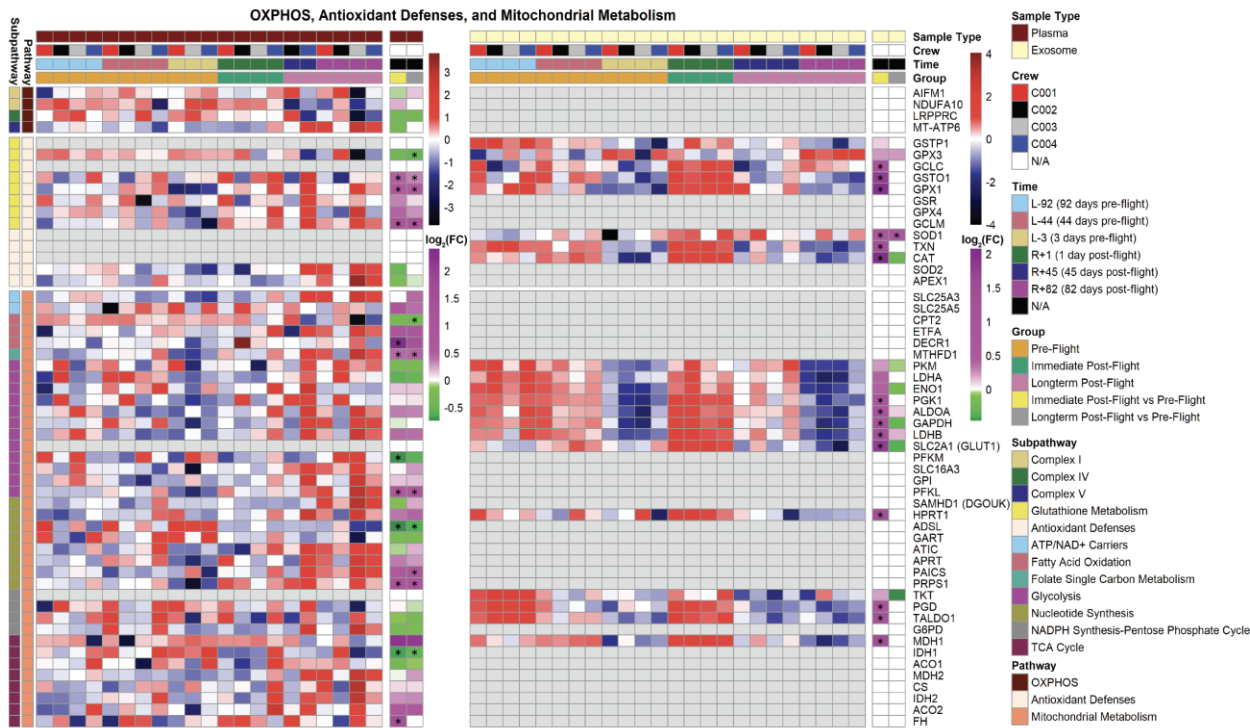
Supplementary Figure 2. Validation of PF4 change in abundance in plasma and EVPs using ELISA.

a. ELISA of PF4 in plasma isolated from cell preparation tubes (CPT). **b.** ELISA of PF4 in EVPs isolated from plasma.

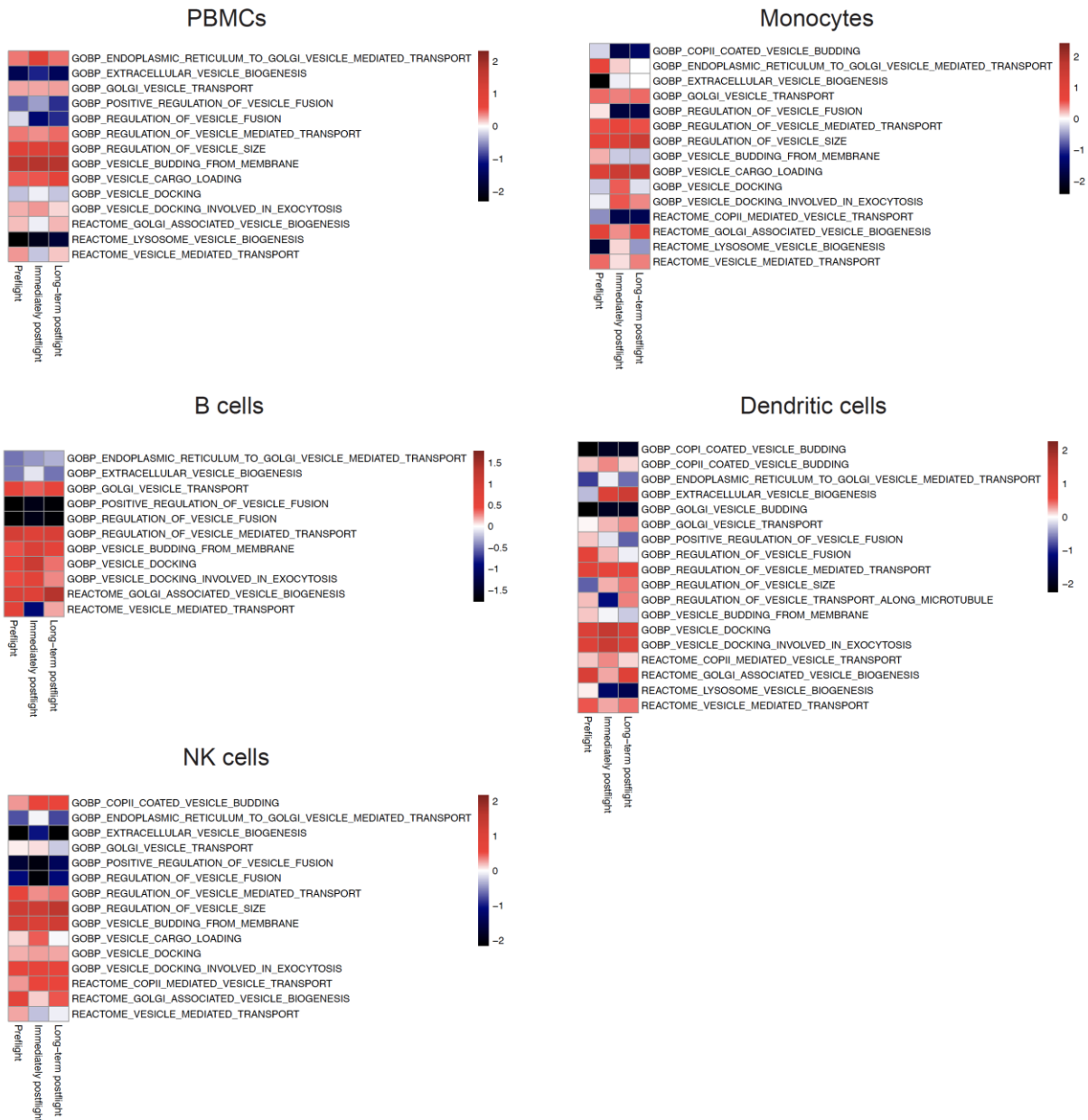


Supplementary Figure 3. Heatmap of plasma and EVP proteins involved in metabolic pathways.

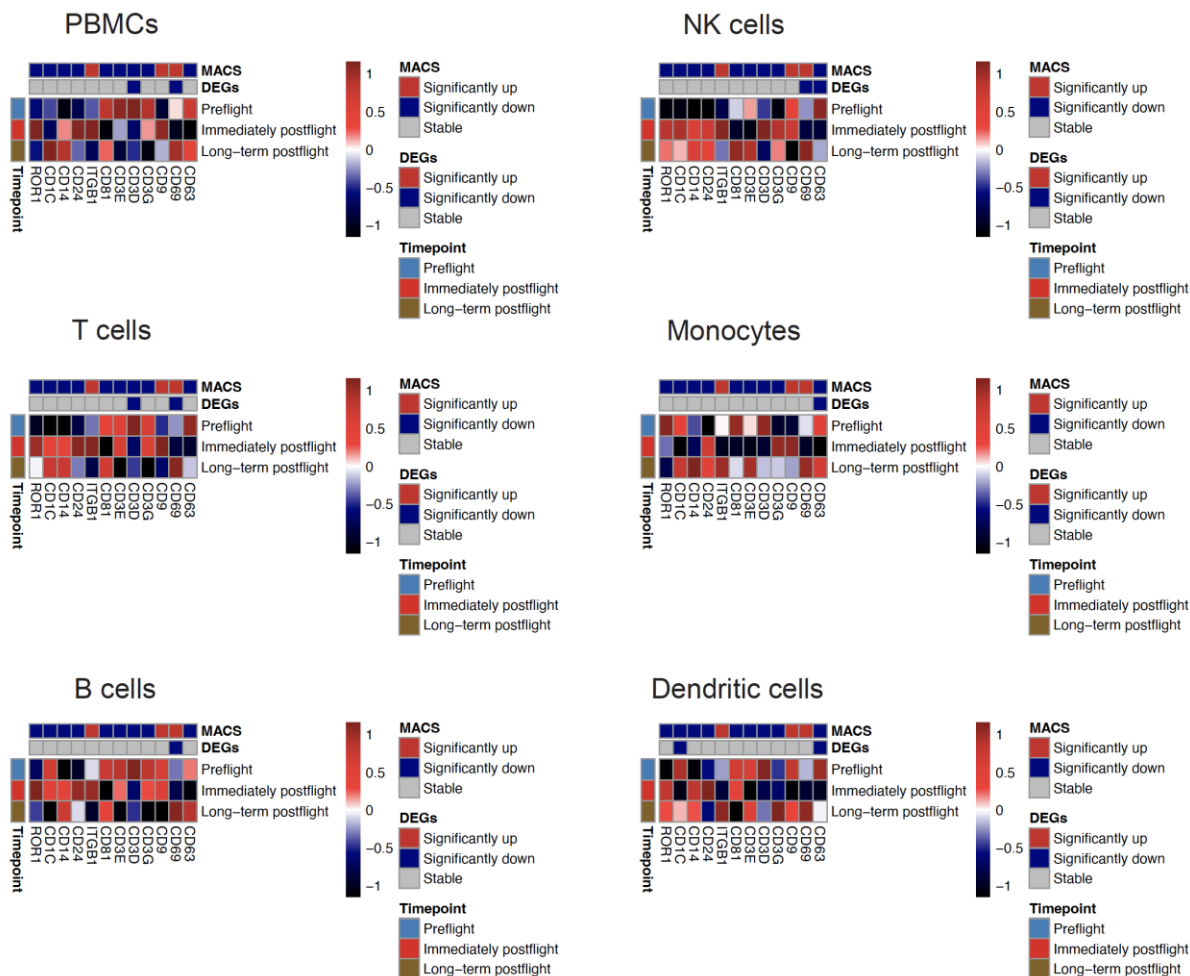
Heatmaps for all I4 astronauts over time (i.e., pre-flight to multiple time points post-flight) for the individual proteins and $\log_2(\text{fold-change})$ for the mitochondrial OXPHOS, antioxidant, and custom metabolic pathways. The individual protein expression values are represented in red (upregulated proteins) and blue (downregulated proteins). The fold-change values are determined by $\log_2(\text{fold-change})$ comparing immediate and long-term postflight changes to preflight values. The $p\text{-value} < 0.05$ is indicated in each cell with a *. This is displayed for plasma (left heatmap) and EVP (right heatmap) samples.



Supplementary Figure 4. Fold change (R+1/pre-flight) of the selected secretome-enriched pathways normalized score in immune cells.

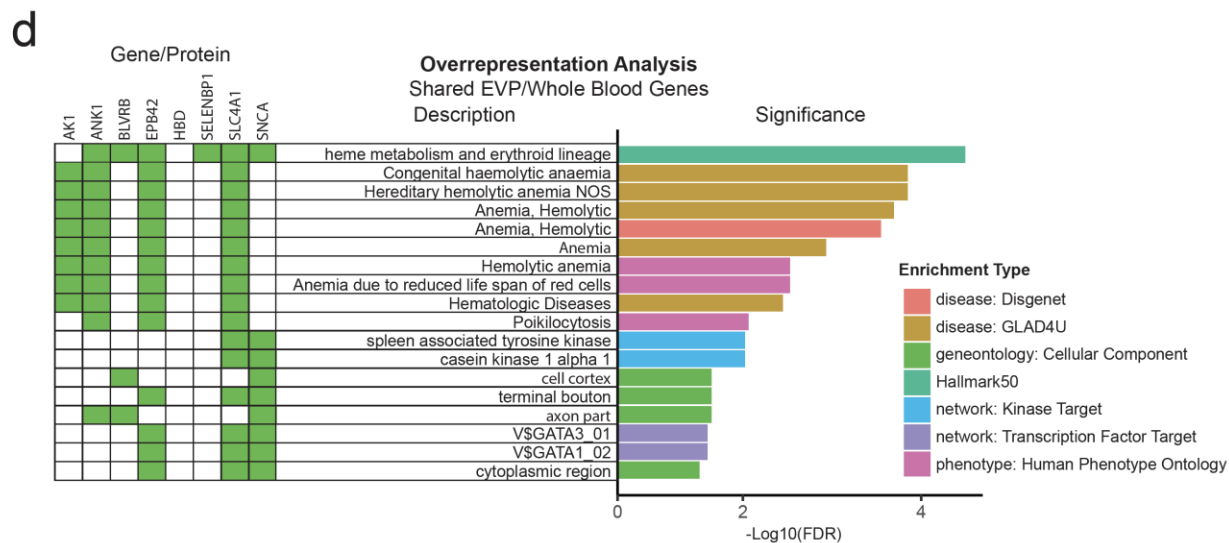
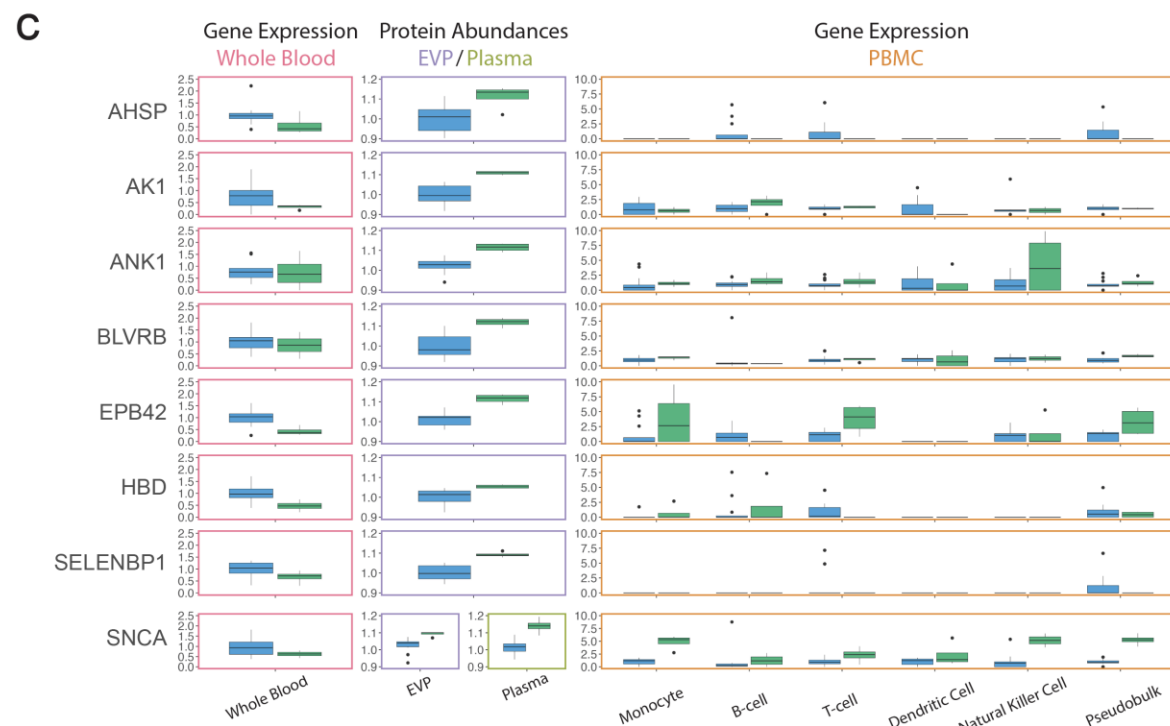
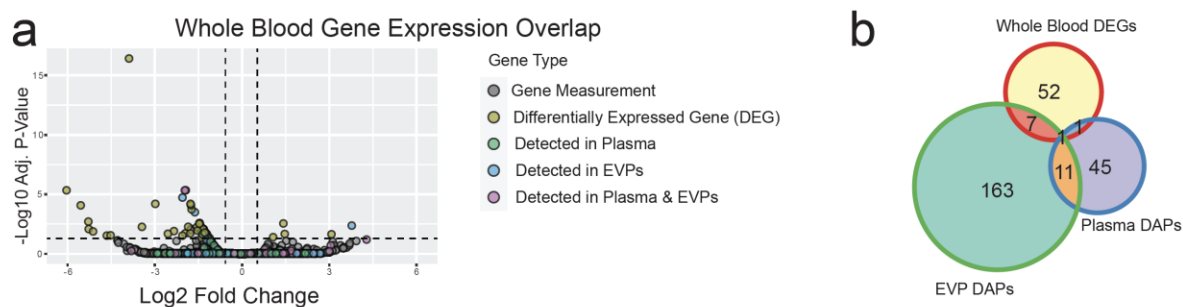


Supplementary Figure 5. Expression of MACSplex EVP surface markers in immune cells.
Heatmaps represent the protein expression levels of indicated immune markers on the surface of
EVPs, at the indicated timepoints, normalized to negative controls. The values were normalized
by centering and dividing by the standard deviations.



Supplementary Figure 6. Blood cells do not contribute to secretome changes after spaceflight.

a. Volcano plot of differentially expressed genes from whole blood. Genes are colored according to whether they are differentially abundant in the proteomics data. The horizontal line marks the 0.05 threshold for adjusted p-value. The vertical lines mark the $|0.5|$ threshold for logFC. **b.** Overlap between differentially expressed genes in whole blood and differentially abundant proteins in plasma and EVPs. **c.** Whole blood gene expression, protein abundances, and PBMC gene expression for the 8 genes that are differentially expressed/abundant among whole blood and EVPs (AHSP, AK1, ANK1, BLVRB, EPB42, HBD, SELENBP1, SNCA). **d.** Overrepresentation analysis for differentially expressed/abundant genes in the whole blood gene expression and EVP proteomics data. Green boxes (left panel) display the genes/proteins included in the overrepresented set. Enrichment database types are displayed (right panel) if their FDR < 0.05.



96 **Supplementary table**

97 **Supplementary table 1. Data availability on NASA Open Science Data Repositories**

98 **(OSDR)**

Biospecimen	Assay(s)	OSDR Identifier
Blood Plasma	<ul style="list-style-type: none">● Proteomics (Seer Proteograph)● Proteomics of blood extracellular vesicles and particles● Proteomic assay of blood plasma metabolome● Metabolomics data● Cell-free RNA	OSD-571
	<ul style="list-style-type: none">● Direct RNA-seq	OSD-569

99