Supplementary Figures for

Negative effect of sphingosine 1-phosphate on red blood cell storage quality mediated by increased activation of glycolysis at the expense of the pentose phosphate pathway

Ariel Hay,[1,*] Travis Nemkov,[2,*] Fabia Gamboni, [2] Monika Dzieciatkowska, [2] Alicia Key,[2] Matthew Gailbraith,[3] Kyle Bartsch,[3] Kaiqi Sun,[4] Yang Xia,[5] Mars Stone,[6,7] Michael P. Busch, [6,7] Philip J. Norris, [6,7] James C. Zimring,[1] Angelo D'Alessandro[2, *]

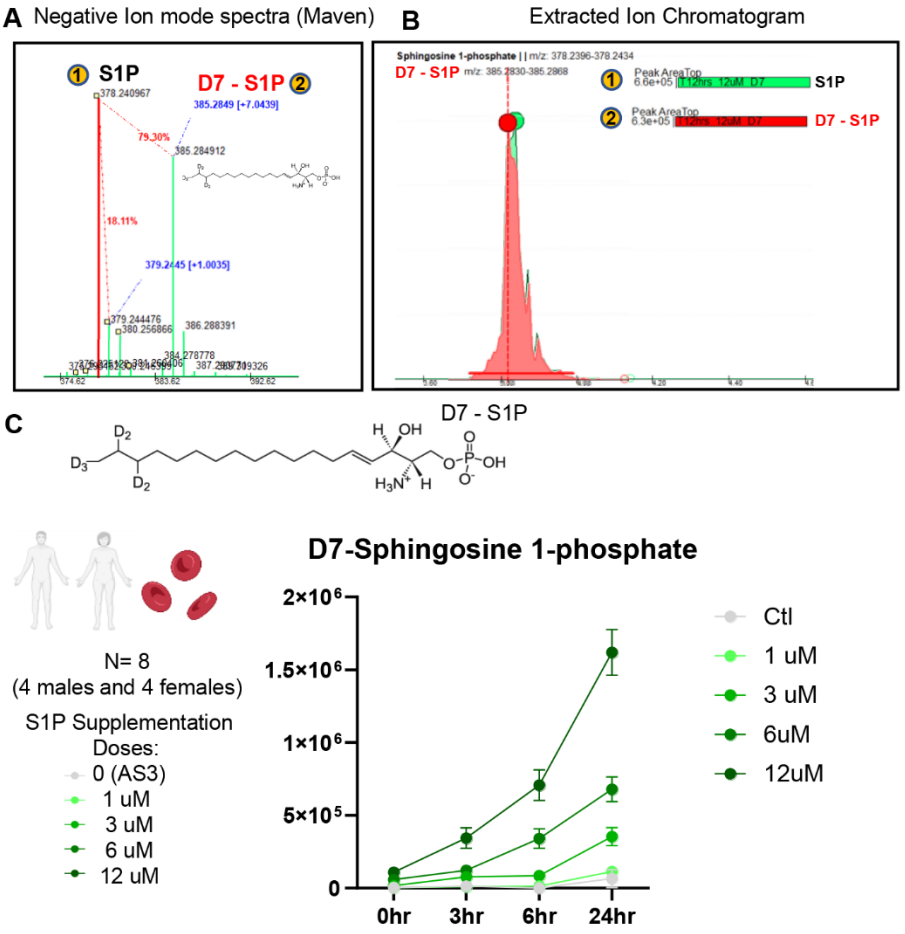
1) Department of Pathology, University of Virginia, VA, USA
2) Department of Biochemistry and Molecular Genetics, University of Colorado Denver – Anschutz Medical Campus, Aurora, CO, USA
3) Linda Crnic Institute for Down syndrome, Anschutz Medical Campus, Aurora, CO, USA
4) Metis Therapeutics, Shanghai, China
5) University of Changsha, Hunan, China
6) Vitalant Research Institute, San Francisco, CA, USA
7) Department of Laboratory Medicine, University of California, San Francisco, CA, USA

***Correspondence:**
Angelo D'Alessandro, PhD
Department of Biochemistry and Molecular Genetics
University of Colorado Anschutz Medical Campus
12801 East 17th Ave., Aurora, CO 80045
Phone # 303-724-0096
E-mail: angelo.dalessandro@cuanschutz.edu

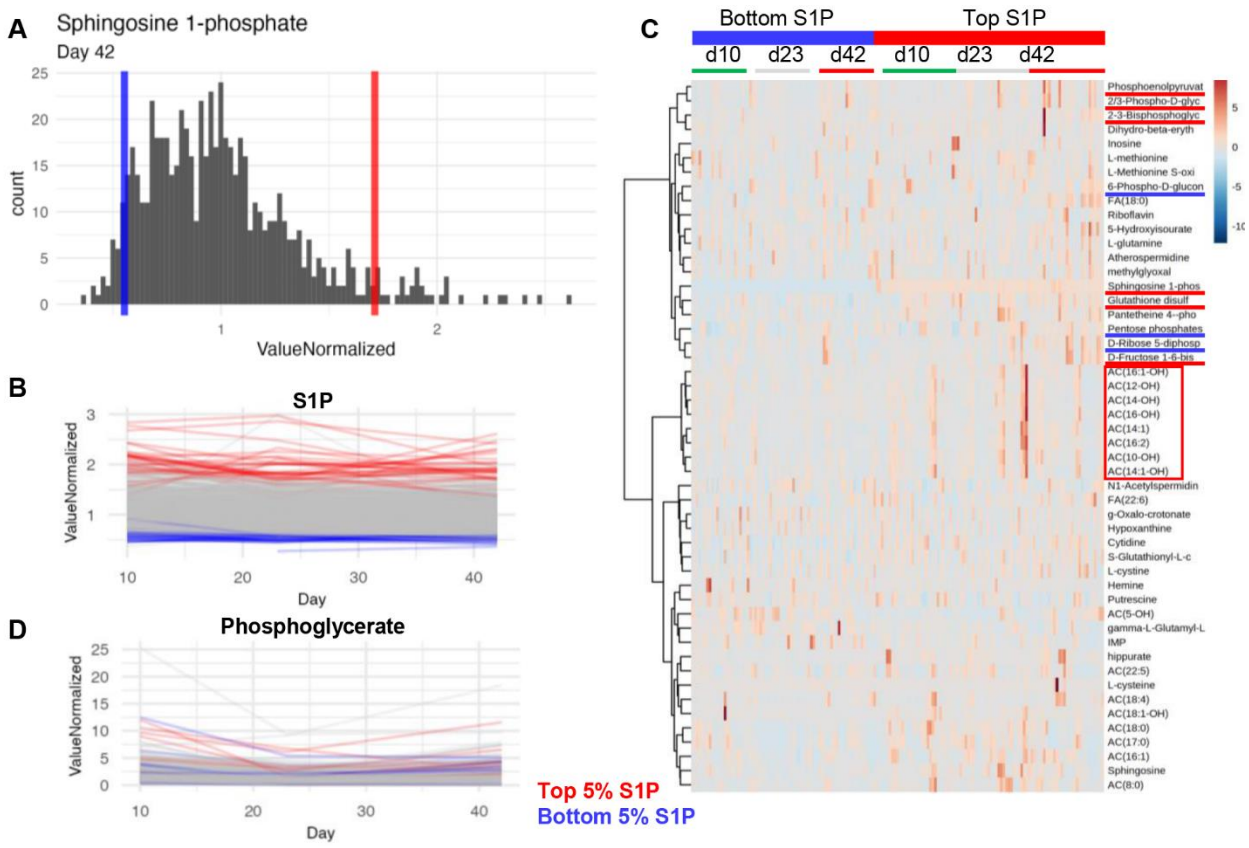
* *These authors contributed equally and share the first authorship*

Running title: ***S1P in stored RBCs***

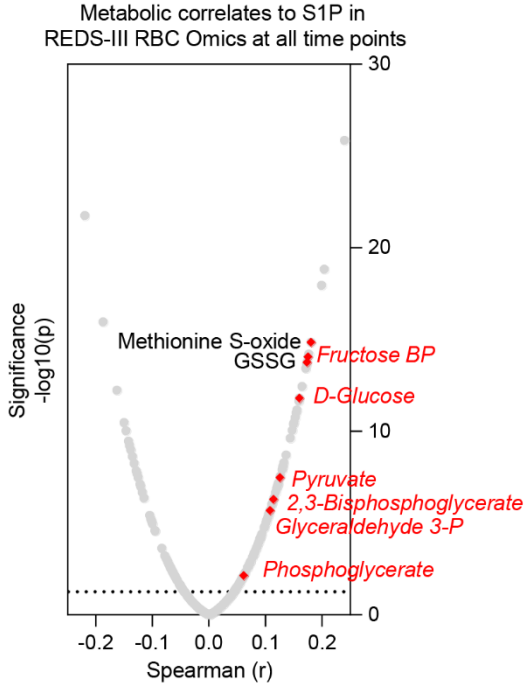
TABLE OF CONTENTS



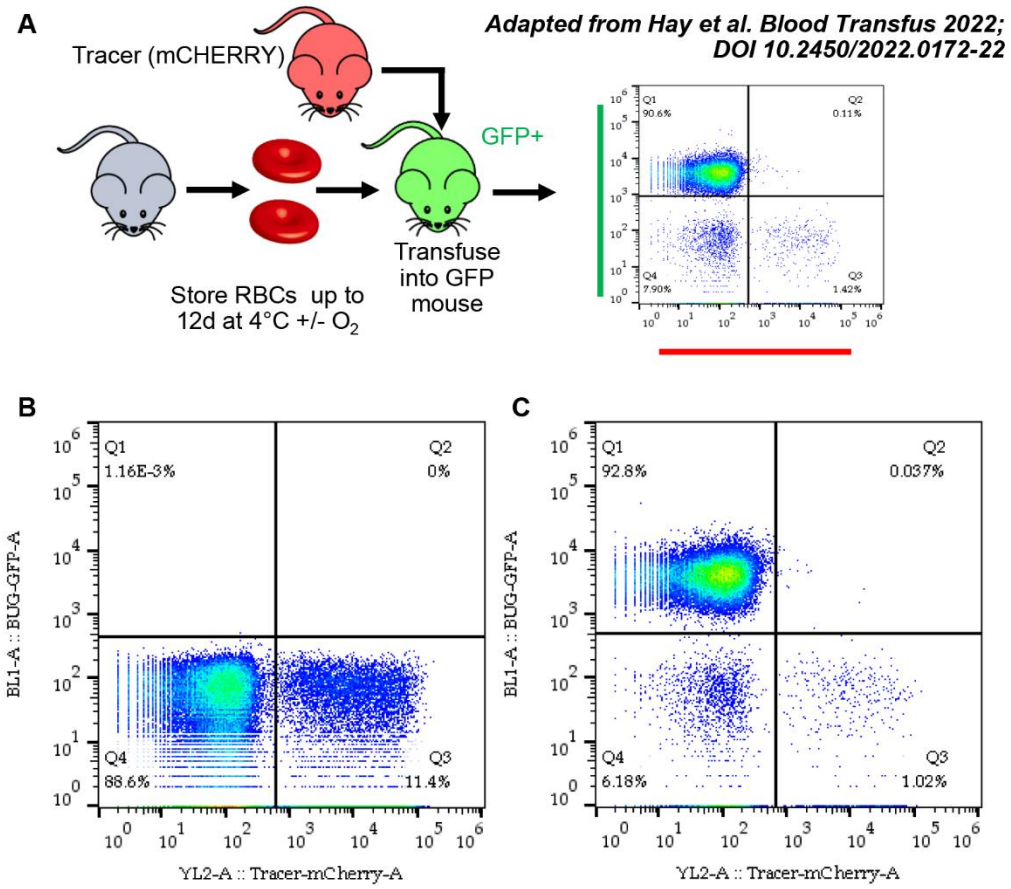
Supplementary Figure 1 – Sphingosine 1-phosphate detection by UHPLC-MS. A representative intact mass spectrum for S1P (endogenous) versus a D7-S1P (stable isotope labeled standard – **A**) and related overlapping extracted ion chromatograms (green for unlabeled S1P and red for labeled D7-S1P; Retention time 3.81 min - **B**). Incubation of human RBCs (n=8, 4 males and 4 females) with increasing doses of D7-S1P (1, 3, 6 and 12 uM) results in the dose-response uptake from the supernatants, as determined by intracellular RBC metabolite measurements over time after incubation at 37°C for up to 24h (**C**).



Supplementary Figure 2 – Distribution of S1P levels in day 42 units from the REDS-III RBC Omics recalled donor study (A). Specifically, blue and red bars indicate thresholds for the bottom and top 5% of the S1P levels detected in any units (n=643) at storage day 42. In **B**, line plots indicate S1P levels in these two sub-groups (top = red and bottom = blue 5% of S1P levels). Based on these groupings, donors were monitored at storage day 10, 23 and 42 to determine the top metabolites positively or negatively associated with S1P levels. Results clearly indicate a positive association with blood unit S1P levels and metabolites in glycolysis or oxidant stress (underlined in red), and a negative association with metabolic markers of the pentose phosphate pathway (in blue in the heat map in **C**). In **D,** line plots for a representative glycolytic metabolites show that donors with the highest levels of S1P (red lines) tended to show the highest levels of glycolytic metabolites like phosphoglycerate.



Supplementary Figure 3 – Metabolic correlates to S1P in the REDS-III RBC Omics study at all time points tested (storage day 10, 23 and 42) in 1,929 samples from 643 blood donors.



Supplementary Figure 4 – Summary representation of flow cytometry plots from a murine post-transfusion recovery study (A). Specifically, in **B** a representative plots from a post-transfusion recovery study shows the result from the input blood. This is a mixture of non-fluorescent test blood (SphK1-, B6, etc.) mixed with an mCherry "tracer" that allows us to track baseline clearance of healthy unmanipulated RBCs in comparison to our test population - this mixture is what was transfused into the GFP recipients. In **C**, the plot represents a 24-hour PTR bleed of the GFP recipients. Recipient GFP blood is shown on the upper quadrant on Y-axis, while the mCherry tracer shifts on the right quadrant along the X-axis