With or without you?
Red Blood Cell storage under hypoxic conditions

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Trauma Hemostasis and Oxygenation Research Network
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Disclosures

FUNDING

University of Colorado
Anschutz Medical Campus

- *R01 HL146442 (NHLBI) (D'Alessandro) PI*
- *R01 HL149714 (NHLBI) (D'Alessandro) PI*
- *R01 HL148151 (NHLBI) (Spitalnik, D'Alessandro, Karafin, Zimring) MPI*
- *R01 HL161004 (NHLBI) (Buehler, D'Alessandro, Irwin) MPI*
- *R01DK136945 (NIDDK) (D'Alessandro, Marygold, Perrimon, Tennessen) MPI*
- *RM1 GM131968 (NIGMS) (Silliman, Jones, Hansen, Moore, Cohen, D'Alessandro) MPI*
- *BARDA – MATIC II (Spinella, Leeper, D'Alessandro, Wahed, Wisniewski)*
- *R21 HL150032 (NHLBI) (D'Alessandro) PI*
- *REDS IV-P (NHLBI) – Central Lab – Sub-contract PI*
- Webb-Waring Early Career Award – Boettcher Foundation 2017 *(D'Alessandro) PI*
- *NBF Early Career Grant 2016*

Director of Metabolomics Core

- School of Medicine – UC Denver – Anschutz Medical Campus;
- Cancer Center – UC Denver – Anschutz Medical Campus; *(P30 CA046934-17)*
- *Trauma Research Center; (RM1GM131968)*

PATENTS

- PCT/US2019/032472 - "Compositions and Methods for Storage of Blood and Components Thereof"
- WO 2017/223377 A1 - "Methods for managing adverse events in patient populations requiring transfusion"
- US patent no. 11090331 - "Methods and treatment for reducing the risk of an inflammatory response"
- U. S. Provisional Patent Application Serial No. 63/147,438 "Use of BLRVB inhibitors to allow malaria eradication in patients with G6PD deficiency"

DISCLOSURES

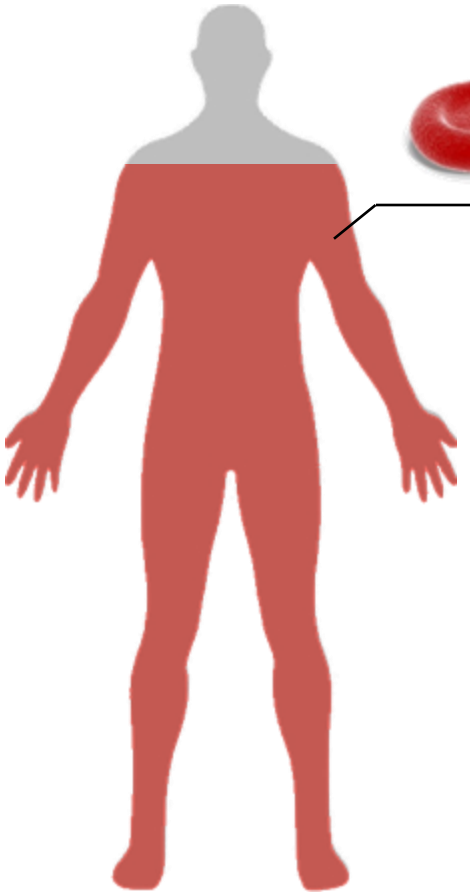
- Omix Technologies Inc - CSO;
- Altis Biosciences LLC - CSO;
- SAB for Hemanext Inc (formerly New Health Sciences Inc)
- SAB for Macopharma Inc

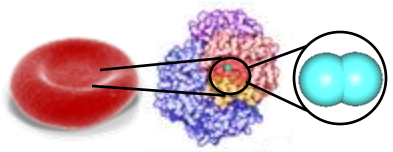
OMIX TECHNOLOGIES

SBIR FUNDING

- *PHASE I - R43GM130268*
- *PHASE II - R44 GM130268*
- *PHASE I - R43 CA239872*
- *PHASE II - R43 HL123074*
- *PHASE II - R44 HL127843*
- *PHASE I - R43 HL146014*
- *PHASE II - R44 HL132172*
- *PHASE II - R44 HL149579*

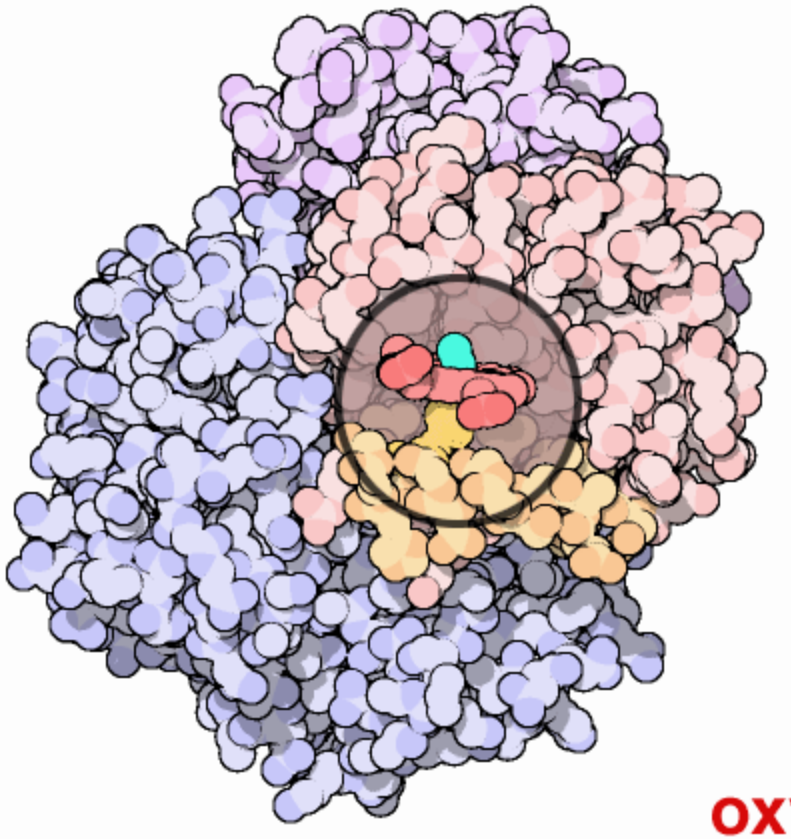
Red Blood Cells – Abundant, Simple, Central to Human Metabolism

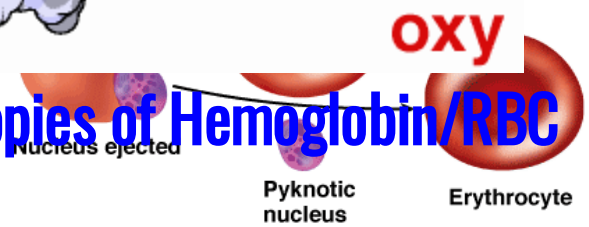




Key numbers

- RBCs ~83%
- 25 x 10^{12} RBCs/body
- Lifespan of ~120 days
- We spend a lot of energy to remove and generate 0.2 trillion RBCs/day



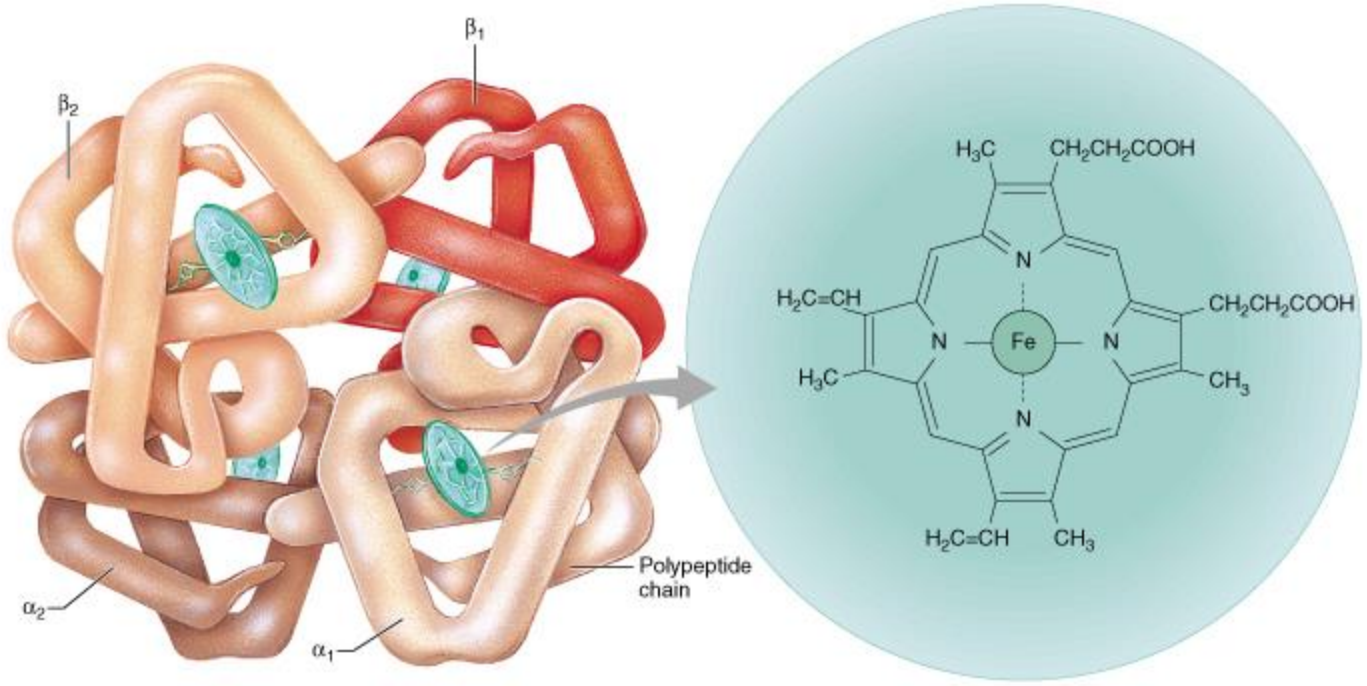


250 million copies of Hemoglobin/RBC

Source: Mescher AL: *Junqueira's Basic Histology: Text and Atlas, 12th Edition:* http://www.accessmedicine.com

Nemkov et al. Exp Rev Proteomics 2019

Red Blood Cells – Perfect Model to study Energy and Redox Metabolism



(a) Hemoglobin (b) Iron-containing heme group

Fenton Reaction

$$Fe(II) + H_2O_2 \longrightarrow Fe(III) + HO^- + HO^\bullet$$

Haber–Weiss Reaction (Superoxide Driven Fenton Reaction)

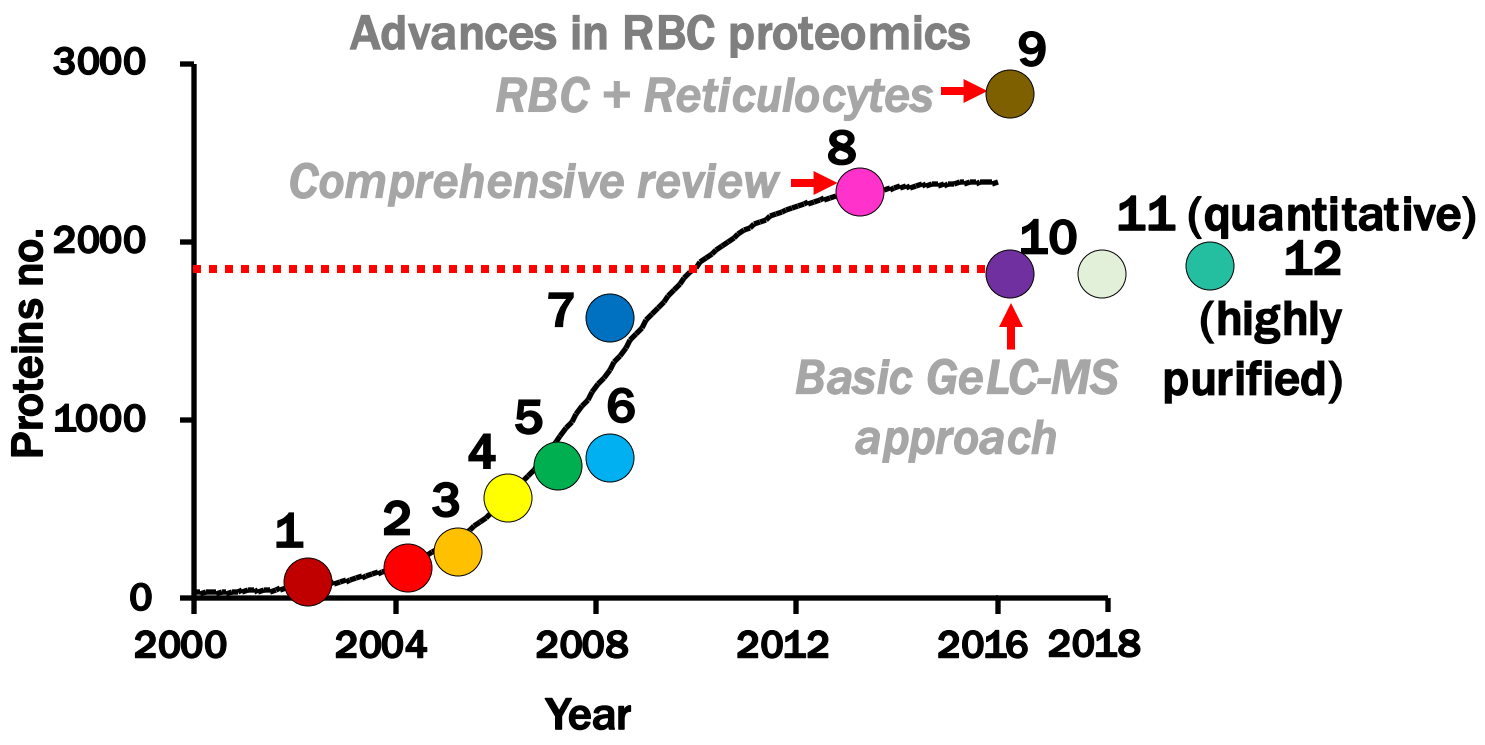
$$H_2O_2 + Fe(II) \longrightarrow Fe(III) + HO^- + HO^\bullet$$

$$O_2 \qquad O_2^{\bullet-}$$

Haber–Weiss Net Reaction

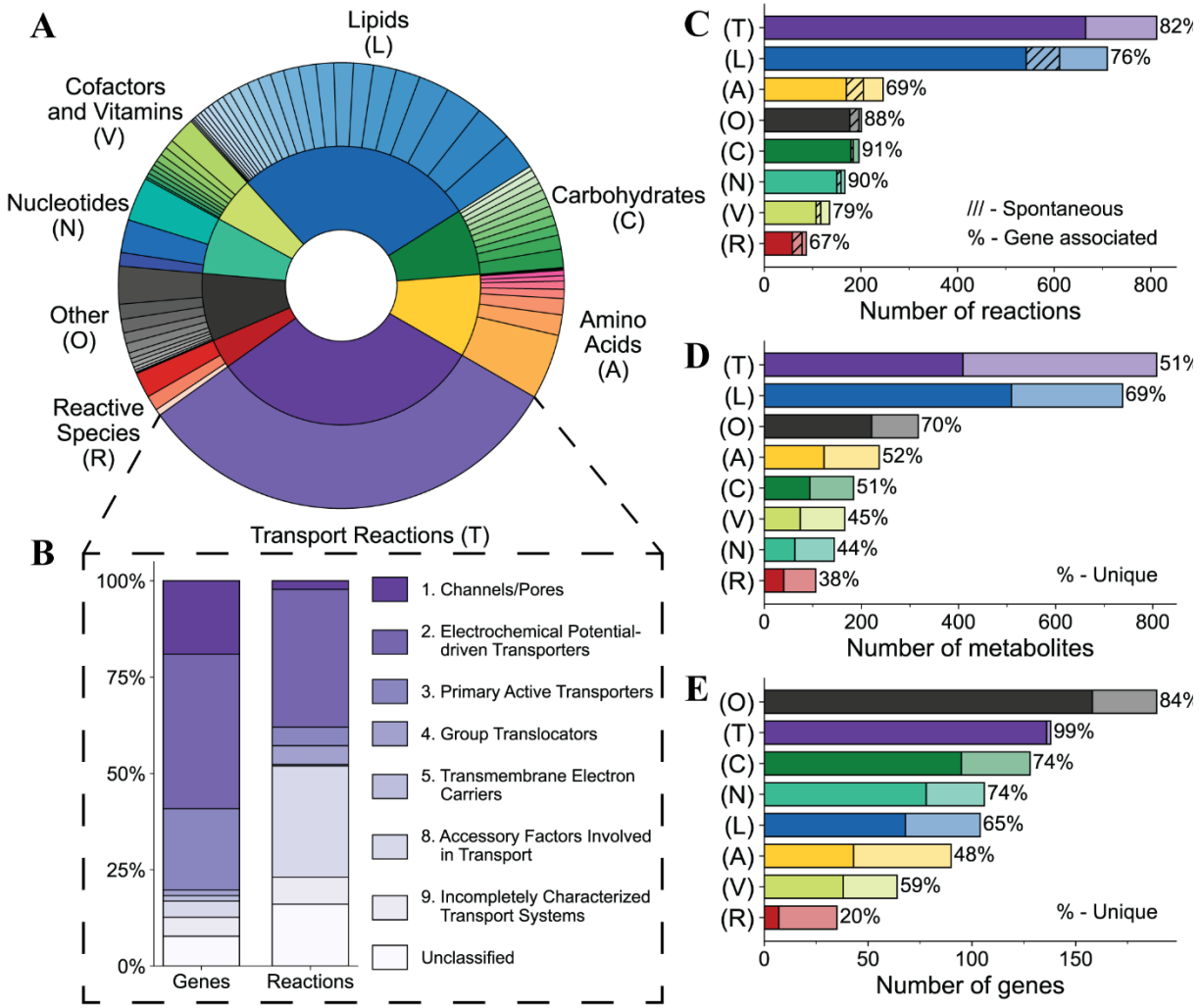
$$O_2^{\bullet-} + H_2O_2 \xrightarrow{Fe(II)/Fe(III)} O_2 + HO^- + HO^\bullet$$

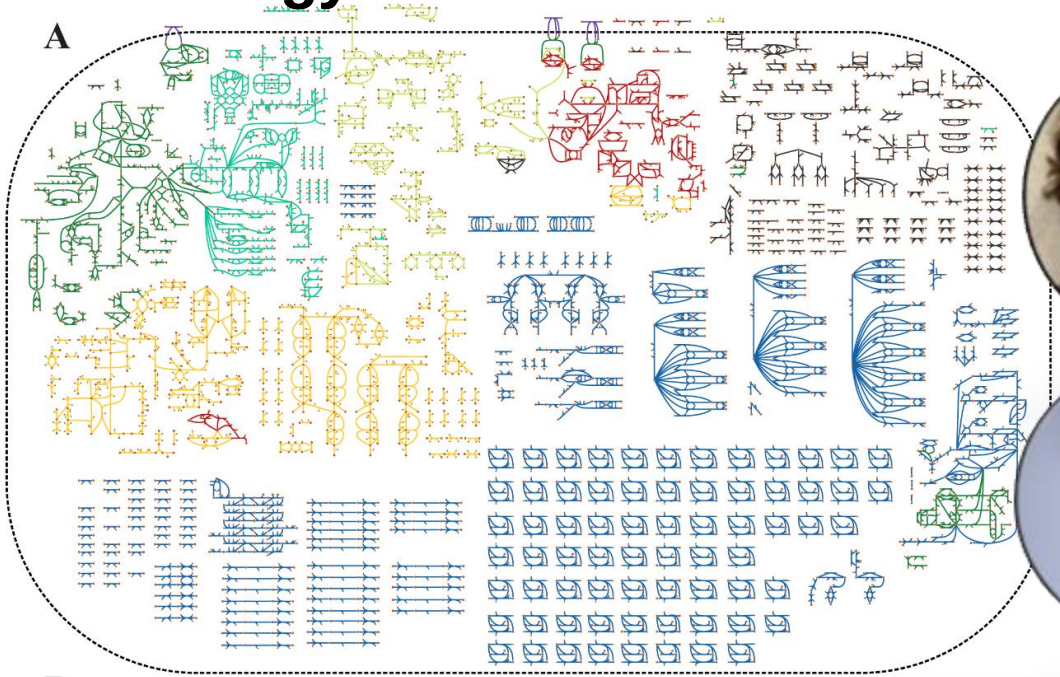
Red Blood Cells – Not so simple after all

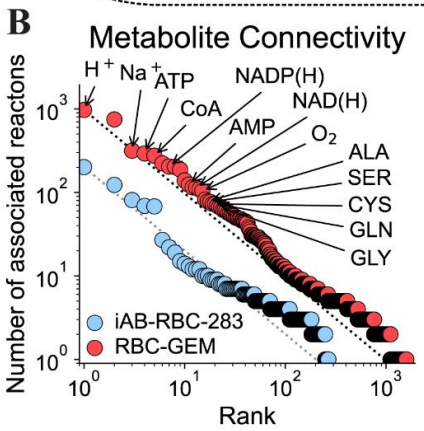


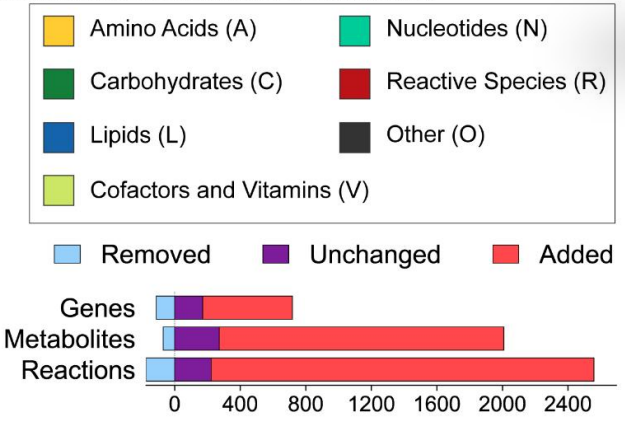
12 Gaultier et al
11 Bryk et al
10 D'Alessandro et al
9 Wilson et al.
8 Goodman et al.
7 Roux-Dalvai et al.
6 Bachi et al.
5 Goodman et al.
4 Pasini et al.
3 Kakhniashvili et al.
2 Tyan et al.
1 Low et al.

RBC-GEM: a Knowledge Base for Systems Biology of Human RBC Metabolism



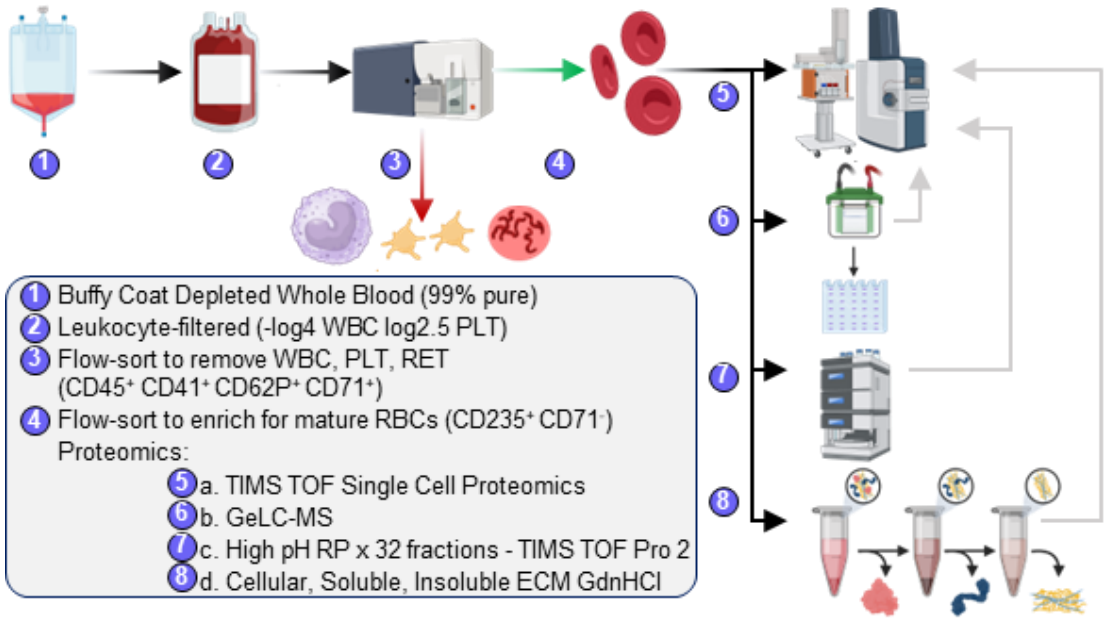






Haiman et al BioRxiv 2024 – under review

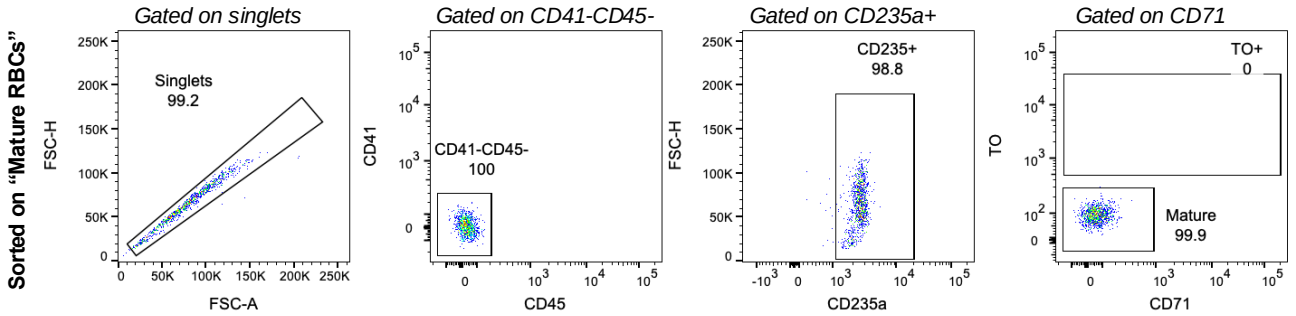
RBC Proteome Updated – is PFKP even expressed in ultra-pure mature RBCs?

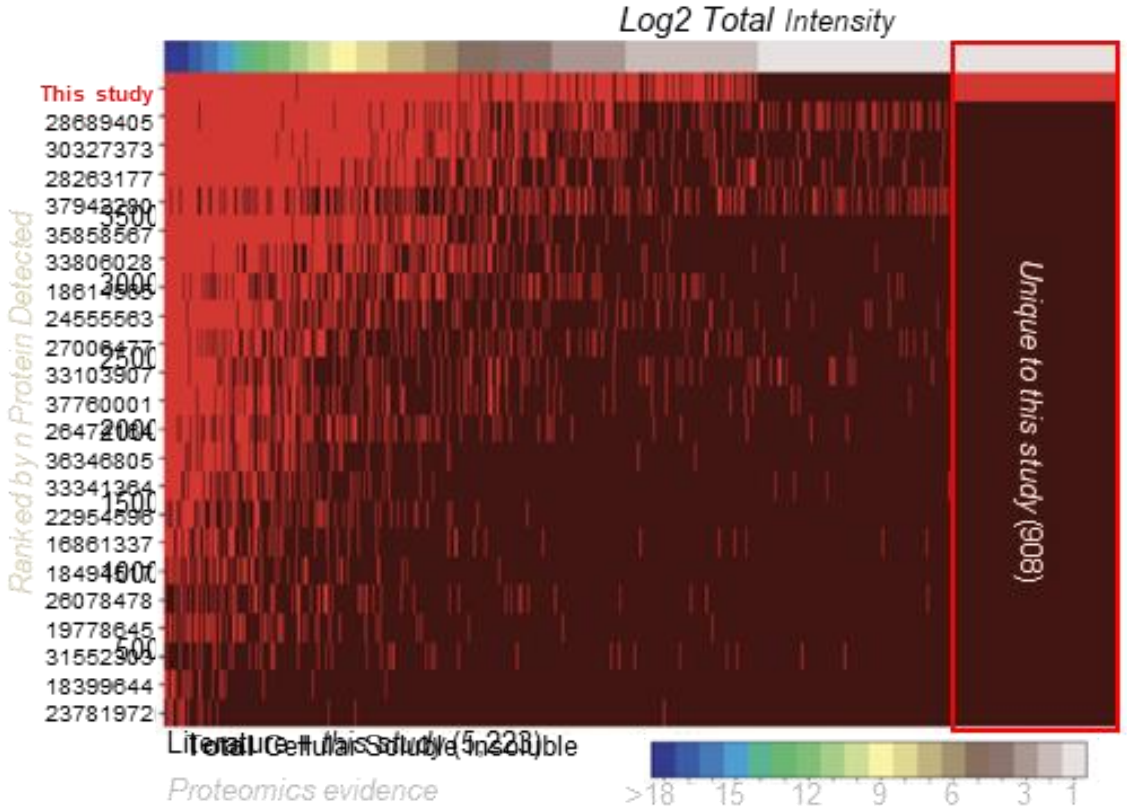


1. Buffy Coat Depleted Whole Blood (99% pure)
2. Leukocyte-filtered (-log4 WBC log2.5 PLT)
3. Flow-sort to remove WBC, PLT, RET (CD45⁺ CD41⁺ CD62P⁺ CD71⁺)
4. Flow-sort to enrich for mature RBCs (CD235⁺ CD71⁻)

Proteomics:

5. a. TIMS TOF Single Cell Proteomics
6. b. GeLC-MS
7. c. High pH RP x 32 fractions - TIMS TOF Pro 2
8. d. Cellular, Soluble, Insoluble ECM GdnHCl





D'Alessandro et al. In preparation

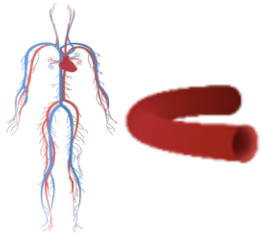
RBC as an organ: a window on systems-wide metabolic health

GAS TRANSPORT

- Oxygen/Carbon dioxide Transport
- Nitric oxide metabolism

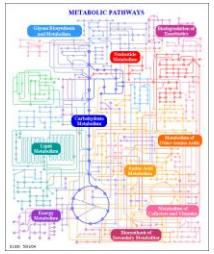
ION HOMEOSTASIS

- Chloride and bicarbonate homeostasis
- Ion homeostasis (Ca^{++}, Na^{+}, K^{+})
- Iron metabolism (Fe^{++})



VASODILATION

- Sequestration and release of Nitric Oxide
- Generation and release of purinergic agonists
- Response to adenosine/purinergic stimuli



SYSTEM METABLOLISM

- Glucose metabolism and lactatemia
- Redox (and glutathione/sulfur/iron)
- Amino acid uptake and metabolism
- Scavenging/Response to neurotransmitters

DETOXIFICATION

- Transamination reactions
- Glutathionylation of oxidized proteins/lipids
- Uptake and metabolism of drugs
- Uptake and release of dietary mediators (e.g dietary lipids)

IMMUNOMODULATION

- Direct interaction through “eat me”/”do not eat me” signals
- Uptake and release of cytokines
- Generation and release of immunomodulatory
  - ATP, lactate
  - Uptake/release of succinate and carboxylic acids
  - Eicosanoids and oxylipins, Sphingosine 1-phosphate
  - Tryptophan and catabolites (kynurenine, NAD)
  - Arginine and catabolites (e.g. NO and polyamines)
  - heme and iron metabolism in phagocytosing macrophages
  - miRNA in phagocytosing cells

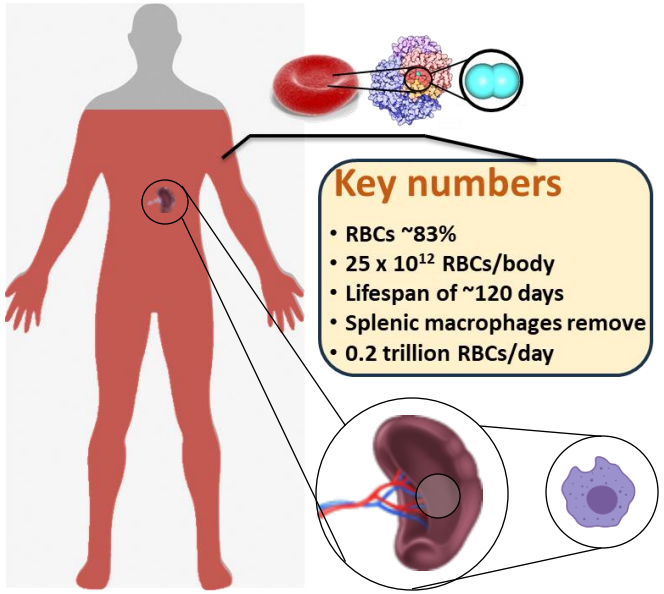
CROSS-TALK with other RBCs or other cells

- Membrane antigens mediating cell-cell interactions
- Release of small molecule mediators
- Vesiculation
- Release of mediators of target cell physiology upon hemolysis
- Scavenging/release of metabolic substrates for other cells

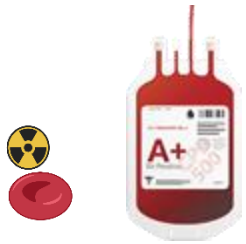
Nemkov et al 2008

Haiman et al. BiorXiv 2024

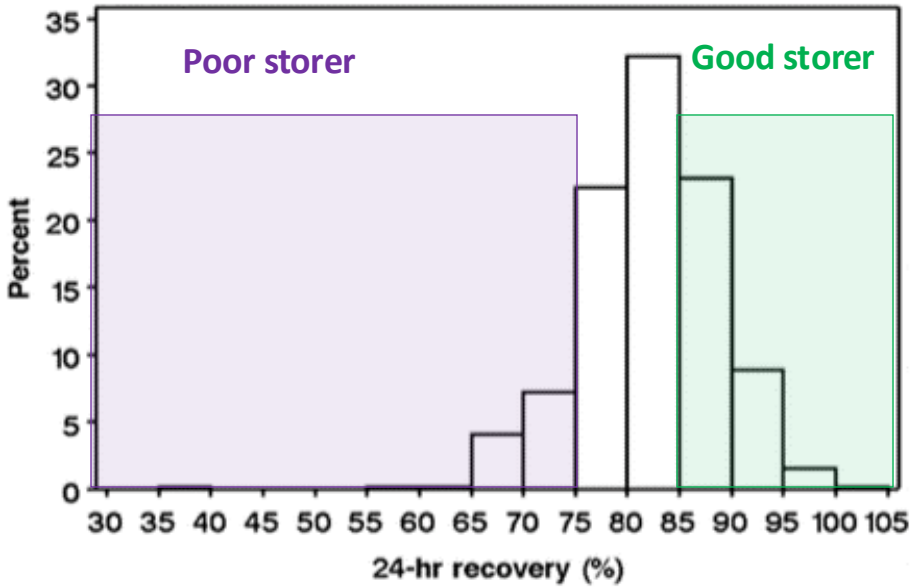
Extravascular hemolysis of Red Blood Cells is a process essential to human biology and transfusion medicine



100 Million units of pRBC transfused every year
Most common in hospital procedure after vaccination

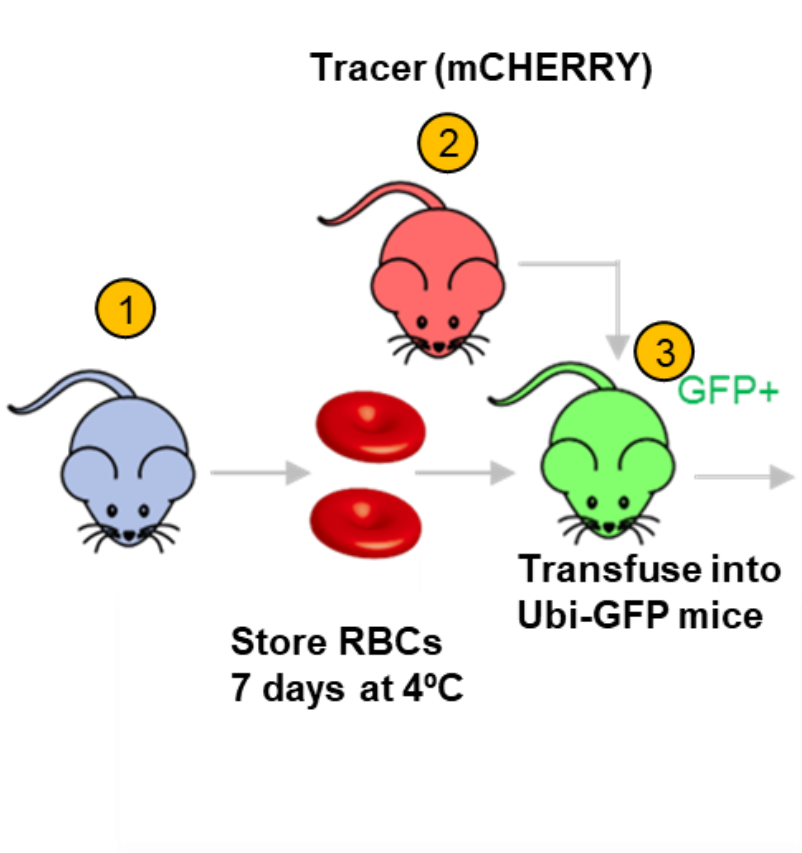


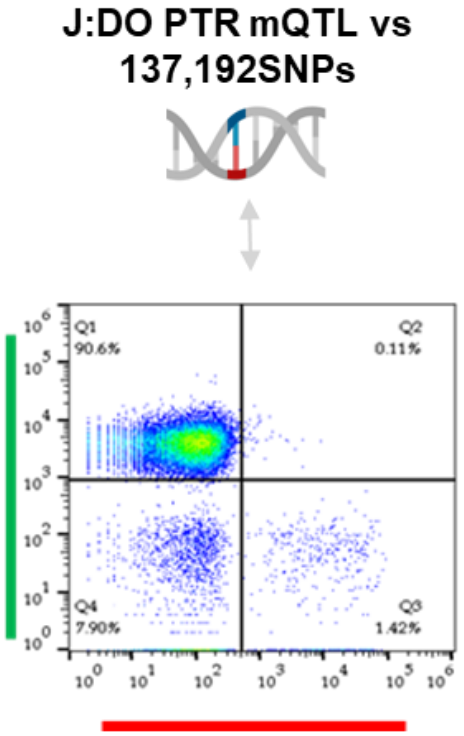
^{51}Cr labeling for Post Transfusion Recovery

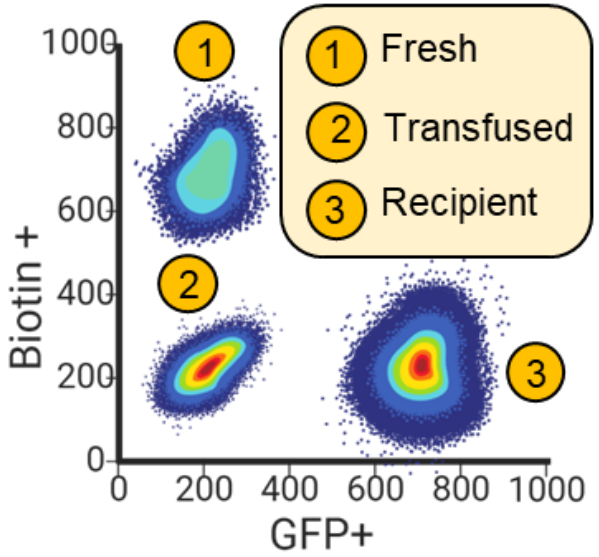


Dumont and Aubuchon. Transfusion 2007; Roussel et al. Blood 2021; D'Alessandro Blood 2021

Modeling diversity in RBC storability in mice

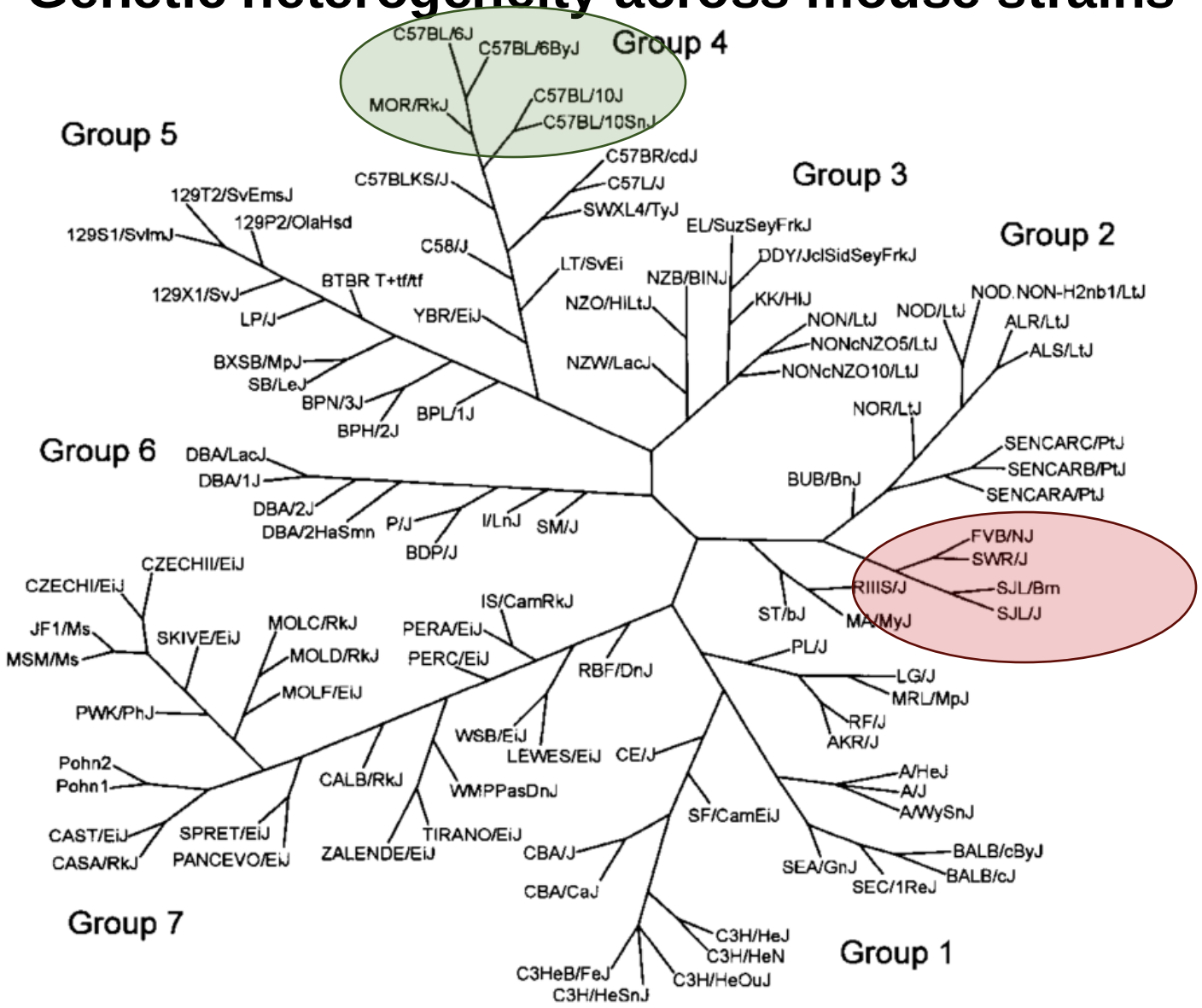






D'Alessandro et al. *Blood 2024 – just accepted*

Genetic heterogeneity across mouse strains

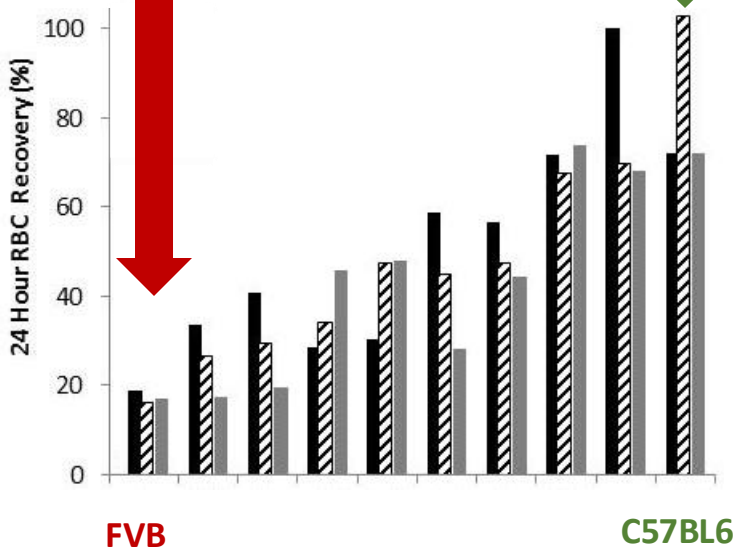


C57BL6/J

FVB/J

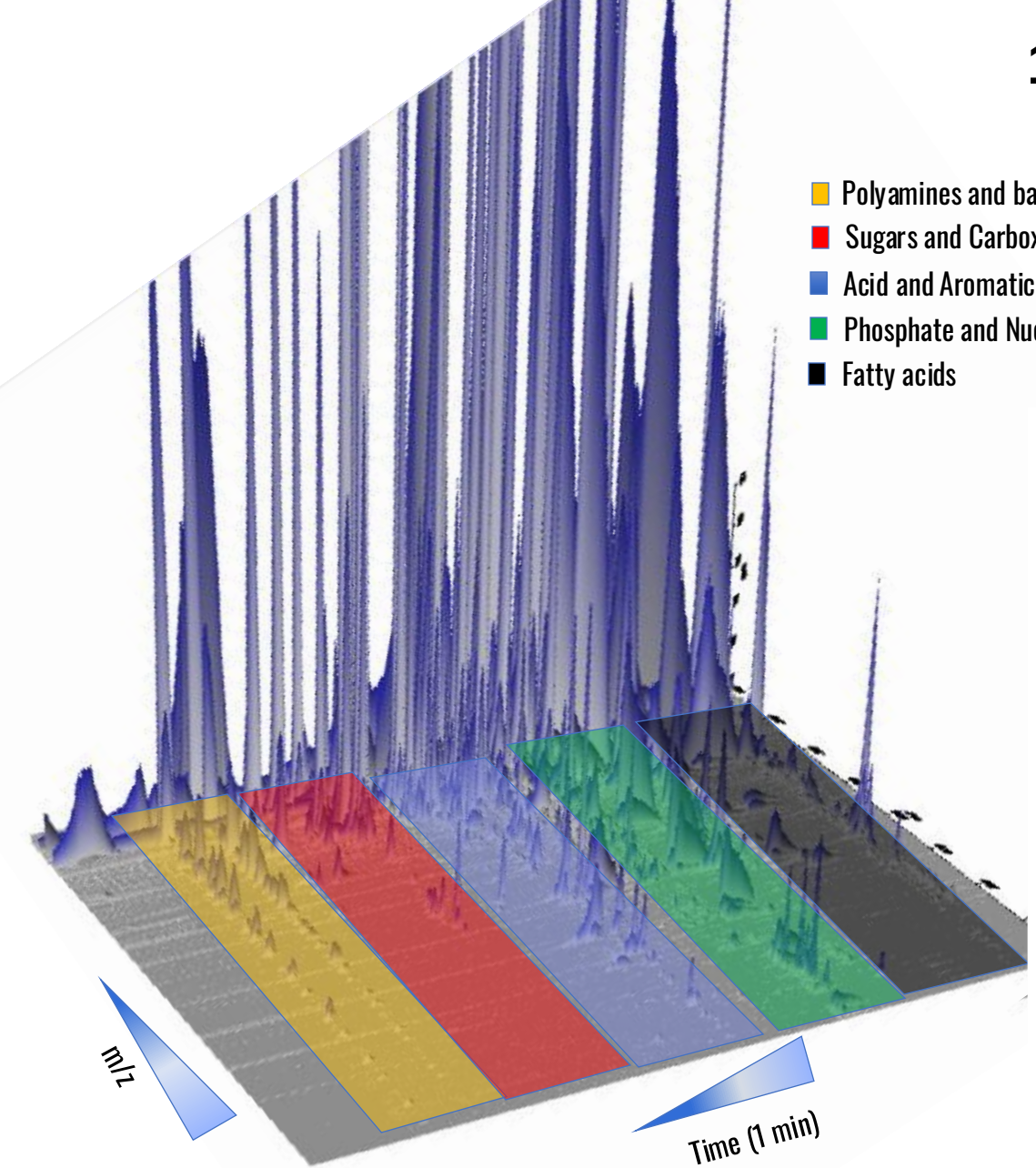
Poor storer

Good storer



Zimring et al Transfusion 2012; Hay et al Blood Transfusion 2023

1 min METABOLOMICS and 5 min LIPIDOMICS



» Life Sciences » Systems Biology and Bioinformatics

Methods in Molecular Biology

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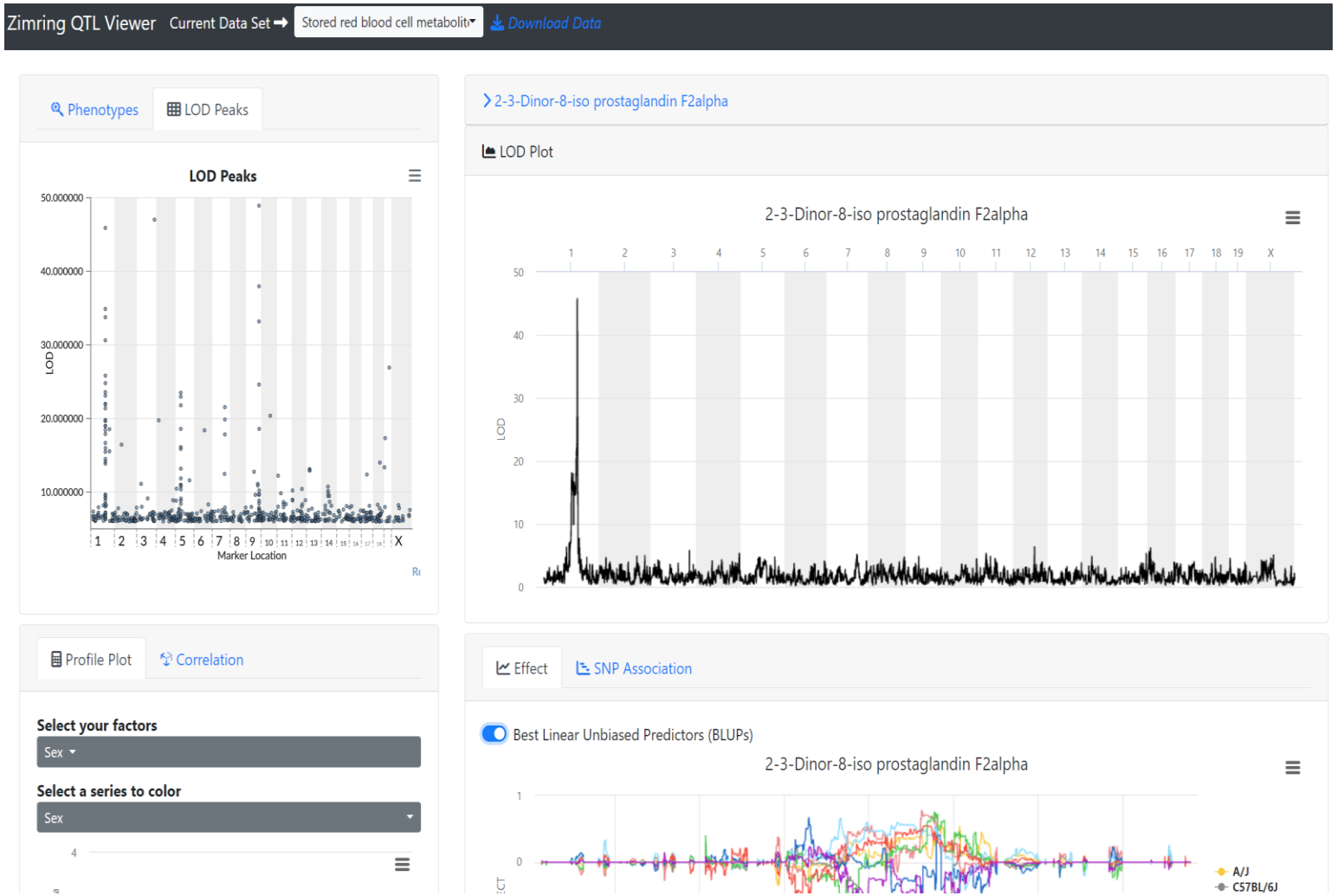
High-Throughput Metabolomics

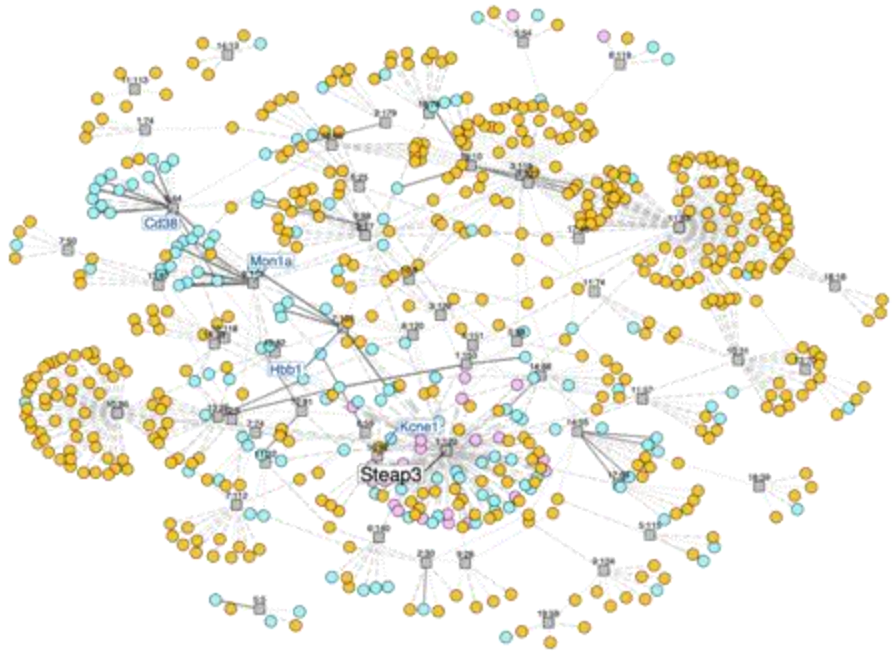
Methods and Protocols

Editors: **D'Alessandro**, Angelo (Ed.)

Nemkov et al. *Front Physiol 2023; Nemkov et al. Meth Mol Biol 2019*

Modeling diversity in RBC storability in mice

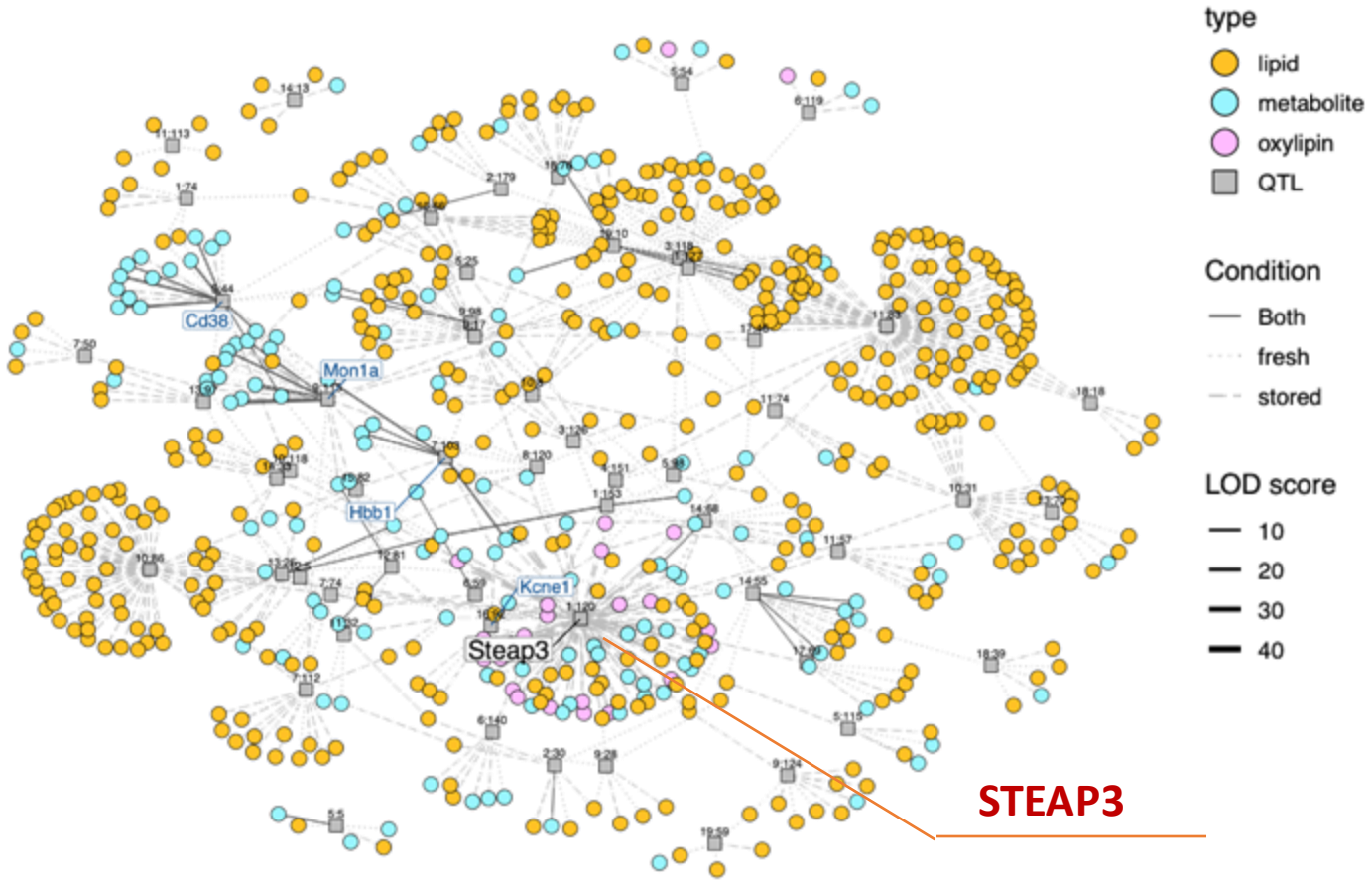


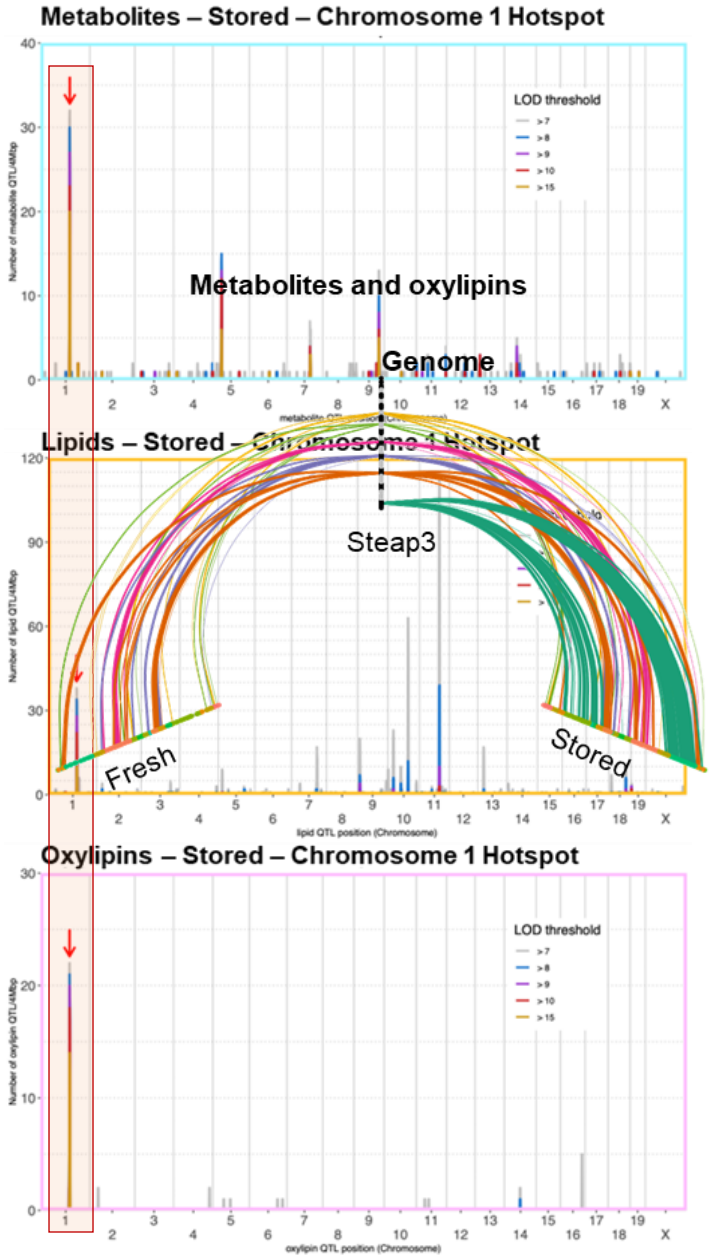


QTL Viewer

D'Alessandro et al. *Blood 2024 – just accepted*

Modeling diversity in RBC storability in mice

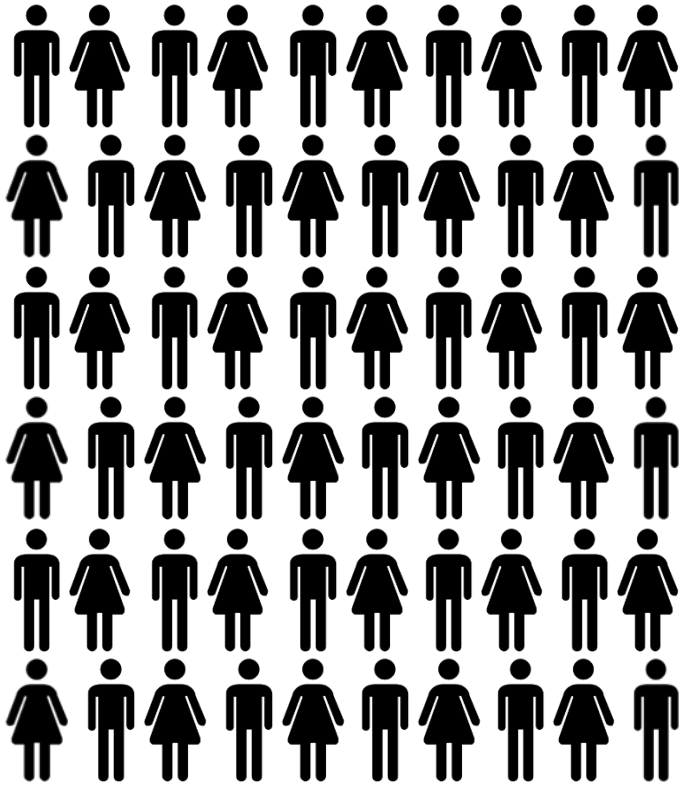




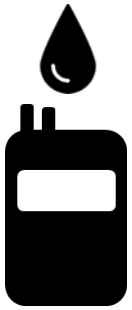
D'Alessandro et al. *Blood 2024 – just accepted*

Recipient Epidemiology and Donor Evaluation Study – REDS RBC Omics

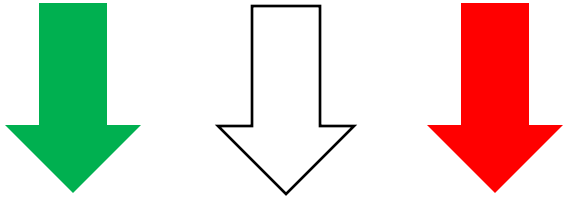
Donor effect on Storability
(n=13,403)



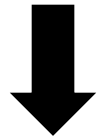
Blood storage for 42 days
+ hemolysis

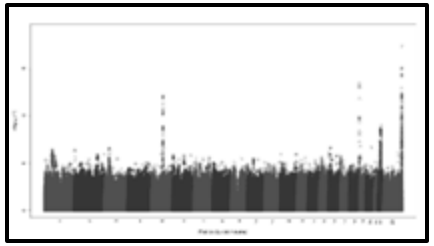


n=12,753 12,799 10,476



Genotyping
(SNPs)

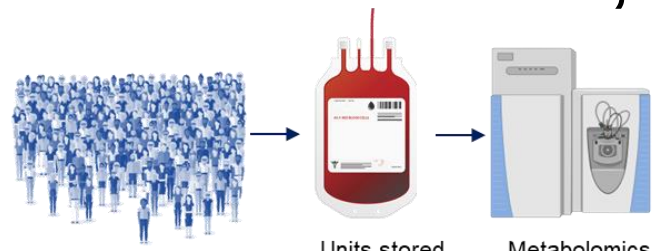


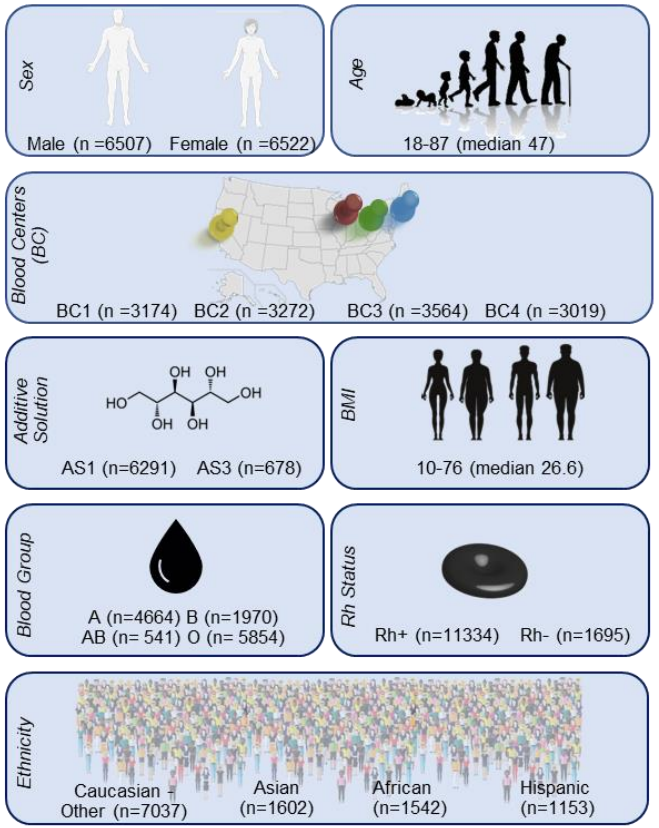


Correlation

Endres-Dighe et al. Transfusion 2019; Lanteri et al. Transfusion 2019
Josephson et al Transfusion 2022

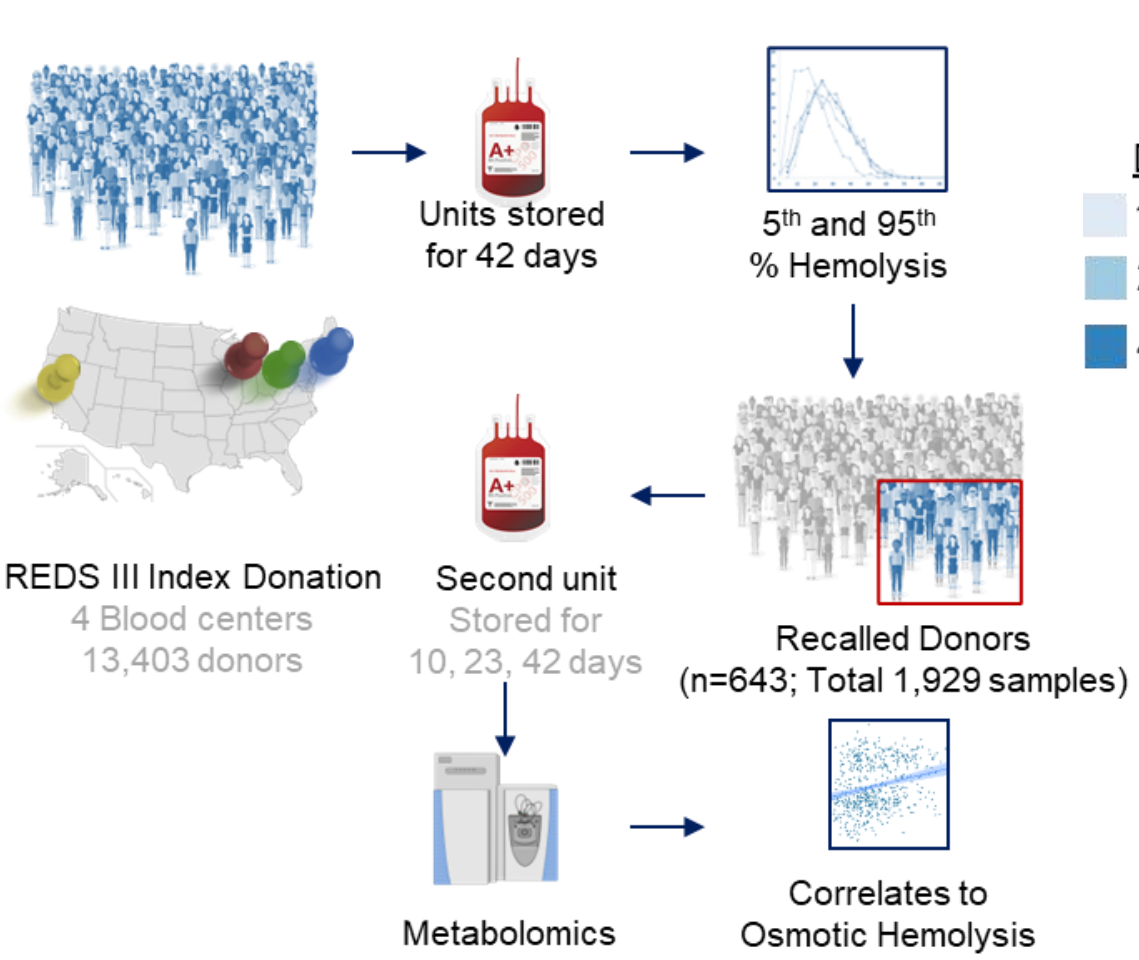
Metabolomics of 13,091 Blood Donors

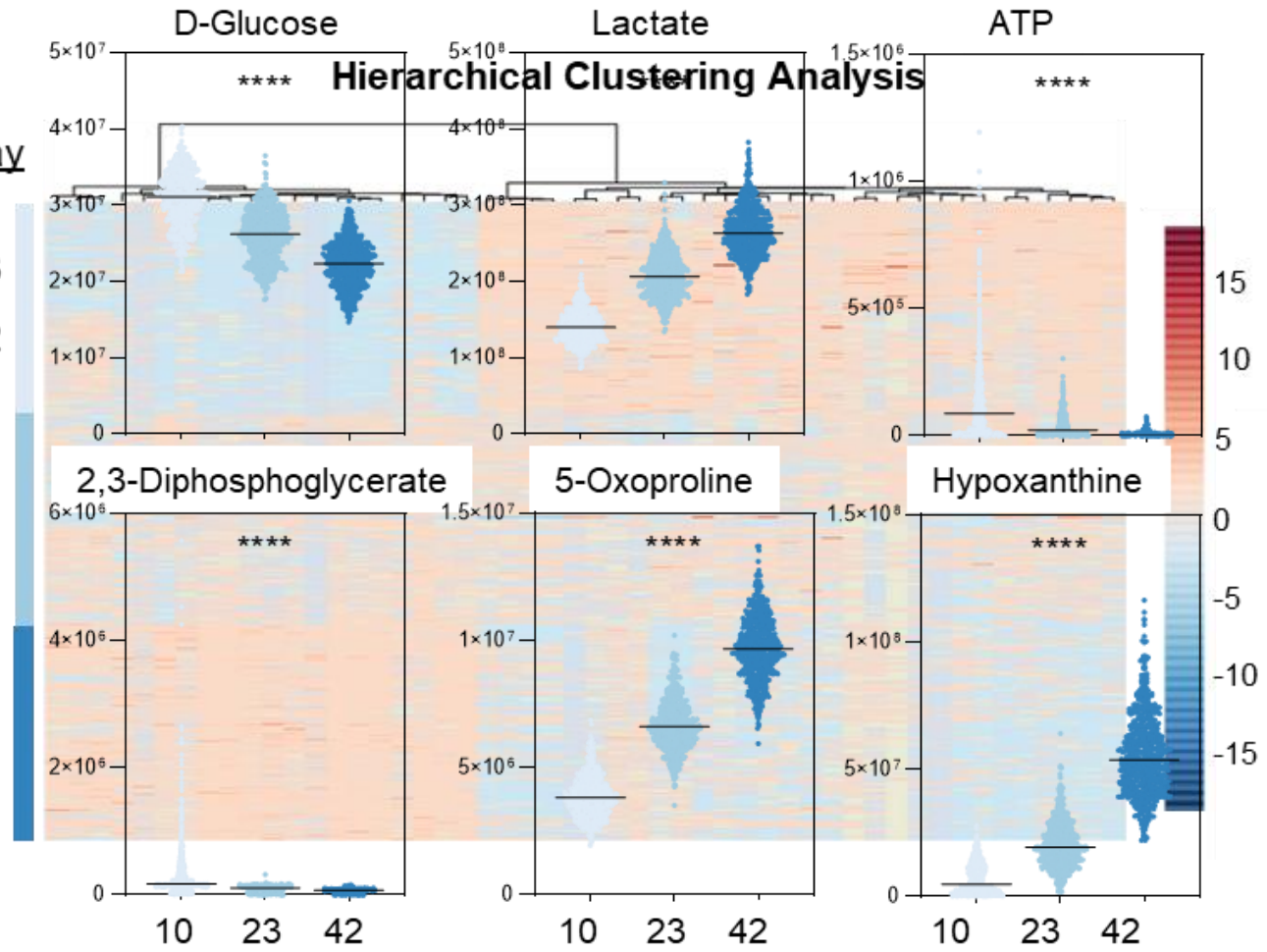




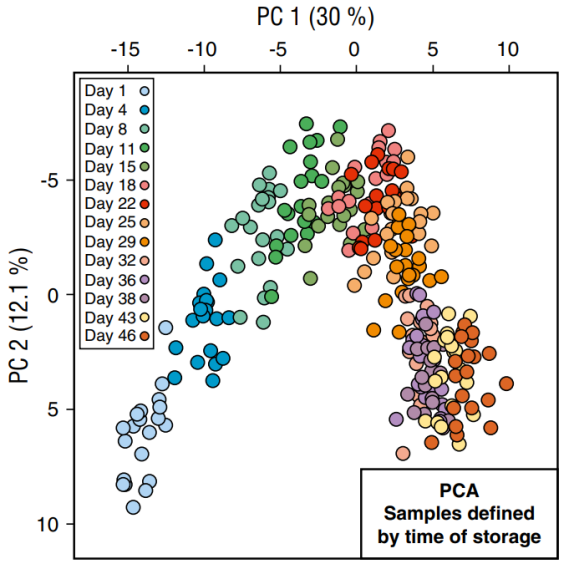
Nemkov et al. *Blood 2024*

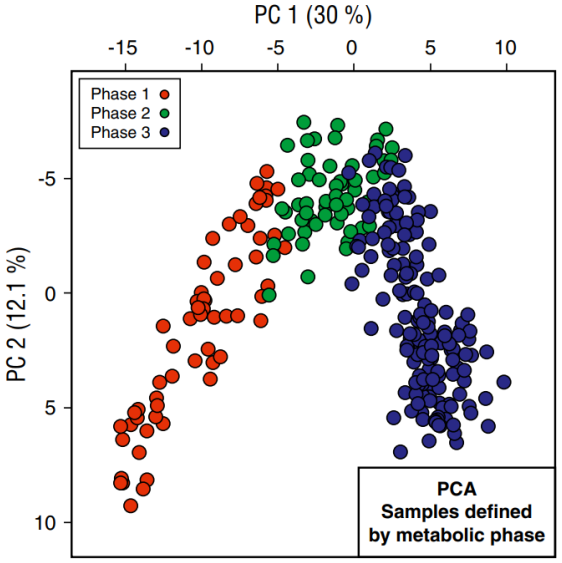
RBC metabolism changes with storage

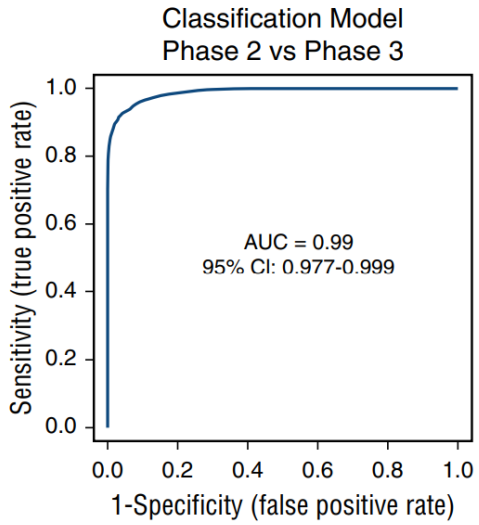


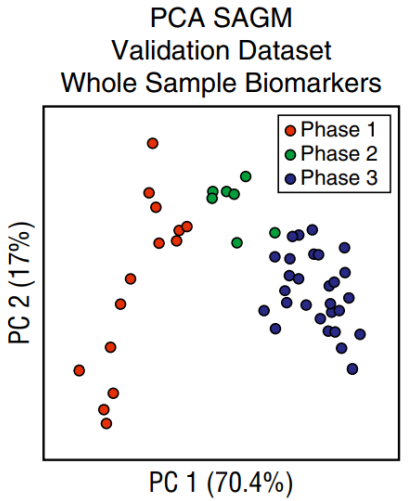


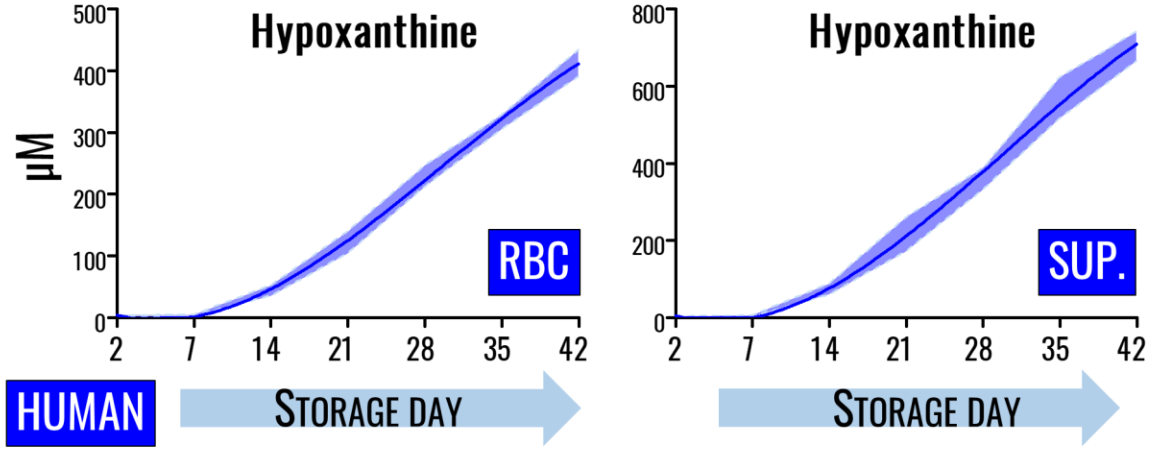
Biomarkers of the RBC Metabolic Age

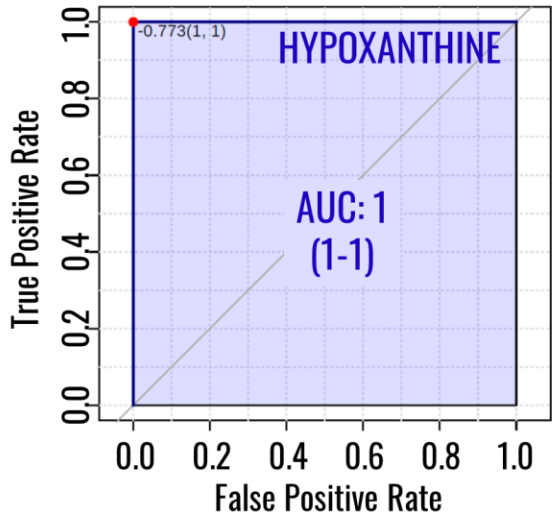


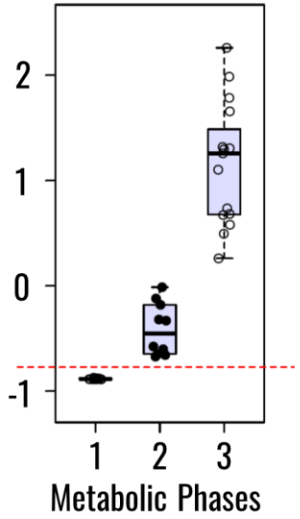


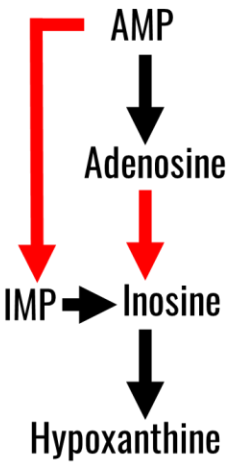






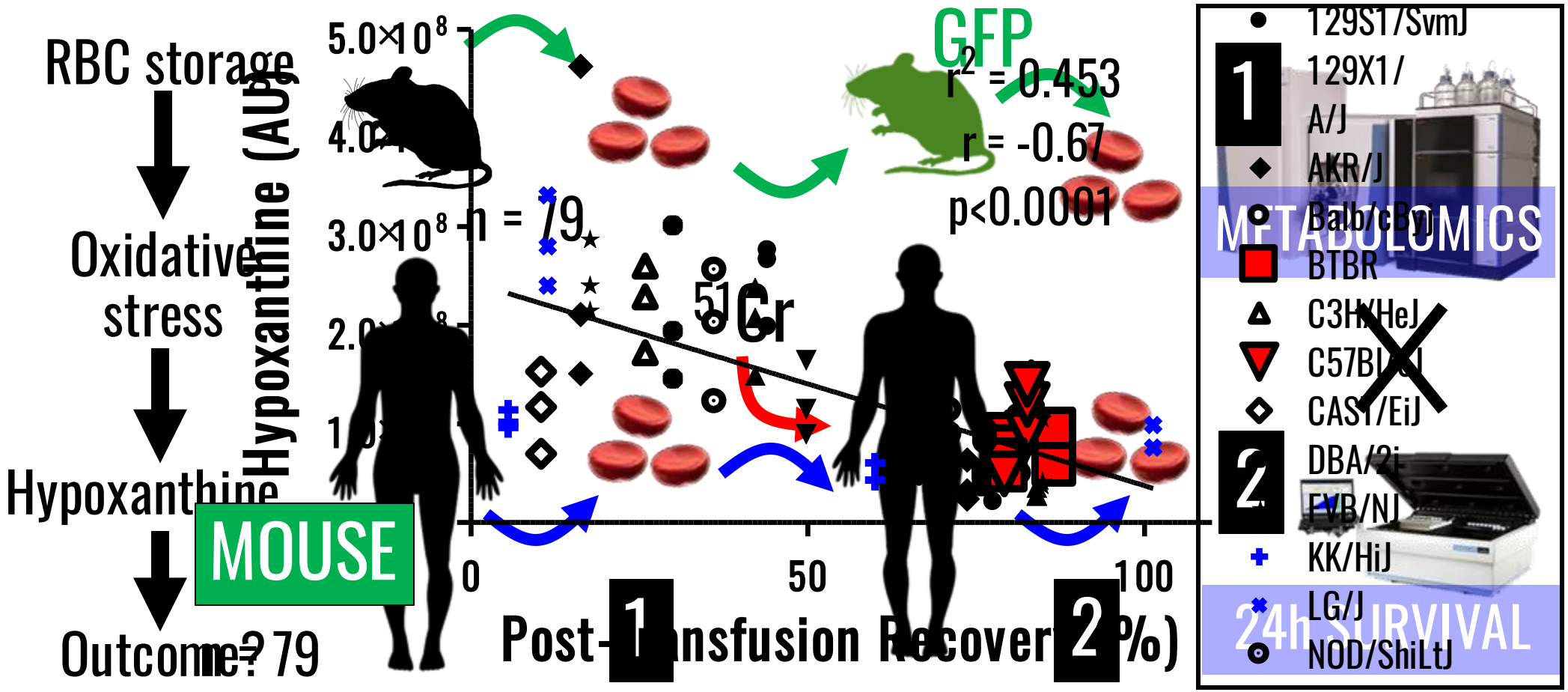






D'Alessandro et al. Haematologica 2012; D'Alessandro et al. Transfusion 2016; Paglia, D'Alessandro et al Blood 2016; Nemkov et al. Haematologica 2018

High hypoxanthine (ATP breakdown and oxidation) correlates to low PTR in mice and humans

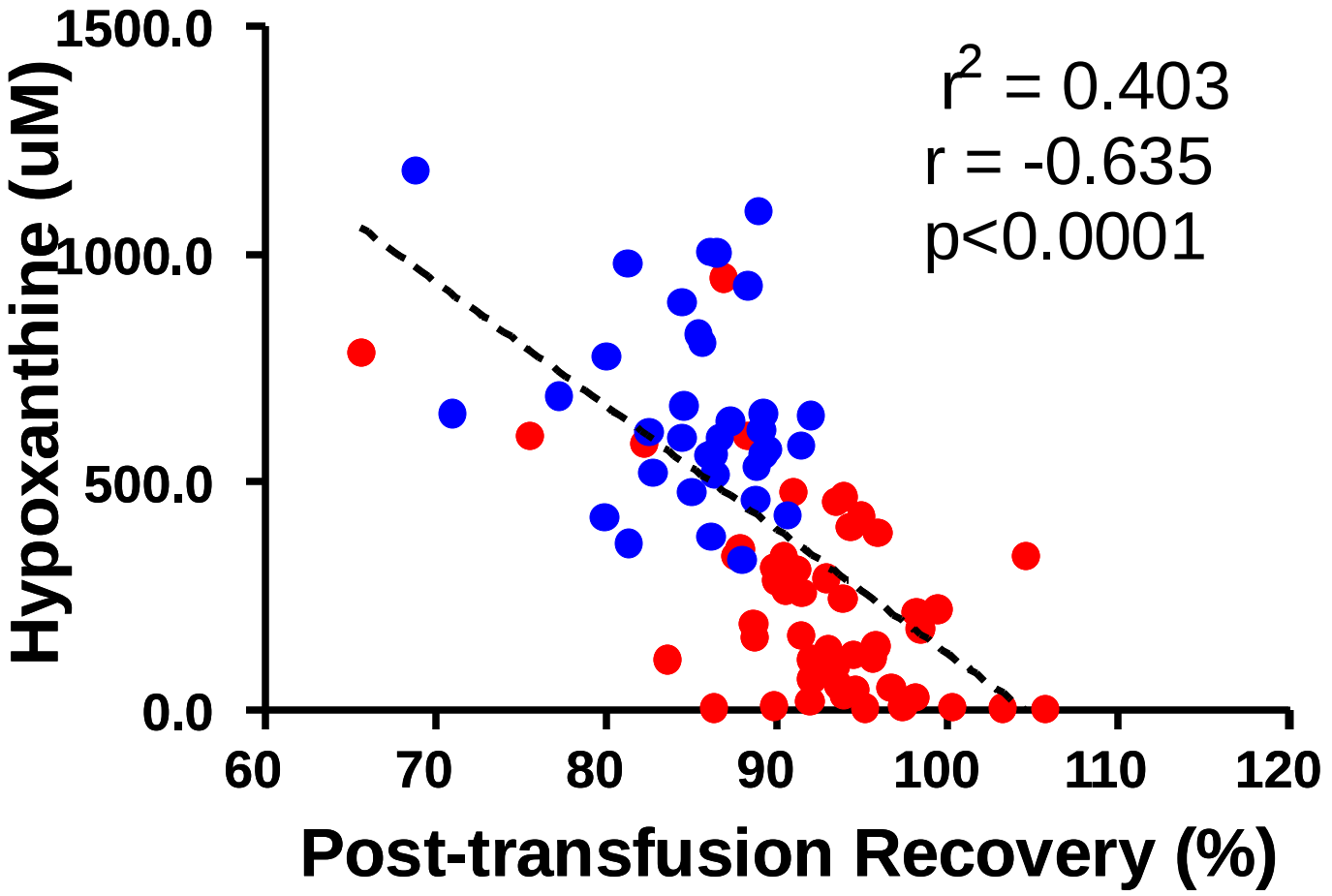


Nemkov et al. Haematologica 2018; Howie et al. Blood Advances 2019

High hypoxanthine (ATP breakdown and oxidation) correlates to low PTR in mice and humans

HUMAN

n = 52
(all storage time points)

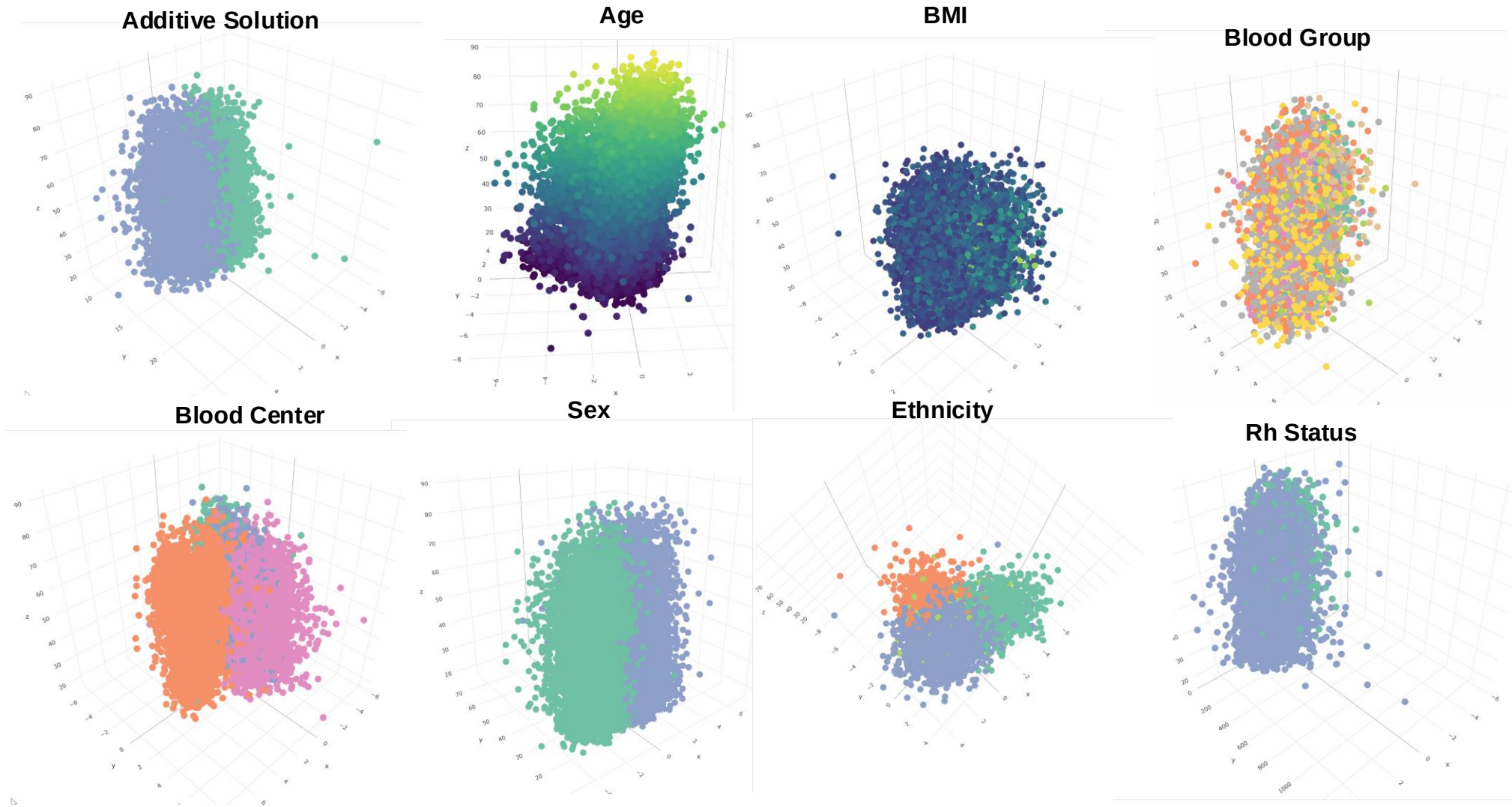


HUMAN

n = 37
(day 42)

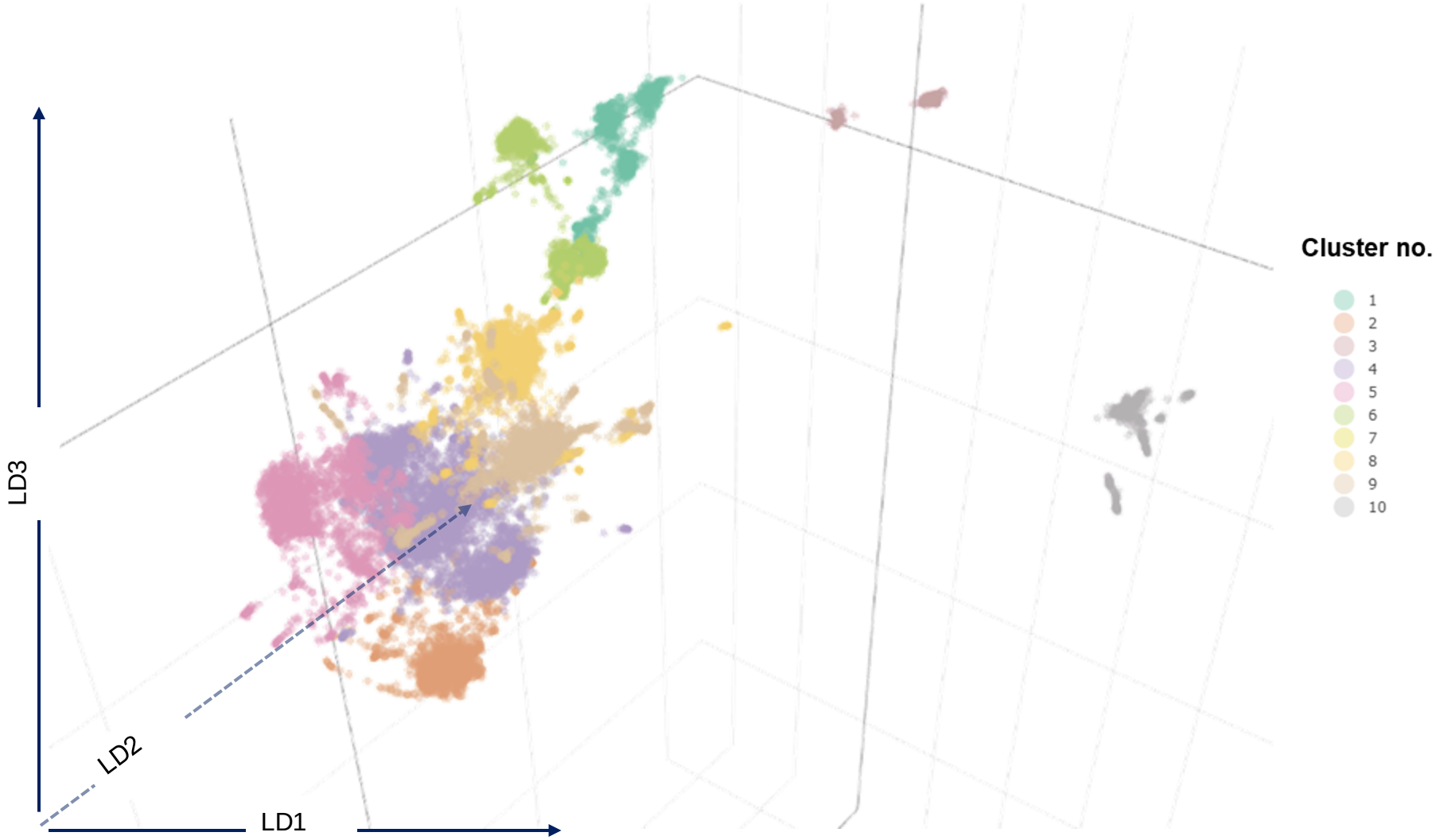
Nemkov et al. Haematologica 2018; Francis, D'Alessandro et al. JCI 2022;

Many iatrogenic and biological variables impact the metabolome of stored RBCs

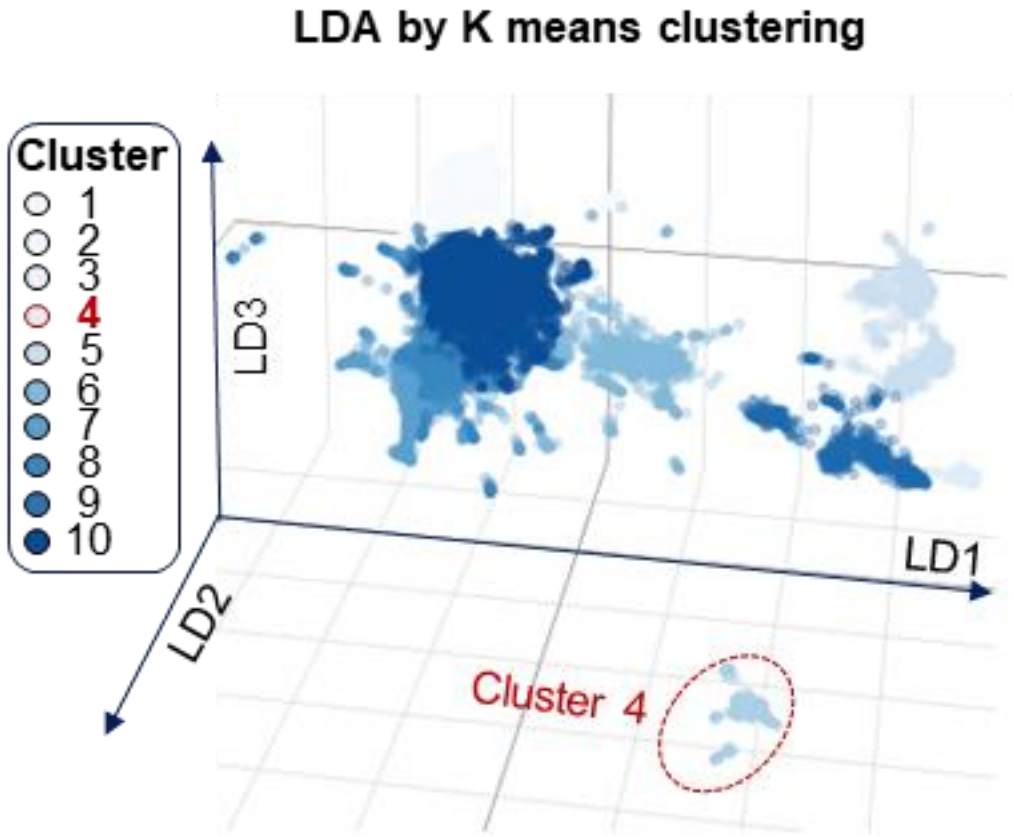


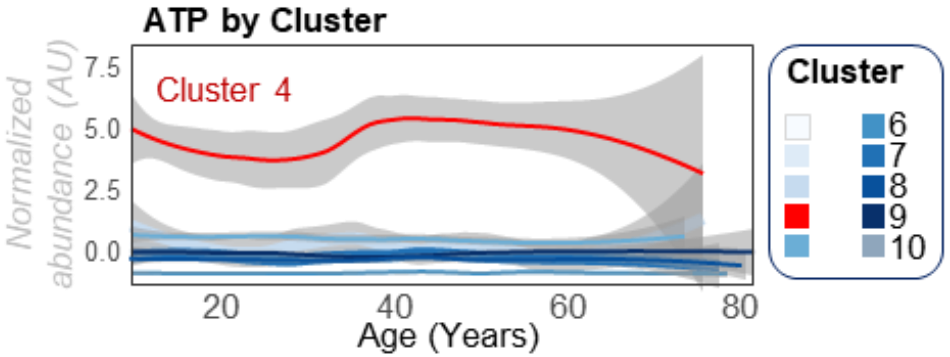
Nemkov et al. *Cell Metabolism 2024*

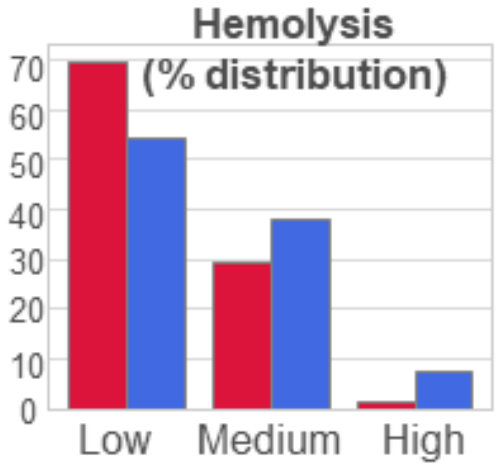
Data analysis guides interpretation! Kmeans clustering + Linear Discriminant Analysis



Finding the SUPER-DONORS (high energy metabolism, low oxidant stress)!





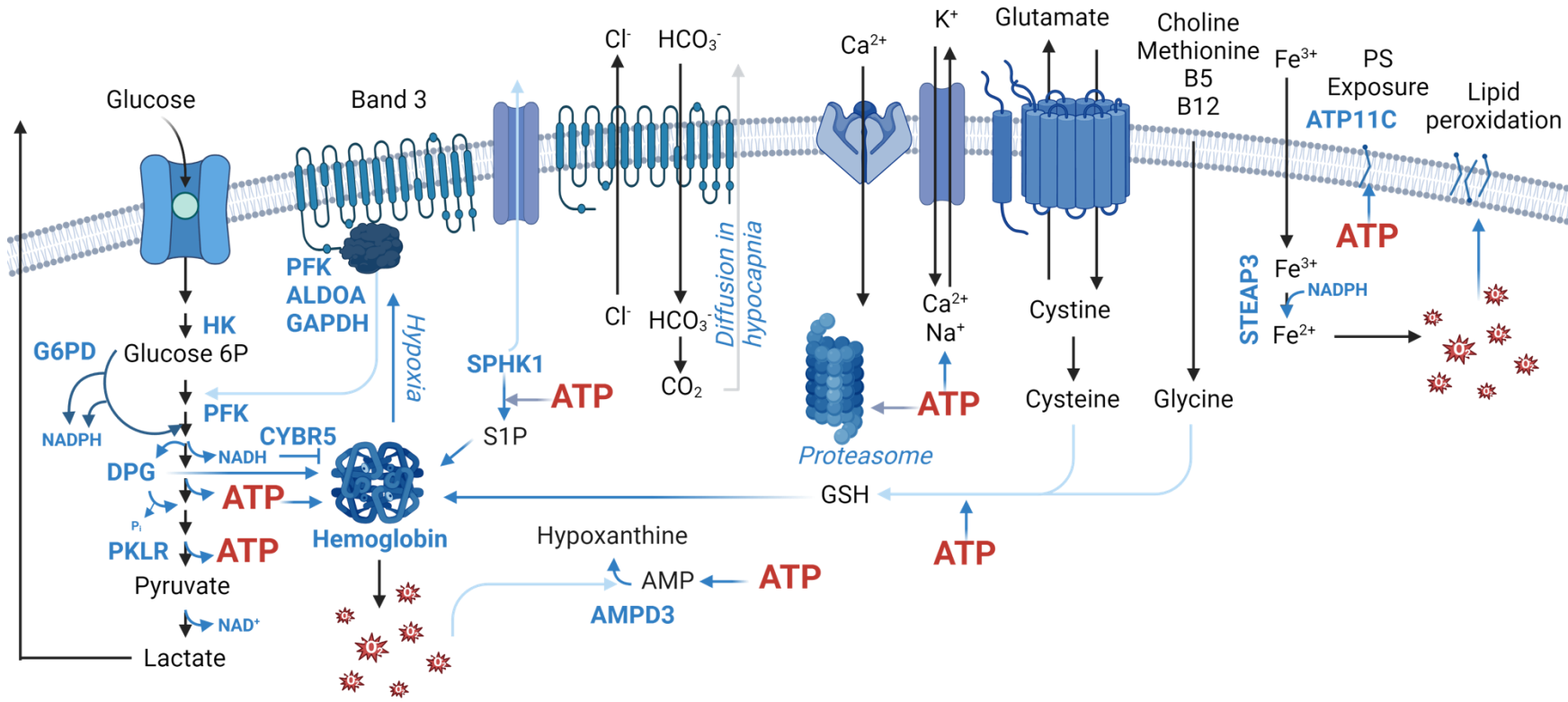


Cluster 4 vs Other Clusters Combined

ATP: a central lynchpin in RBC metabolism, deformability, function

ATP regulates:

- Hemoglobin allostery with DPG and O_2 kinetics
- Proton pumps
- Membrane protein phosphorylation
- Structure and deformability
- Phospholipid asymmetry
- Proteasome activity
- Vesiculation
- Intra- or Extra-vascular Hemolysis

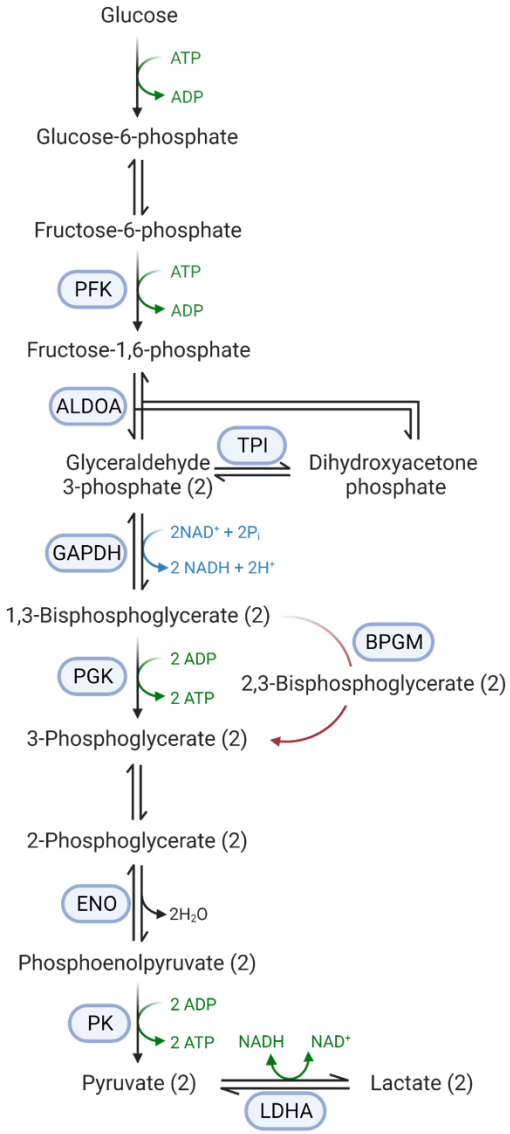


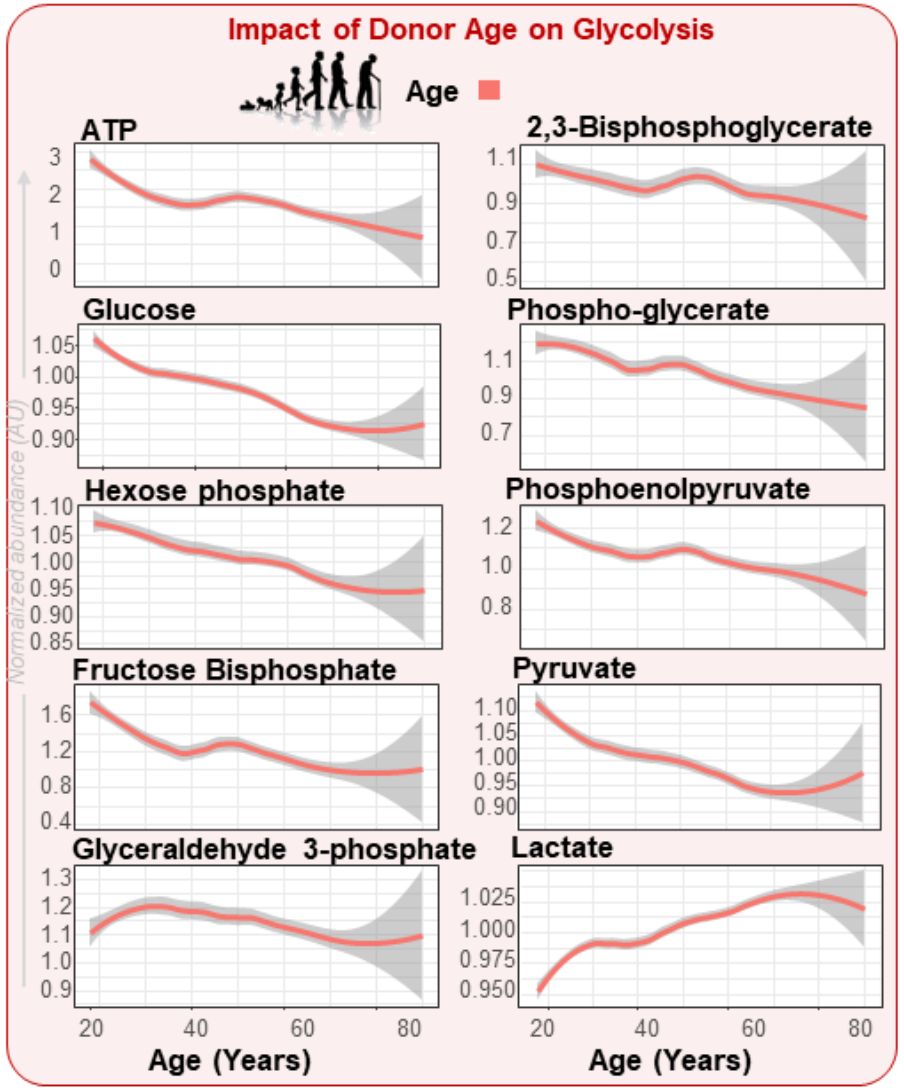
The energy-less red blood cell is lost: erythrocyte enzyme abnormalities of glycolysis

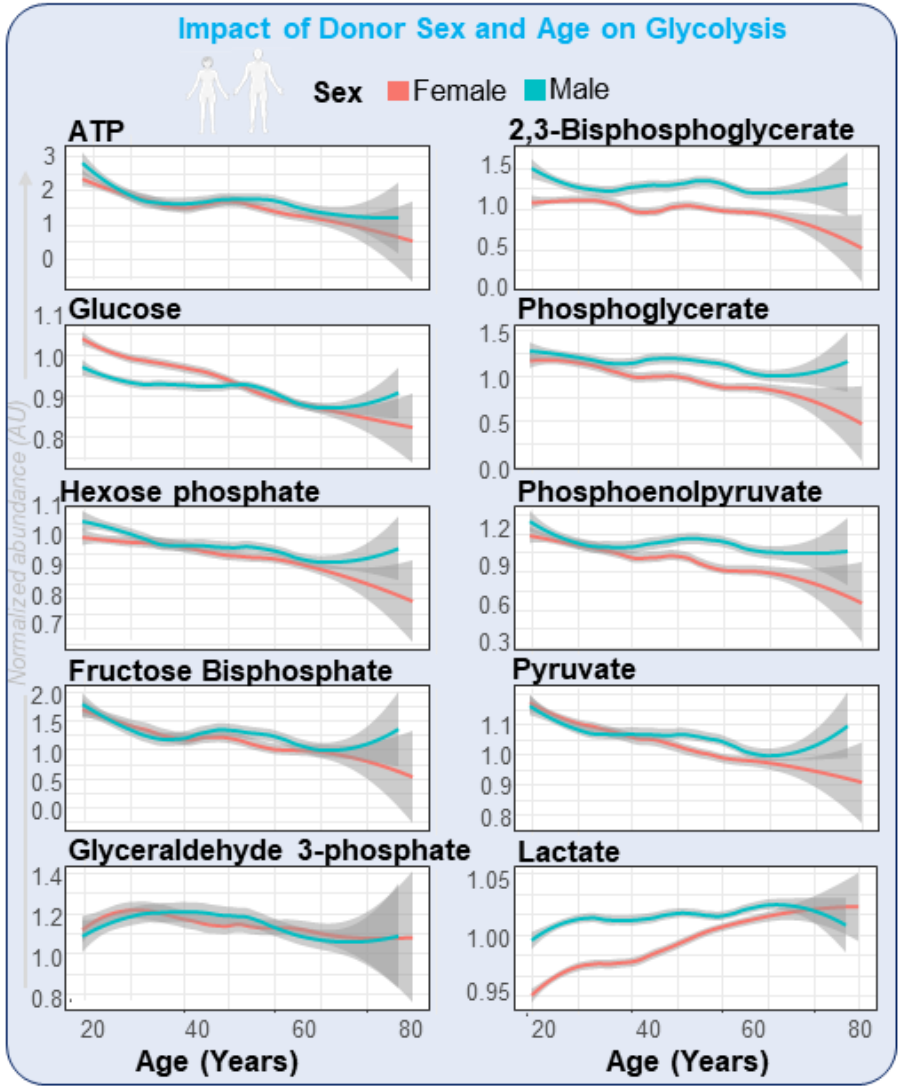
Richard van Wijk and Wouter W. van Solinge

van Wijk and van Solinge Blood 2005

Age and Sex impact RBC energy metabolism

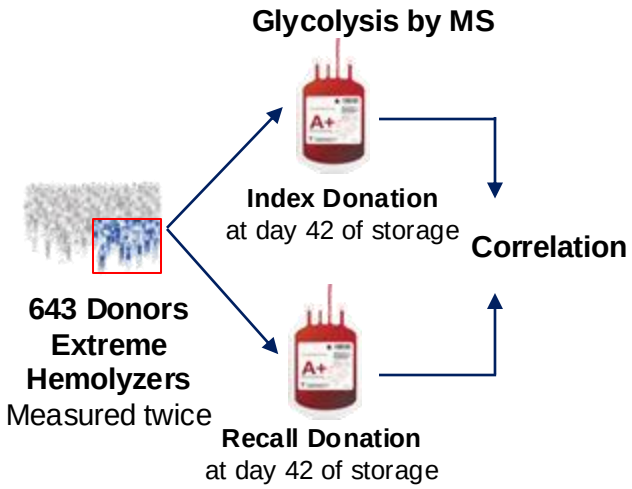


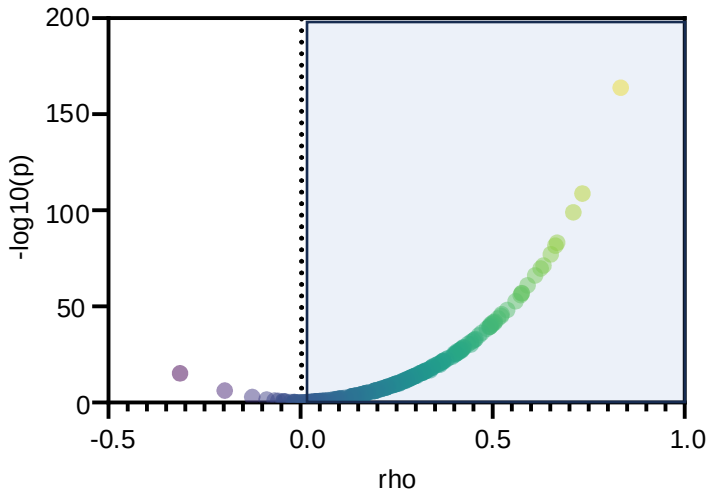


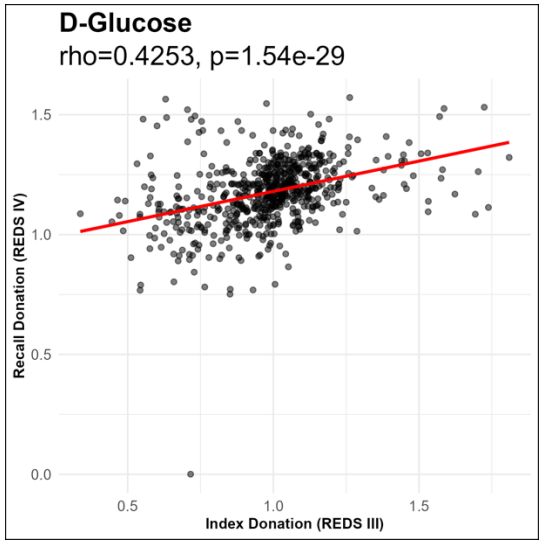


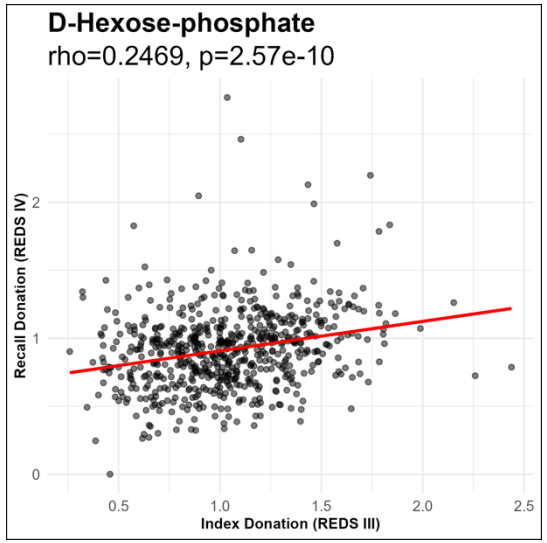
Nemkov et al. *Cell Metabolism 2024*

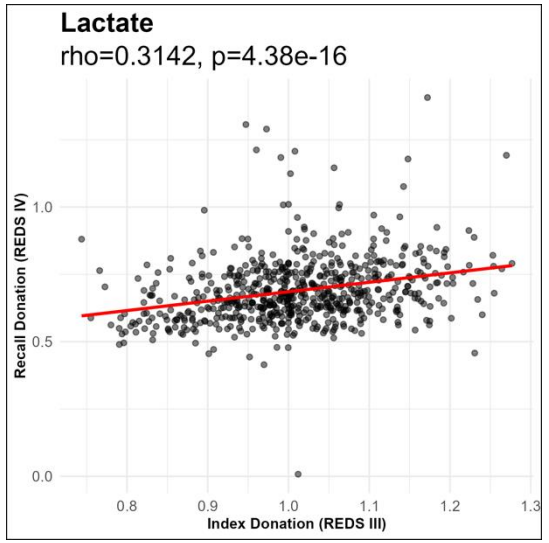
Levels of glycolytic metabolites are reproducible across donations





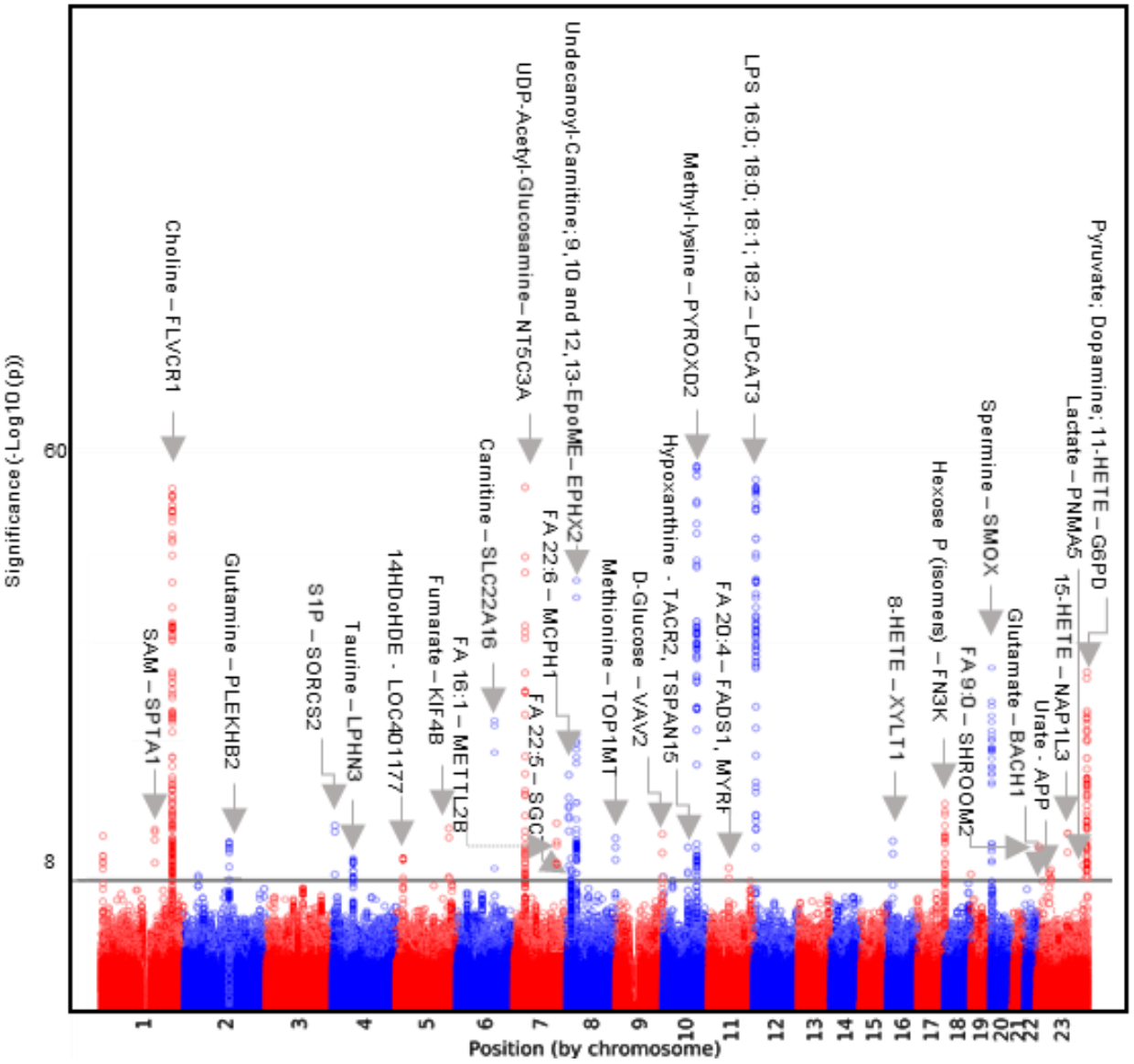


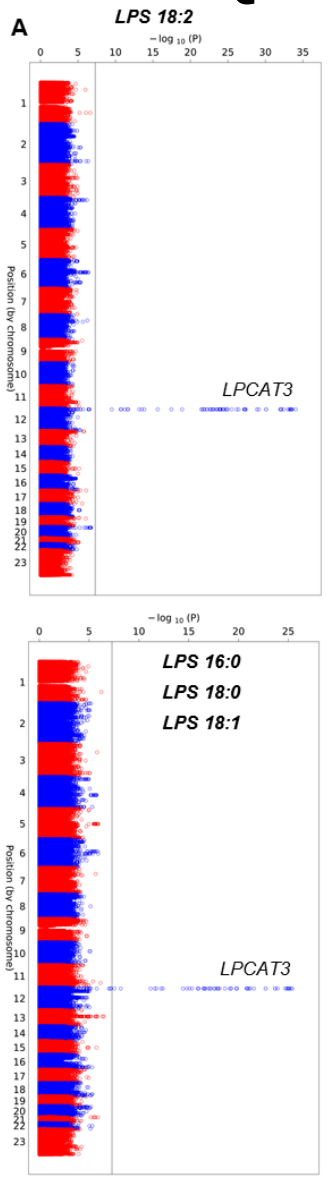


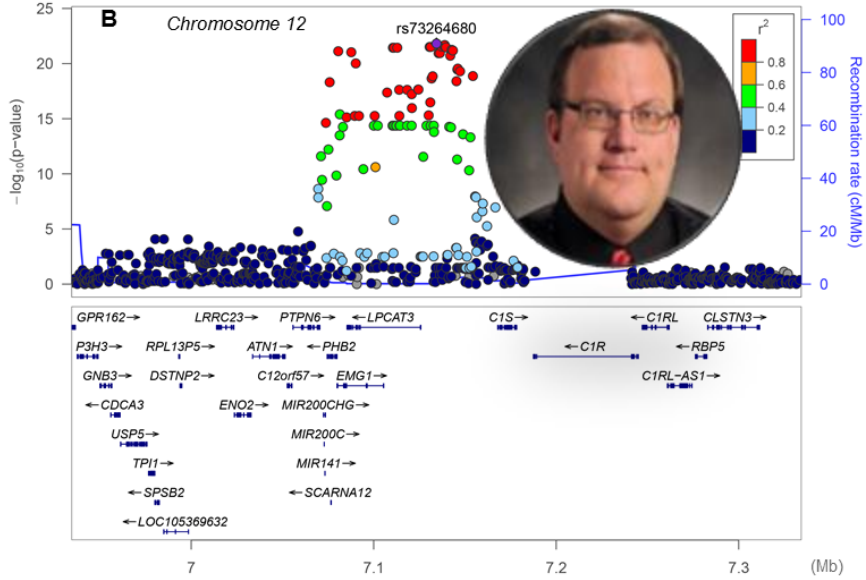


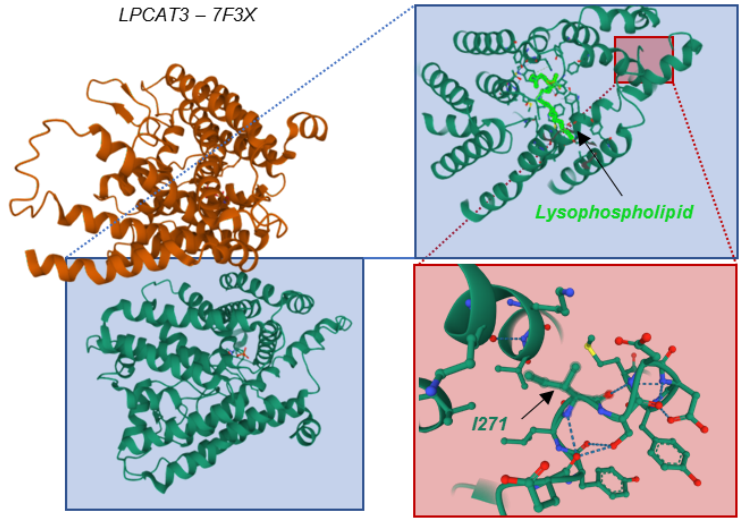
Nemkov et al. *Blood 2024a* and *2024b*

Metabolite heterogeneity encrypted in our DNA: mQTL

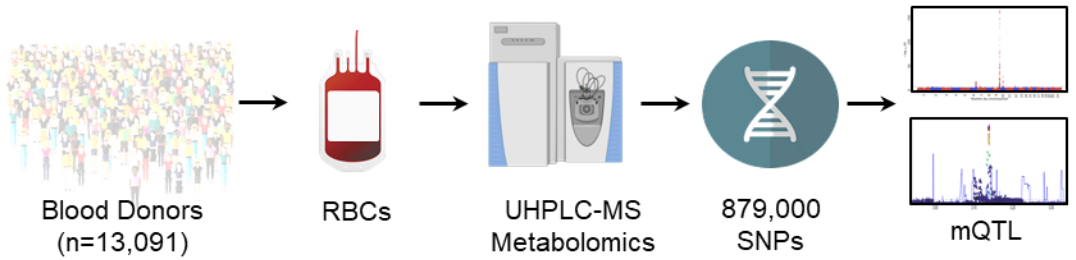


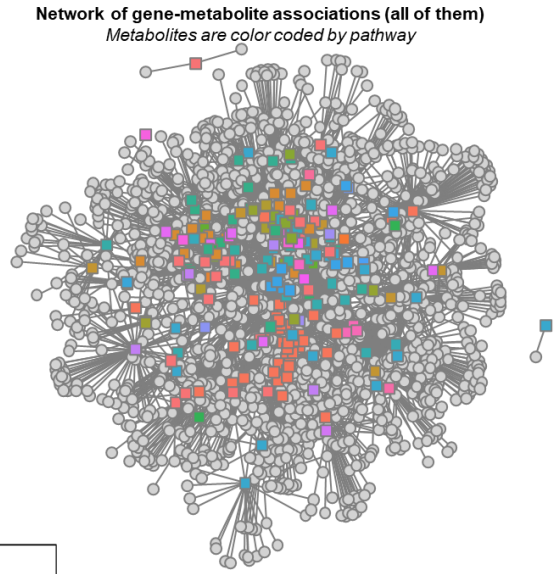


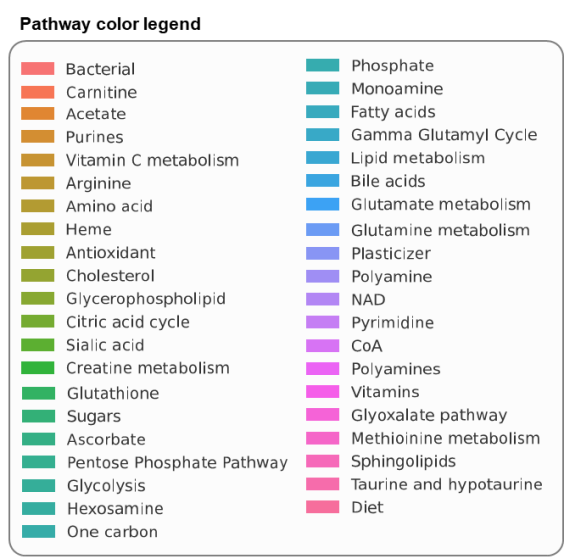


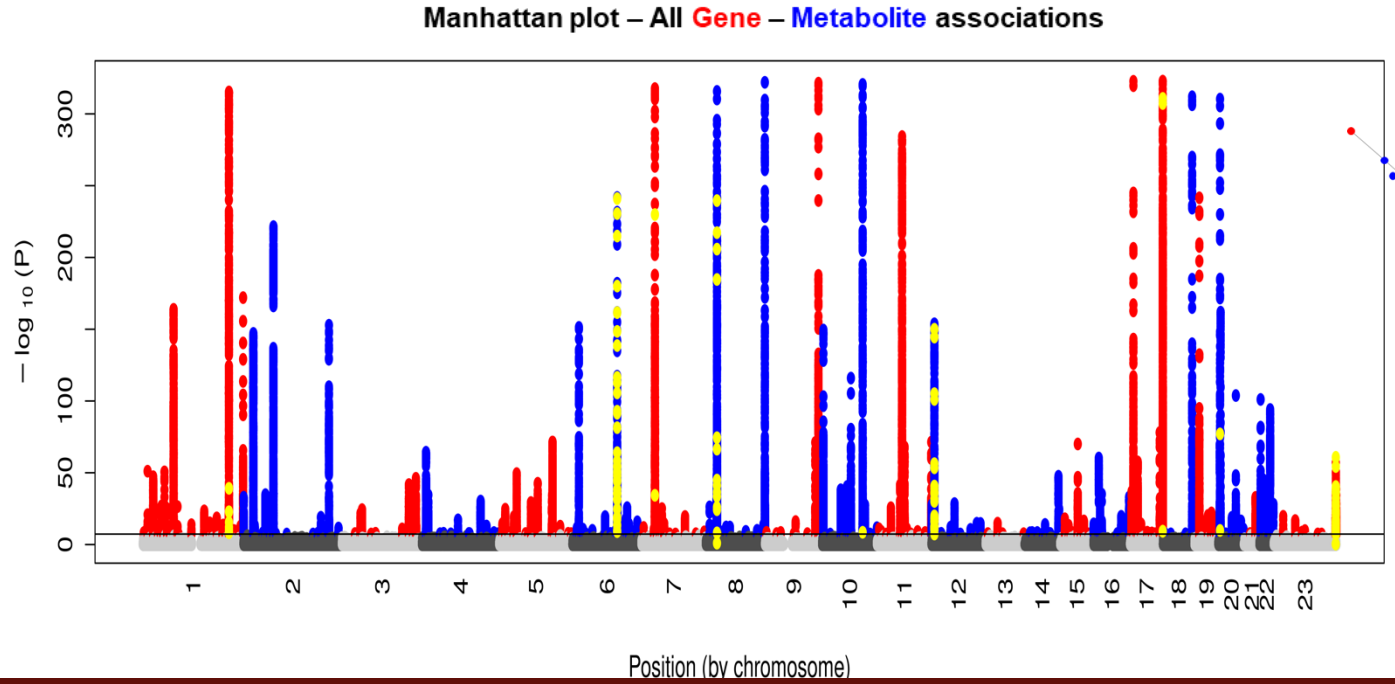


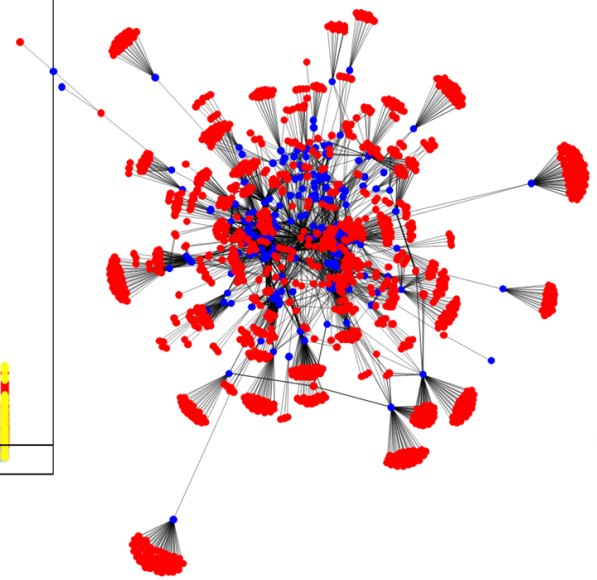
Genetic underpinnings of metabolite levels in donated blood
>1 Million gene-metabolite associations

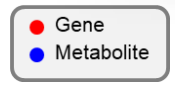






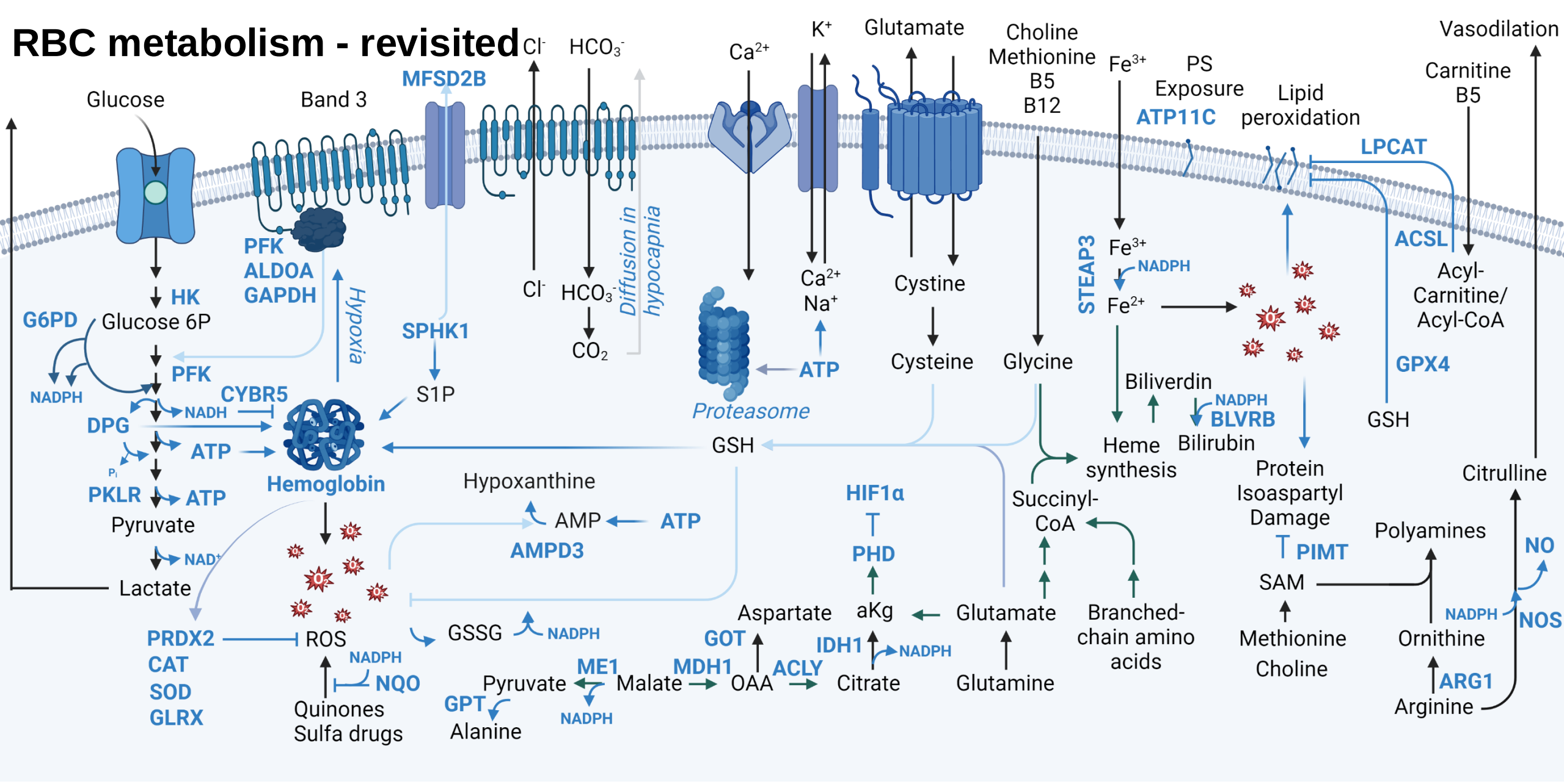




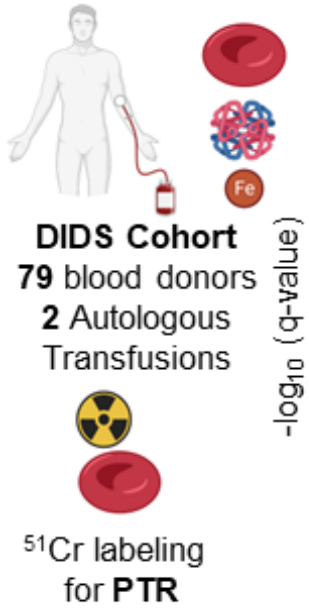


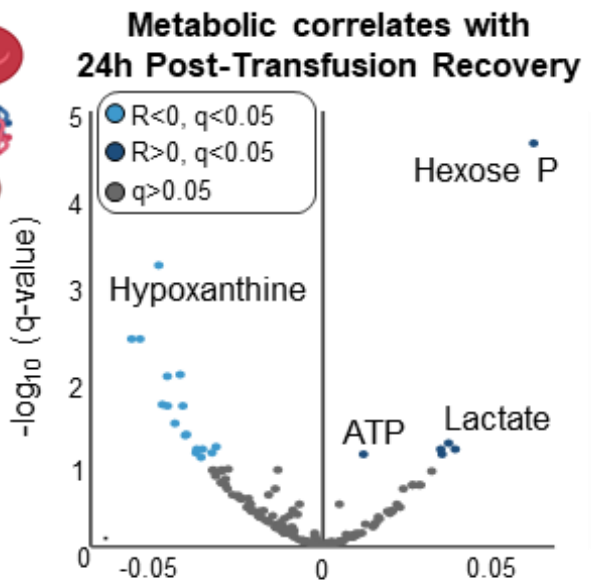
Earley et al. *In preparation;* Moore et al. *J Biol Chem 2022*

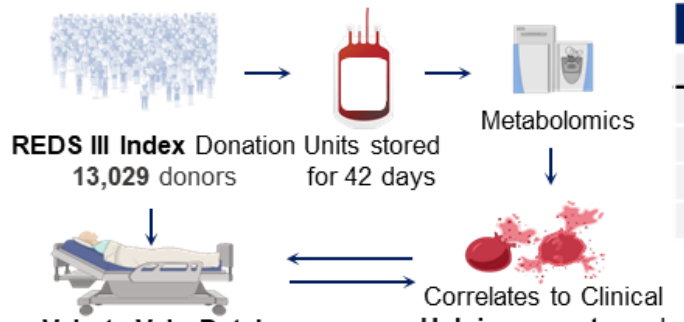
RBC metabolism - revisited

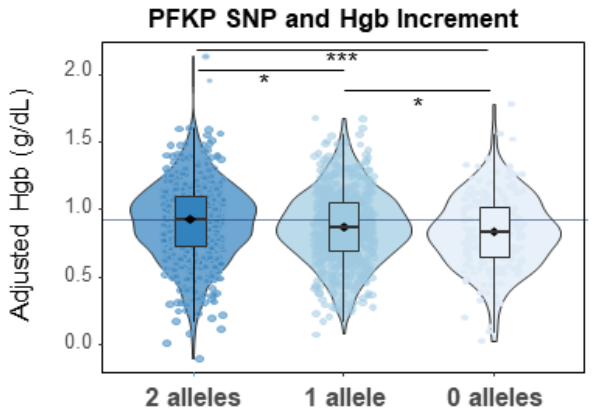


ATP and Hypoxanthine correlate with PTR and hemoglobin increments in vivo in healthy autologous or ill heterologous recipients

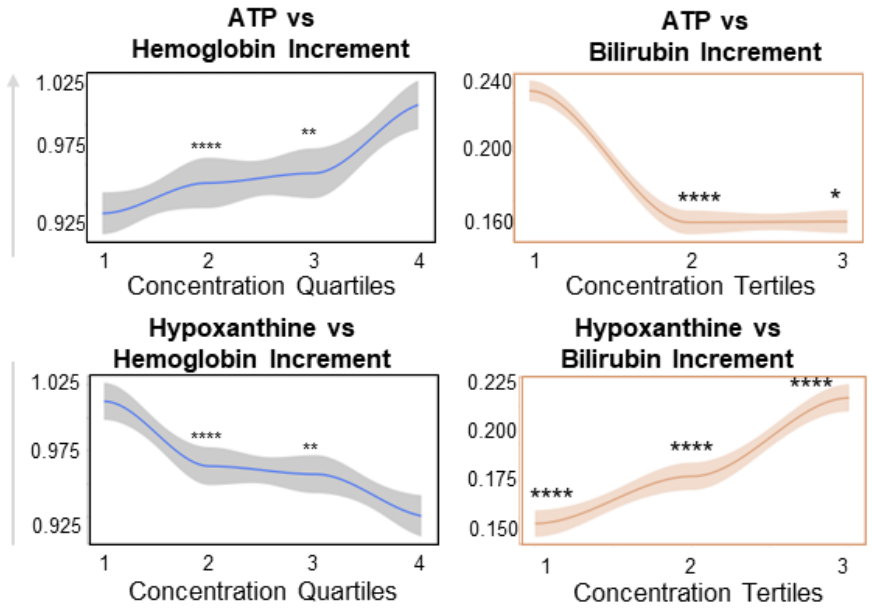






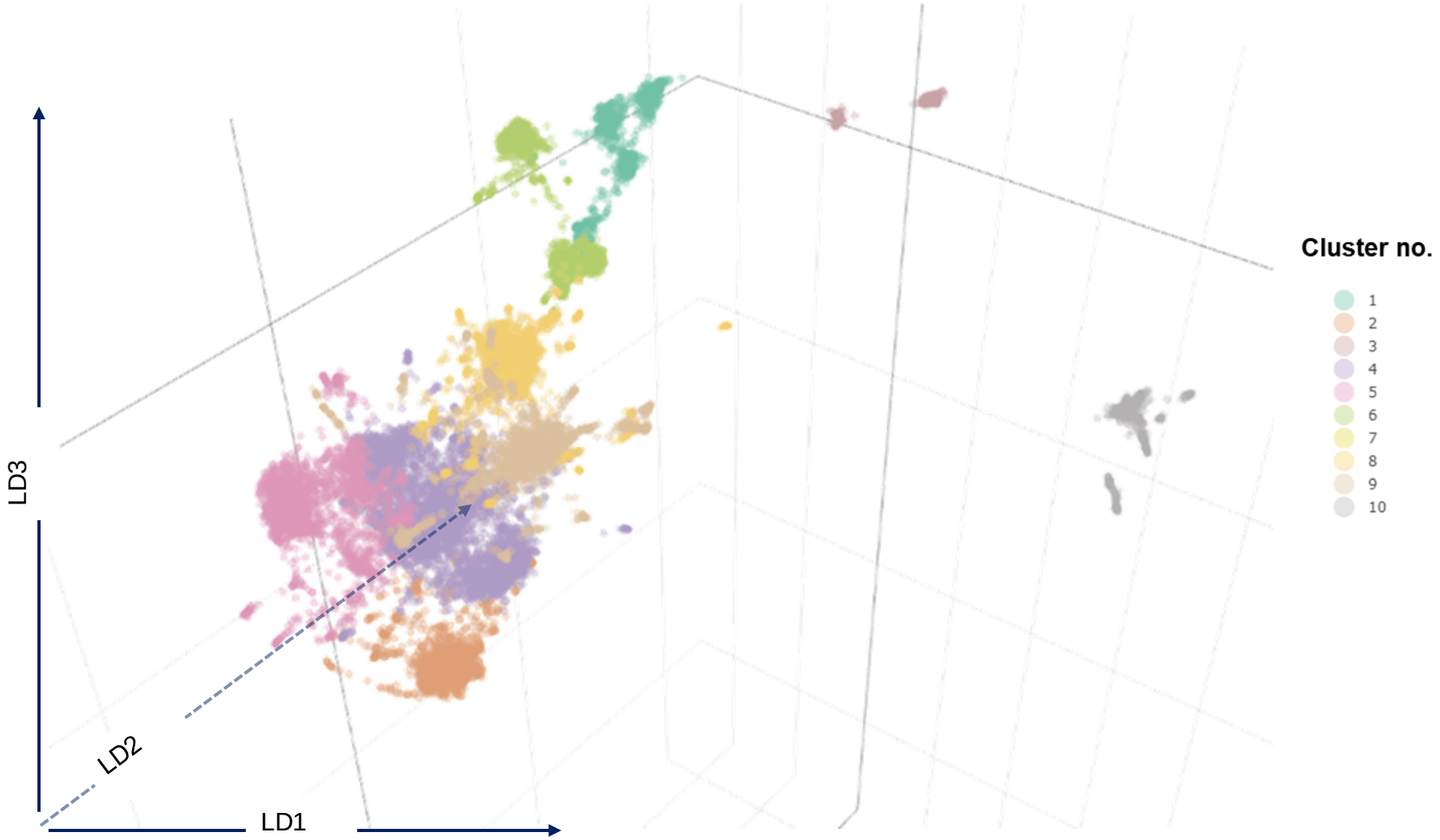


Change in hemoglobin increment after RBC transfusion for ATP and Hypoxanthine quartiles				
Values (g/dL)	1st quartile	2nd quartile	3rd quartile	4th quartile
Hypoxanthine	1.02 [0.9-1.06]	0.98 [0.94-1.02]	0.97 [0.93-1.01]	0.93 [0.89-0.97]
Adjusted	1.01 (0.28)	0.96 (0.28)	0.95 (0.29)	0.92 (0.29)
ATP	0.96 [0.92-1.00]	0.98 [0.94-1.03]	0.97 [0.93-1.02]	1.02 [0.98-1.07]
Adjusted	0.94 (0.30)	0.96 (0.29)	0.97 (0.28)	1.01 (0.28)

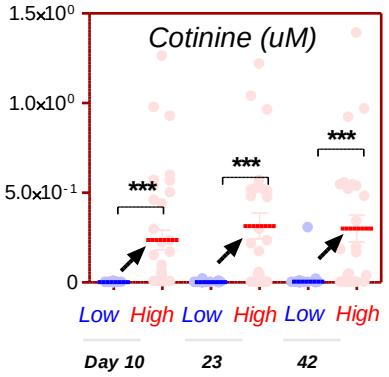


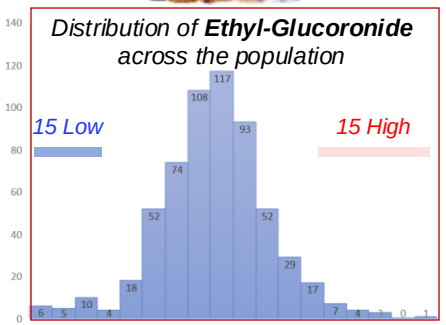
Nemkov et al. *Cell Metabolism in press*

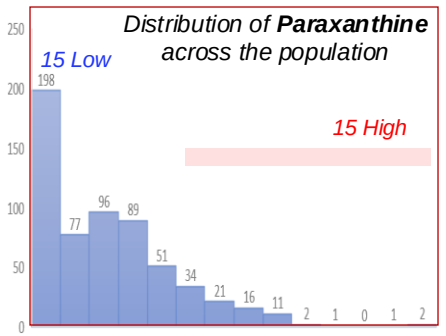
There’s more that defines a donor than just storage, genetics and demographics

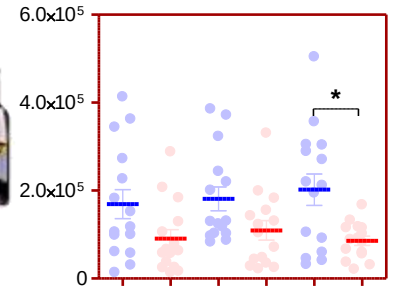


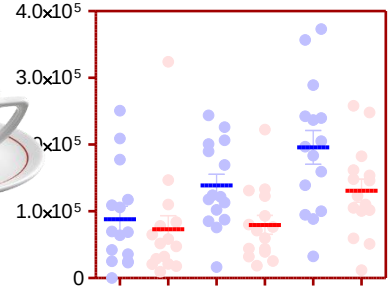
Donor habits matter!!! Smoking, drinking (alcohol or coffee), diet!

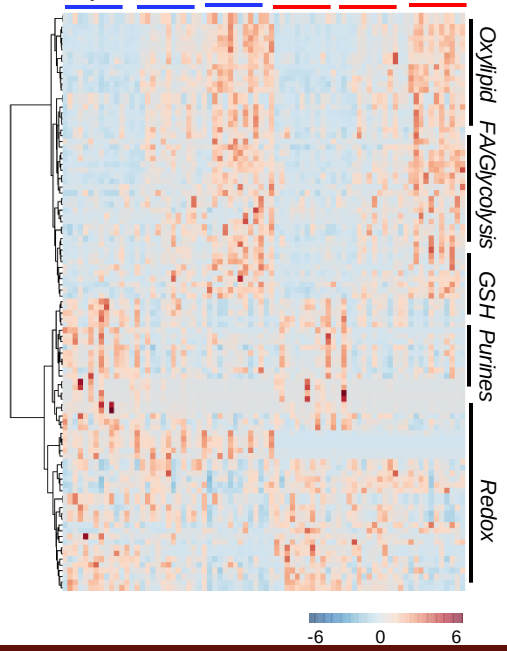


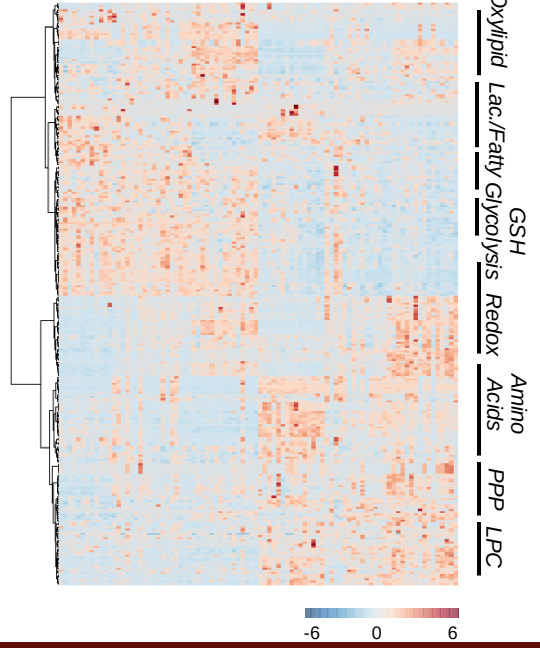


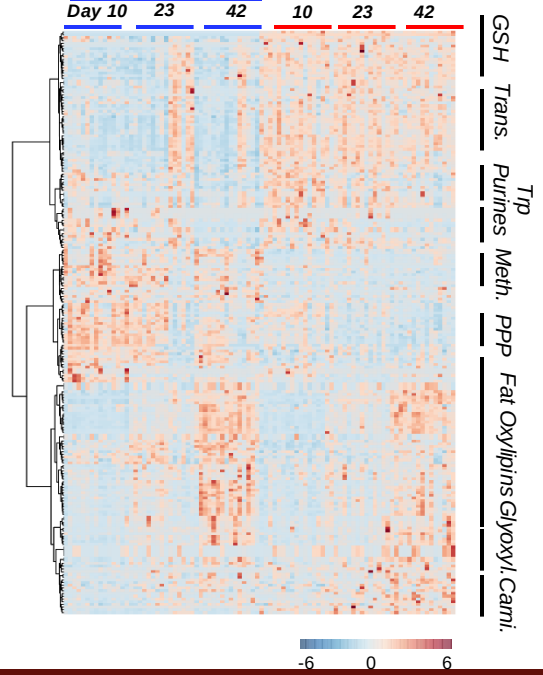






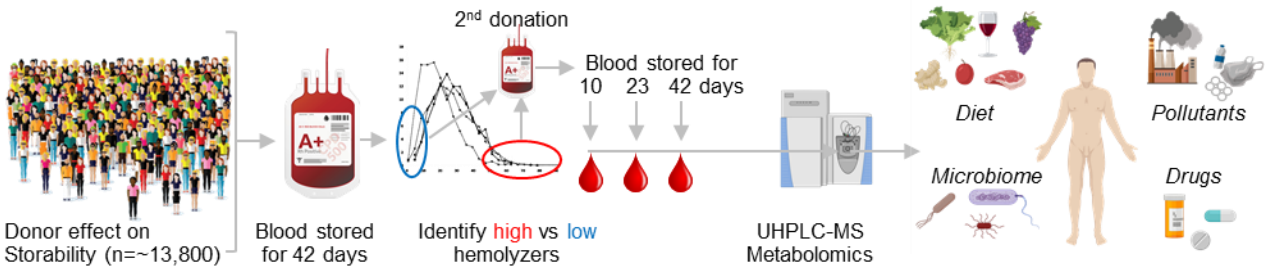


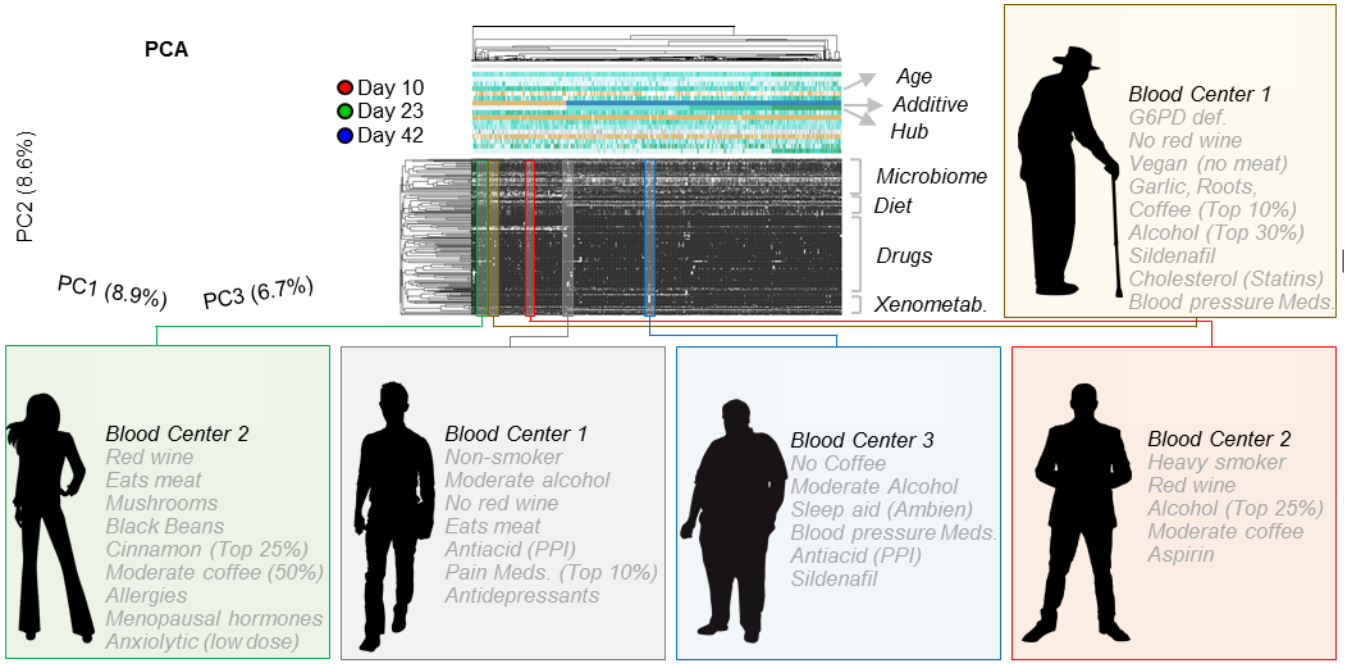


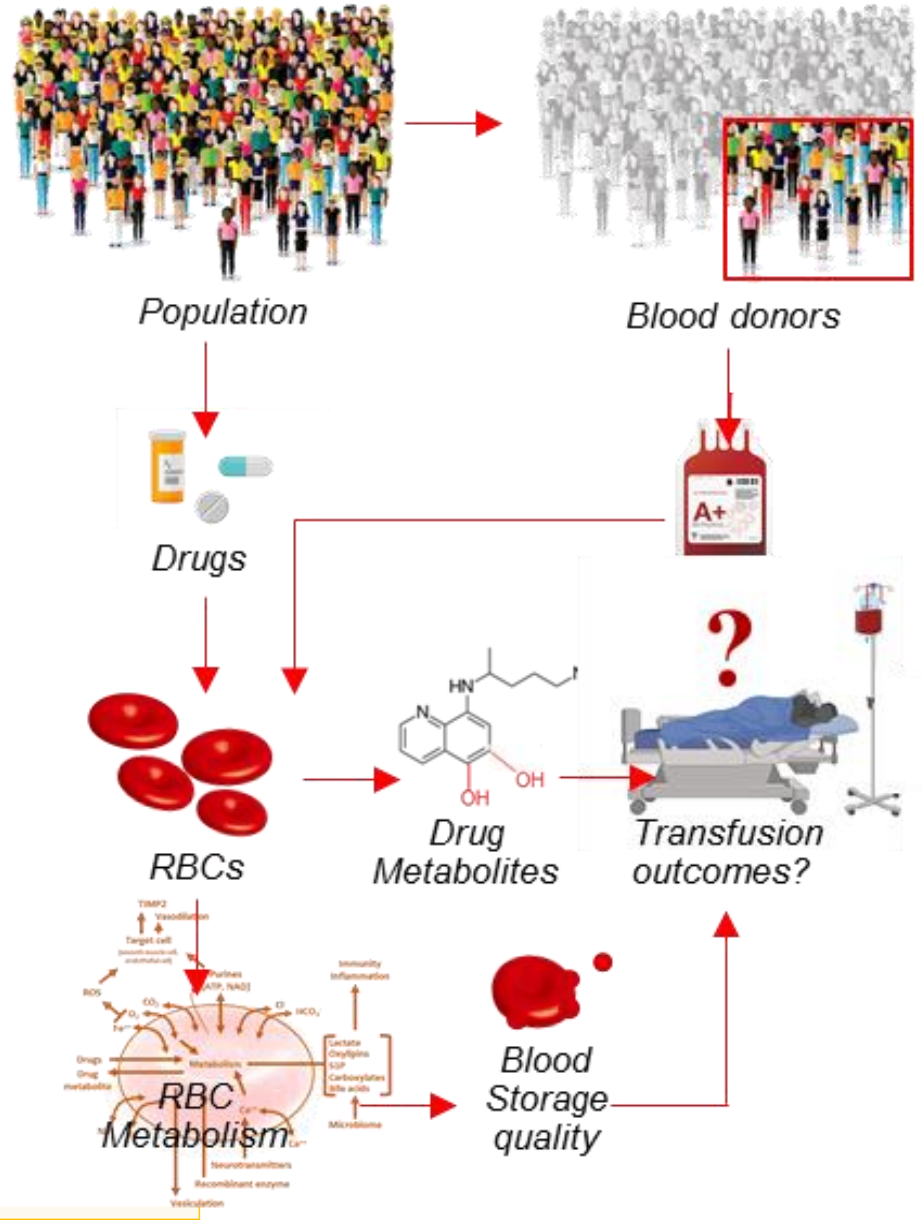


D'Alessandro et al. Transfusion 2020a, b; Stefanoni et al. Transfusion 2020

DONOR EXPOSOME: a PANDORA's box

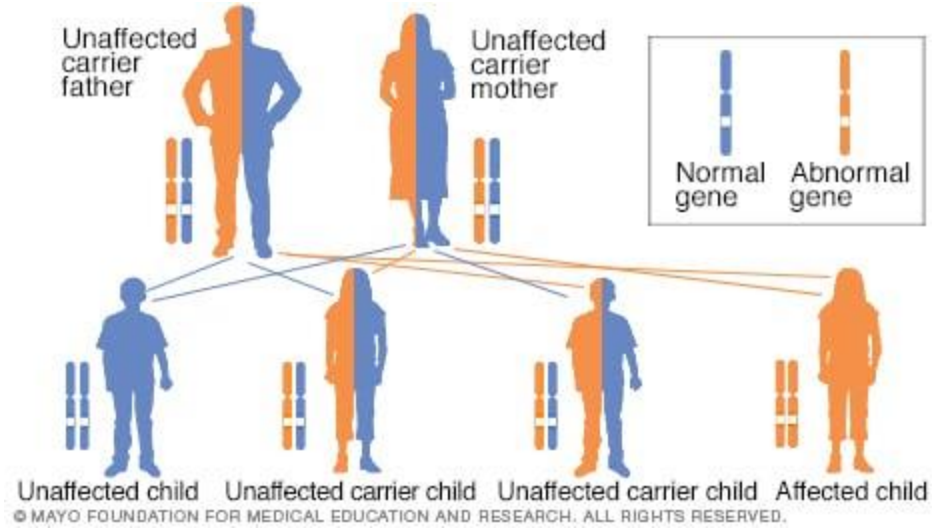


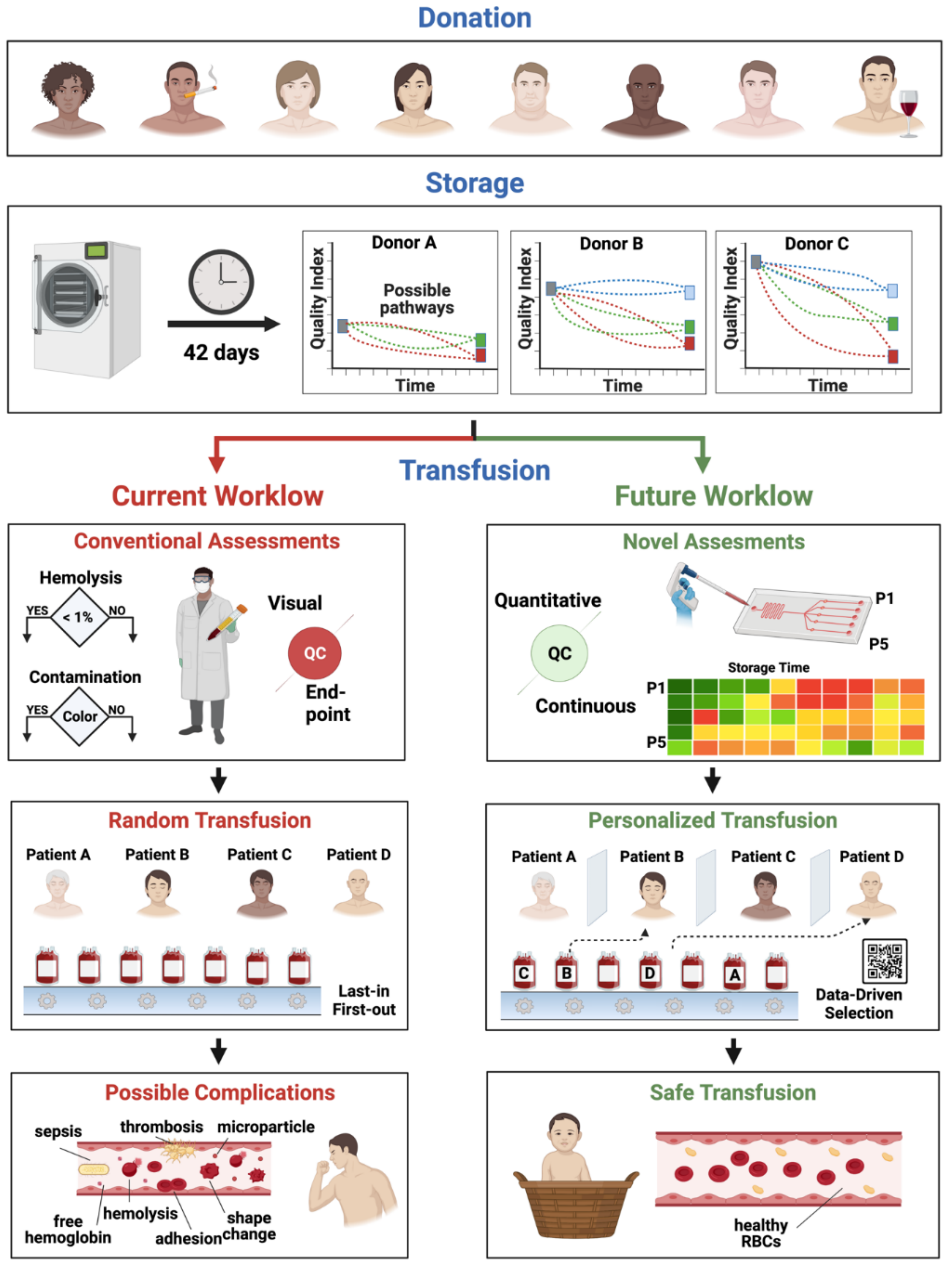




Nemkov et al. JCI Insight 2021

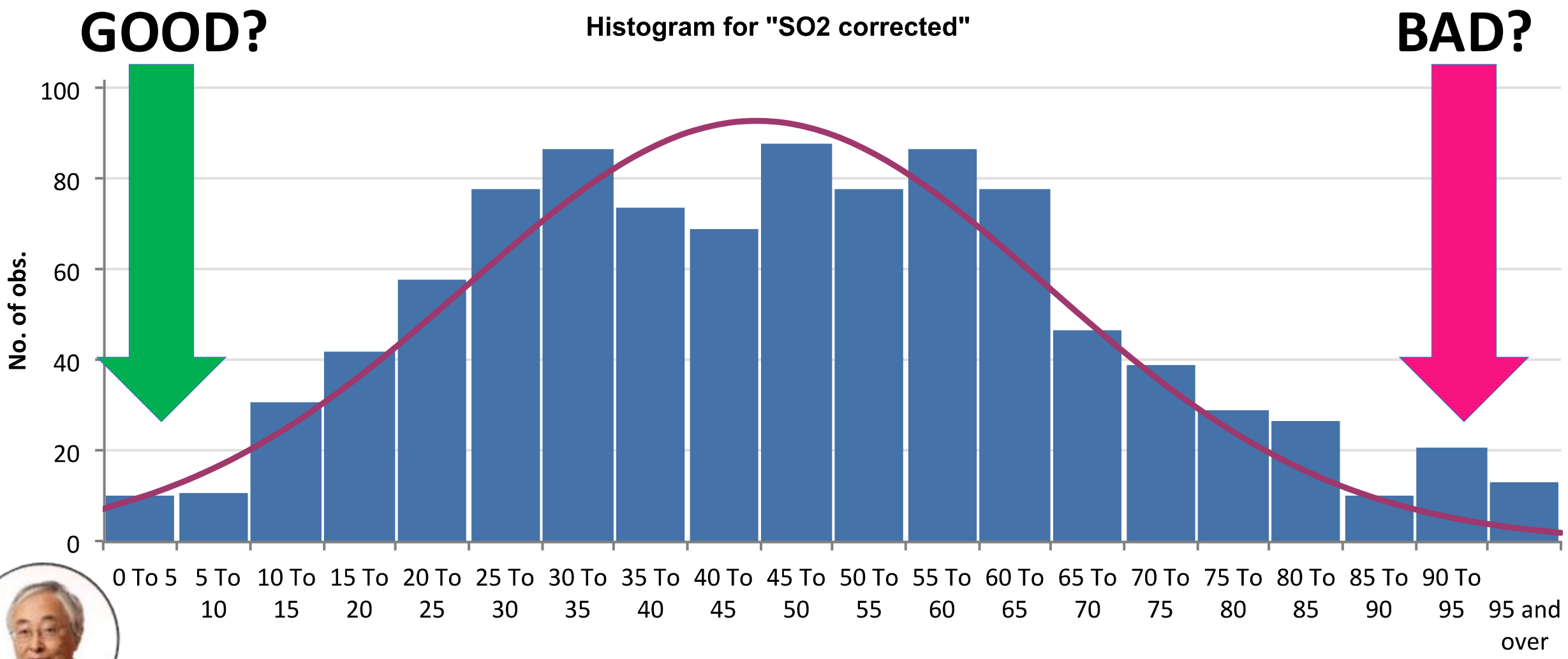
Chemical Individuality: from Molecular Epidemiology to Precision Transfusion Medicine





Sir Archibald Garrod, 1908; Isiksacan, D'Alessandro et al. PNAS 2023

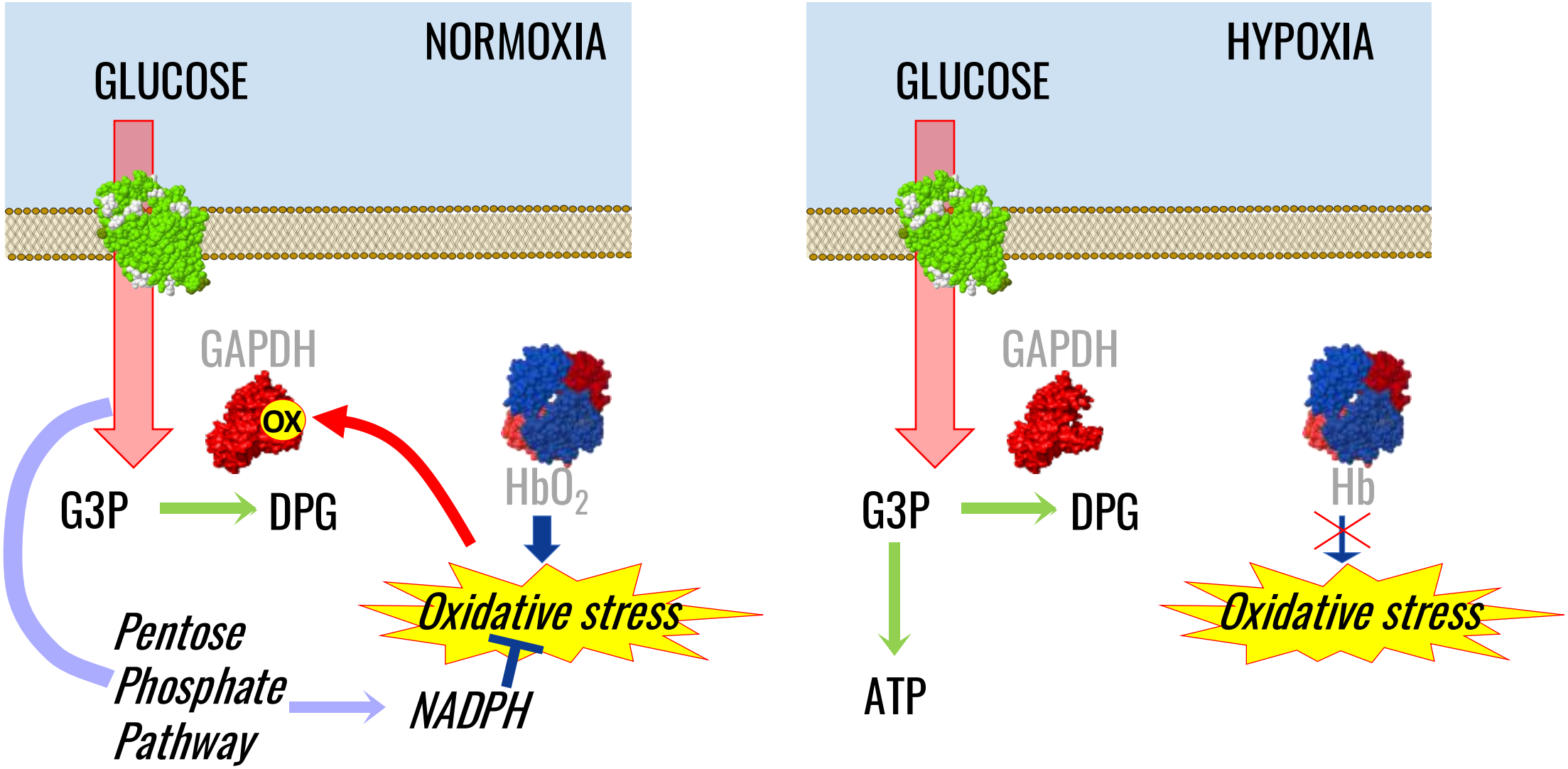
From energy to redox: stored RBCs are challenged with oxidative stress



N=997 - Data kindly provided by Dr. Tatsuro Yoshida, *New Health Sciences inc*

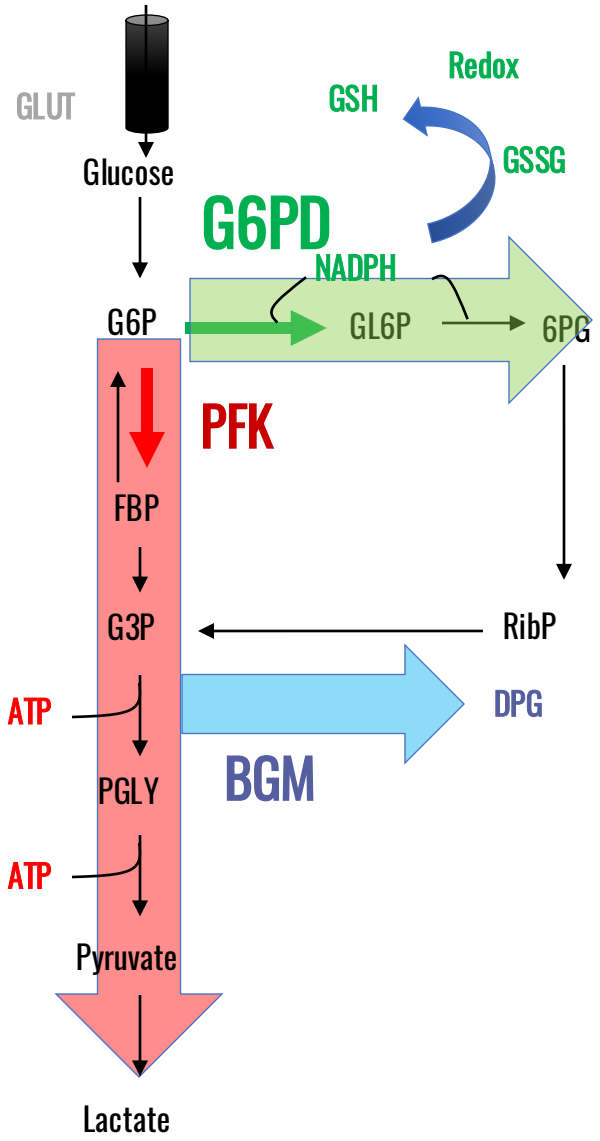
Nemkov et al. Haematologica 2018

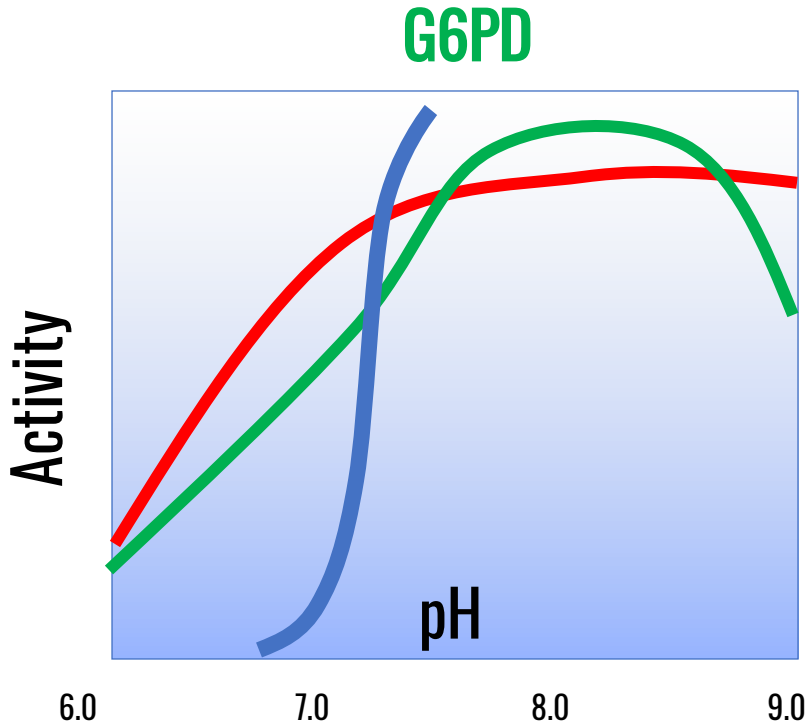
Hypothesis: anaerobic storage should promote glycolysis by decreasing oxidative stress



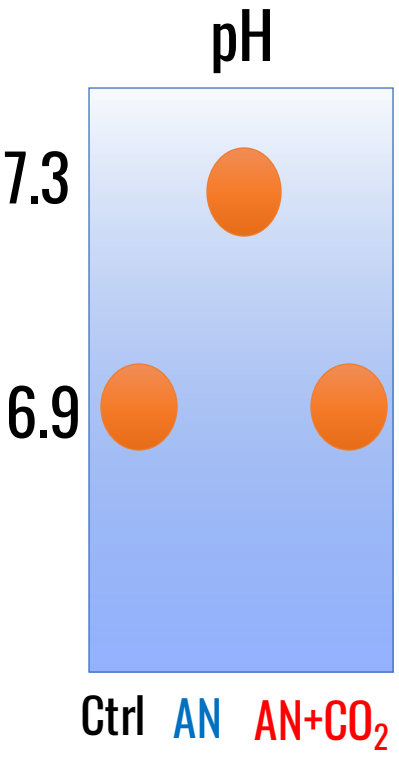
Castagnola et al Blood Transfusion 2010; Rogers et al 2012; Low et al 1992

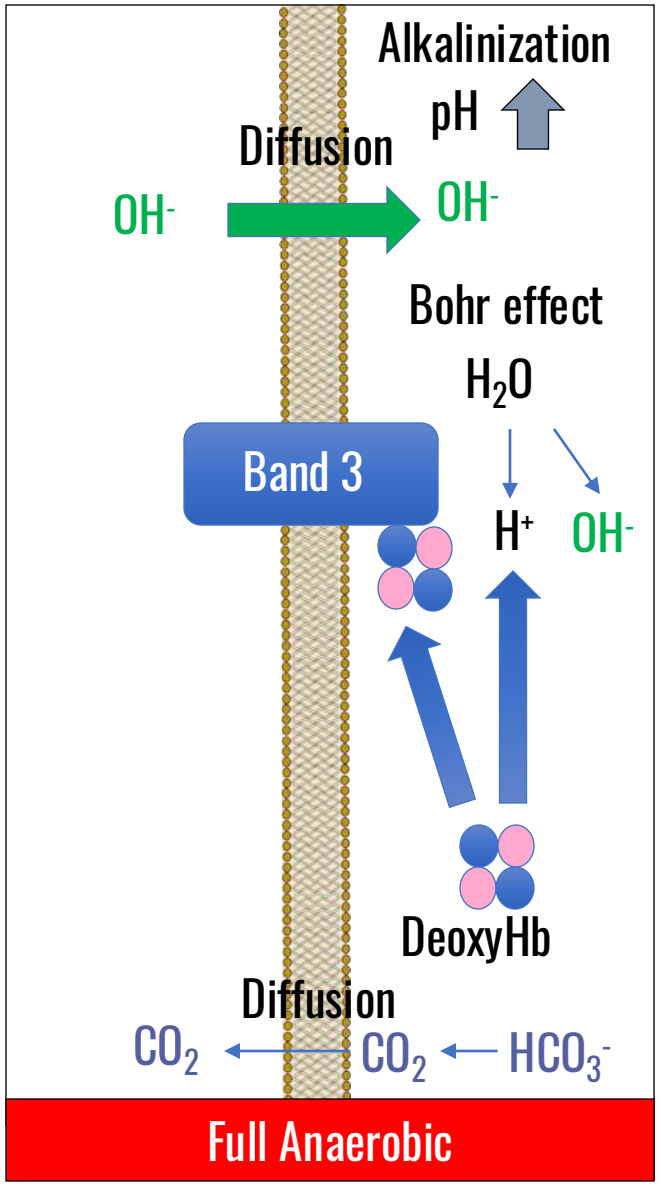
Biochemical rational beyond removing O2: intracellular alkalinization favors glycolysis, PPP and Rapoport-Luebering

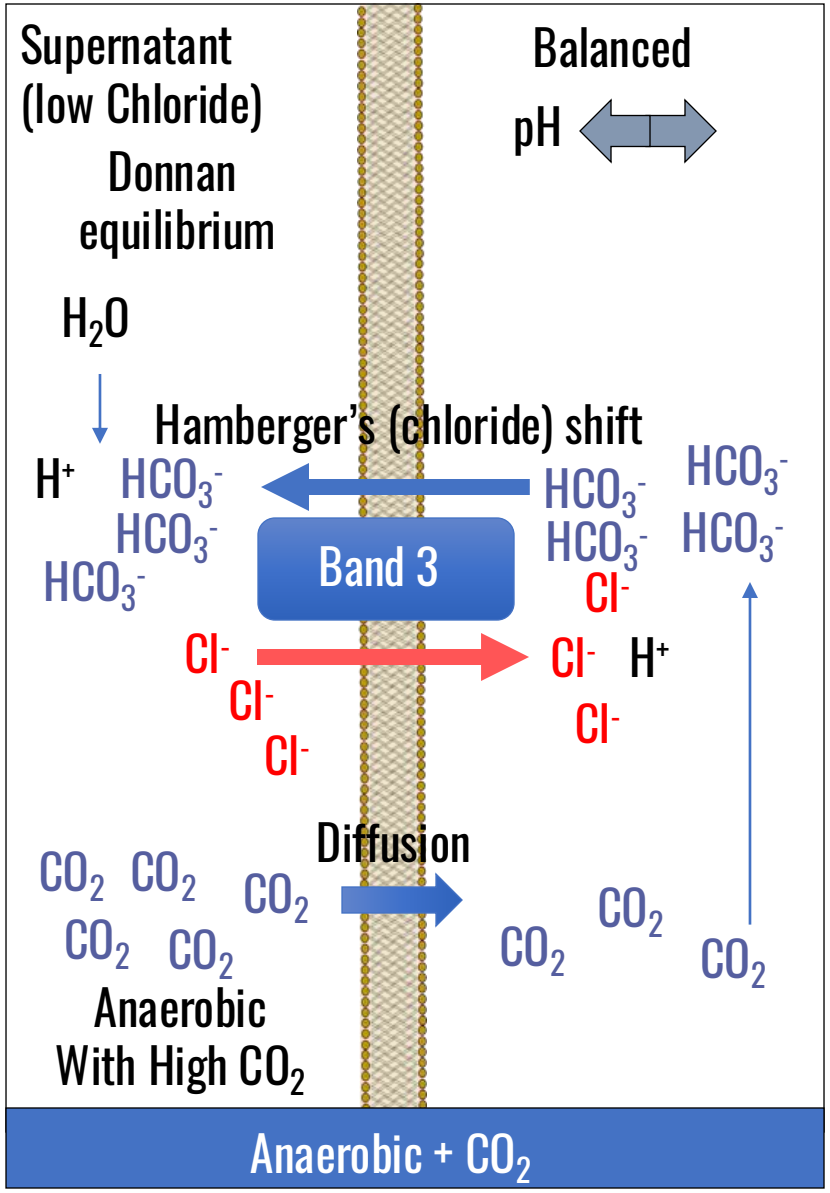




Anerobic storage: are anaerobiosis benefits pH dependent?
Anaerobic storage + 5% CO2



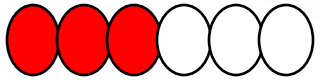




Dumont et al. 2015; Yoshida et al., Transfusion 2007 and 2008; Sparrow, Blood Transfusion 2012; D'Alessandro et al, Mol Biosyst 2012

RBC storage: Hyperoxia promotes the storage lesion, hypoxia ameliorates it

$^{13}C_{1,2,3}$-GLUCOSE



Red Blood Cell *Unit*
(n = 4 per group)

Hyperoxia

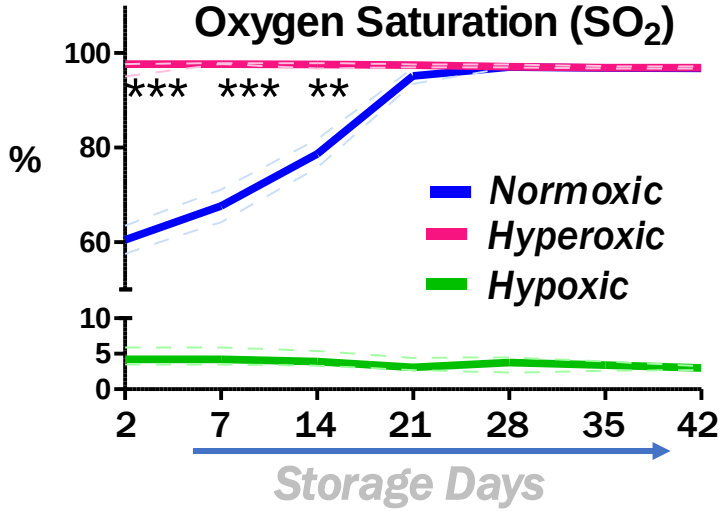
Control

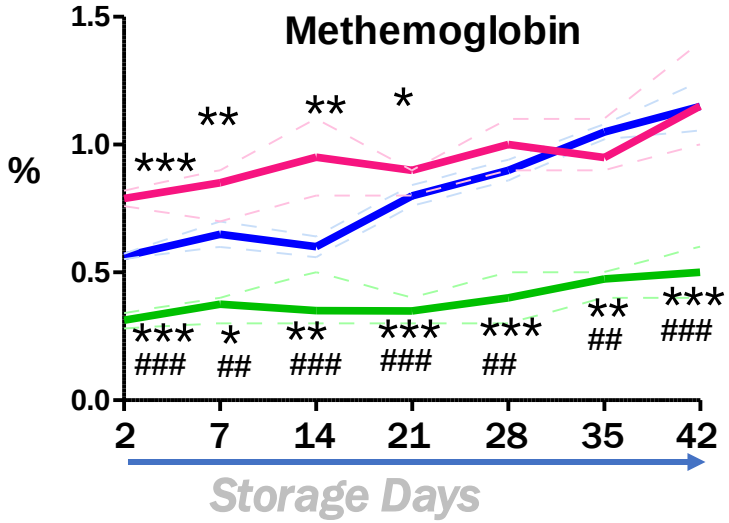
Hypoxia

METABOLOMICS

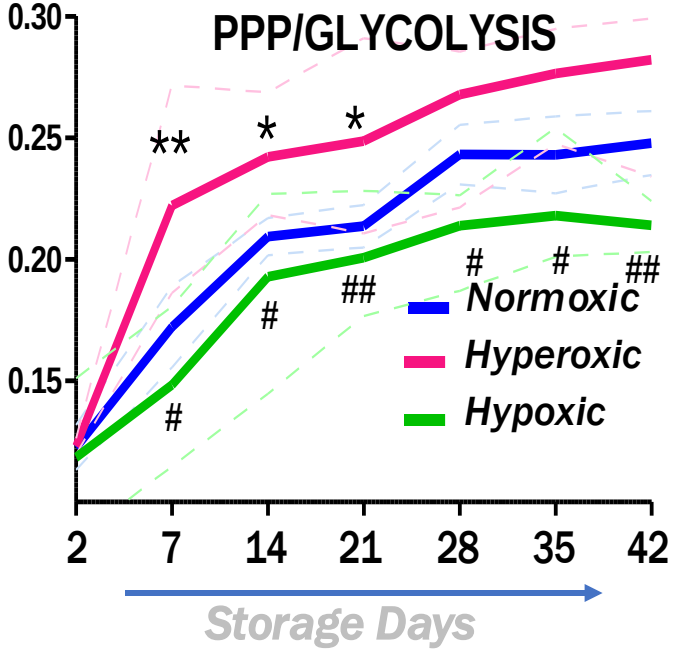
2 7 14 21 28 35 42

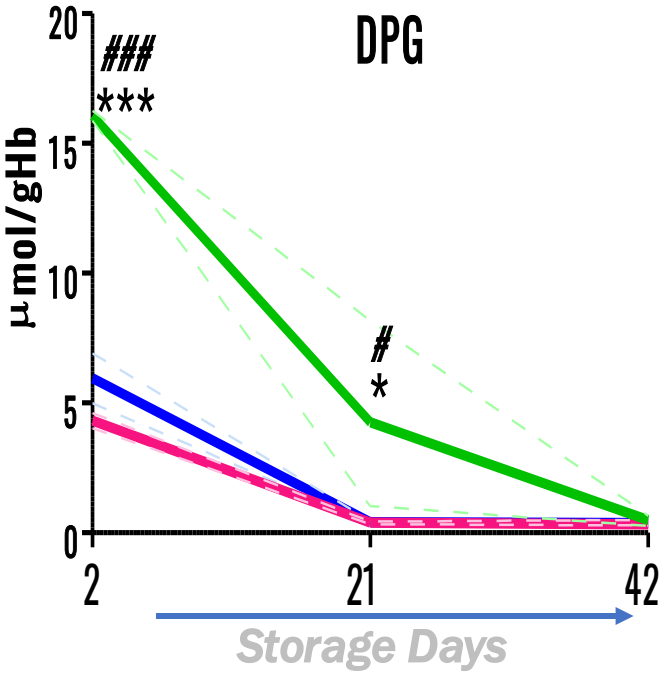
Storage day

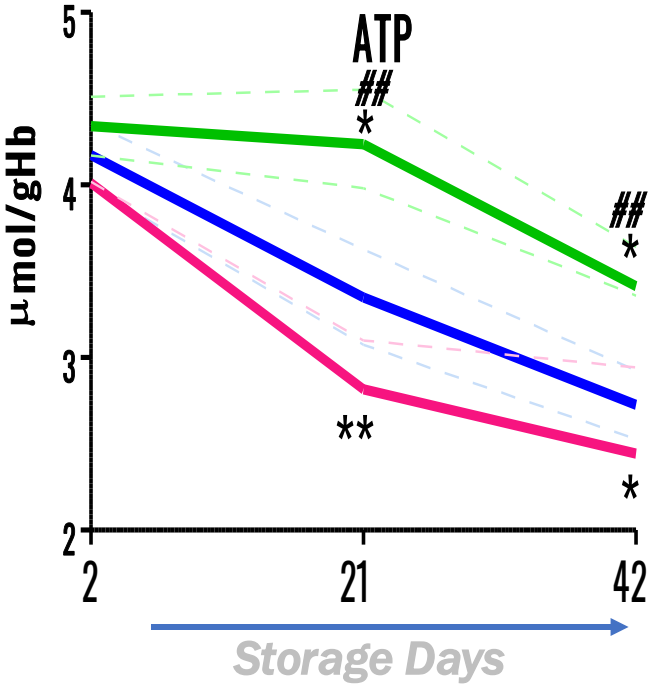




RBC storage: Hypoxia improves energy metabolism

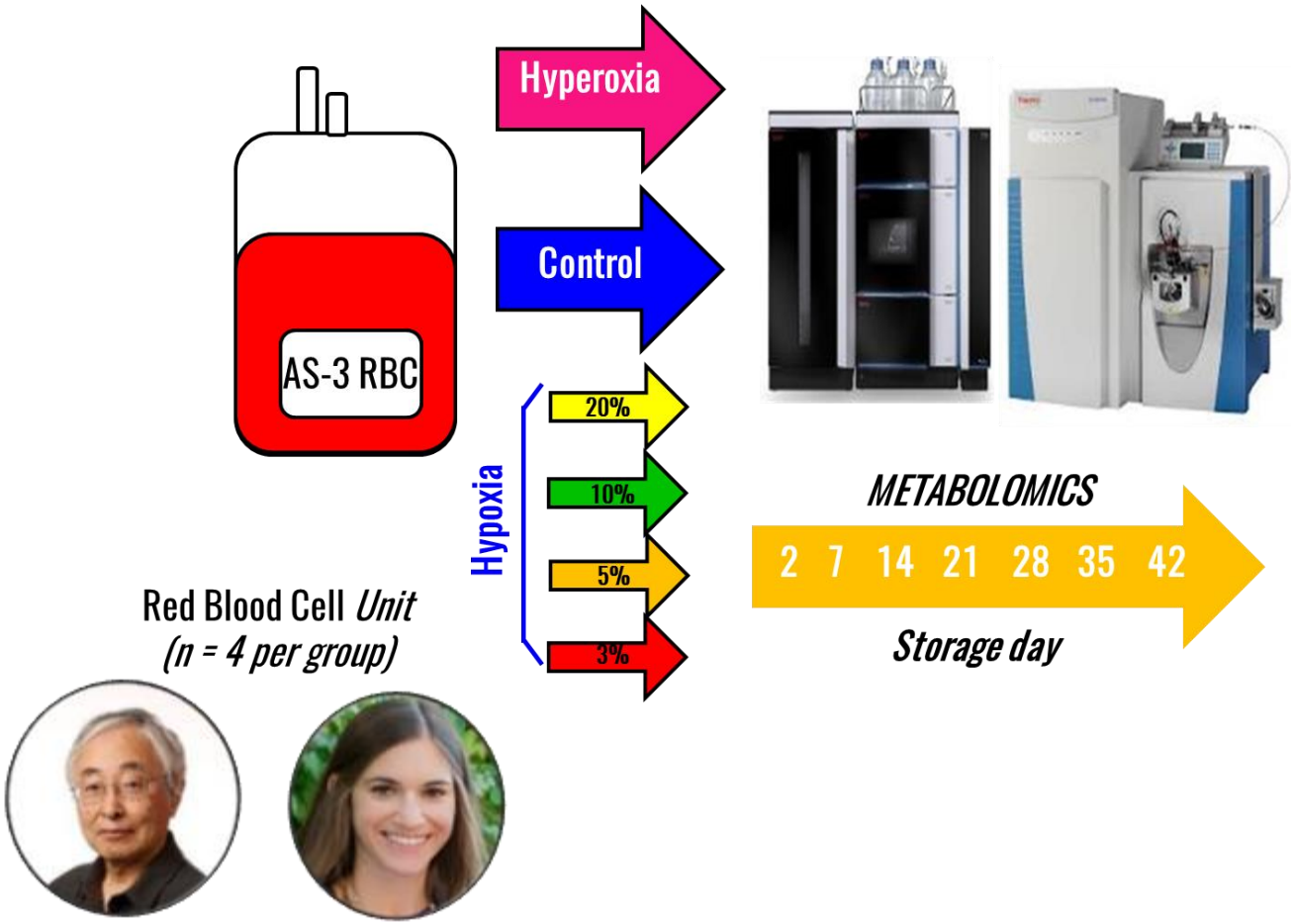


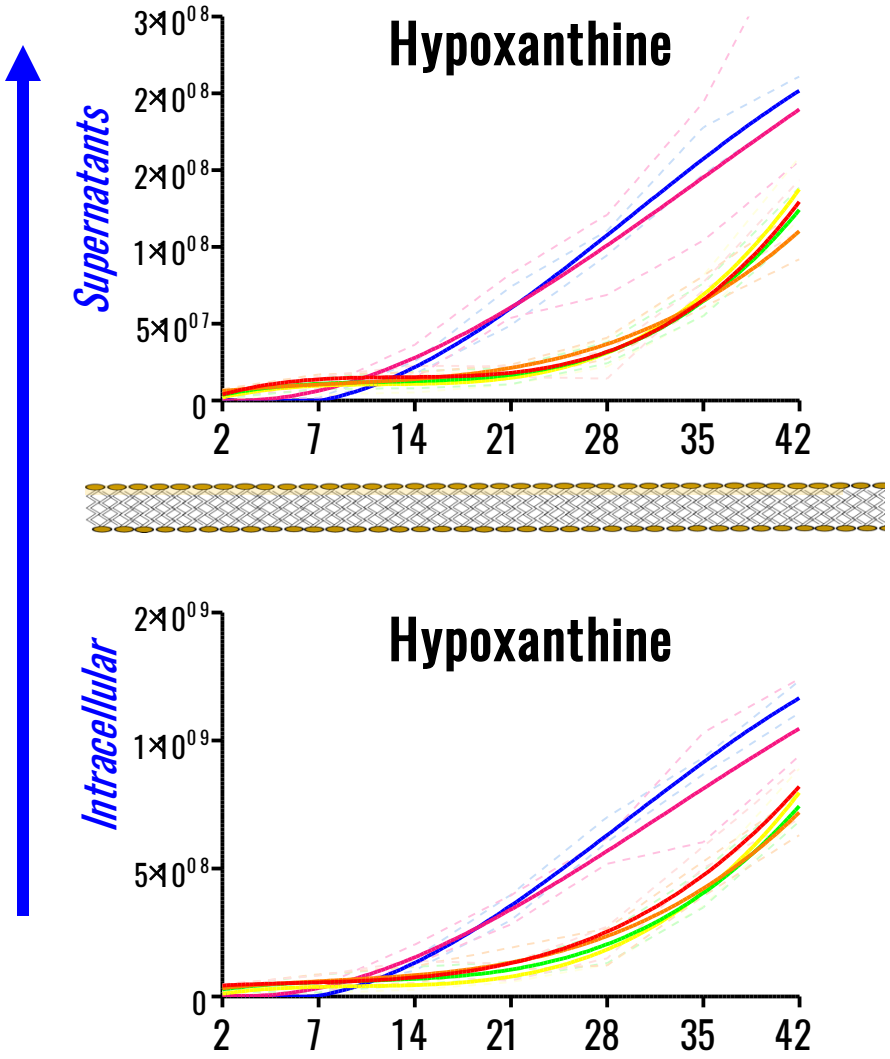




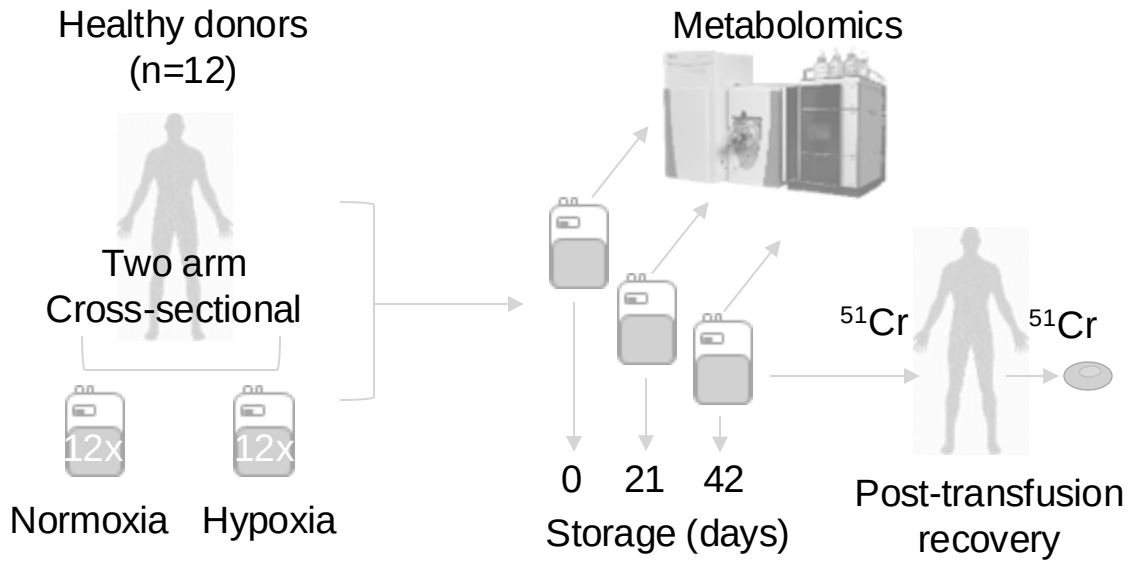
Reisz et al. Blood 2016

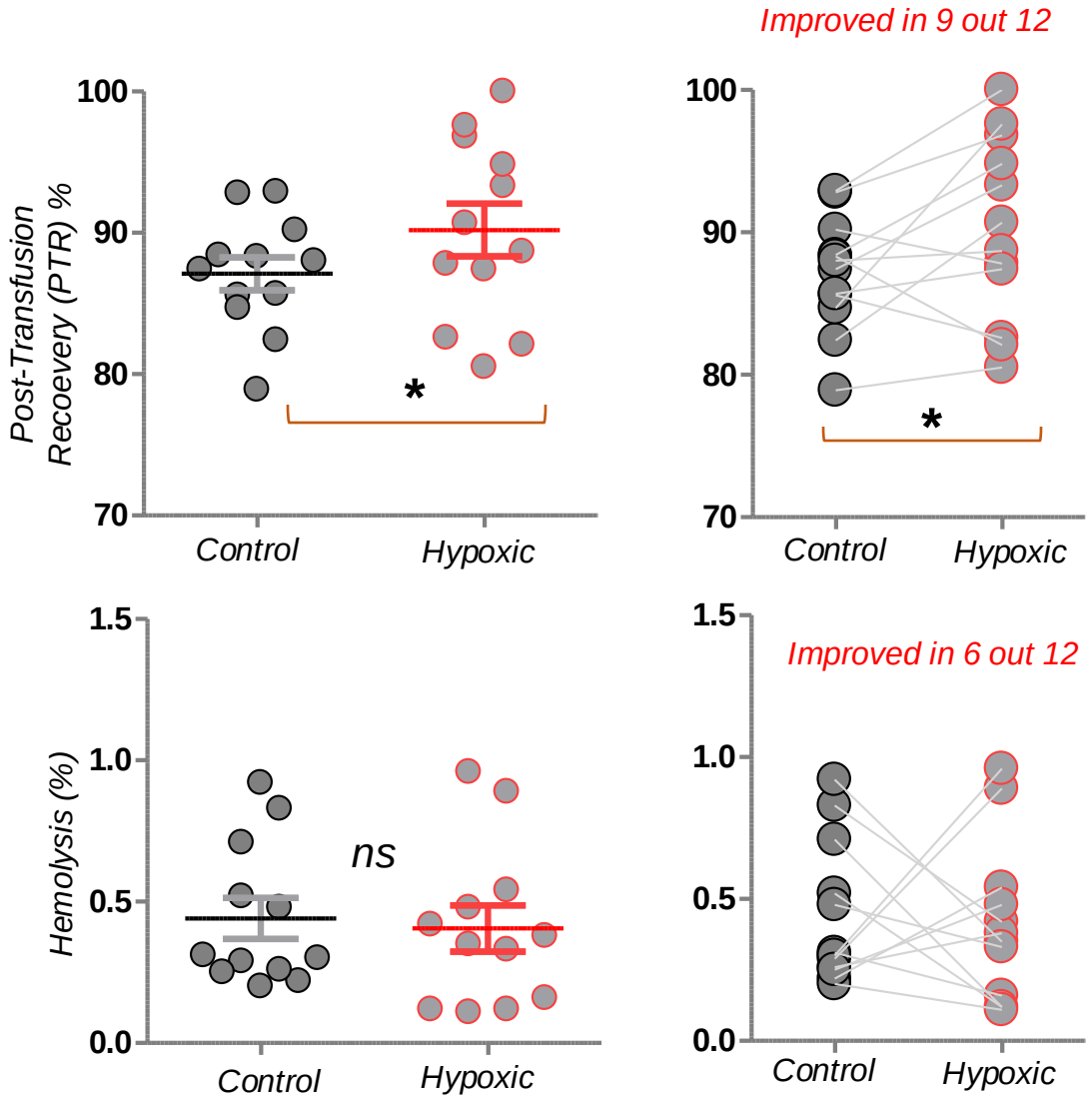
RBC storage: Hypoxia improves energy metabolism and prevents accumulation of hypoxanthine



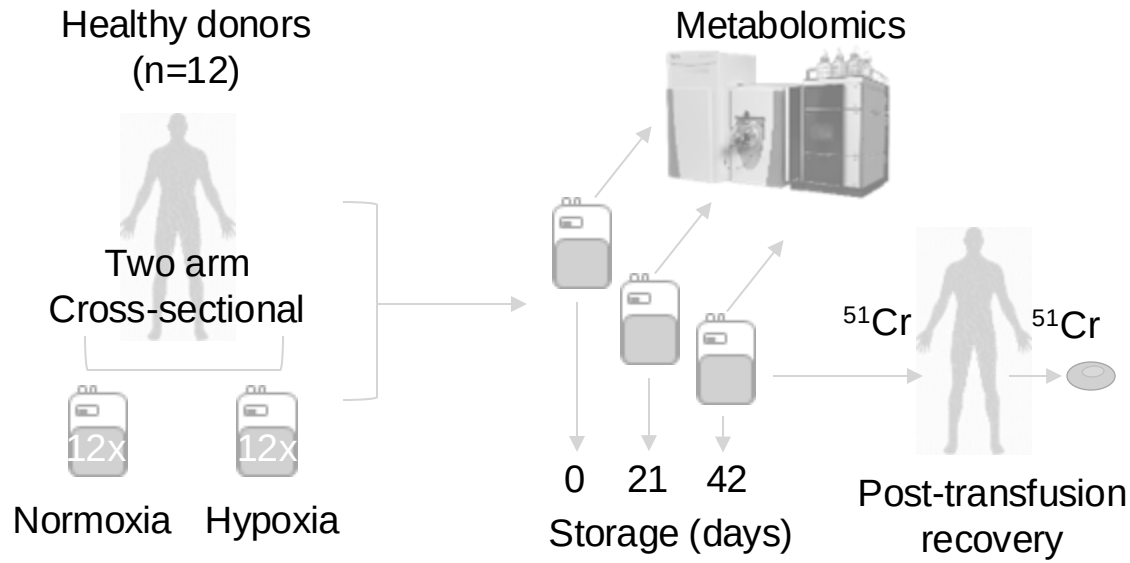


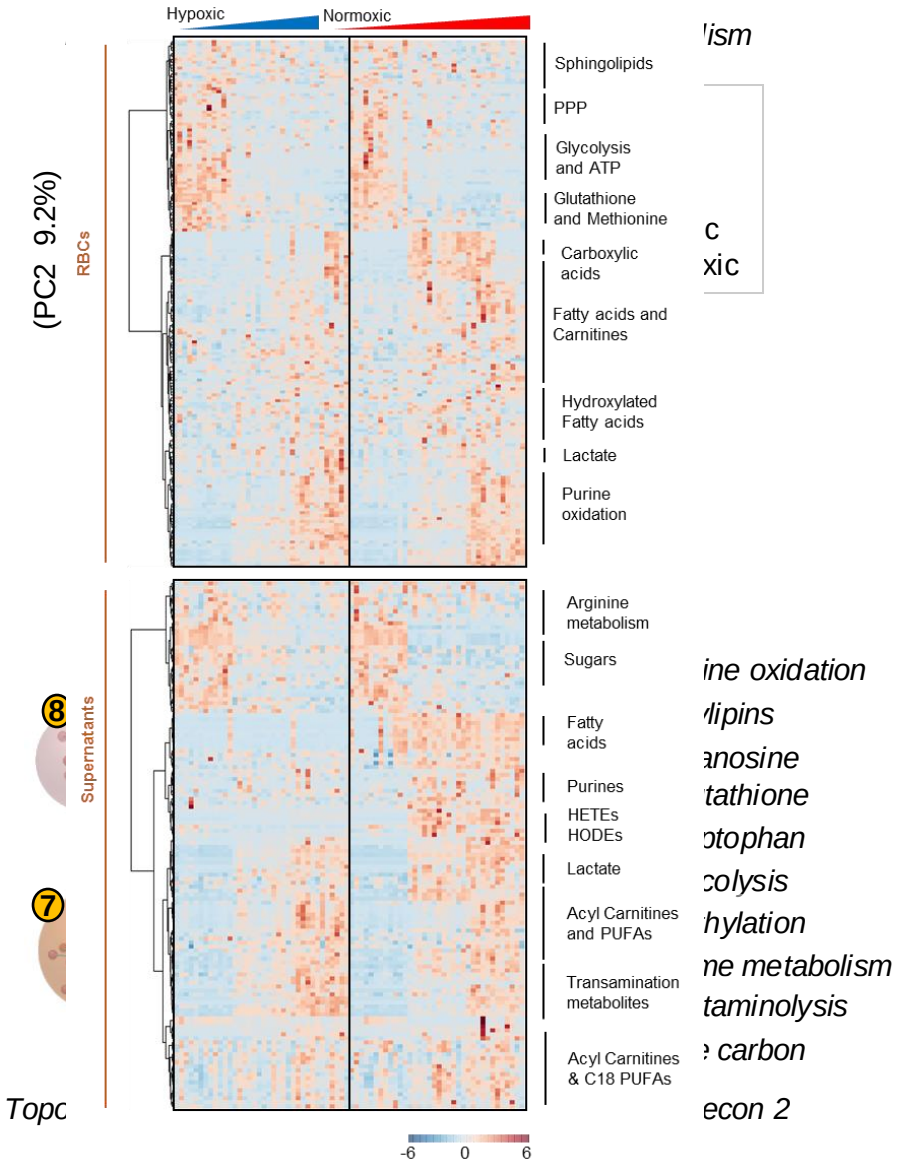
Two-arm cross sectional study: Hypoxia improves PTR





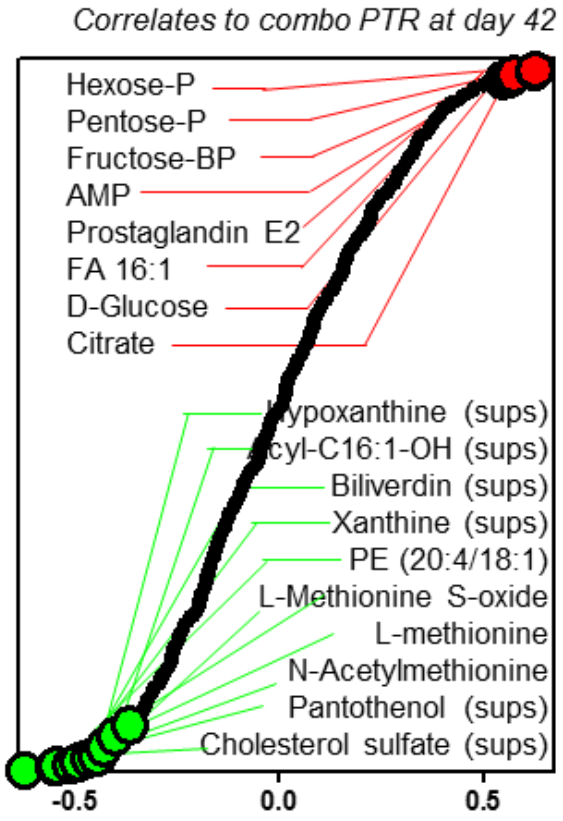
Hypoxia improves metabolism other than PTR

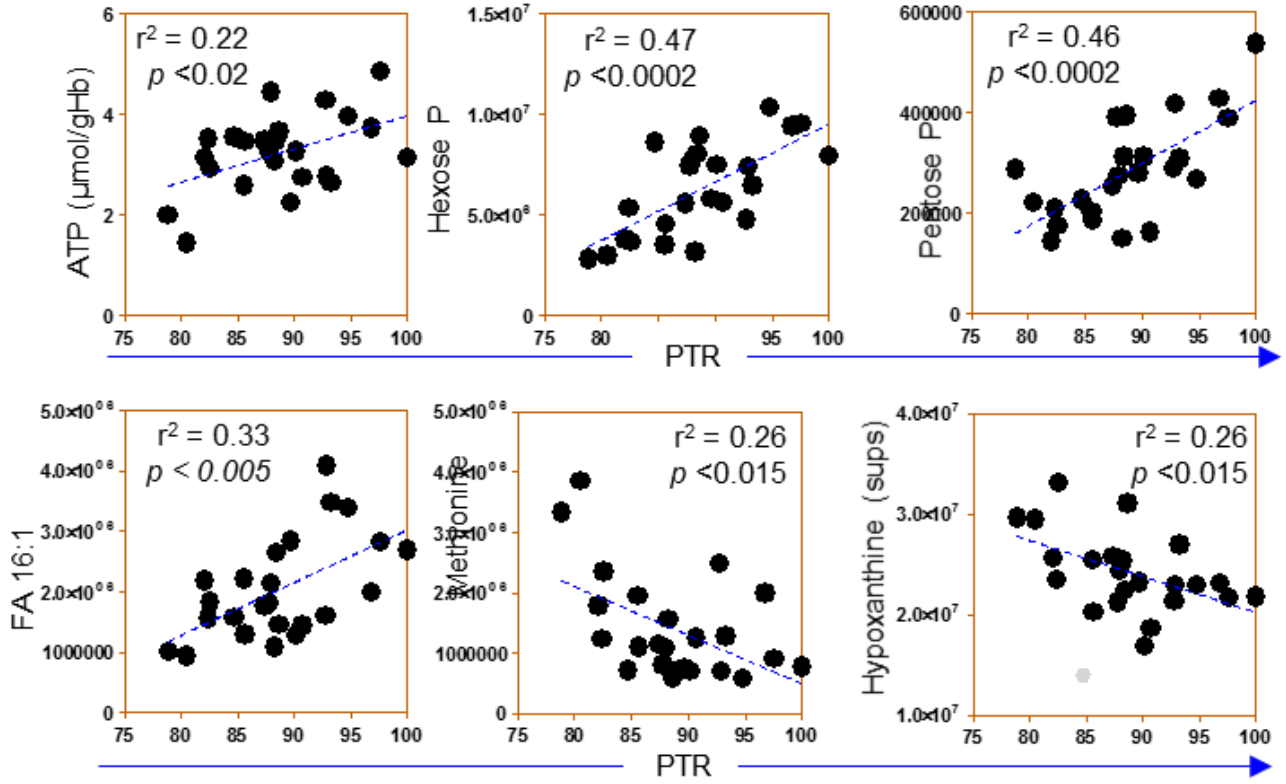




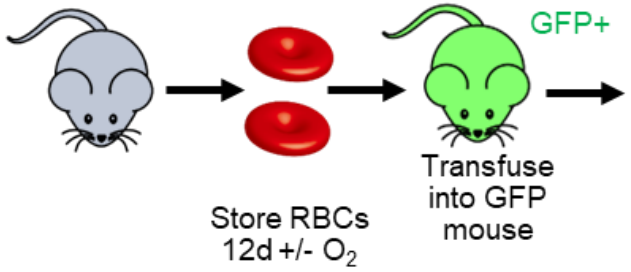
Metabolic correlates to PTR: Glycolysis and PPP

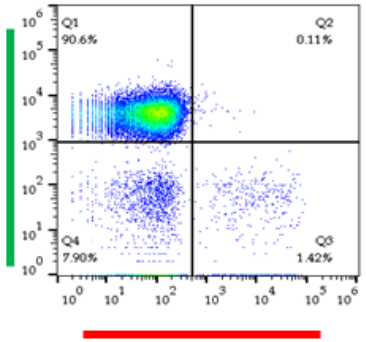
Tatsuro Yoshida, PhD
Hemanext Inc

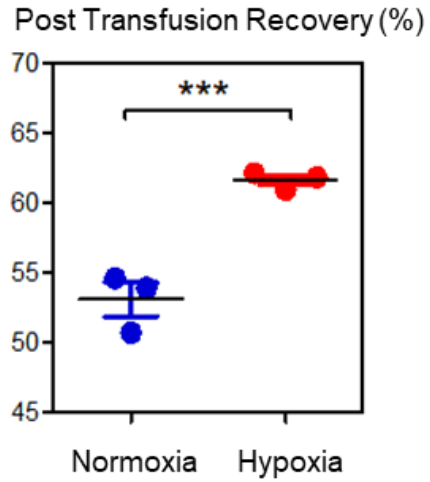


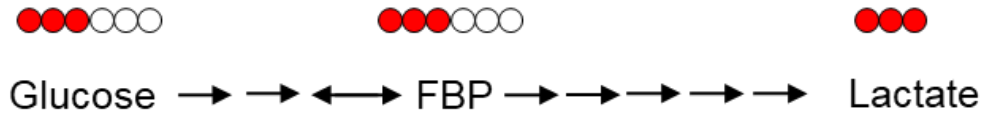


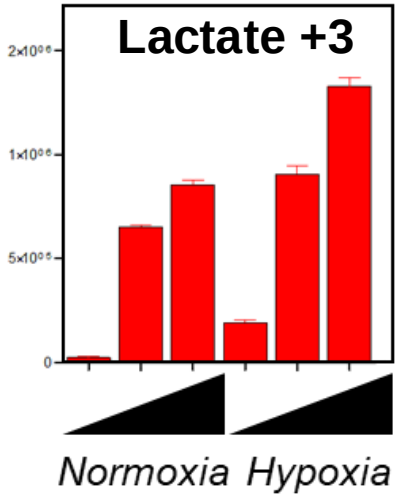
Metabolic and PTR benefits of hypoxic storage are recapitulated in mice



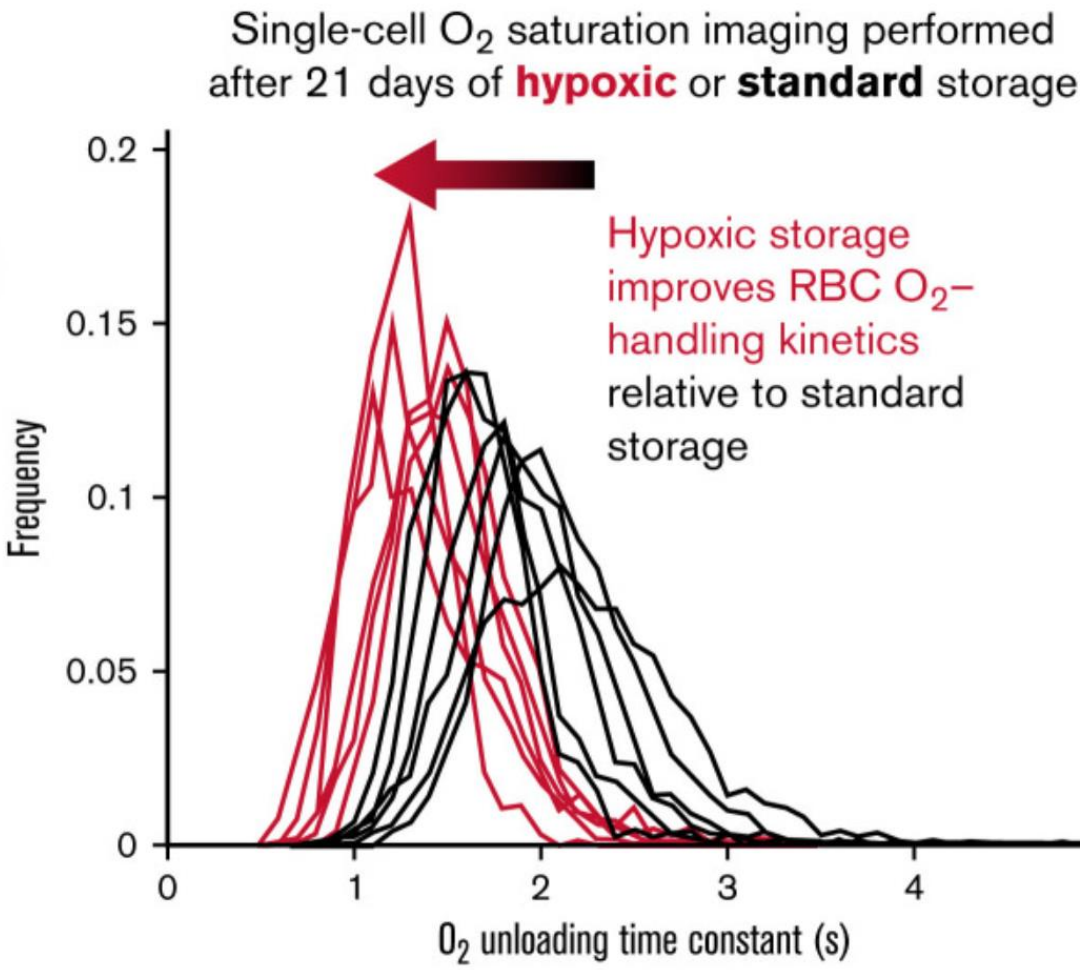


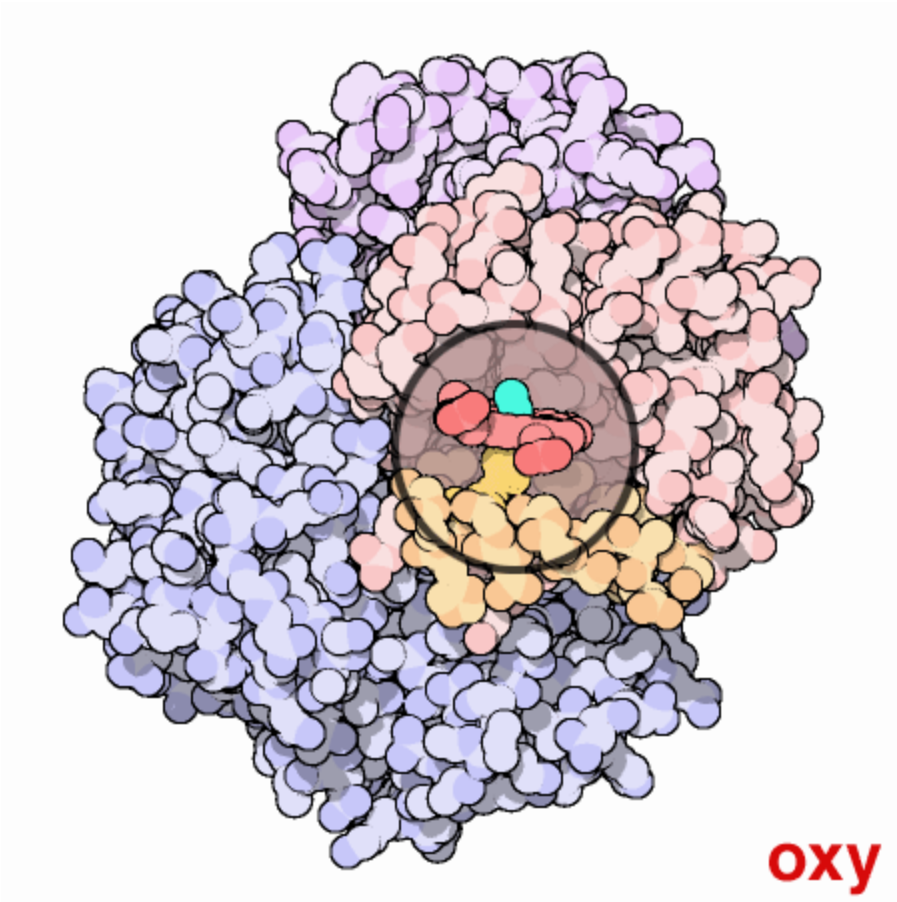






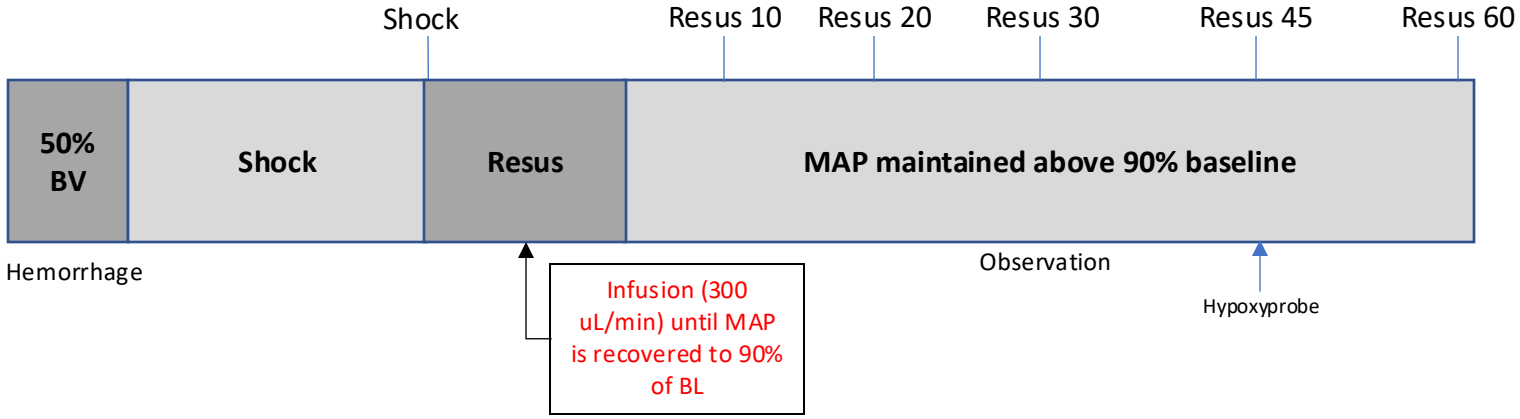
Hypoxic storage boosts DPG and single cell O_2 kinetics



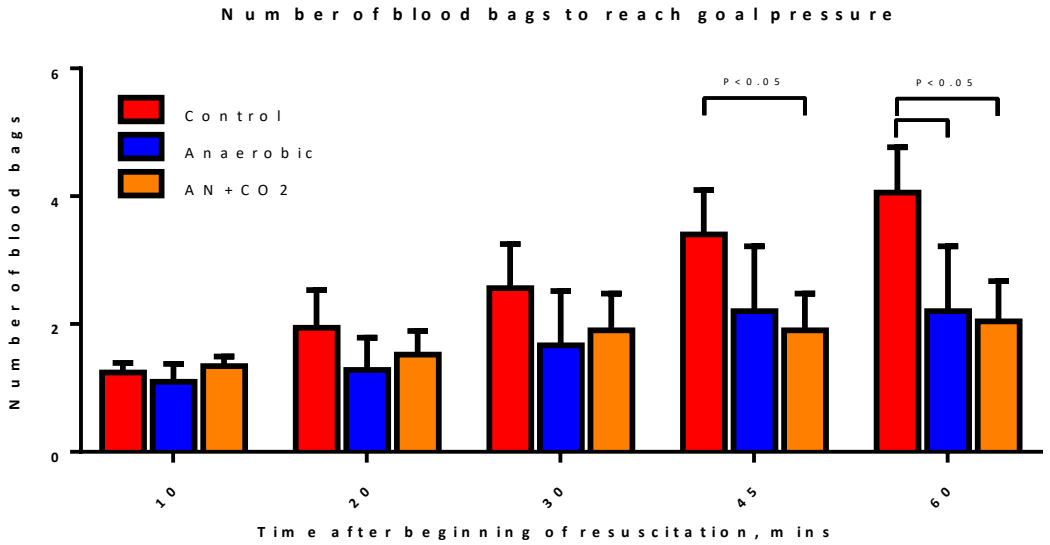


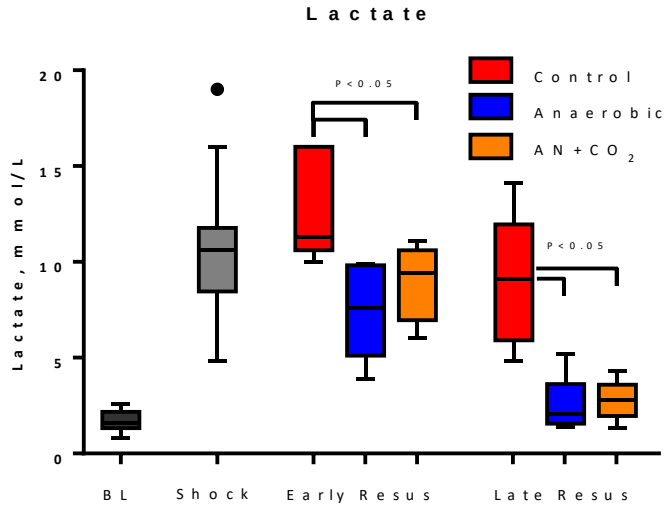
Rabjucka et al. Blood Advances 2022; Haematologica 2022;

Hypoxic RBCs are superior to ctrl end of storage RBCs in hemorrhaged rats



Pedro Cabrales, PhD
Univ. California San Diego





Hypoxic Storage of RBCs

PTR studies in humans

Hypoxia and RBC energy/redox metabolism

Metabolism in hypoxia/hypocapnia

Single Cell O_2 kinetics

Resuscitation in T/HS in rats








D'Alessandro et al. Transfusion 2021; Reisz et al. Blood 2016; Reisz et al. Transfusion 2017; D'Alessandro et al. Mol Biosystems 2012; Dumont et al. Transfusion 2019; Williams et al Shock 2019; Rabjucka et al. Haematologica 2022;

Blood storage studies – THANK YOU for your ATTENTION!

ATP Hypoxanthine

G6PD status

Kynurenine

Carnitine

Funding

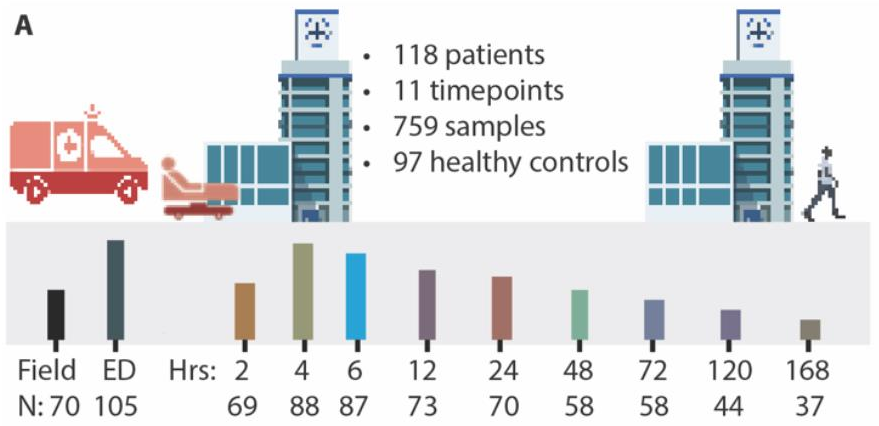
HHSN2682011 -00001/9I
NHLBI – R01HL126130
NHLBI – R01 HL146442
NHLBI – R01 HL148151
NHLBI - R01 HL149714
NHLBI - R01 HL161004
NHLBI - R21 HL150032
NIGMS - RM1 GM131968
NIGMS - F32GM124599
NIGMS - R01GM067945
NCI - R35CA209896
NIDDK - R01DK136945
BARDA - 75A50123C00047

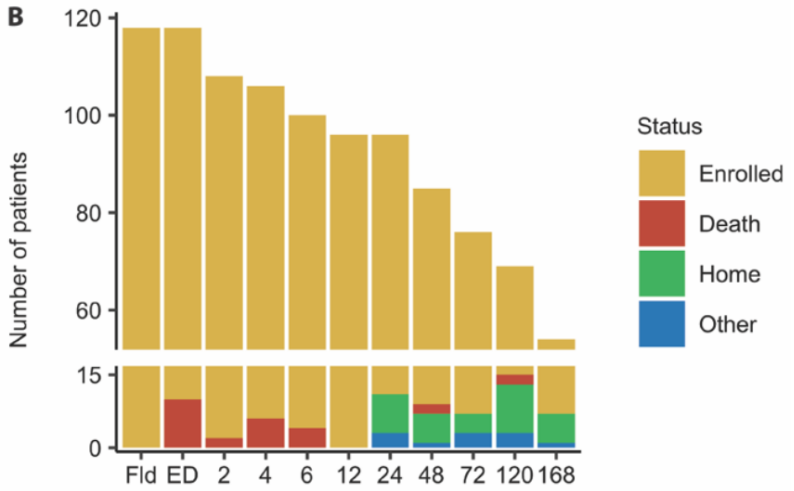
NIH National Heart, Lung, and Blood Institute

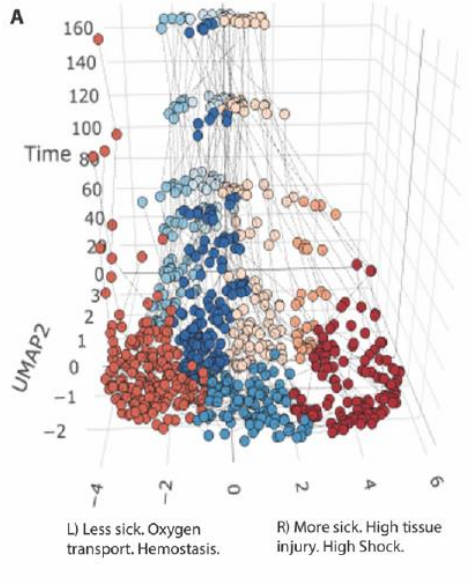
REDS-IV-P Optimizing Transfusion Across the Lifespan

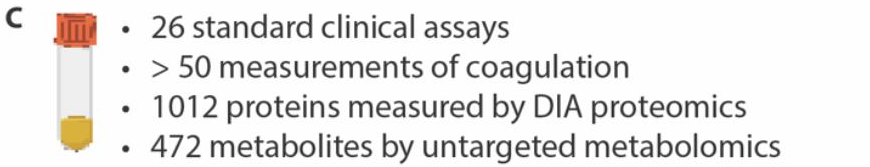
D'Alessandro et al. Transfusion 2021; Reisz et al. Blood 2016; Reisz et al. Transfusion 2017; D'Alessandro et al. Mol Biosystems 2012; Dumont et al. Transfusion 2019; D'Alessandro et al. Haematologica 2022; Nemkov et al. Cell Metabolism 2024; Nemkov et al. Blood 2024a and 2024b

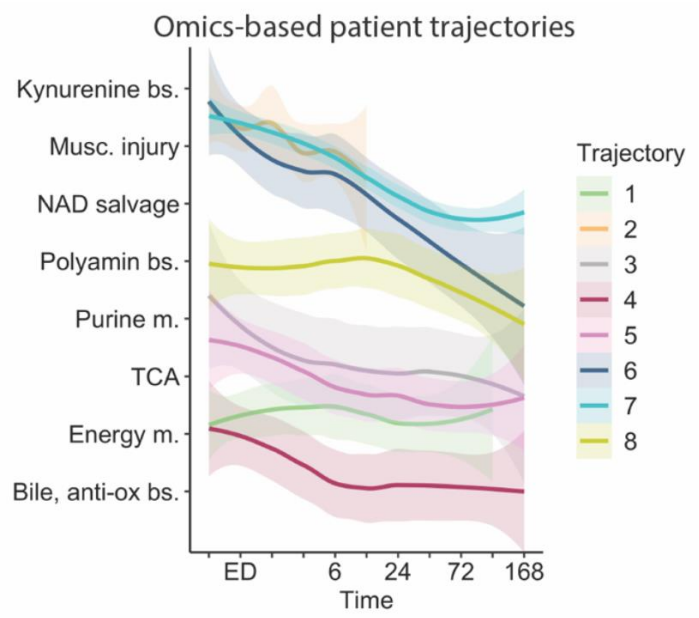
Metabolic changes in Trauma/Hemorrhagic shock in humans: COMBAT







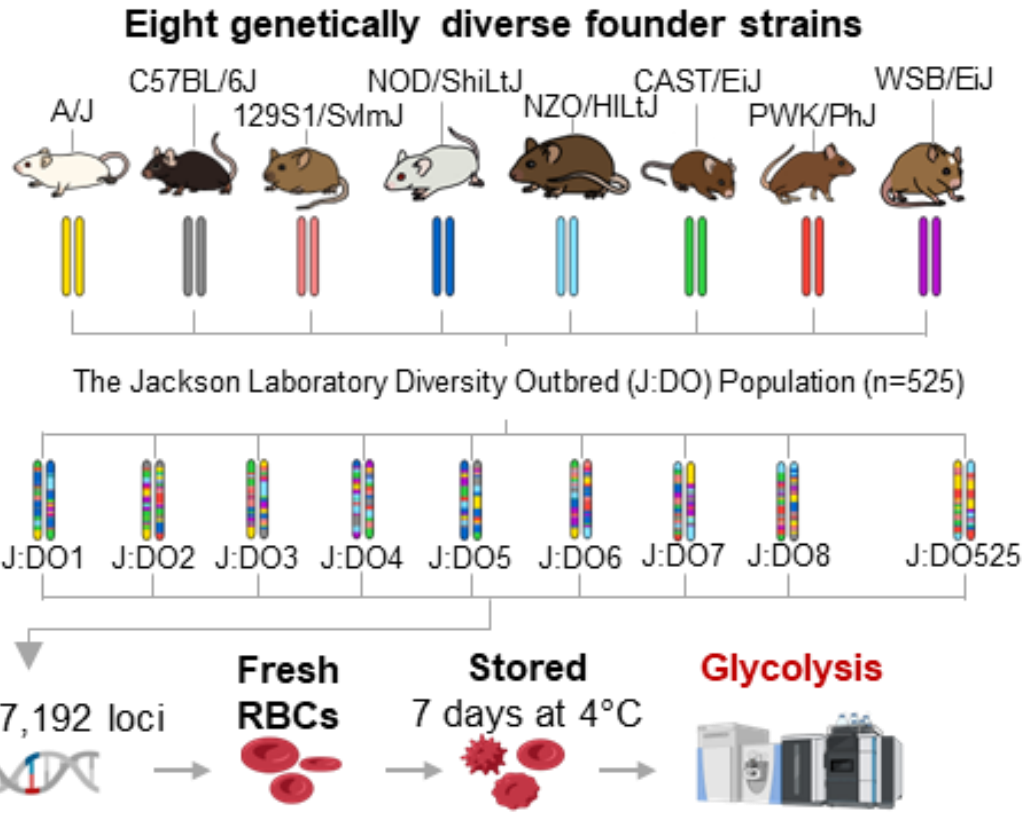


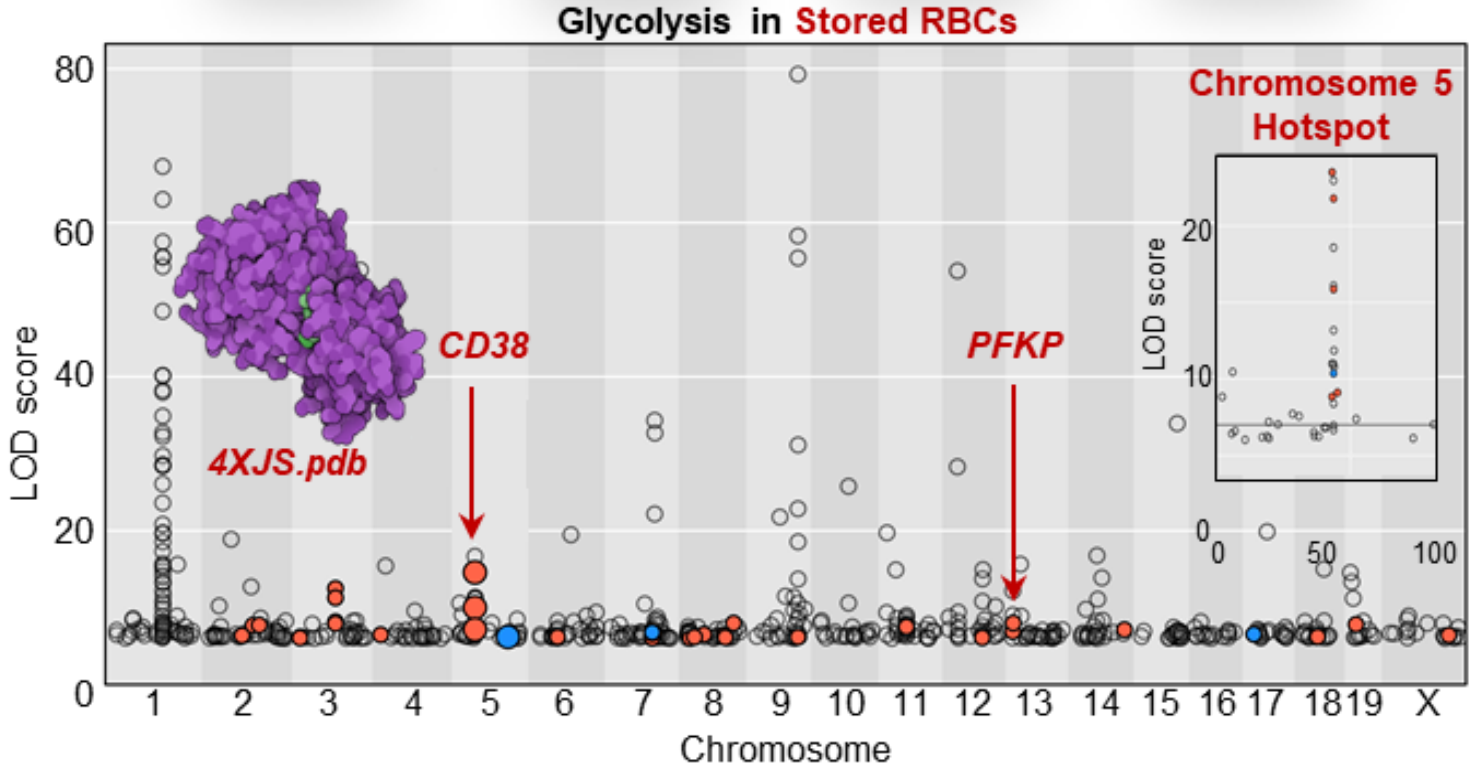


Cohen, Erickson et al. *BiorXiv*

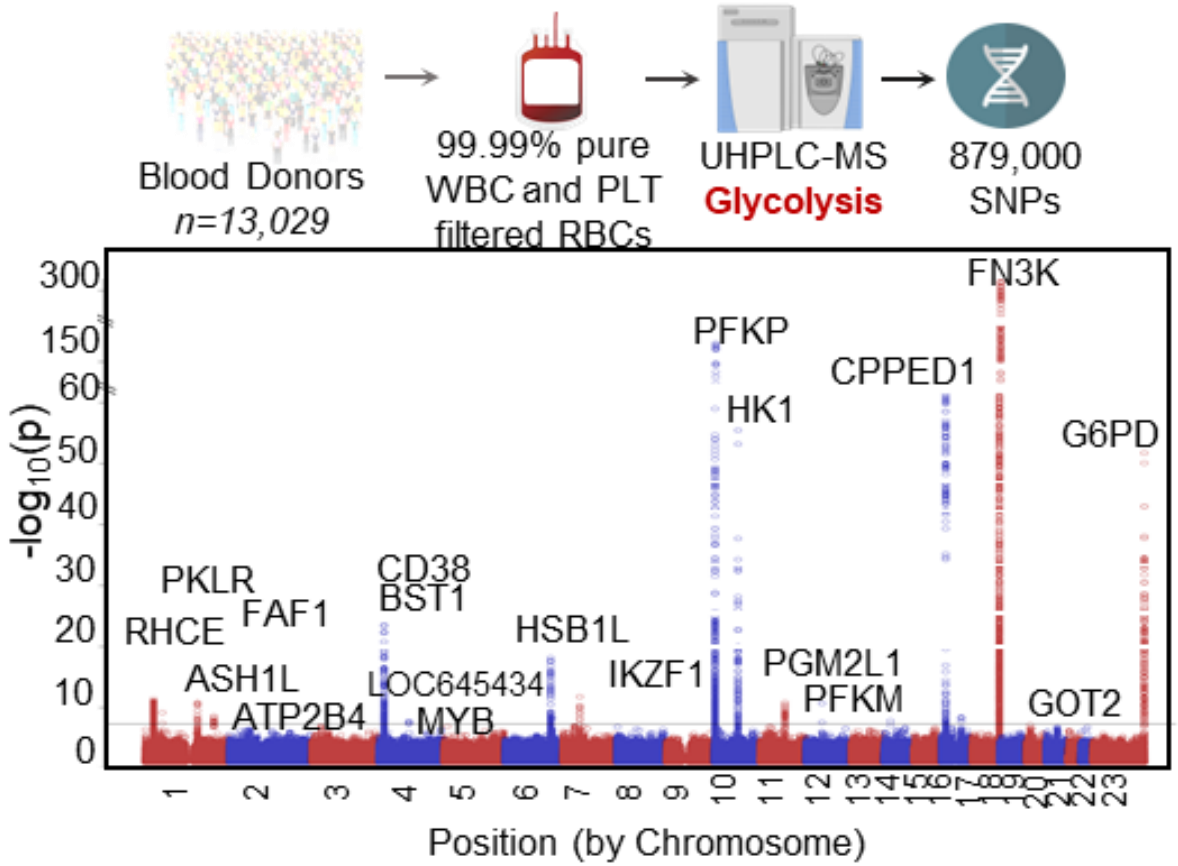
EXTRA SLIDES

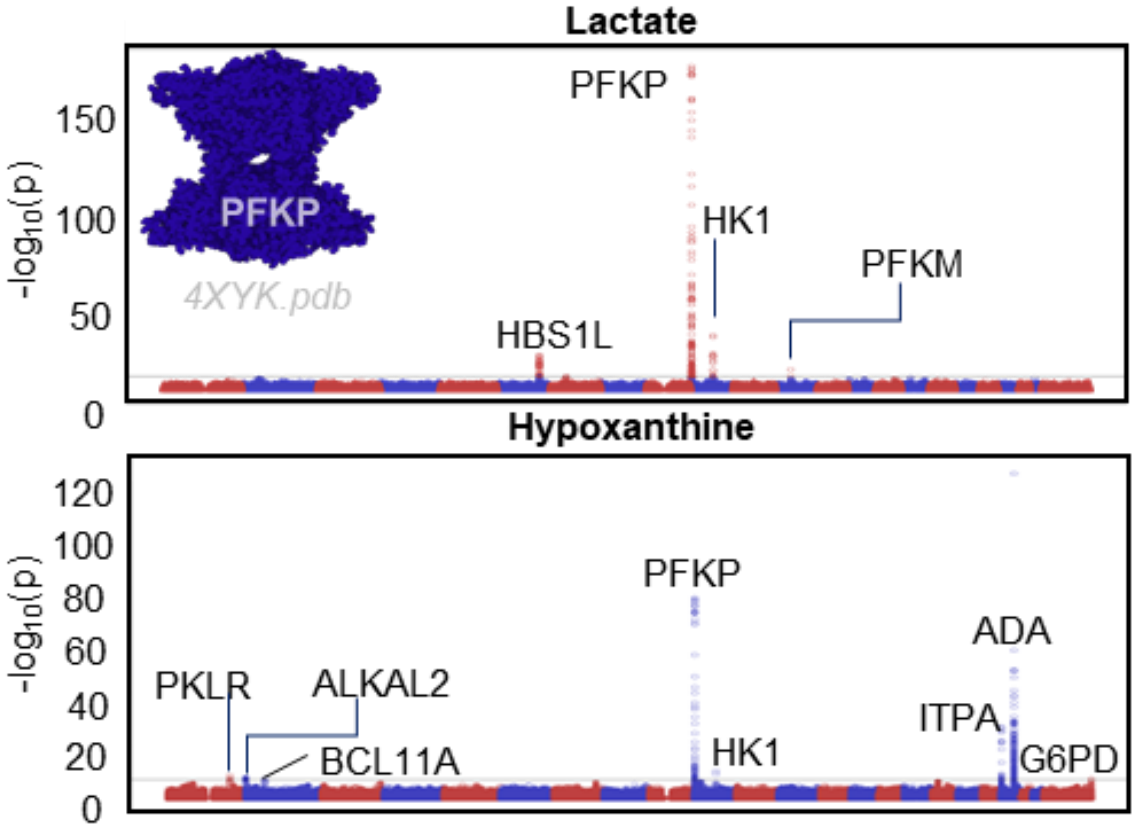
Genetic regulation of (fresh and stored) RBC energy metabolism in mice



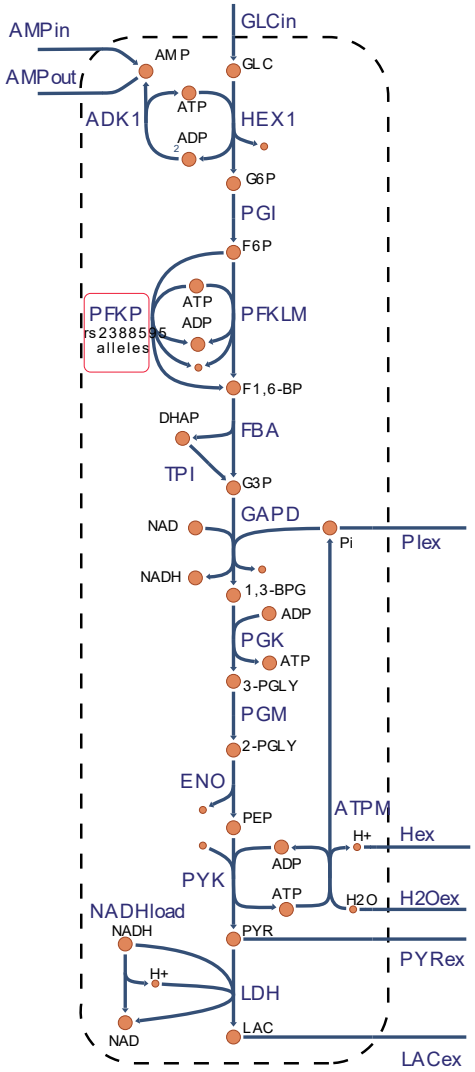


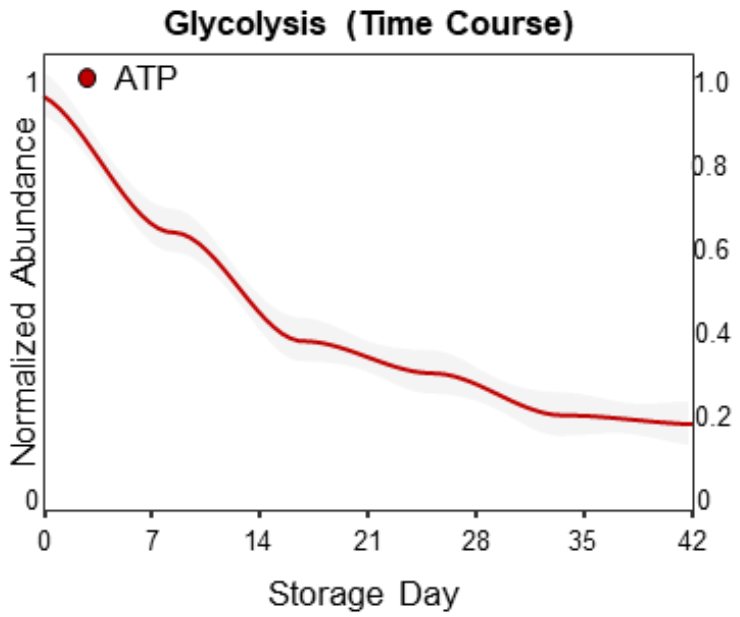
Genetic regulation of (stored) RBC energy metabolism in humans

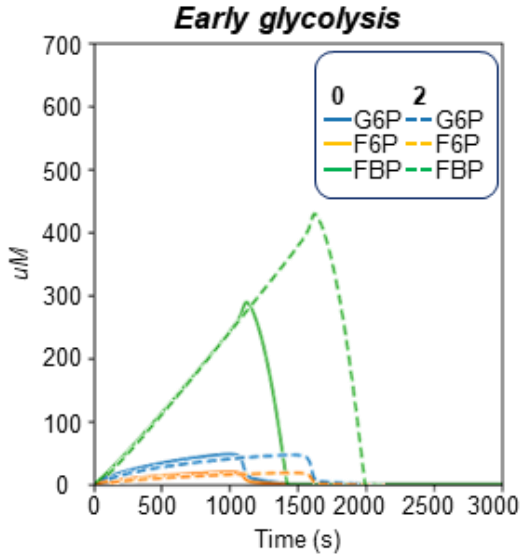


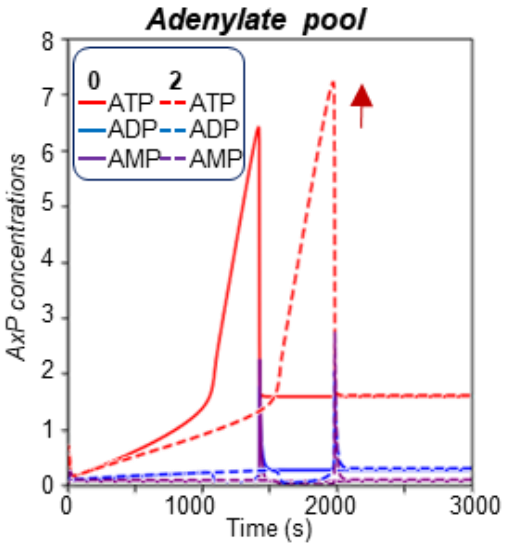


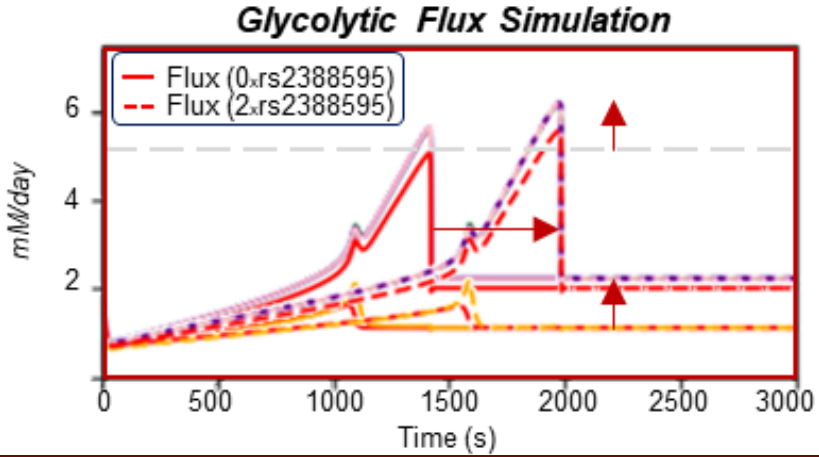
PFKP is less inhibited by ATP and its Km / substrate specificity promote glycolysis when ATP is low

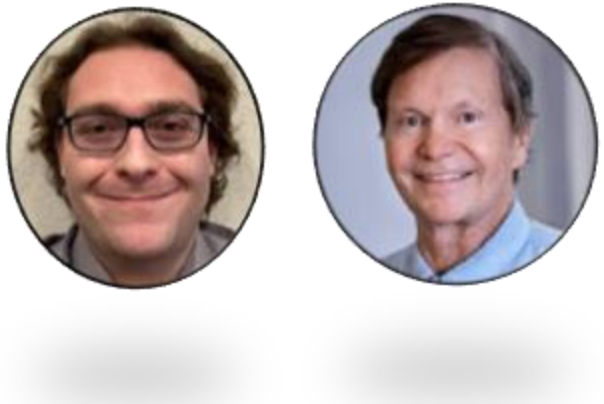






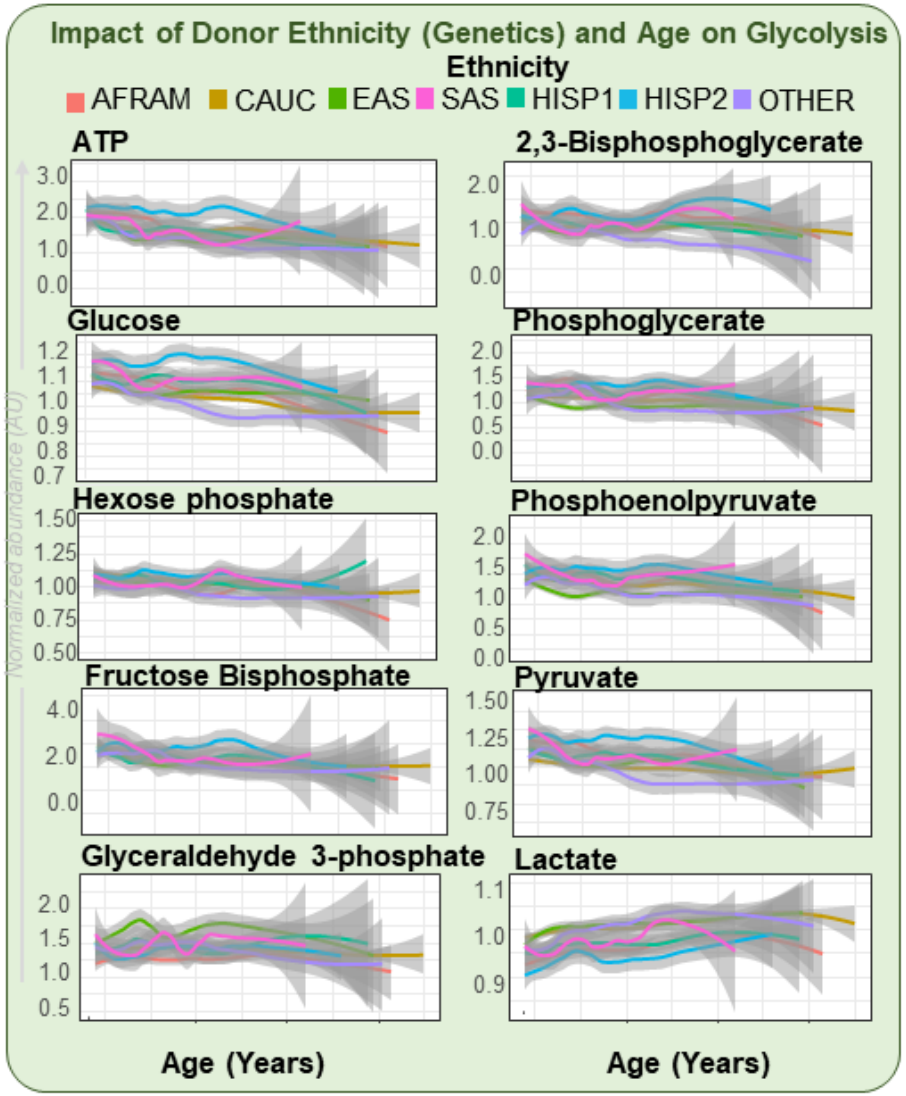




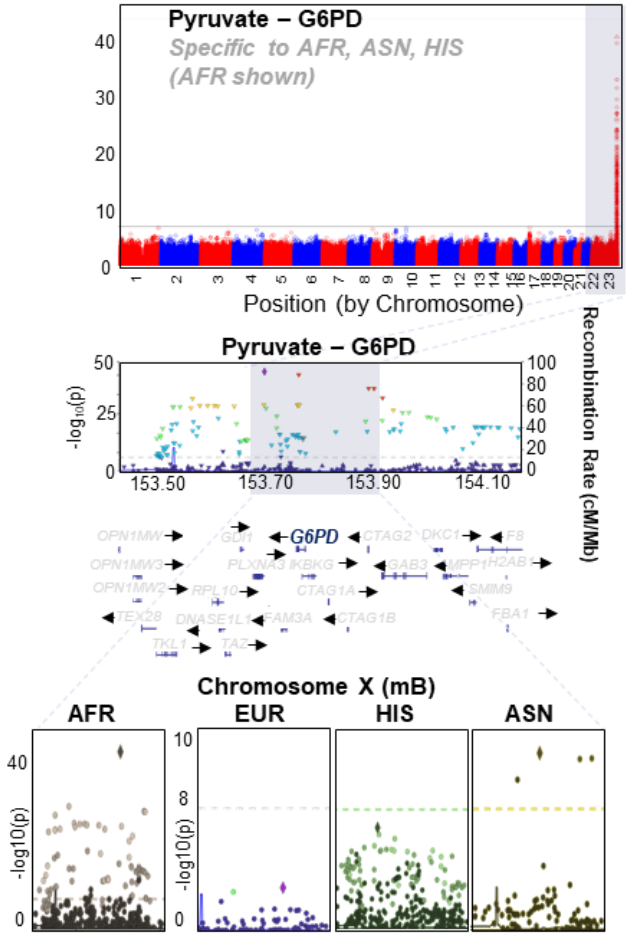


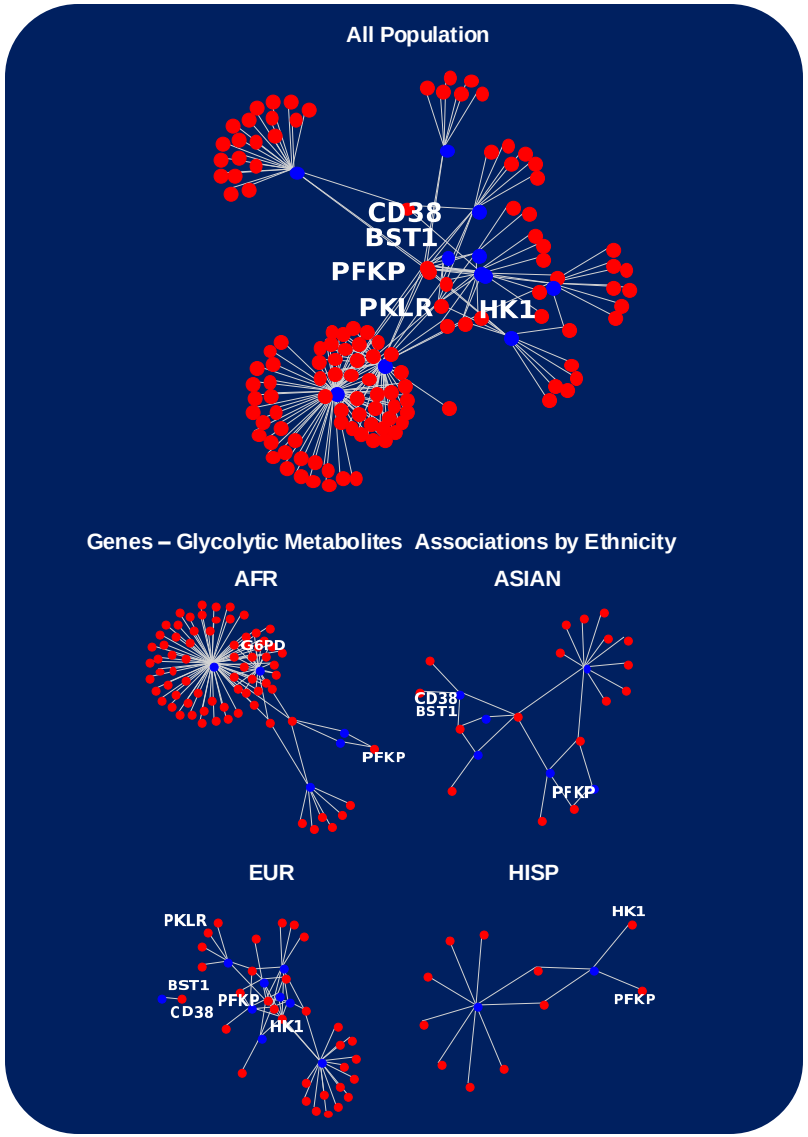
Nemkov et al. *Cell Metabolism* 2024

Impact of genetic ancestry on gene-metabolite association

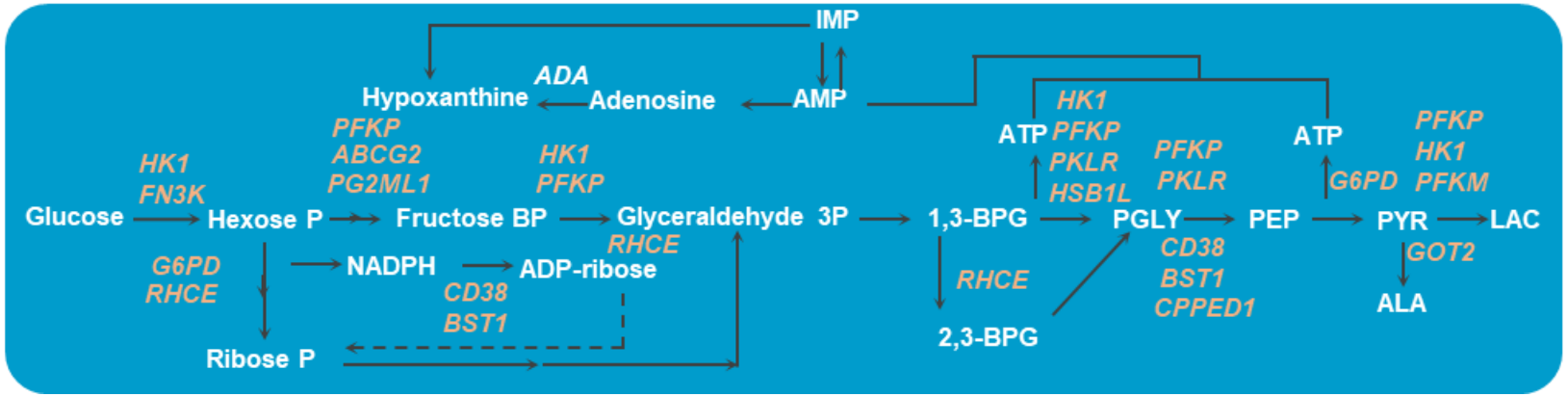


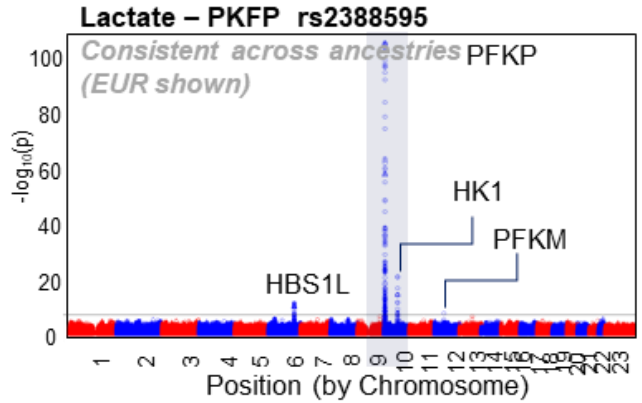
G6PD-pyruvate and donor African ancestry



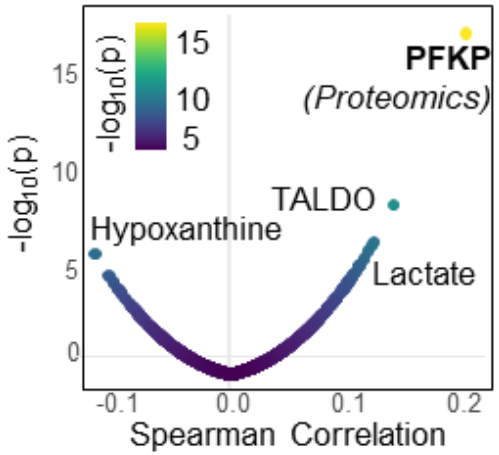


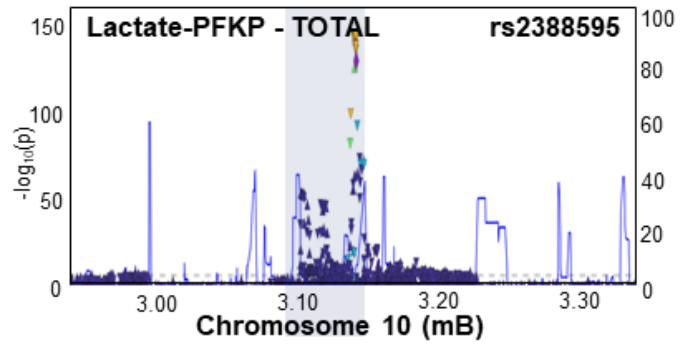
Pathway overview – Focus on PFKP – consistent across ancestries

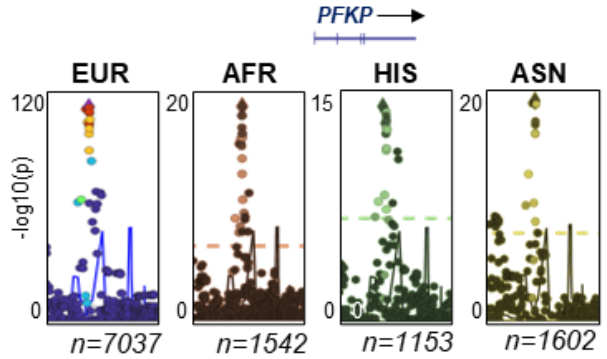




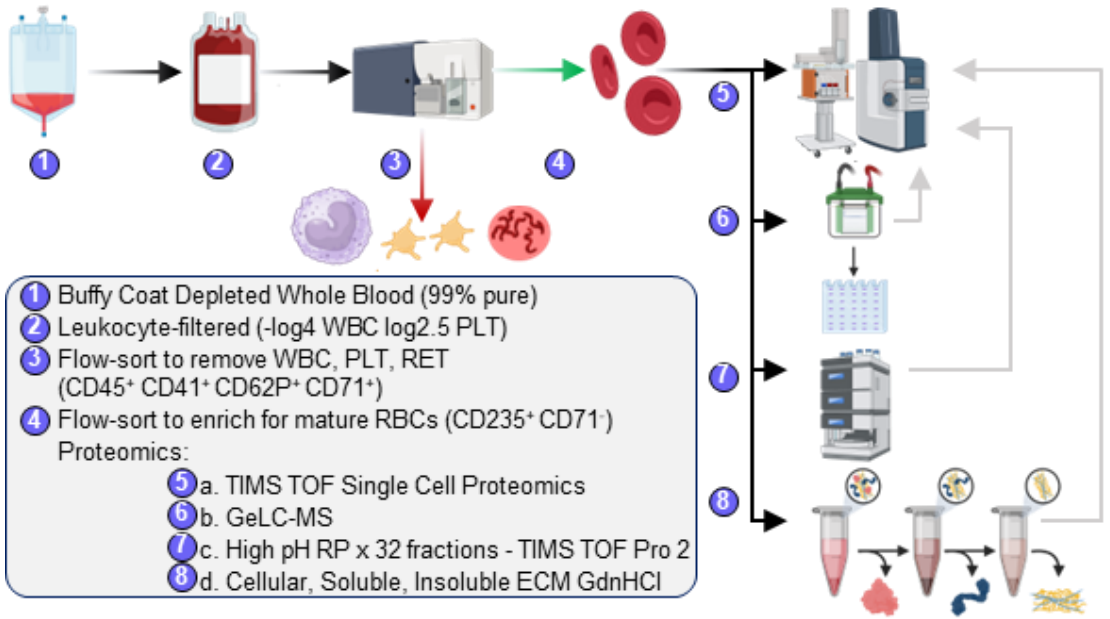
Multi-Omics Correlates with PFKP SNP
from 643 **Recalled Donors**
at storage day 10, 23, 42







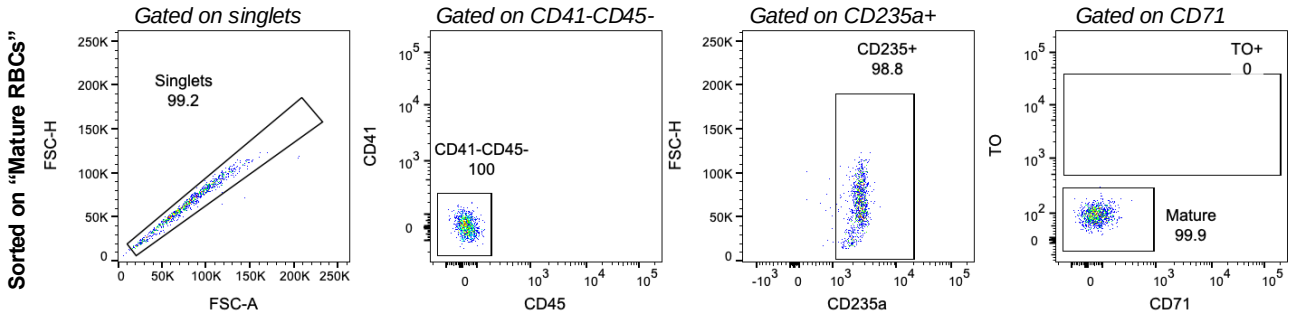
RBC Proteome Updated – is PFKP even expressed in ultra-pure mature RBCs?

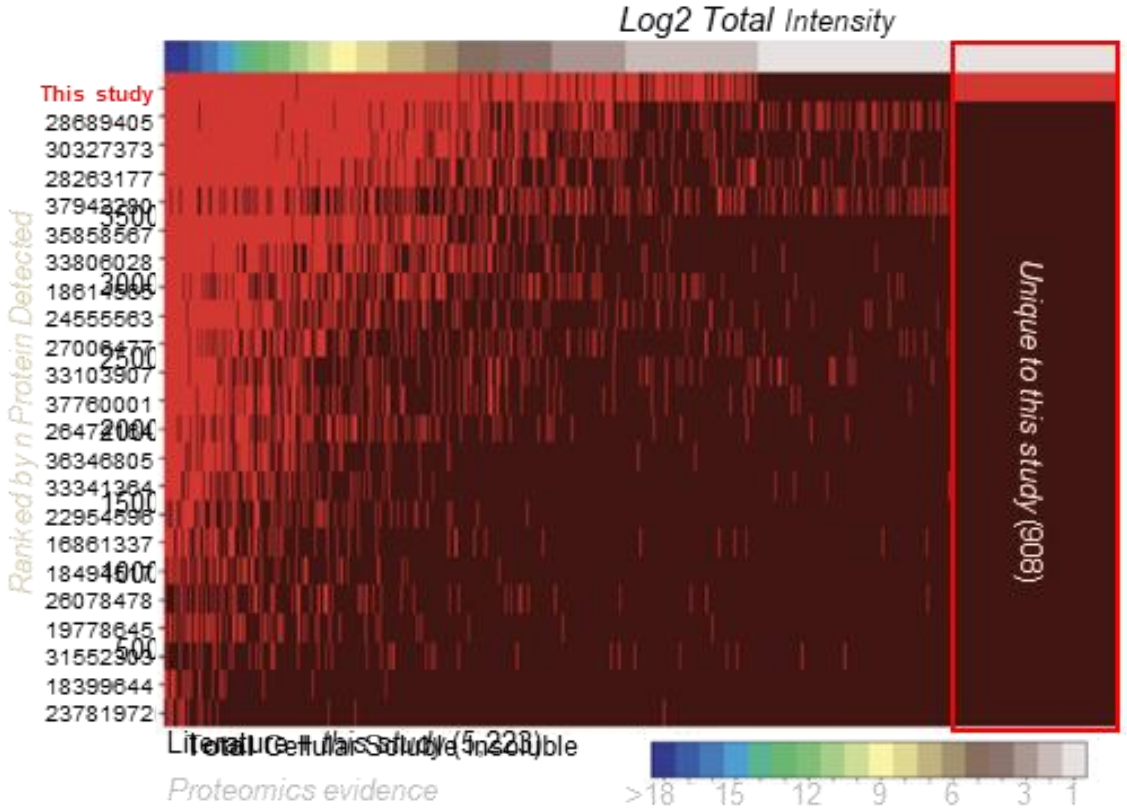


1. Buffy Coat Depleted Whole Blood (99% pure)
2. Leukocyte-filtered (-log4 WBC log2.5 PLT)
3. Flow-sort to remove WBC, PLT, RET (CD45⁺ CD41⁺ CD62P⁺ CD71⁺)
4. Flow-sort to enrich for mature RBCs (CD235⁺ CD71⁻)

Proteomics:

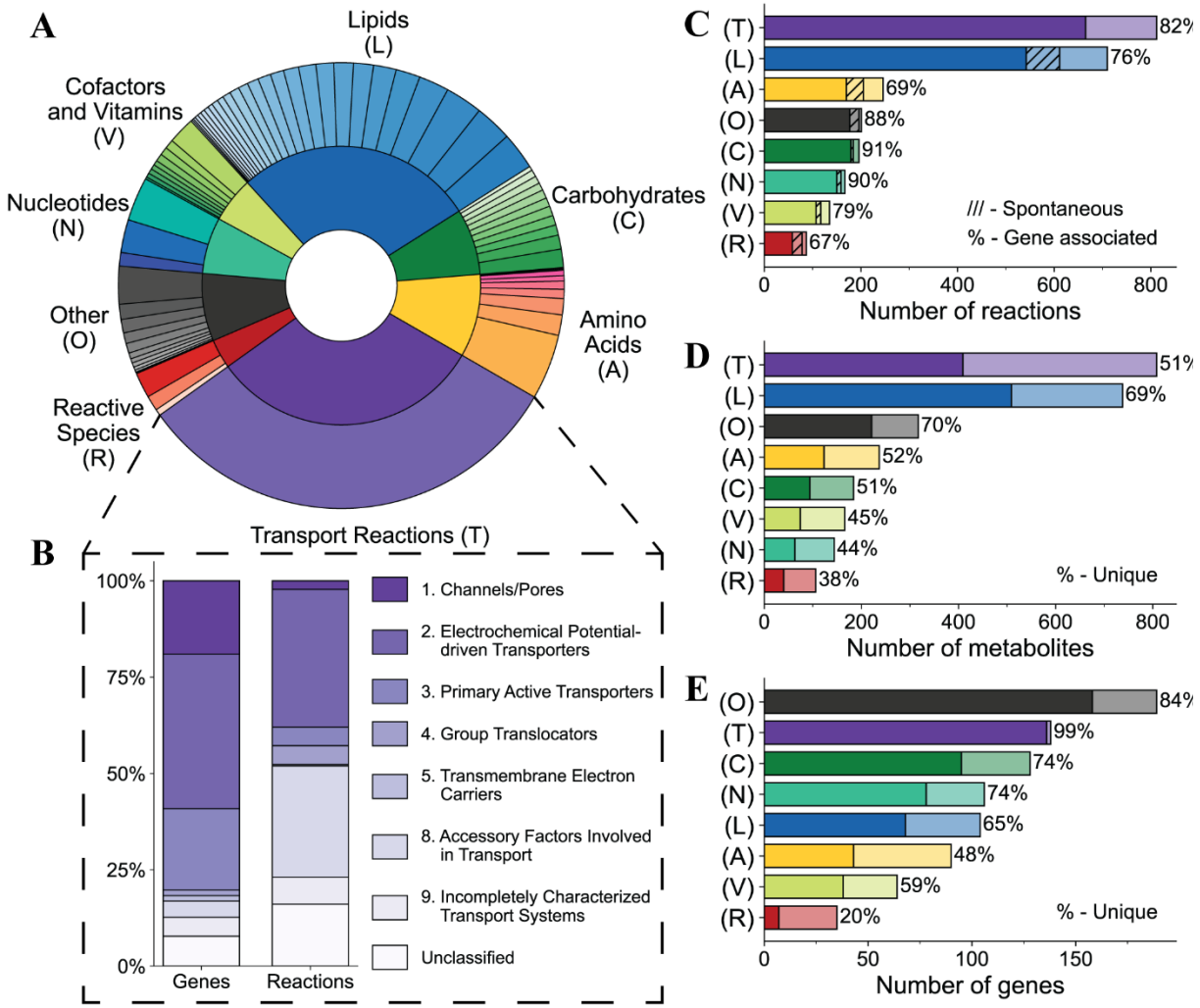
5. a. TIMS TOF Single Cell Proteomics
6. b. GeLC-MS
7. c. High pH RP x 32 fractions - TIMS TOF Pro 2
8. d. Cellular, Soluble, Insoluble ECM GdnHCl

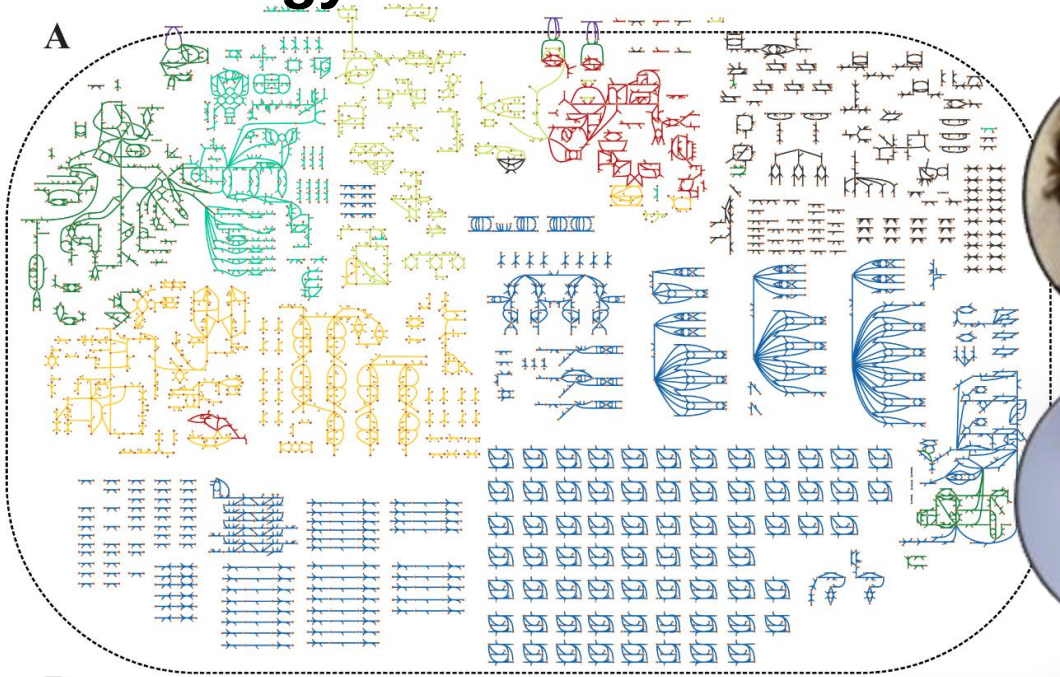


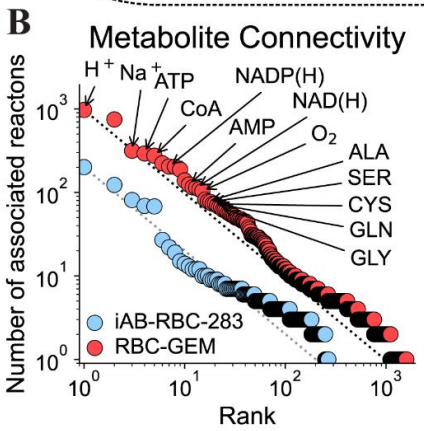


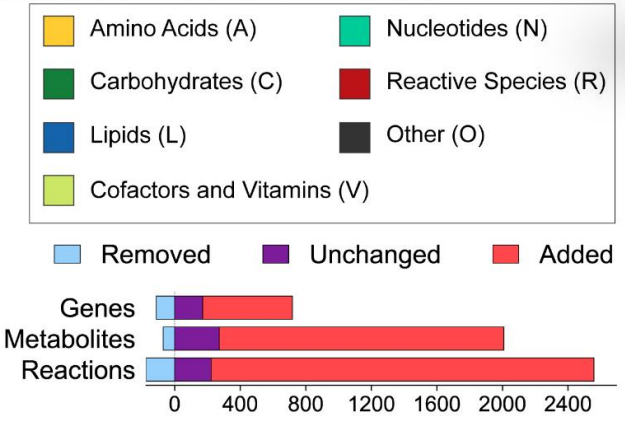
D'Alessandro et al. In preparation

RBC-GEM: a Knowledge Base for Systems Biology of Human RBC Metabolism









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PFKP is expressed in ultra-pure mature RBCs, at 30% higher levels in donors in cluster 4