

# Cold-stored Platelet Metabolism and Function: Targets for Modulating Hemostatic Capacity

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# Acknowledgements

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Macky Neal, MD

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**All of the  
Donors!**

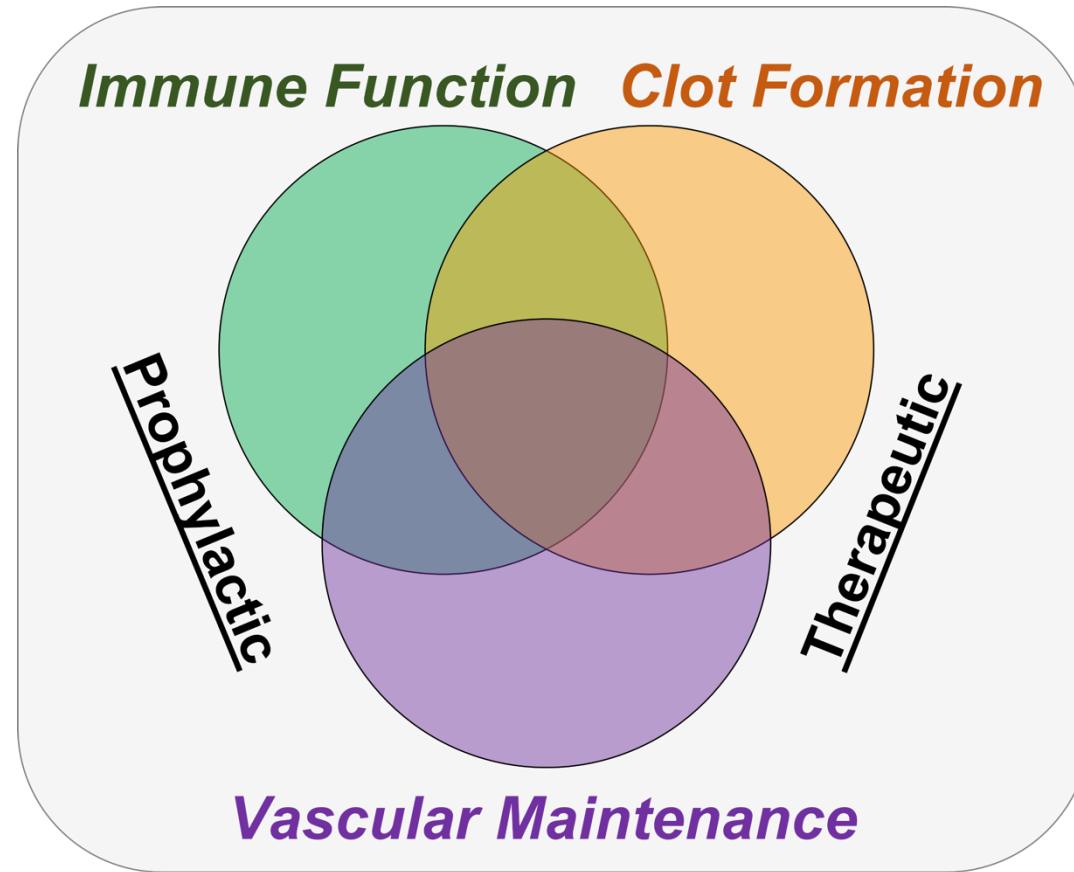


**CD41 (Platelets)**  
**VWF**

## ImpactLife

(formerly Mississippi Valley Regional Blood Center)

# PLATELETS



KEY:

Platelet Function:   

Indication for Transfusion

# Cold Stored Platelets (CS-PLT) for Active Bleeding

- Improved hemostatic function *in vitro*
  - compared to room temperature stored platelets
  - ***not accounting for physiologically relevant flow***
- Potential financial/logistical benefits
  - ▼ bacterial contamination
  - ▲ shelf life
  - ▼ wastage and cost
- Potential medical consequences
  - Increased alloimmunization?
  - Hyper-hemostatic function?

The US Army and Airforce and 3 US blood suppliers have already implemented the use of cold-stored platelets (out to day 14) in actively bleeding patients.

There are  $\geq 8$  CS-PLT clinical trials currently ongoing in the US.

# Unanswered Questions about CS-PLT

1. Do cold stored platelets maintain hemostatic function under conditions of physiologically relevant flow?

Rationale: all current markers of hemostatic function focused on stagnant assays, and platelet function heavily dictated by flow regimes

2. Can platelets be stored cold beyond two weeks (D14) and still be functional?

Rationale: LTOWB stored out to 3 weeks, and now a few reports on D21 platelets

3. Are there key metabolic pathways induced by cold storage that are associated with preserved hemostatic function?

Rationale: platelet function bioenergetically demanding, cold storage reduces metabolic activity

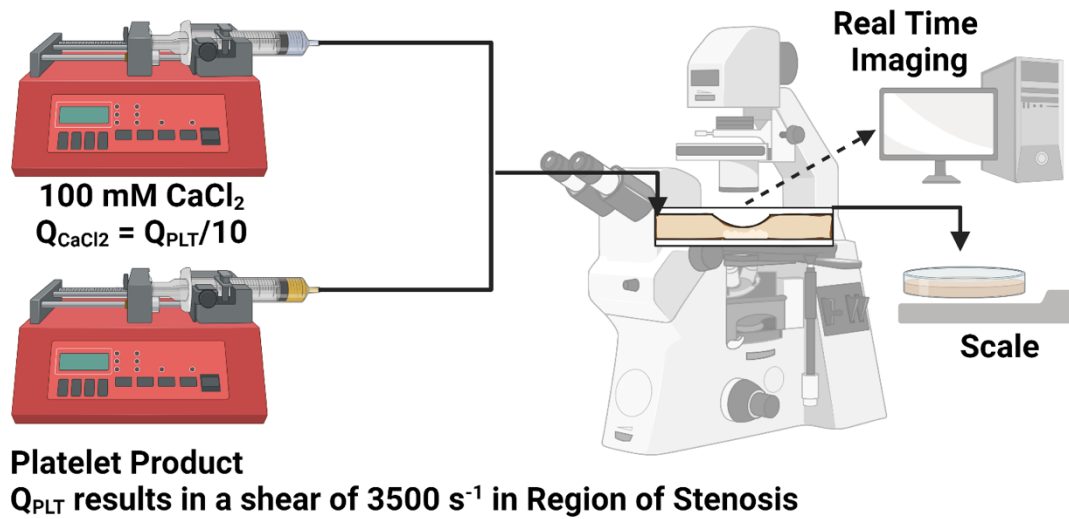
Do platelets stored cold for 21 days maintain hemostatic function under conditions of physiologically relevant flow?

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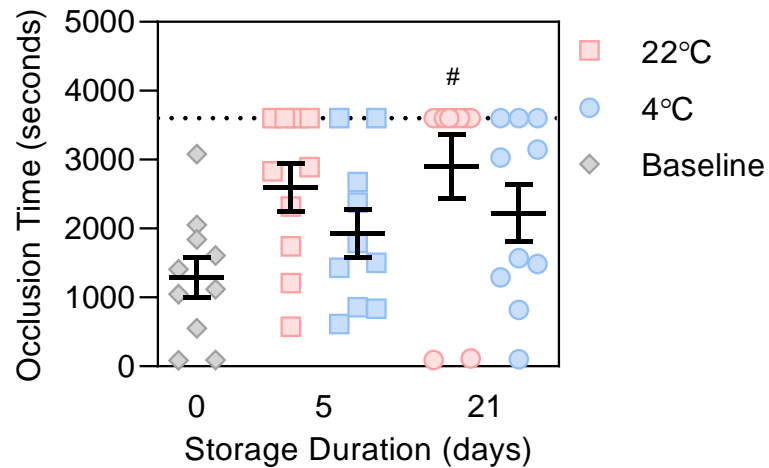
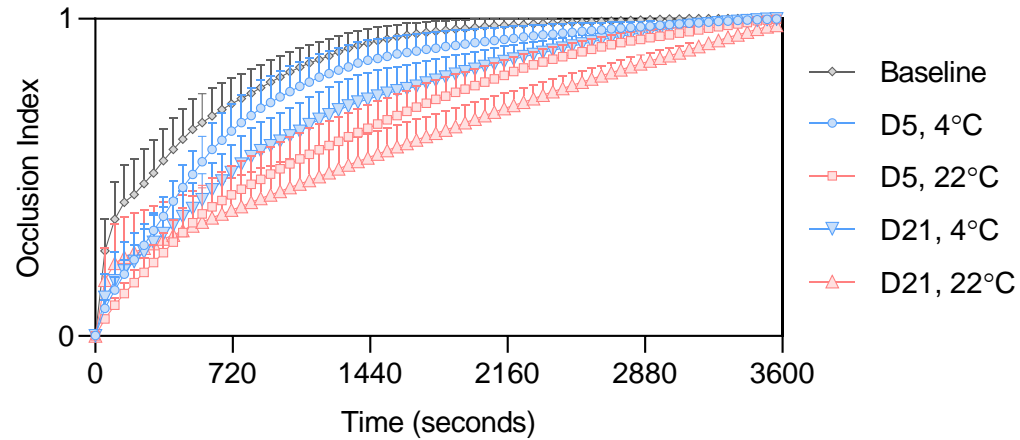
Are there key metabolic pathways induced by cold storage that are associated with preserved hemostatic function?

# Measuring Platelet Function Under Flow





# D21 CS-PLT function as well as D5 RT-PLT under flow

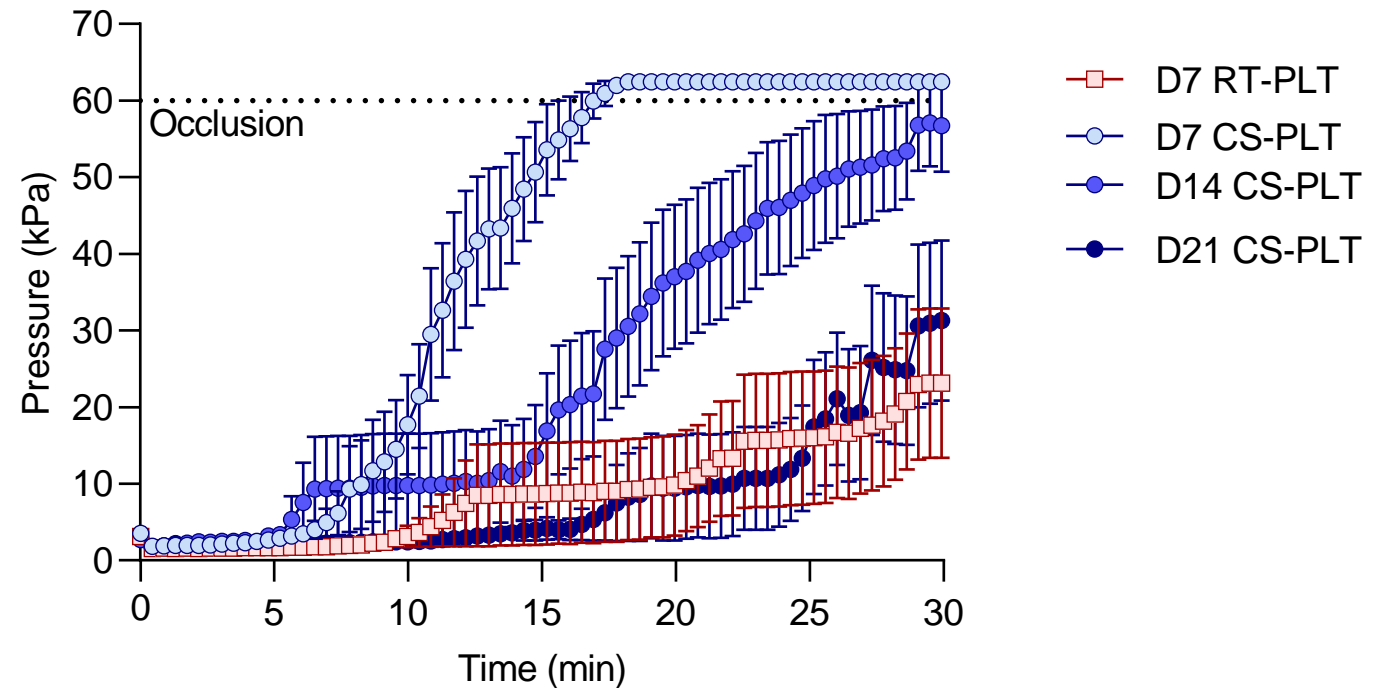


N=10 paired donors, Trima in 100% plasma; data graphed as mean (SEM) in occlusion trace plot, and as median (IQR) in other plots; pound symbols denote degree of significance when comparing the given group to baseline; #, p,0.05; ## p<0.01.

# Third Approach For Measuring Function Under Flow



## Trima Platelets in Isoplate



N=8 paired donors; mean + SEM  
unpublished data

Do platelets stored cold for 21 days maintain hemostatic function under conditions of physiologically relevant flow?

***As well as D5/D7 room temperature counterparts, and measured via different assays to assess thrombus formation under flow***

Are there key metabolic pathways induced by cold storage that are associated with preserved hemostatic function?

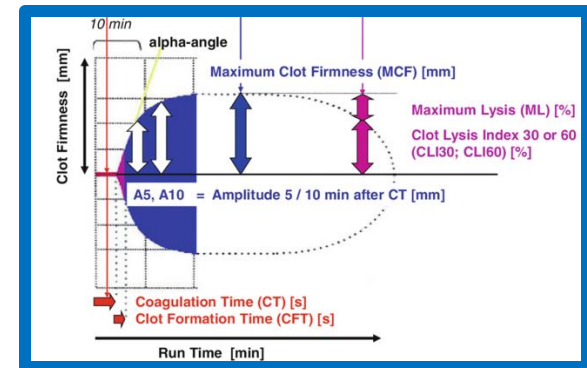
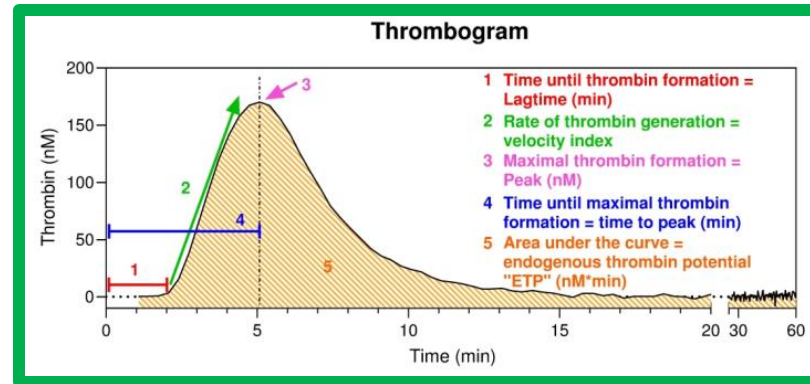
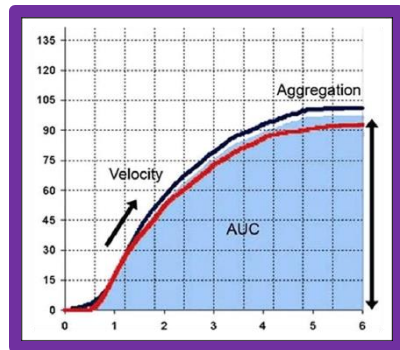
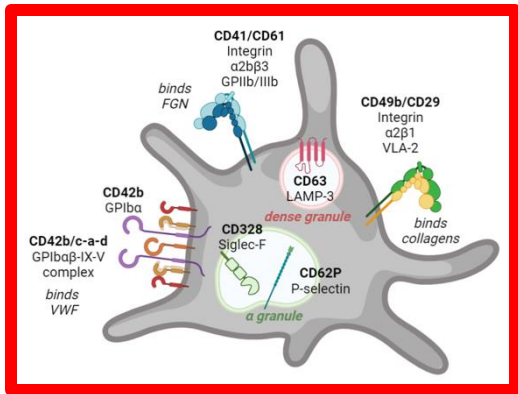
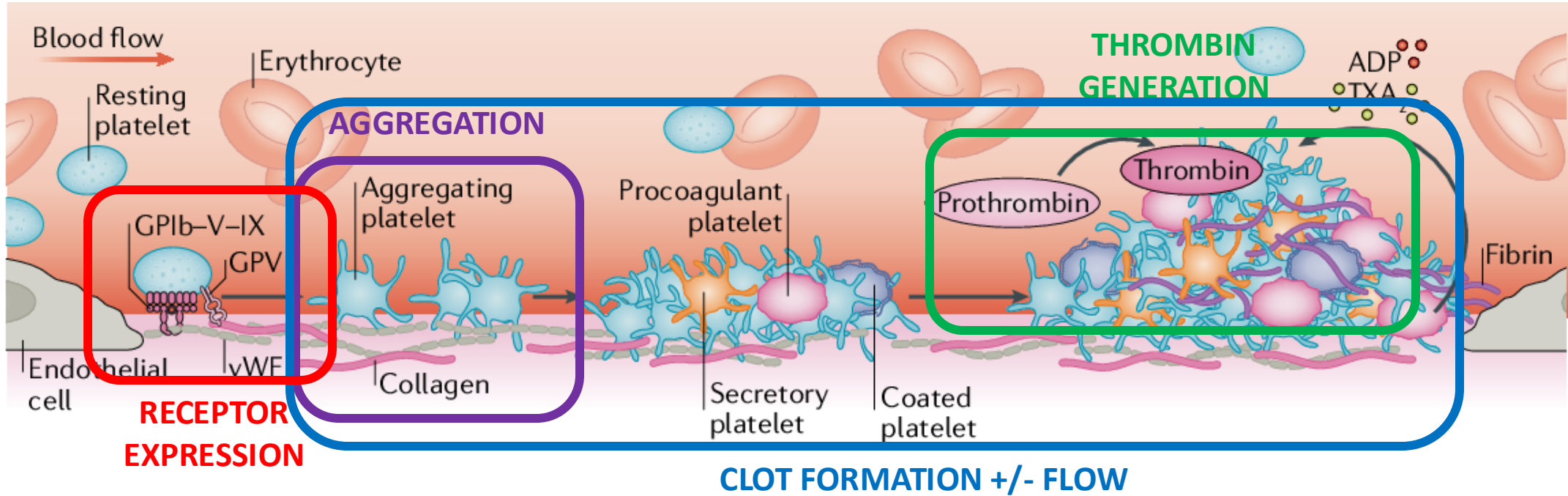
Do platelets stored cold for 21 days maintain hemostatic function under conditions of physiologically relevant flow?

Are there key metabolic pathways induced by cold storage that are associated with preserved hemostatic function?

# Our Stepwise Approach

1. Perform a comprehensive phenotypic and functional assessment of stored platelet products (to include function under flow)
2. Perform metabolic profiling on these same stored platelet products
3. Identify metabolites/metabolic pathways associated with CS-PLT function

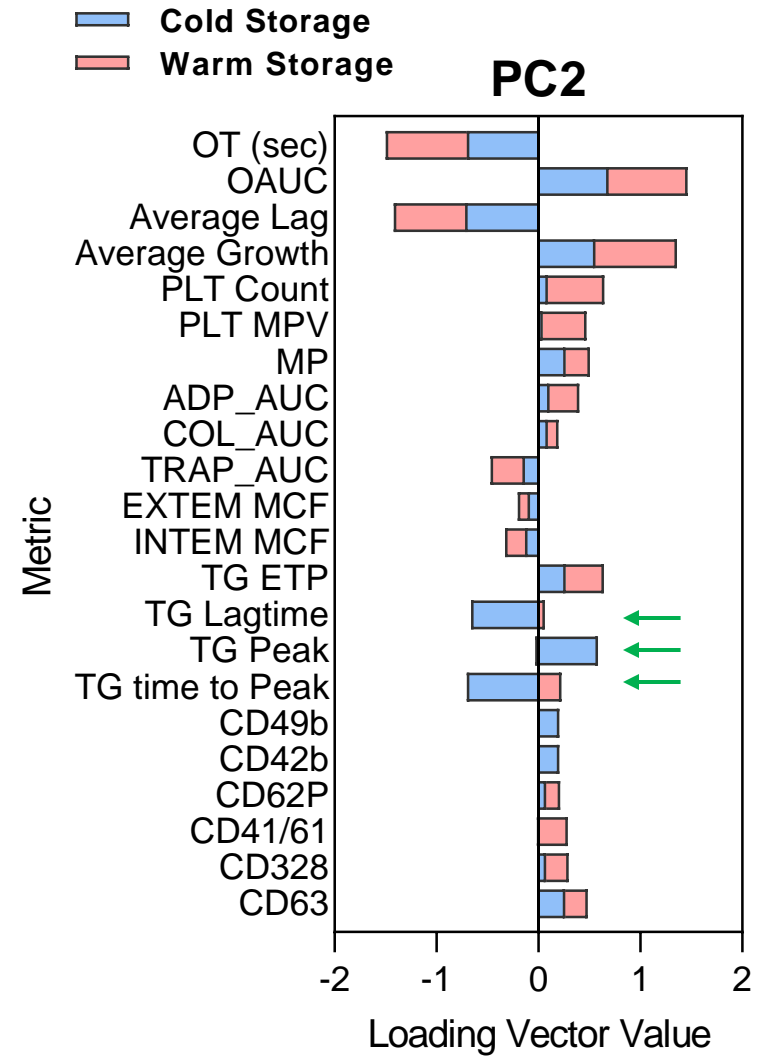
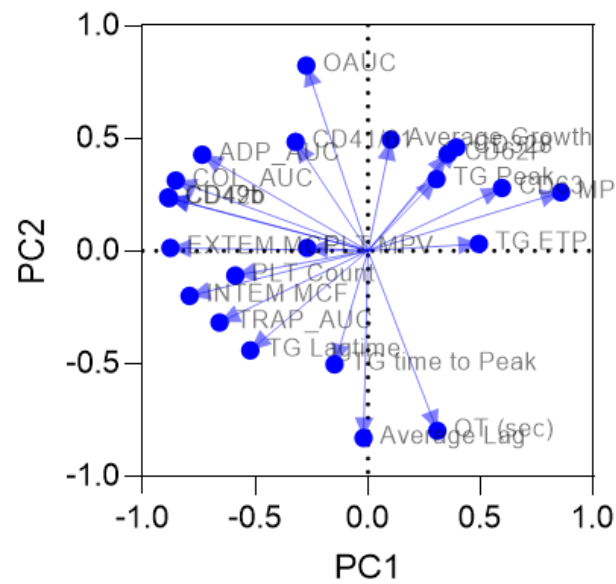
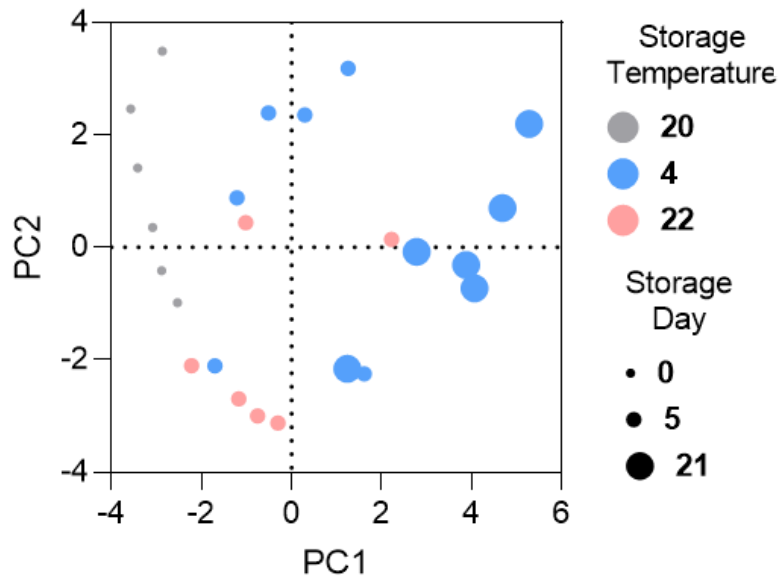
# I. Comprehensive Assessment of Stored PLT Function



# I. Comprehensive Assessment of Stored PLT Function

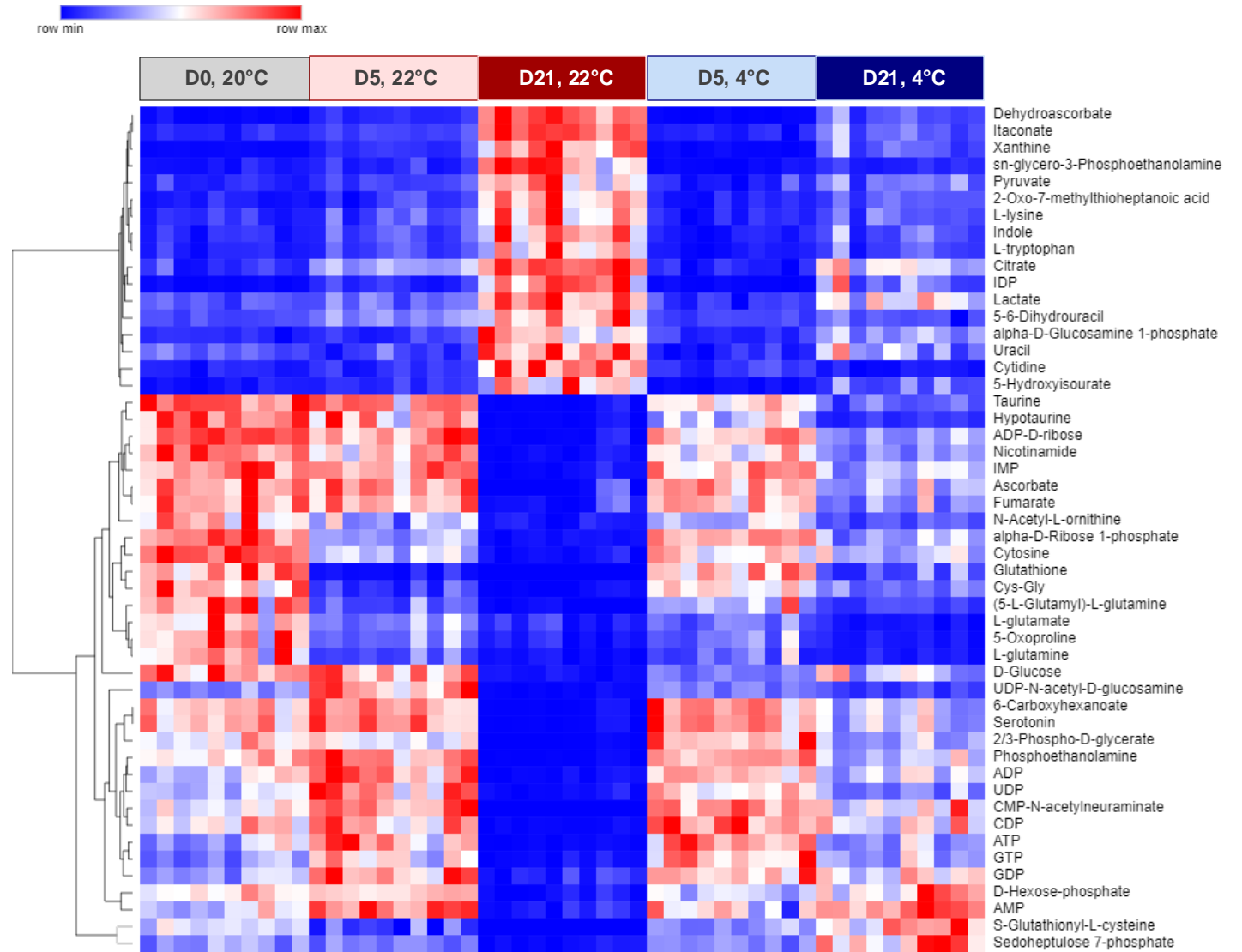
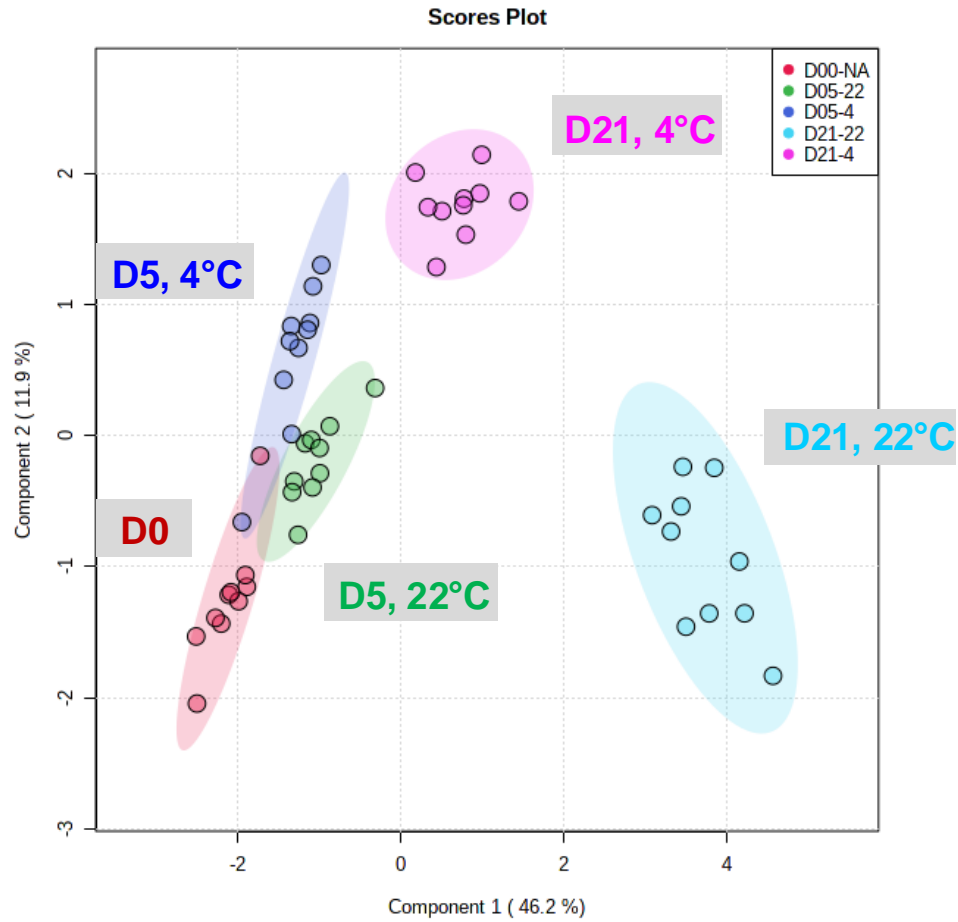
<b>variable</b>	<b>description</b>	<b>units</b>	<b>values</b>	<b>assay</b>	<b>category</b>
Storage Temp	temperature unit was stored at	degrees C	4 = cold 20 = baseline 22 = room temp	n/a	phenotypic
Storage Day	day of storage	days	0, 5, 21	n/a	phenotypic
OT (sec)	occlusion time in seconds	s	>0	microfluidic	functional
OAUC	area under the occlusion trace curve (AUC)	arbitrary	>0	microfluidic	functional
Average Lag	average lag period for clot formation	seconds	>0	microfluidic	functional
Average Growth	average growth period for clot formation	pixels/second	>0	microfluidic	functional
PLT Count	platelet or thrombocyte count	$\times 10^3/\mu\text{L}$	$\geq 0$	cbc	phenotypic
PLT MPV	mean platelet (thrombocyte) volume	fL	$\geq 0$	cbc	phenotypic
MP	thrombolux DLS microparticle (MP) content	%	$\geq 0$	dls	functional
ADP_AUC	area under the curve for adenosine diphosphate (ADP) induced platelet aggregation on Multiplate	U (1U = 10AU*min)	>0	aggregation	functional
COL_AUC	area under the curve for collagen (COL) induced platelet aggregation on	U (1U = 10AU*min)	>0	aggregation	functional
TRAP_AUC	area under the curve for thrombin receptor activating peptide (TRAP) induced platelet aggregation on Multiplate	U (1U = 10AU*min)	>0	aggregation	functional
EXTEM CFT	clot formation time (time to reach 20mm from 2 mm amplitude; CFT) in response to extrinsic activation (extem reagent) on ROTEM	s	>0	ROTEM	functional
EXTEM MCF	maximum clot firmness (maximum amplitude; MCF) in response to extrinsic activation (extem reagent) on ROTEM	mm	>0	ROTEM	functional
INTEM CFT	clot formation time (time to reach 20mm from 2 mm amplitude; CFT) in response to intrinsic activation (intem reagent) on ROTEM	s	>0	ROTEM	functional
INTEM MCF	maximum clot firmness (maximum amplitude; MCF) in response to intrinsic activation (intem reagent) on ROTEM	mm	>0	ROTEM	functional
TG ETP	endogenous thrombin potential in response to 5 pM TF PRP reagent, assessed by calibrated automated thromboscope	nM*min	>0	thrombin generation	functional
TG Lagtime	lagtime (time to initial thrombin generation) in response to 5 pM TF PRP reagent, assessed by calibrated automated thromboscope	min	>0	thrombin generation	functional
TG Peak	peak (maximal amplitude of thrombin generation) in response to 5 pM TF PRP reagent, assessed by calibrated automated thromboscope	nM	>0	thrombin generation	functional
TG time to Peak	time to peak thrombin measurement	min	>0	thrombin generation	functional
CD49b	geometric mean fluorescence intensity of CD49b PerCP Cy5.5 expression as measured by flow cytometry	number	>0	flow cytometry	phenotypic
CD42b	geometric mean fluorescence intensity of CD42b PE Dazzle 594 expression as measured by flow cytometry	number	>0	flow cytometry	phenotypic
CD62P	geometric mean fluorescence intensity of CD62P PE-Cy7 expression as measured by flow cytometry	number	>0	flow cytometry	phenotypic
CD41/61	geometric mean fluorescence intensity of PAC1 647 expression as measured by flow cytometry	number	>0	flow cytometry	phenotypic
CD328	geometric mean fluorescence intensity of CD328 APC Fire 750 expression as measured by flow cytometry	number	>0	flow cytometry	phenotypic
CD63	geometric mean fluorescence intensity of CD63 BV510 expression as measured by flow cytometry	number	>0	flow cytometry	phenotypic

# I. Key Metrics Associated with Cold Storage



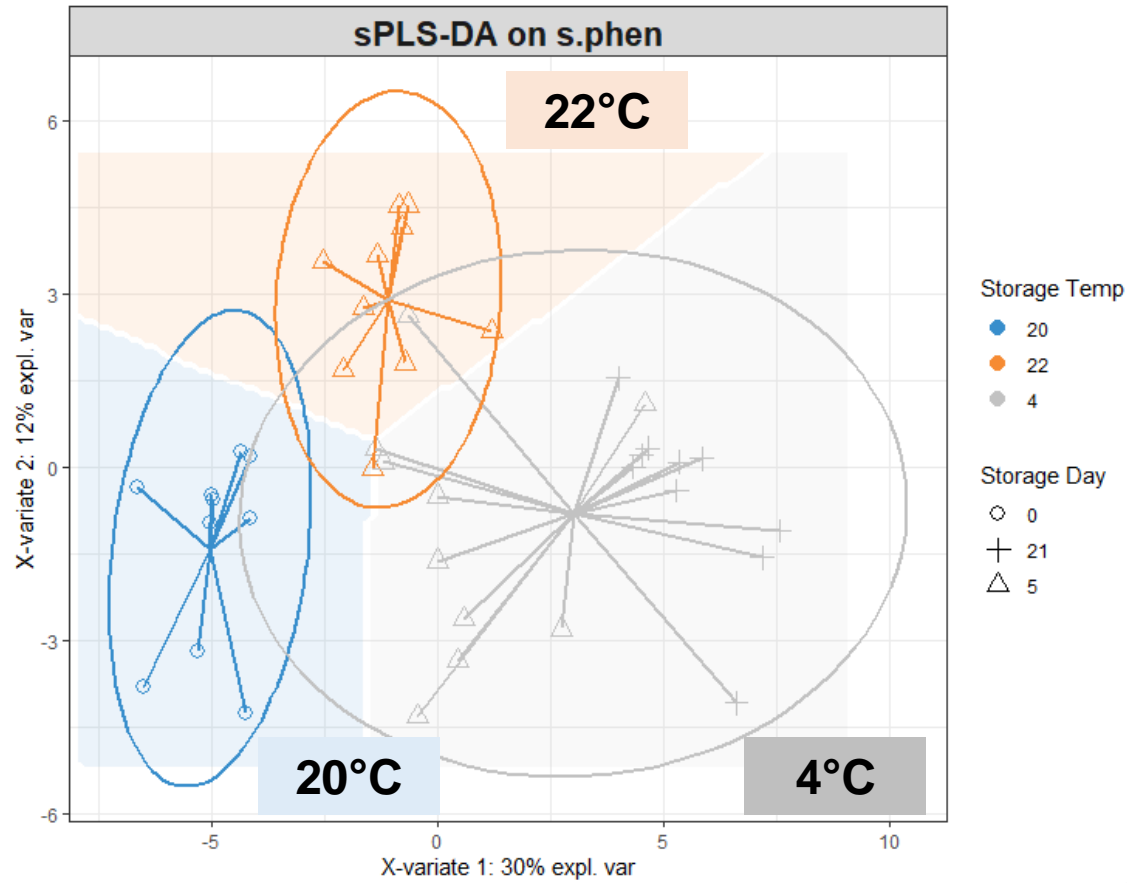


# 2. Metabolic Profiling of Stored Platelet Products

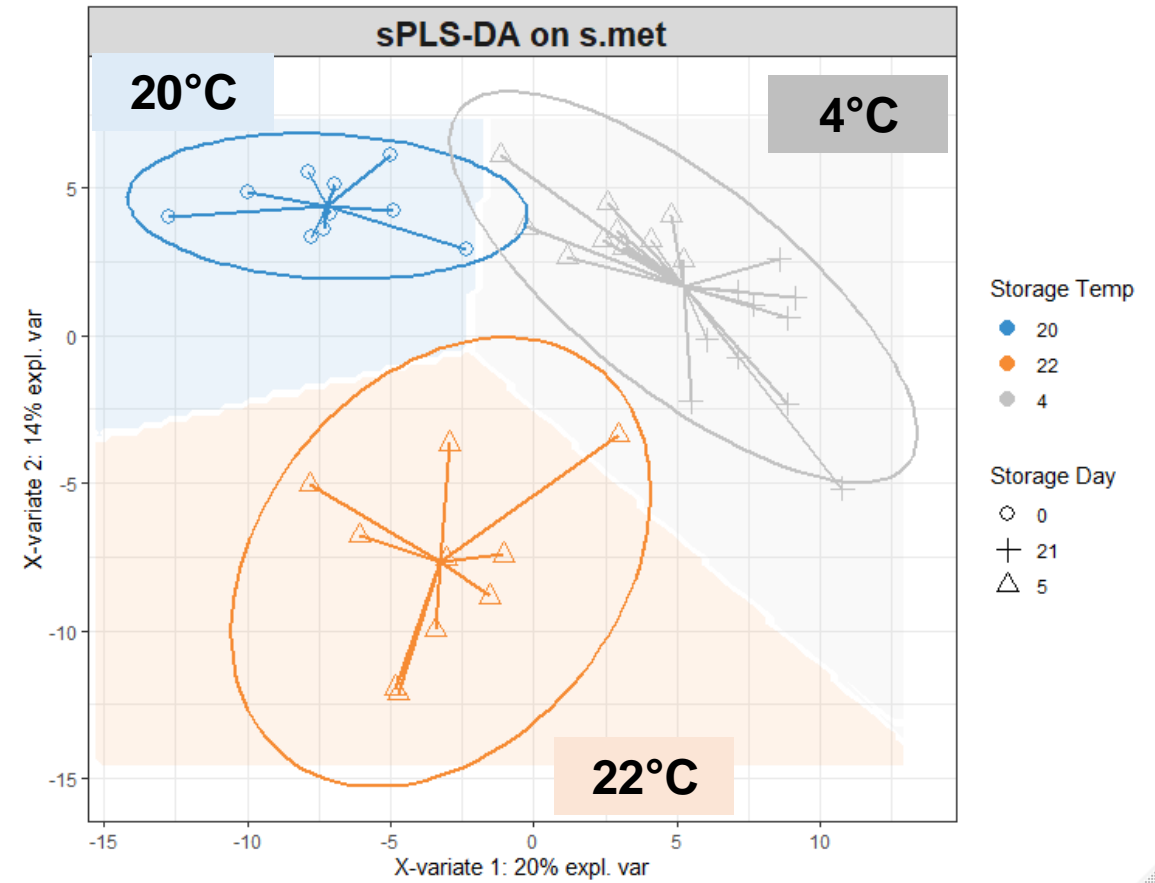


# Storage-dependent relationship between metabolism, phenotype, and function

## Hemostatic Metrics by Storage Condition

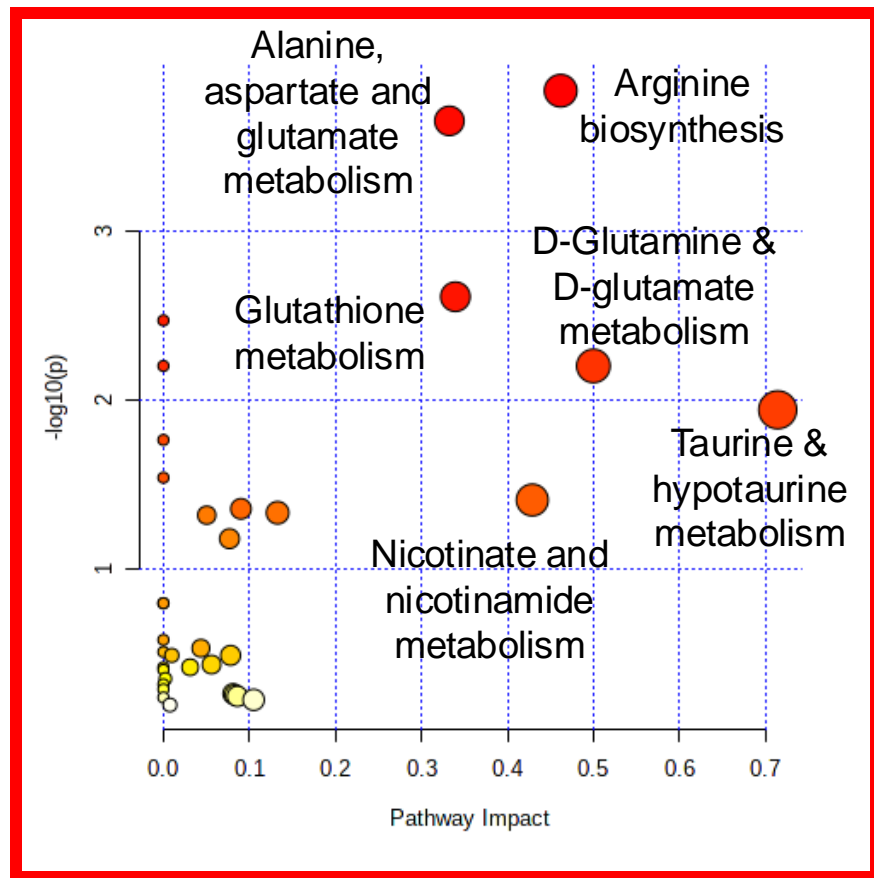


## Metabolic Profiling by Storage Condition

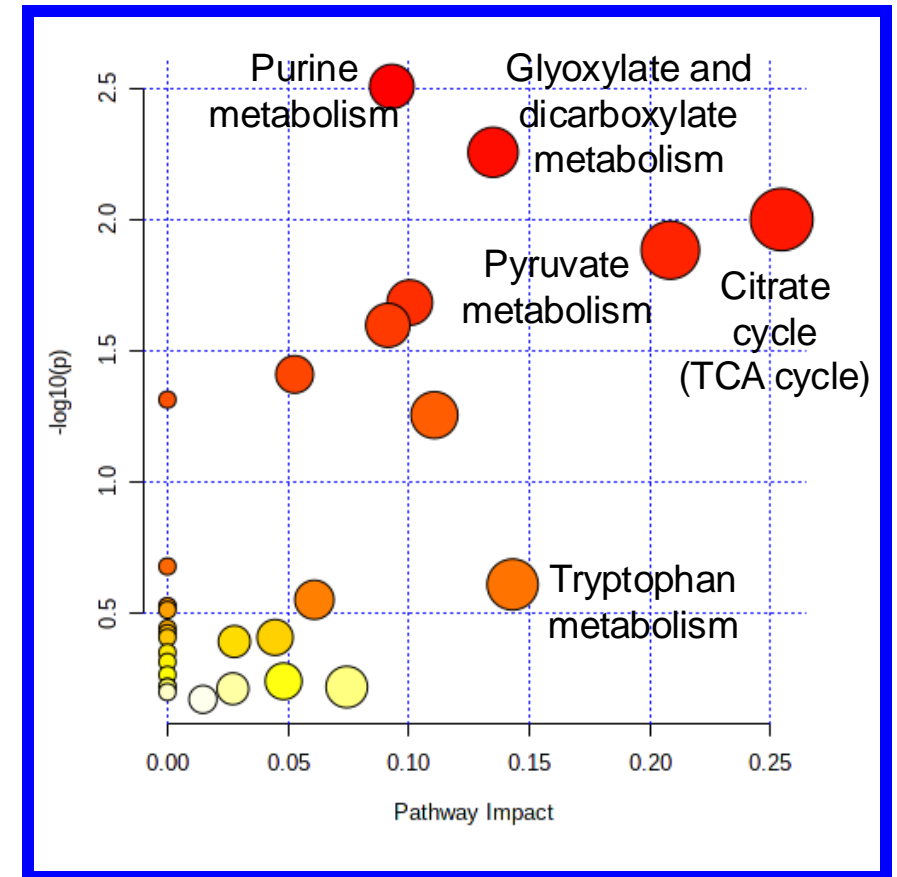
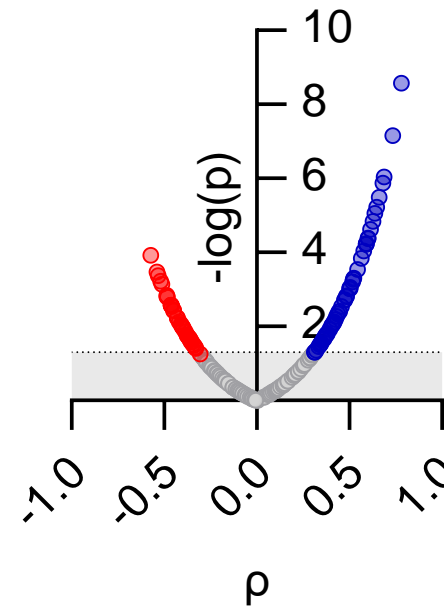


# Metabolites Associated with Metrics

Run metabolites with significant correlations through pathway analysis software MetaboAnalyst

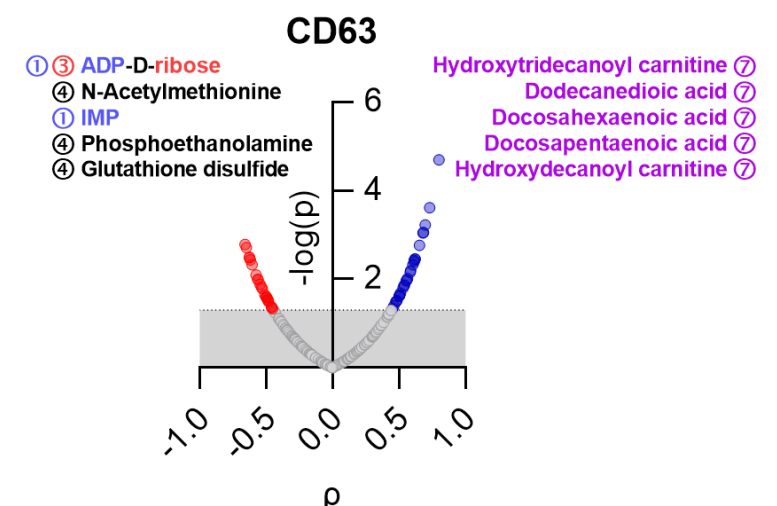
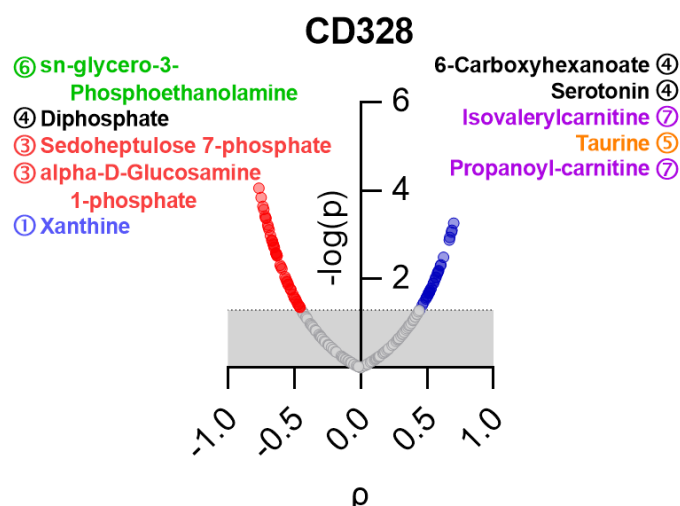
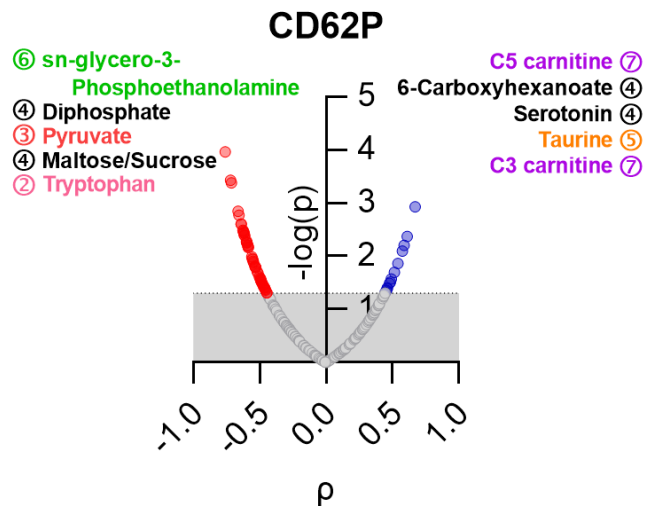
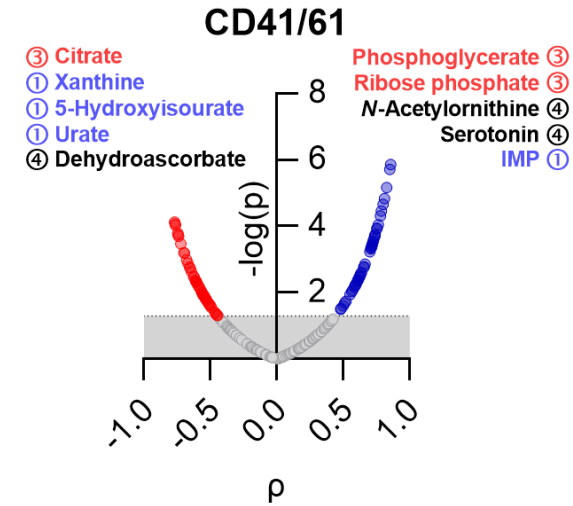
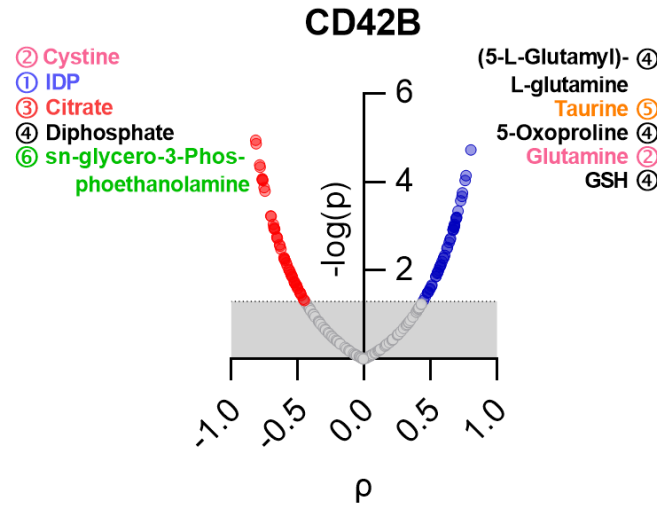
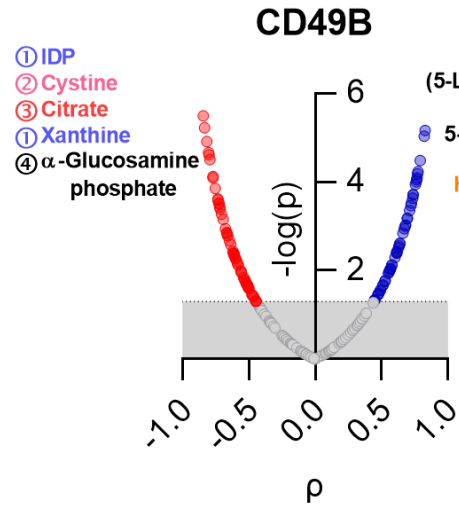


TG ETP



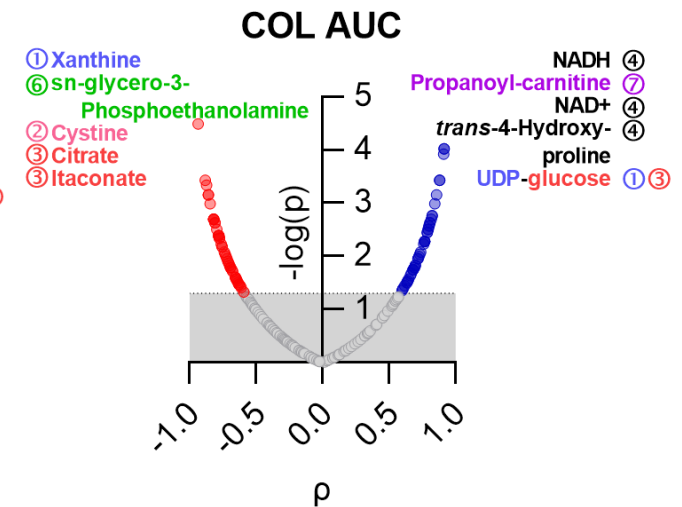
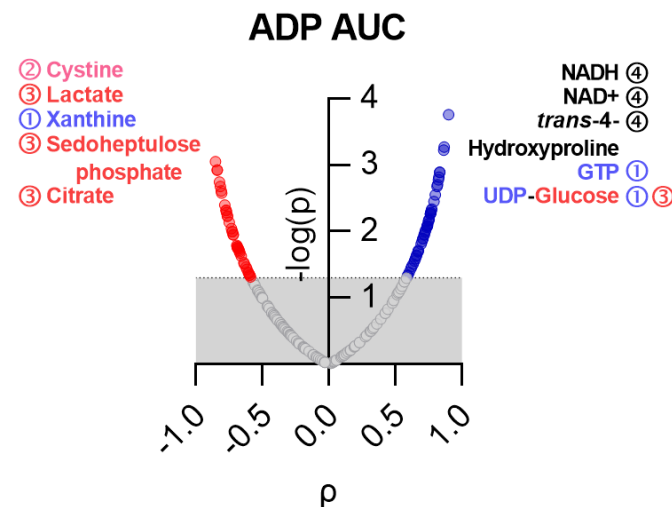
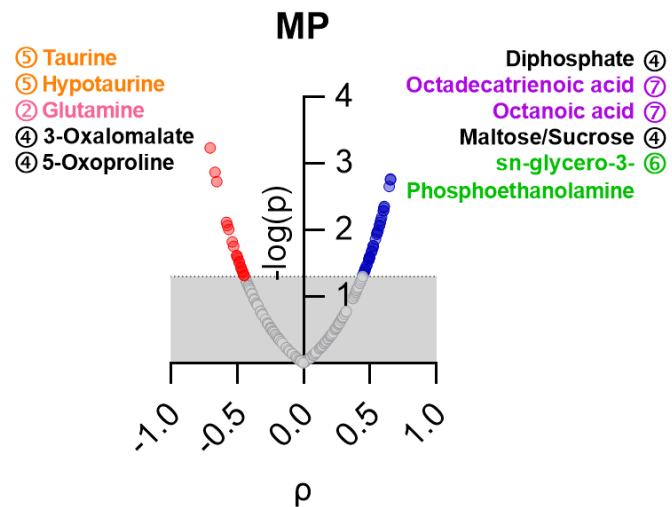
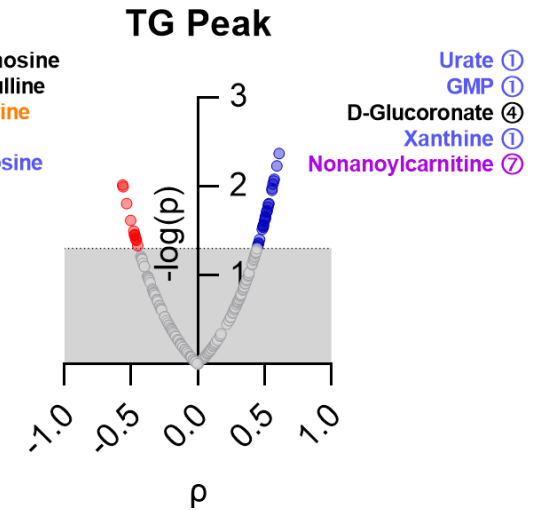
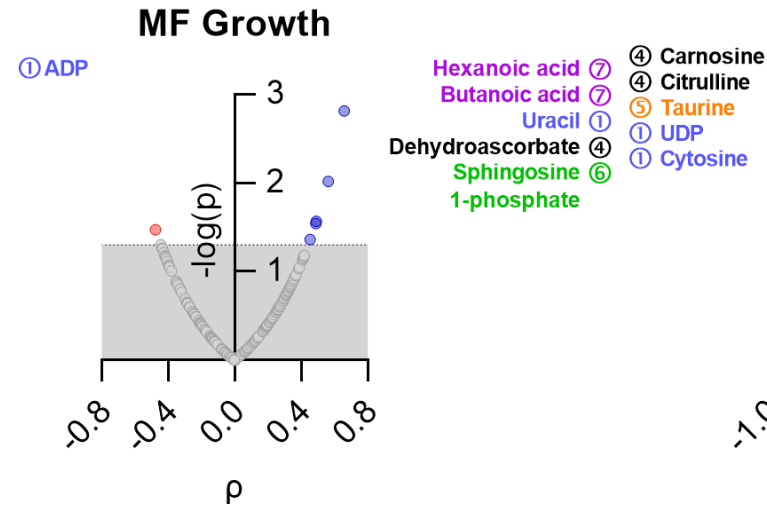
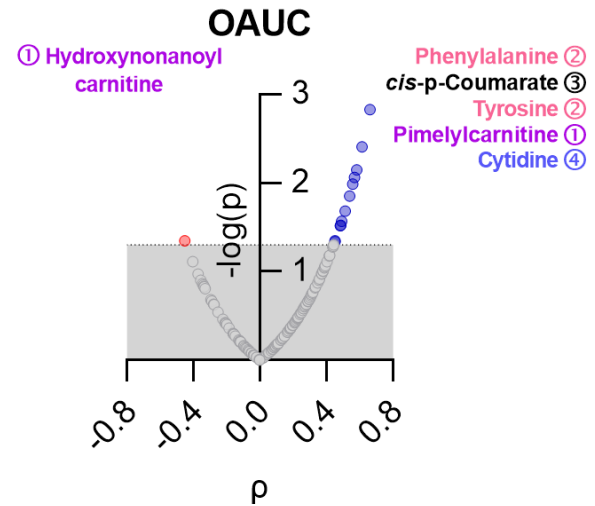
# Metabolites Associated with Metrics

Green – lipid building blocks  
 Purple – acylcarnitines and fatty acids  
 Orange – Taurine/hypotaurine  
 Red – Energy metabolites  
 Blue – nucleotides  
 Pink – amino acids

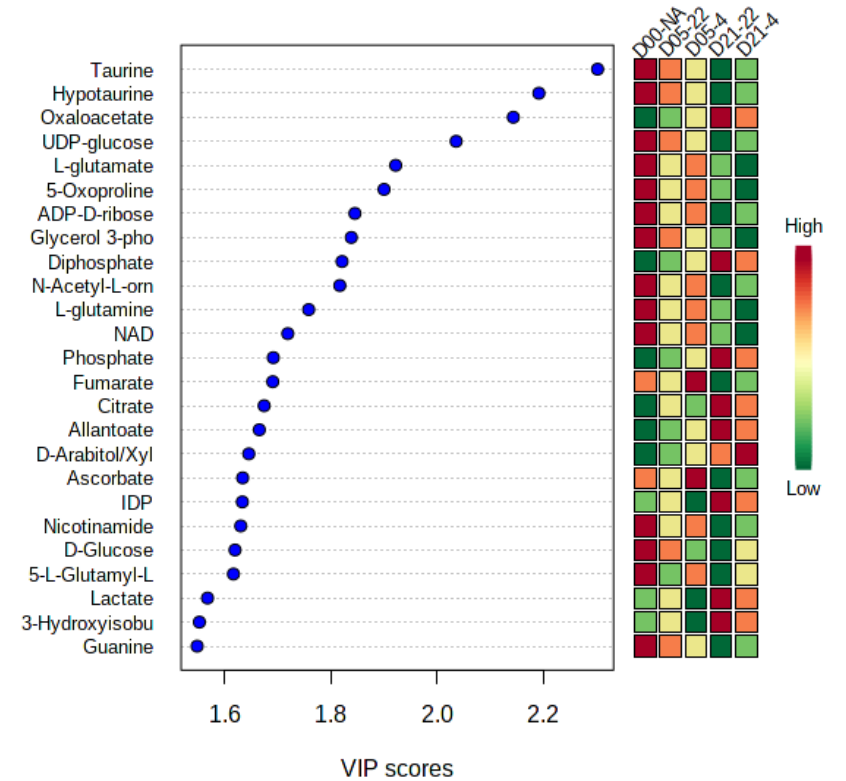
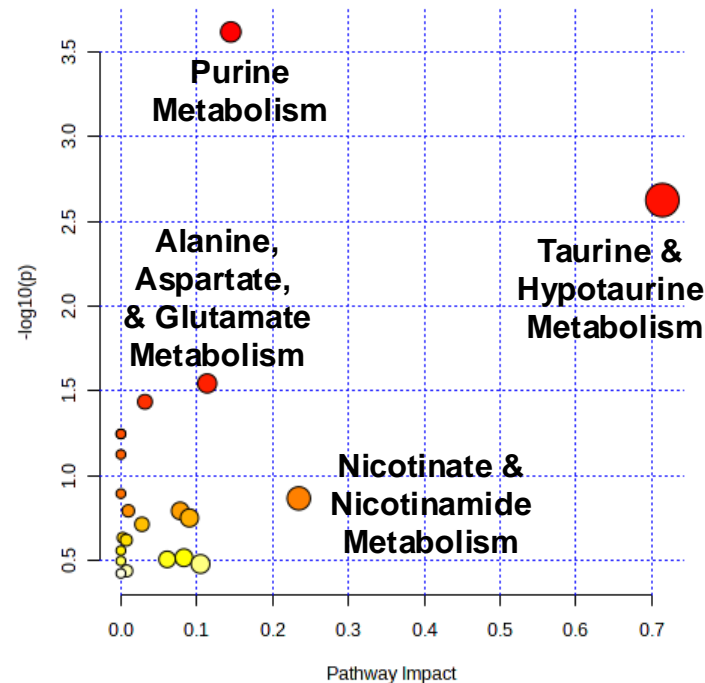
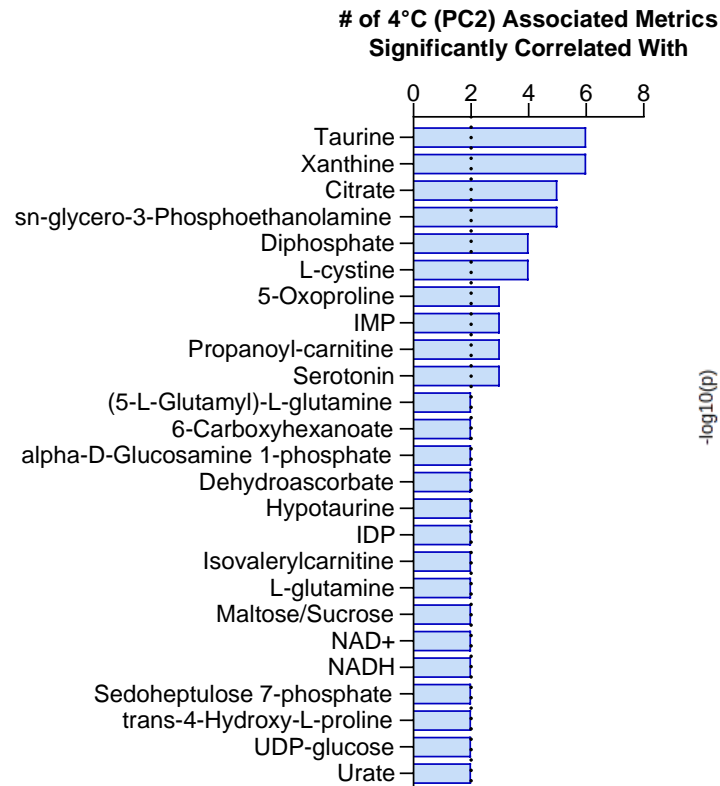


# Metabolites Associated with Metrics

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# Strong Association of Taurine with CS-PLT Function

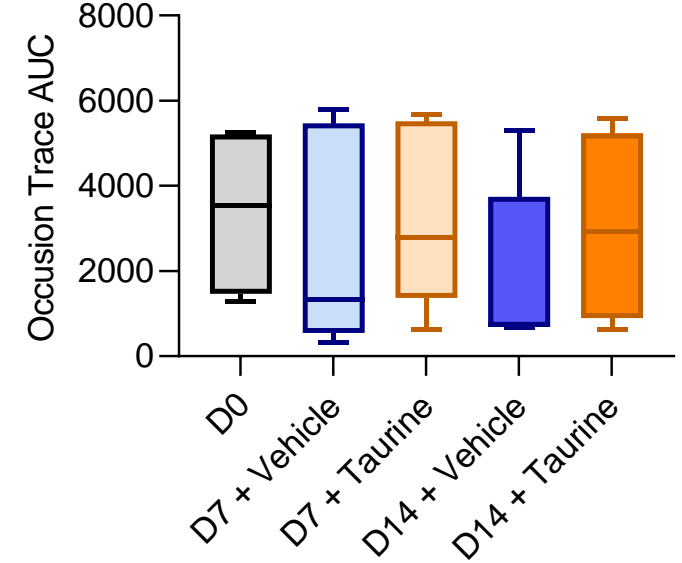
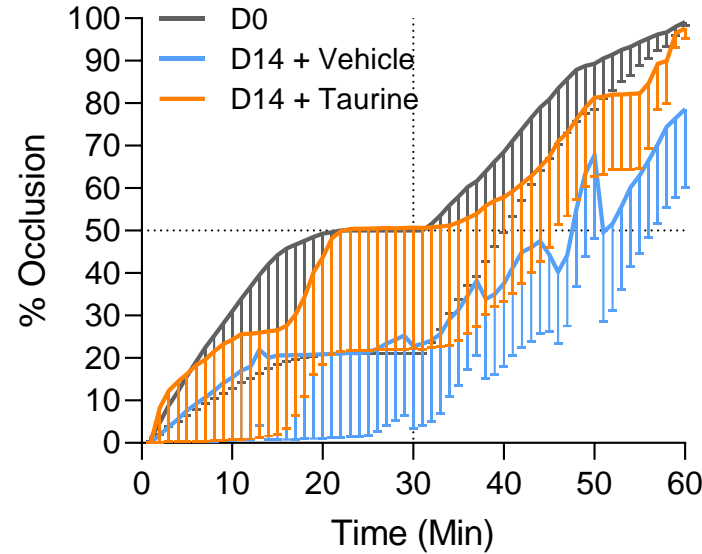
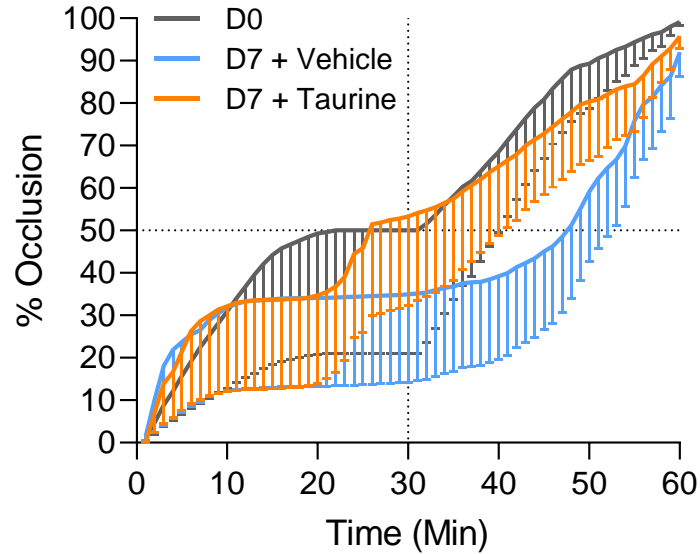


↓ Taurine ↑ Platelet Aggregation

PMID: 3099423, 2729158

***If we supplement CS-PLT products with taurine, will we reverse the hyper-aggregatory phenotype?***

# Taurine Supplementation Restores Ability to Form Thrombus under Flow Conditions



***How is taurine preserving hemostatic function?***

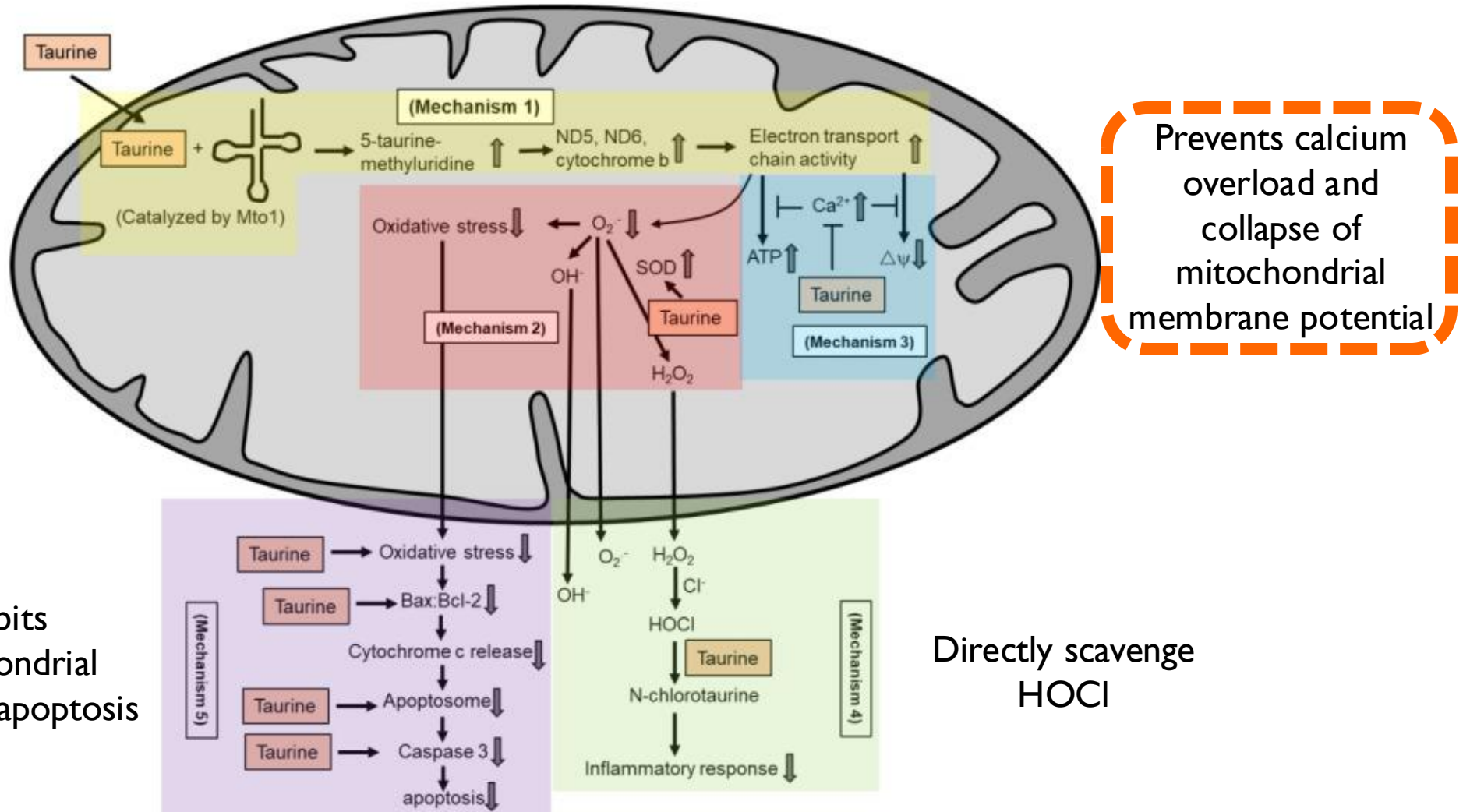
# Taurine – Mechanism of Action in Stored PLT?

Proper synthesis of mitochondrial proteins which regulate complexes in respiratory chain

Reduces superoxides by enhancing intracellular antioxidants

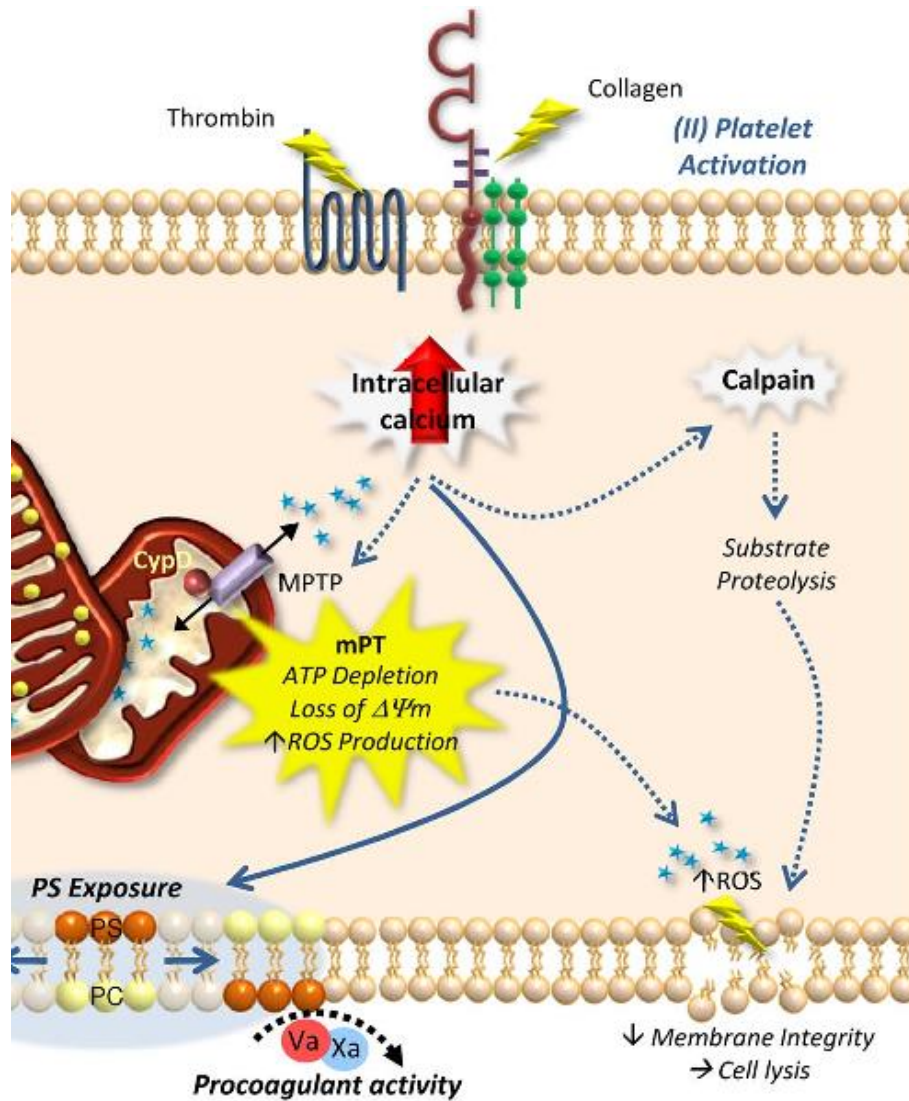
Inhibits mitochondrial mediated apoptosis

Directly scavenge HOCl





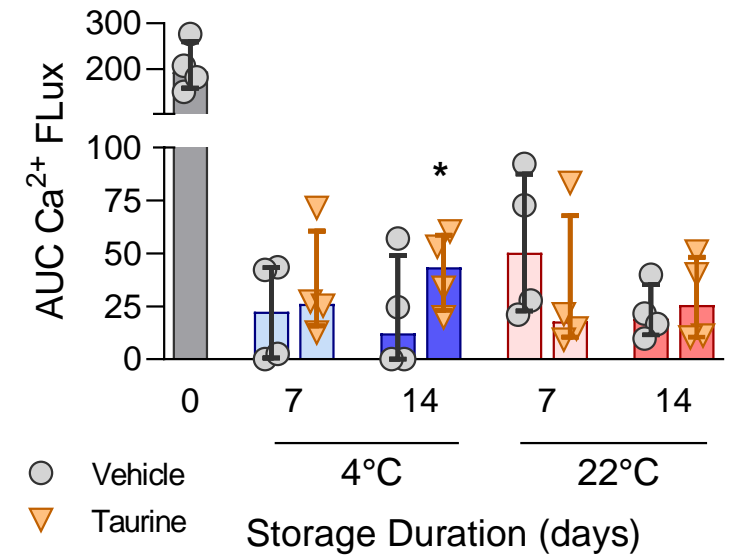
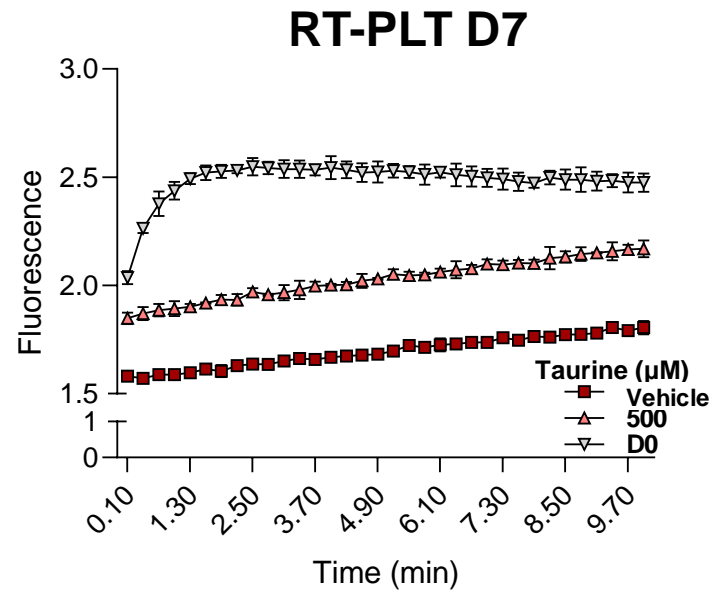
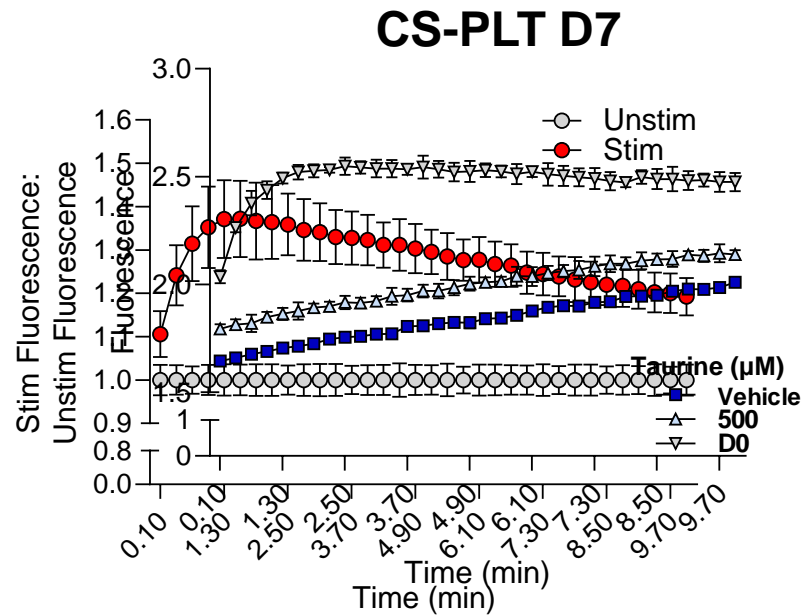
# Taurine – Mechanism of Action in Stored PLT?



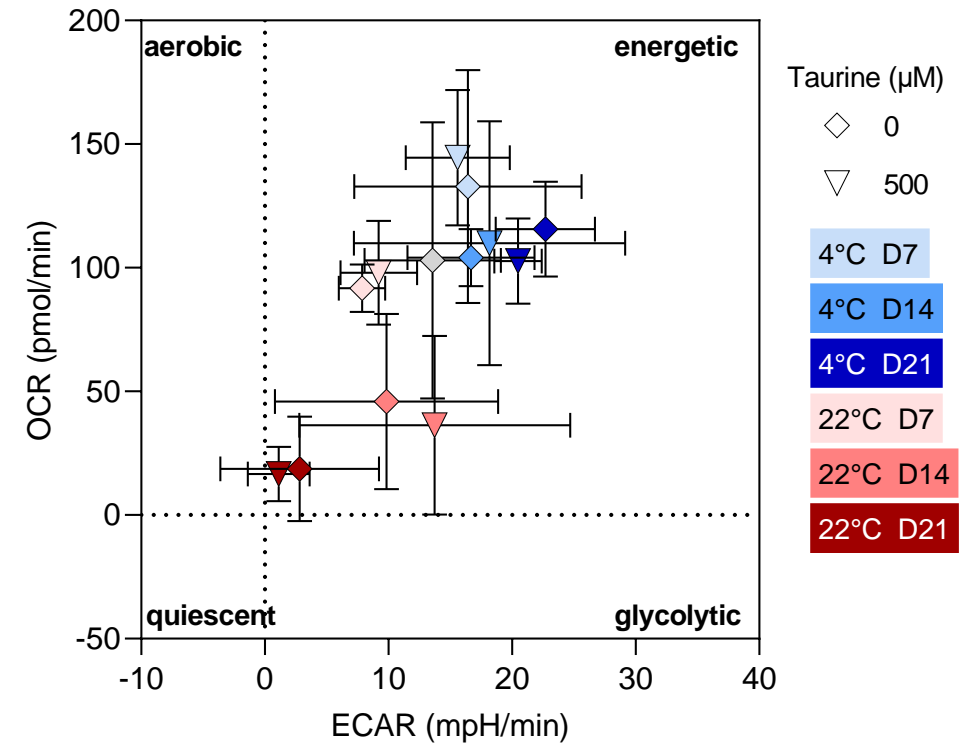
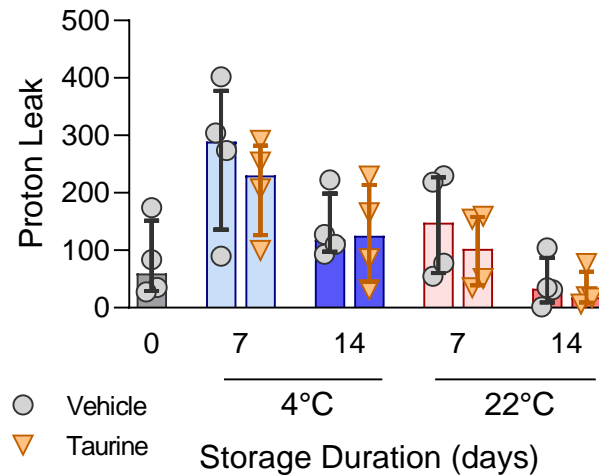
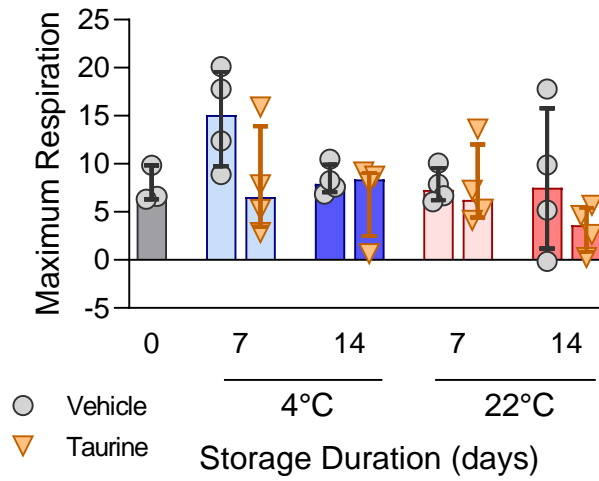
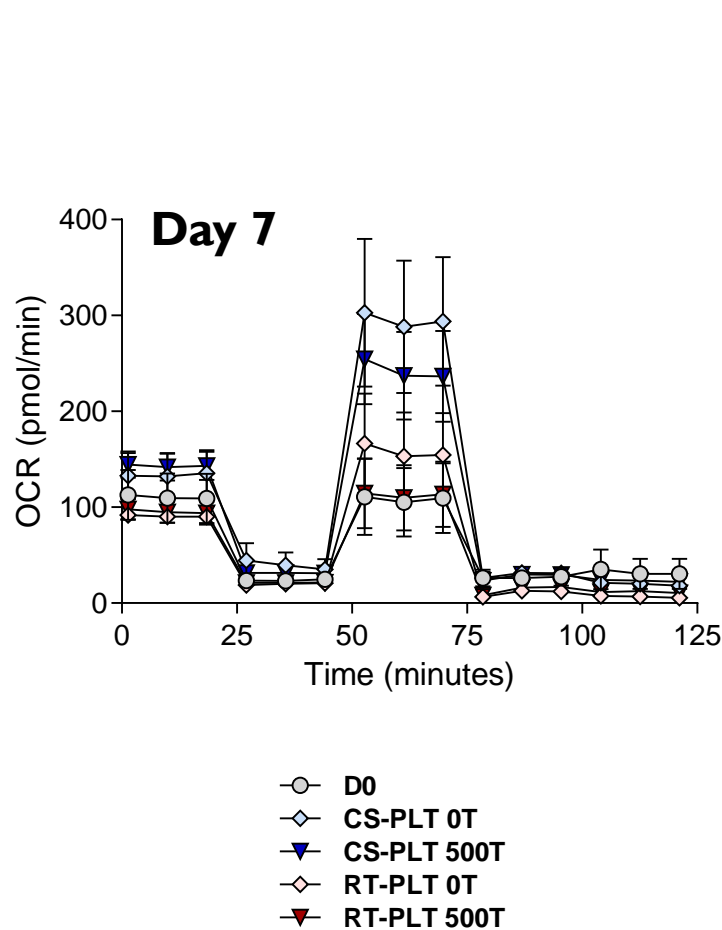
## Taurine Supplementation

1. Reduce  $\text{Ca}^{2+}$  overload
2. Stabilize mitochondrial membranes
3. Reduce procoagulant capacity

# Taurine Supplementation Does not Significantly Affect $\text{Ca}^{2+}$ Flux

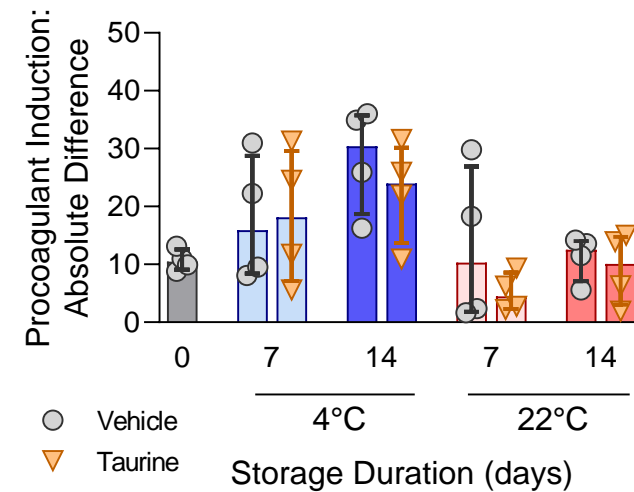
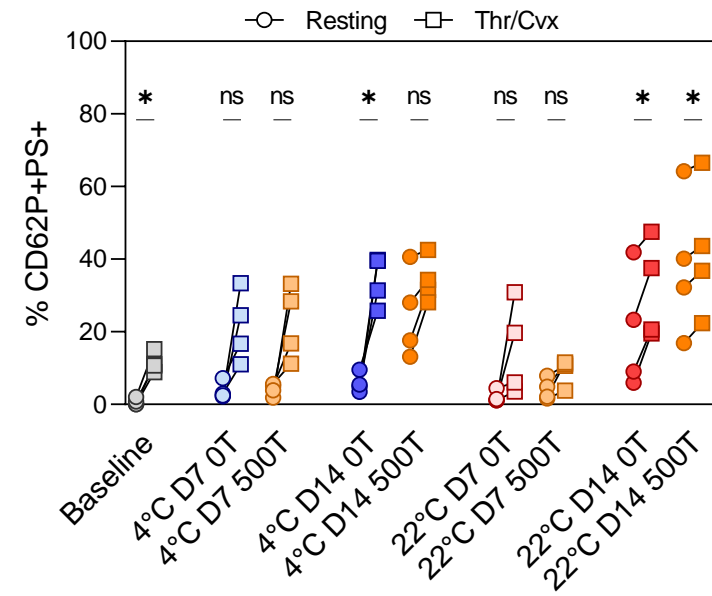
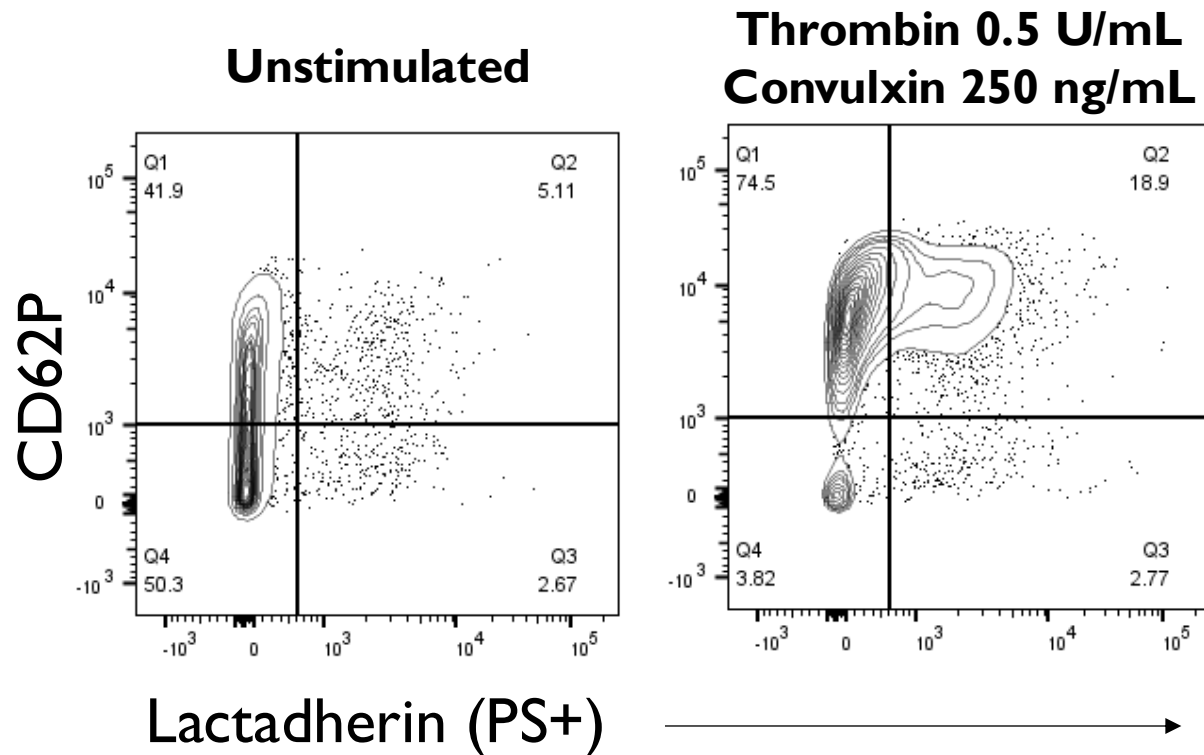


# Taurine Supplementation May Reduce Mitochondrial Stress Responses



N=4 paired donors, data graphed as median (IQR); unpublished data

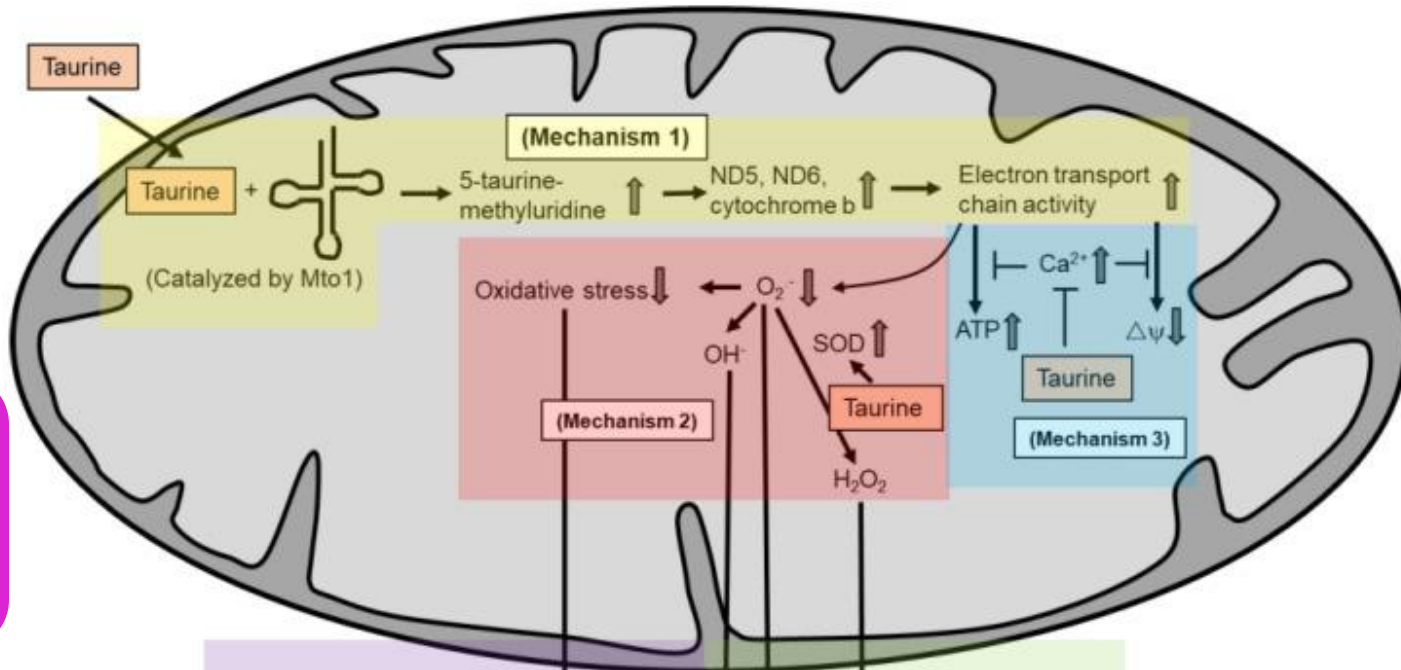
# Procoagulant Function is Not Reduced with Taurine Supplementation



N=4 paired donors, data graphed as individual points (top) and median (IQR) (bottom); paired t-test resting vs Thr/Cvx, \*, p<0.05; *unpublished data*

# Taurine – Mechanism of Action in Stored PLT?

Proper synthesis of mitochondrial proteins which regulate complexes in respiratory chain



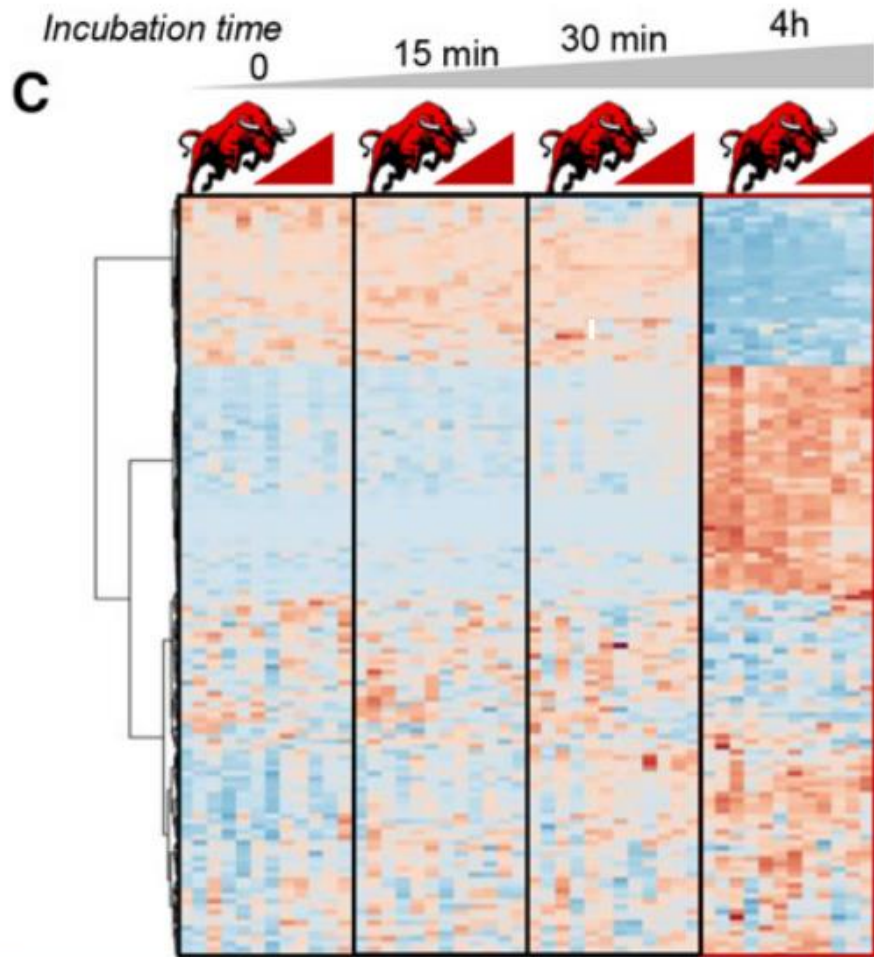
Prevents calcium overload and collapse of mitochondrial membrane potential

Reduces superoxides by enhancing intracellular antioxidants

Inhibits mitochondrial mediated apoptosis

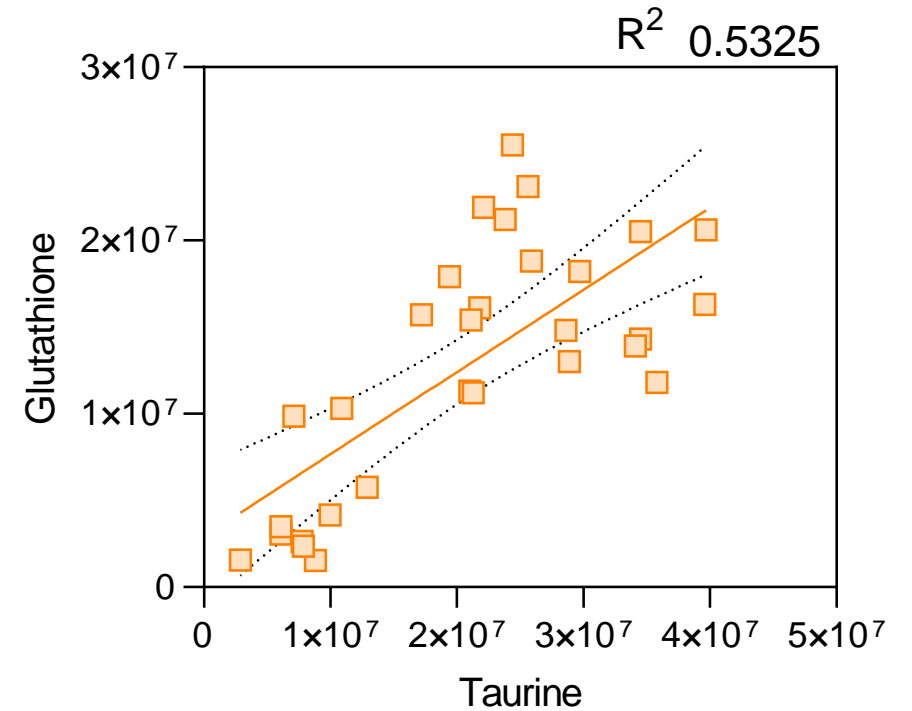
Directly scavenge HOCl

# Taurine is an antioxidant in pRBC



Taurine levels in RBC products correlated with glutathione levels and decreased hemolysis.

Taurine protected against hydrogen peroxide induced oxidant stress.



# Summary

- Intrinsic D21 CS-PLT function is as effective as D5 or D7 RT-PLT under conditions of physiologically relevant flow.
- Taurine was one of the metabolites most prominently associated with CS-PLT function
- Taurine supplementation of PLT products preserves hemostatic function under flow
- Taurine supplementation does not significantly reduce or enhance calcium mobilization, mitochondrial function, or procoagulant induction.

# Next Steps

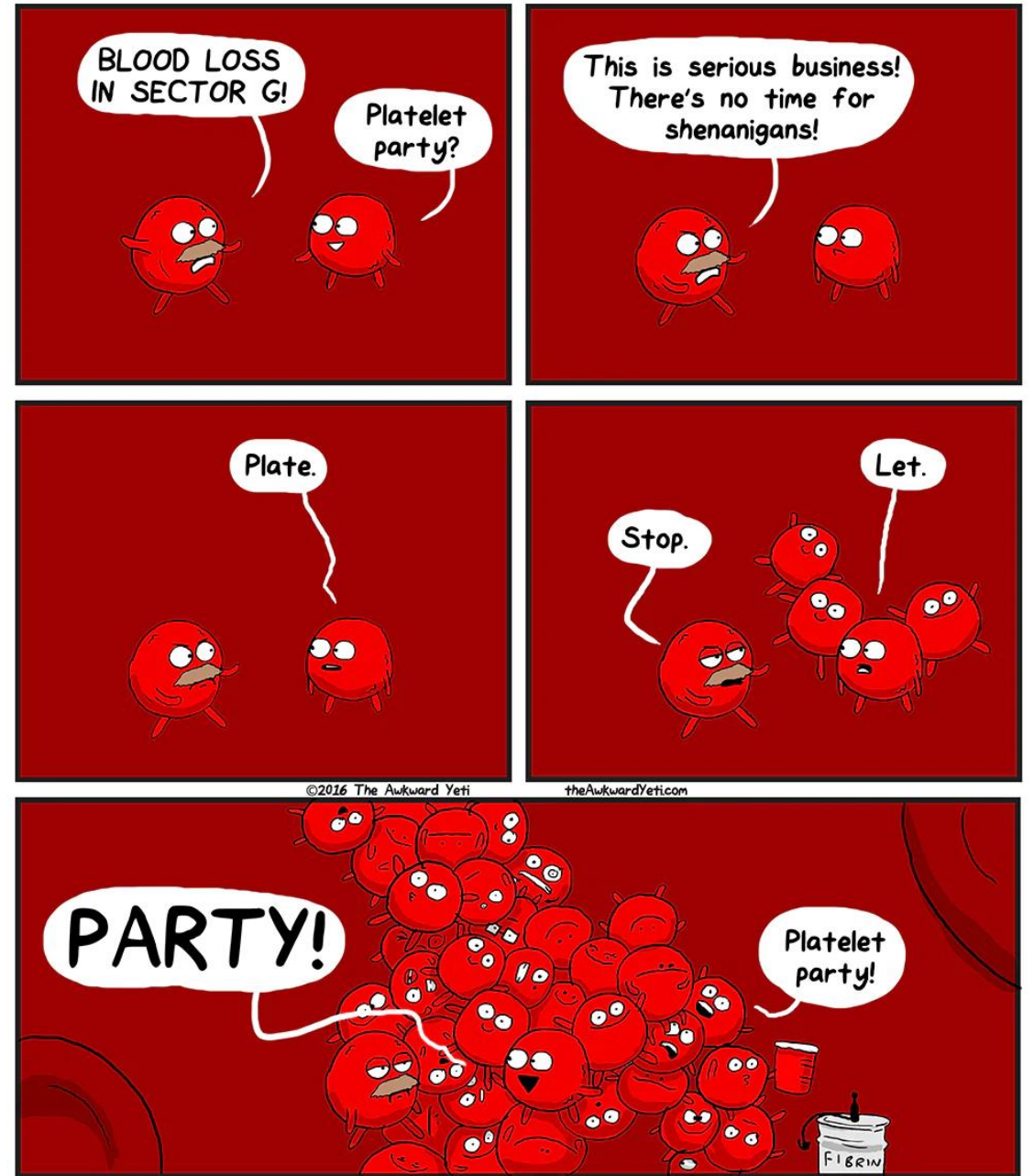
- Identify if taurine promotes antioxidation in a non-mitochondrial fashion in platelets (similar to red blood cells).
- Study platelet function after supplementation with other significantly associated metabolites.
- Determine if CS-PLT ± Taurine have augmented interaction with inflamed and/or damaged endothelium under flow conditions *in vitro*



Questions?  
Comments?  
Airing of grievances?

[kthomas@vitalant.org](mailto:kthomas@vitalant.org)

THANK  
YOU!!!



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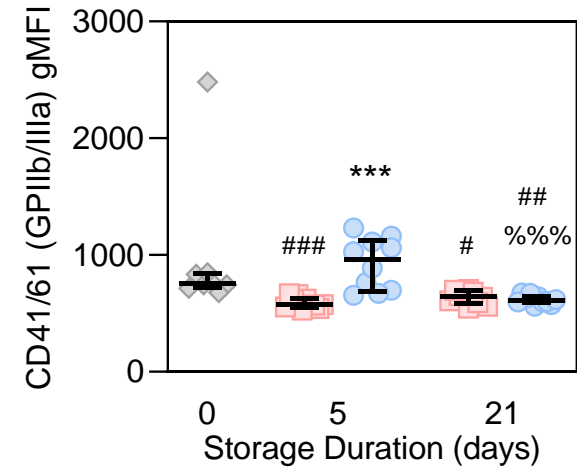
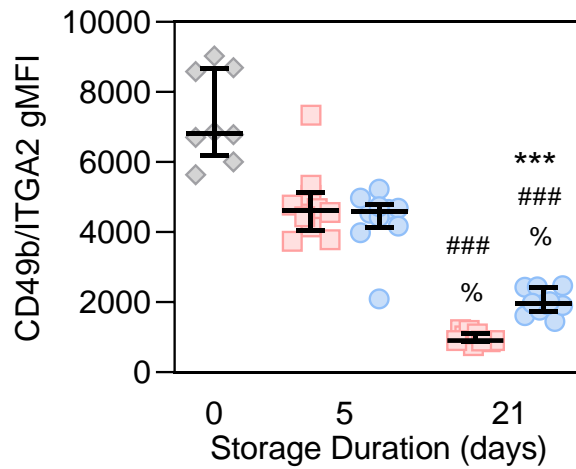
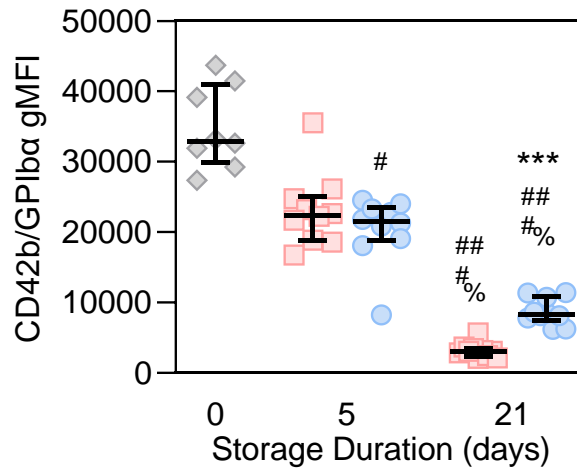
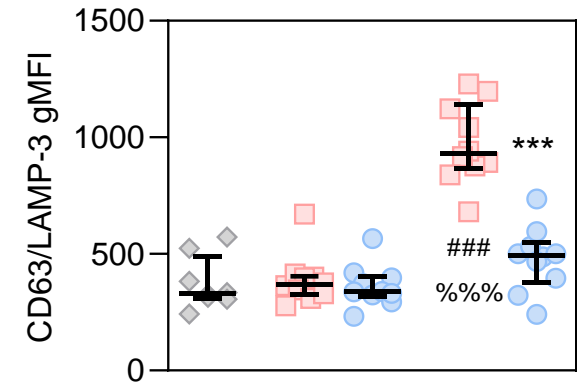
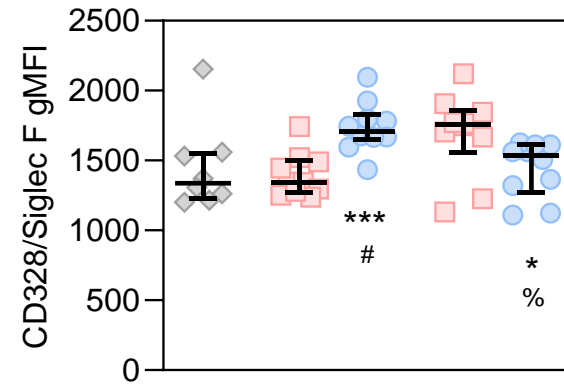
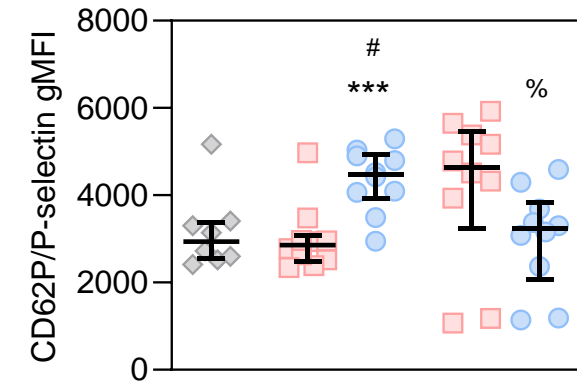
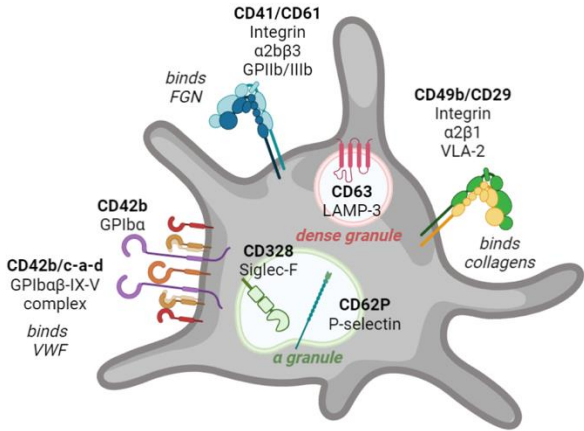


# CS-PLT Clinical Trials

Name	Title	Status	Conditions	Interventions	Locations
<i>CriSP-TBI</i>	Cold-stored Platelet Early Intervention in TBI	Recruiting	Traumatic Brain Injury	Cold Stored Platelets, Standard Care	University of Pittsburgh
<i>CHASE</i>	Extended Cold Stored Apheresis Platelets in Cardiac Surgery Patients	Not yet recruiting	Hemorrhage, Platelets, Defect, Bleeding, Surgical Blood Loss	Cold stored platelets in 100% plasma stored for 10-14 days, Room temperature stored platelets in 100% plasma stored for up to 7 days	Swedish Medical Center - Cherry Hill, Seattle, Washington
<i>RASP</i>	Reversal of Aspirin Antiplatelet Therapy With Cold Stored Platelets	Active, not recruiting	Reversal of Platelet Aggregation Inhibitors	Autologous Room Temperature stored Platelet transfusion, Autologous Cold Stored Platelet transfusion	Bloodworks Northwest
<i>CAPP</i>	Cold Apheresis Platelets in PAS	Unknown status	Platelet Transfusion	Refrigerated storage of apheresis platelets in PAS/plasma, Infusion of aliquot of autologous radiolabeled platelets	Bloodworks Northwest
<i>R-DAPT</i>	Reversal of Dual Antiplatelet Therapy With Cold Stored Platelets	Unknown status	Bleeding, Platelet Dysfunction Due to Drugs, Platelet Dysfunction	Autologous Platelet Transfusion: room-temperature-stored, cold-stored 5 days, cold-stored 10 days, cold-stored 15 days; Aspirin & Clopidogrel	Bloodworks Northwest
<i>Brrr</i>	Assessment of Whole Blood Cold Stored Platelets	Completed	Healthy	Blood Collection and autologous re-infusion	Puget Sound Blood Center
<i>CriSP-HS</i>	Cold Stored Platelet in Hemorrhagic Shock	Recruiting	Trauma, Hemorrhage	Cold Stored Platelets, Standard Care	UCLA, UCSF, University of Mississippi, Baylor College of Medicine, UT HSC Houston
<i>CHIPS</i>	CHILLED Platelet Study "CHIPS"	Recruiting	Acute Blood Loss	Cold Stored Platelets, Room Temperature Platelets	UCLA, UF, Johns Hopkins, Mayo Clinic, Wake Forest, Cincinnati Childrens, CHOP, University of Pittsburgh, Rhode Island Hospital, UT Childrens Medical Center Dallas, UT Medical Center Dallas, Baylor Texas Childrens, University of Wisconsin- Madison

# Cold Storage Preserves Platelet Receptor Expression out to Day 21

## RECEPTOR EXPRESSION

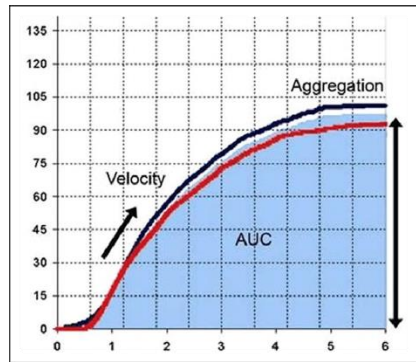


Baseline    22°C    4°C

D21 CS-PLT have same surface level of CD62P, CD328, and CD41.61 as D5 RT-PLT, but reduced CD42B and CD49B.

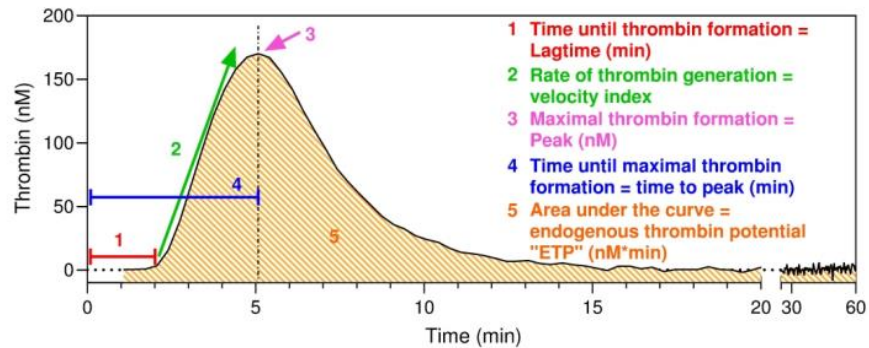
# D2I CS-PLT Maintain Responsiveness To Thrombin

## AGGREGATION

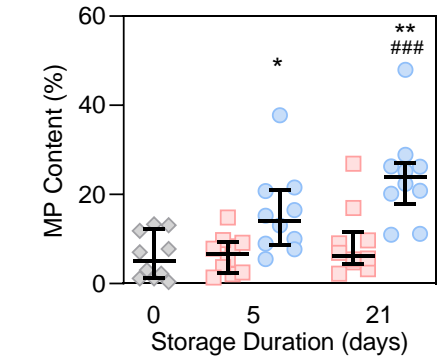
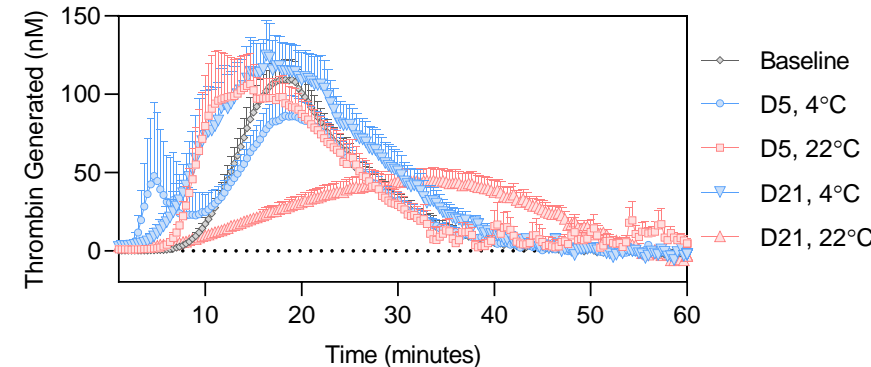
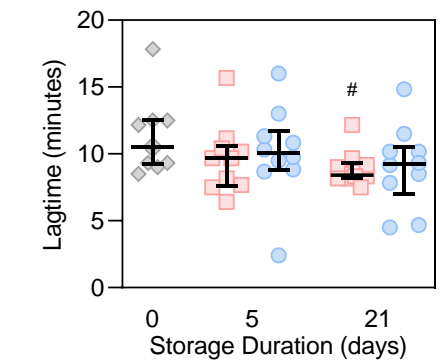
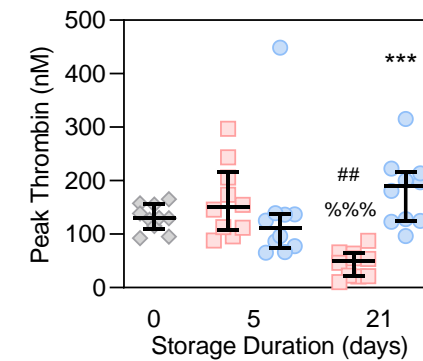
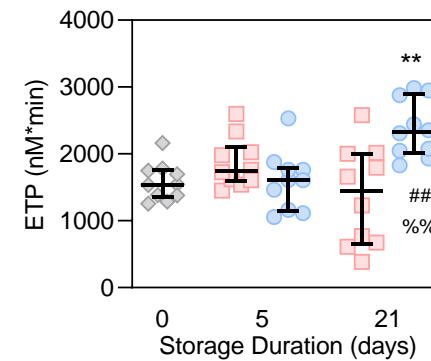
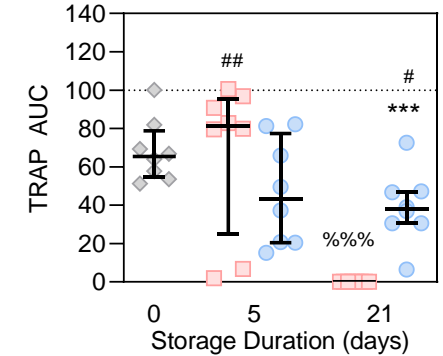
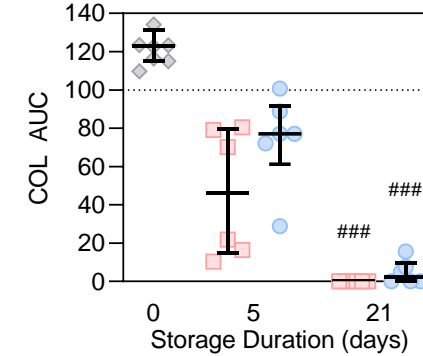
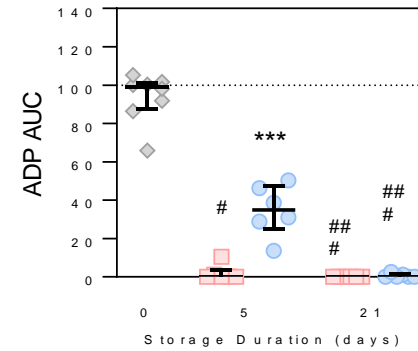


## THROMBIN GENERATION

Thrombogram

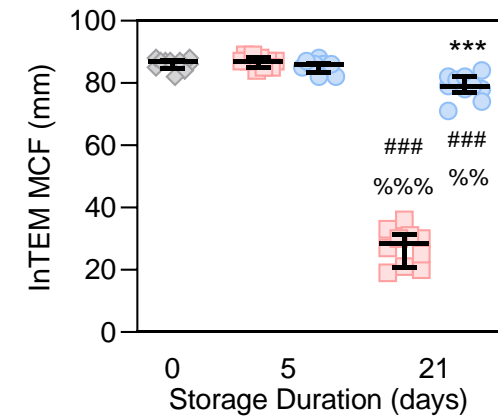
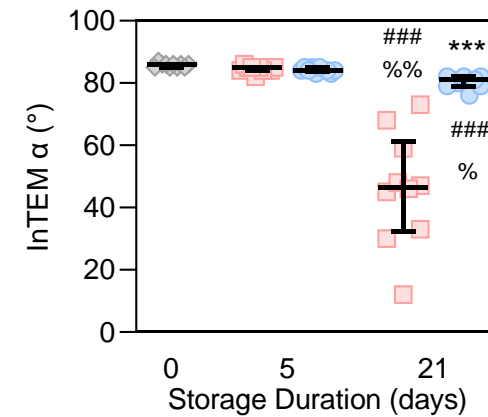
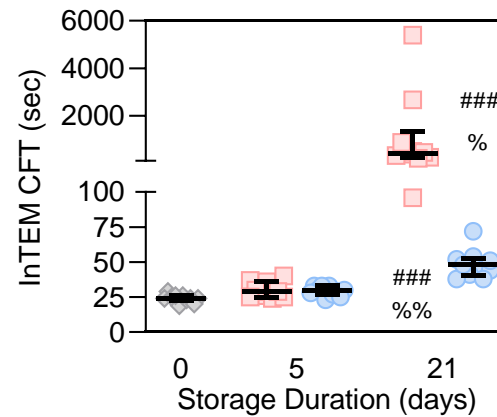
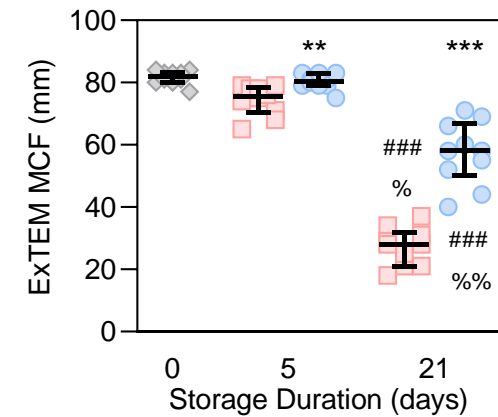
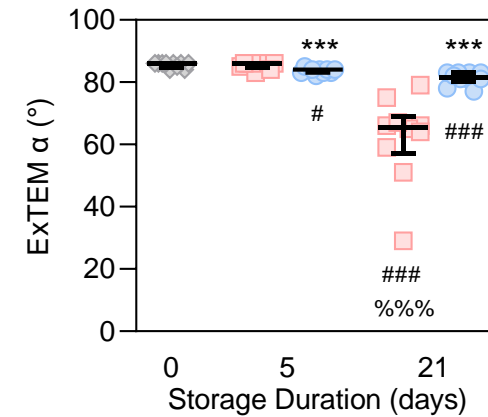
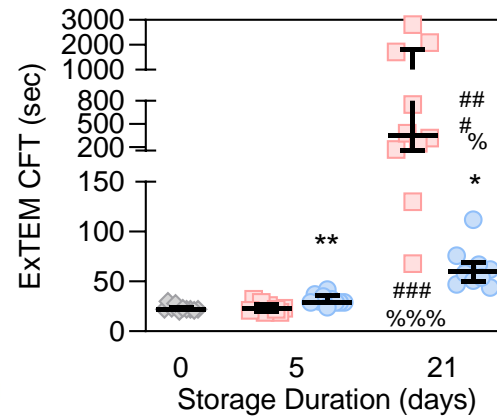
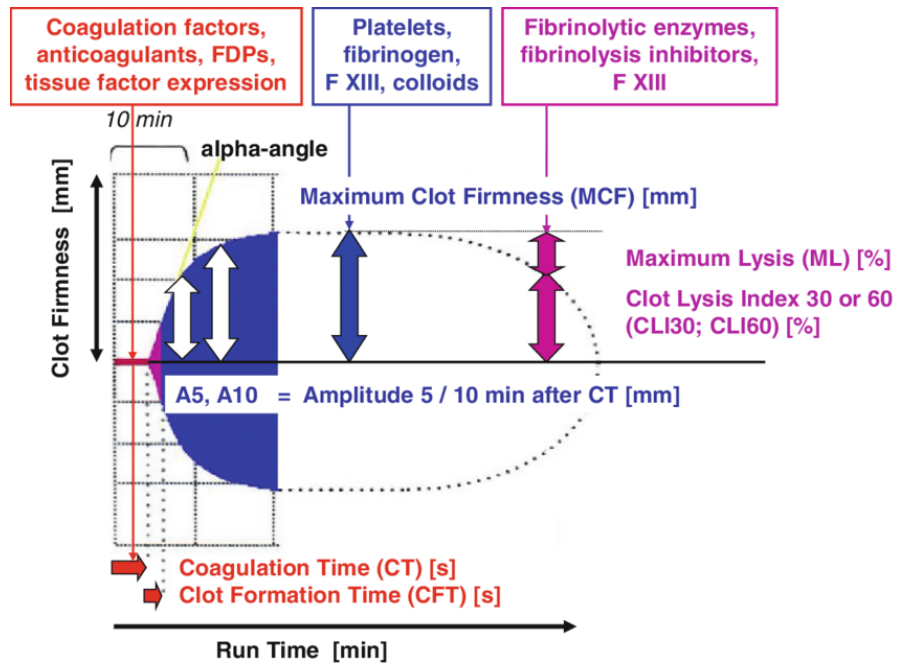


Baseline 22°C 4°C



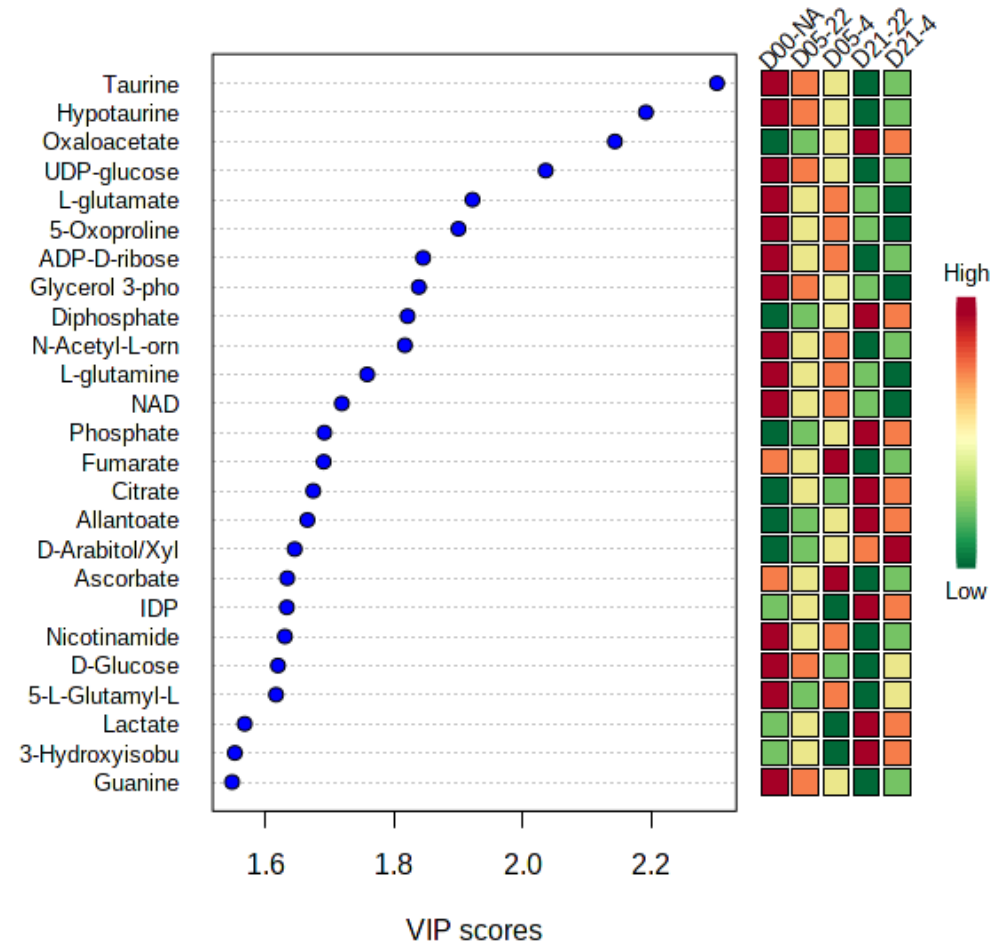
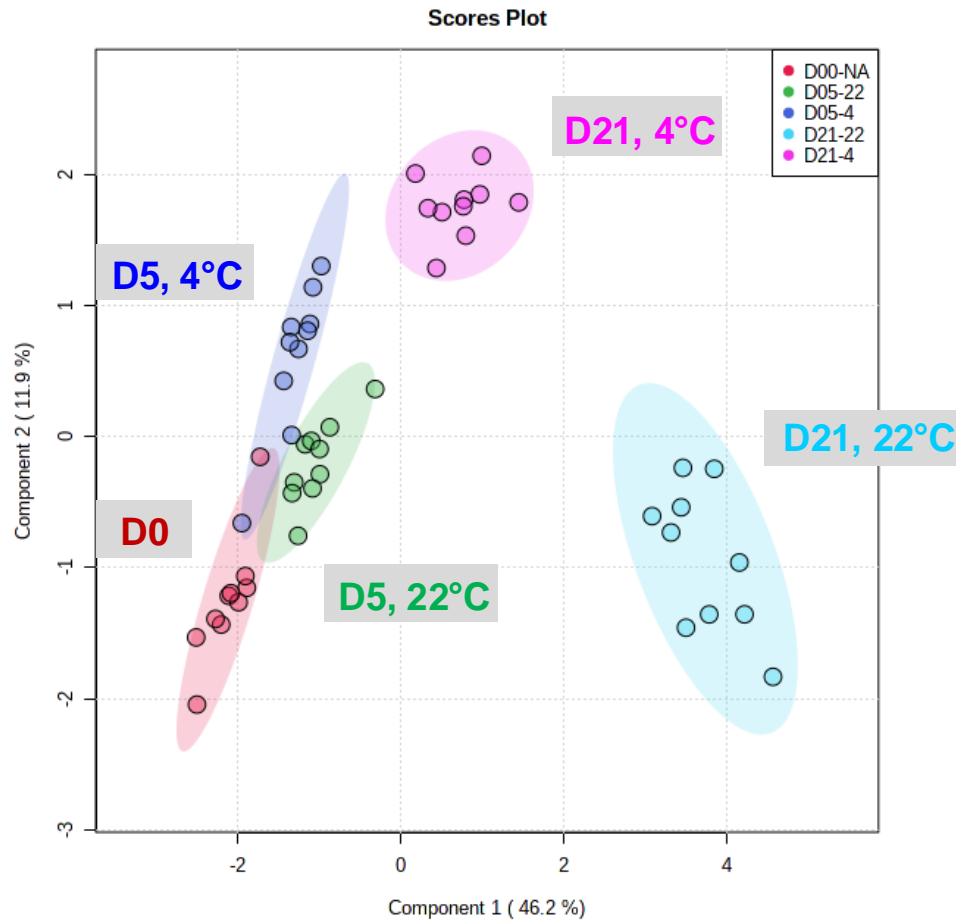
# Cold Storage Preserves Clot Formation in the Absence of Flow

## CLOT FORMATION



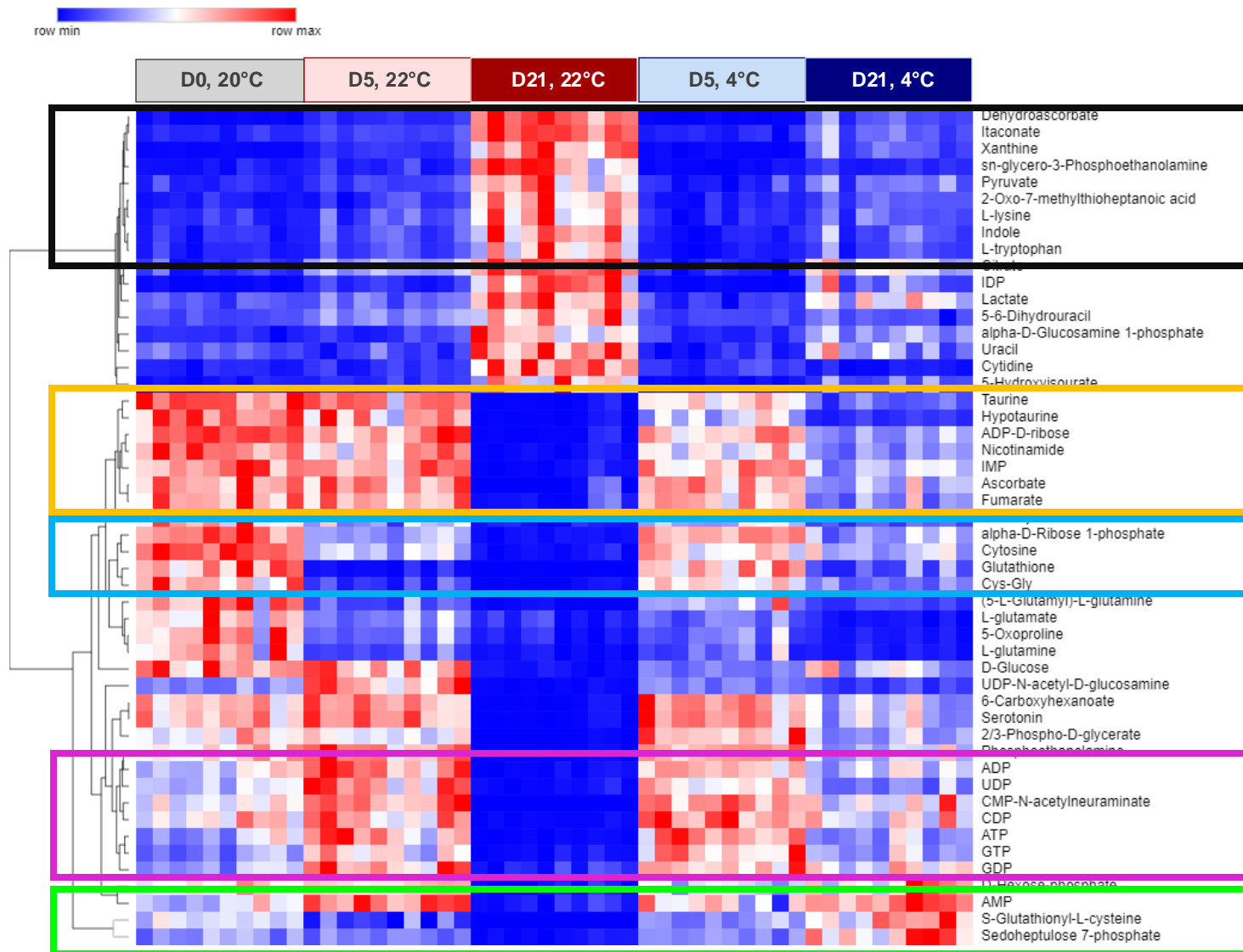
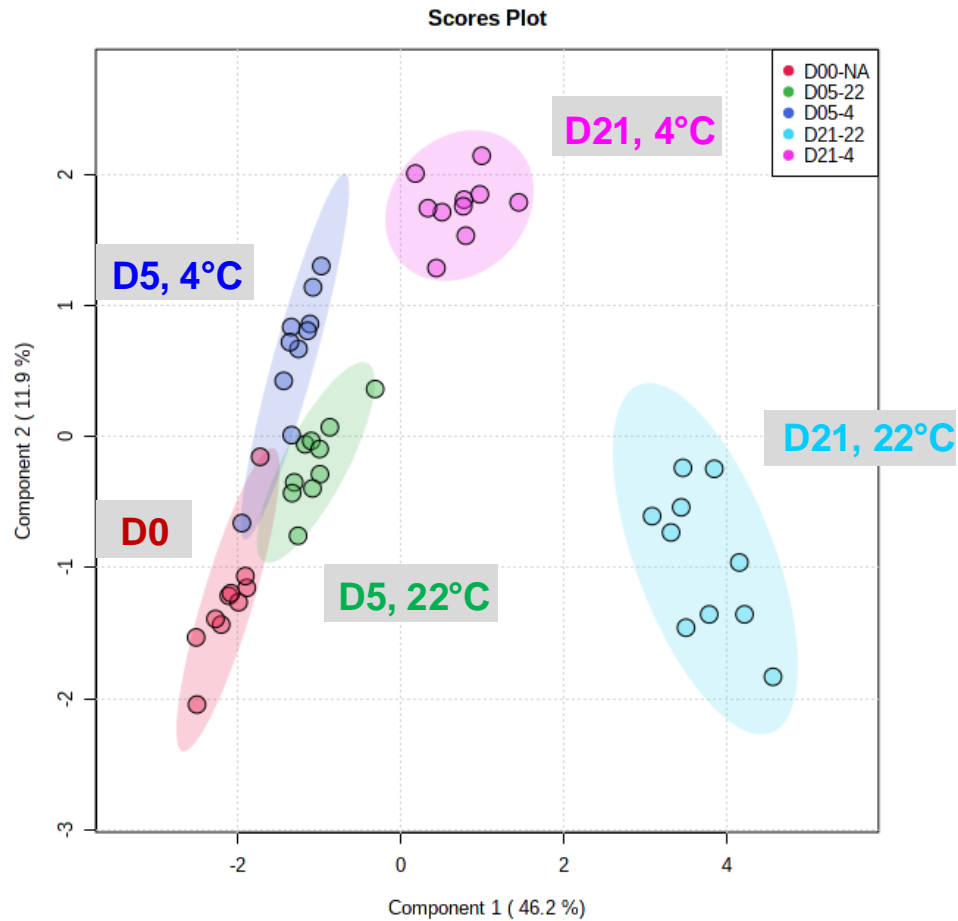
Baseline 22°C 4°C

# Metabolic Profiling of Stored Platelet Products

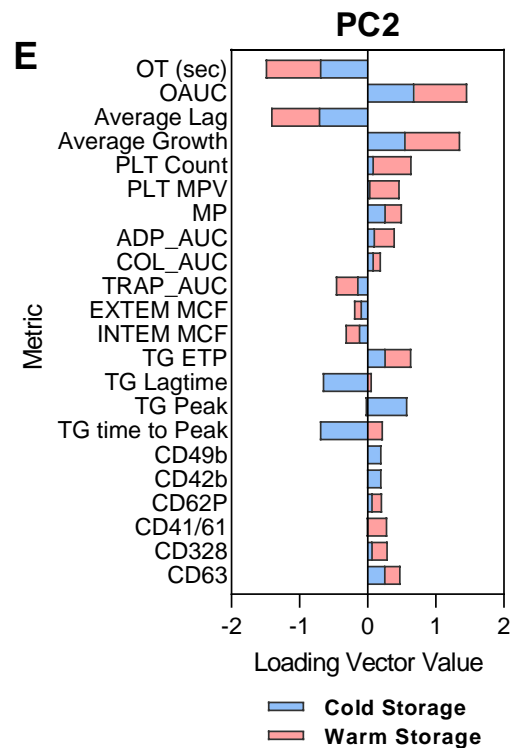
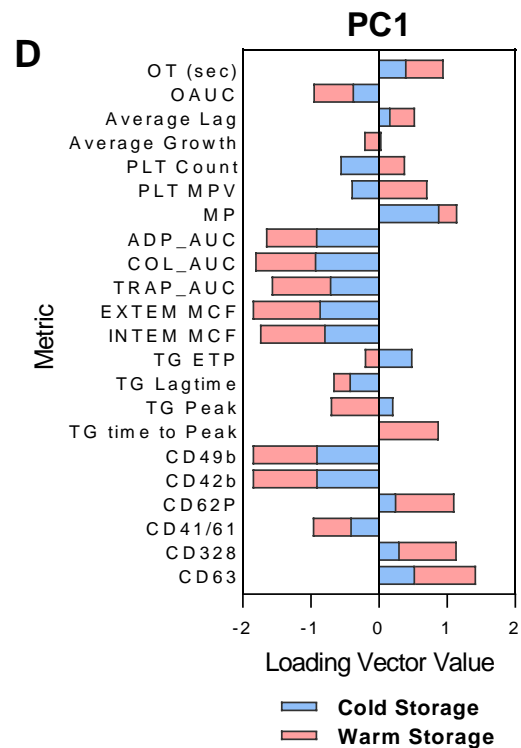
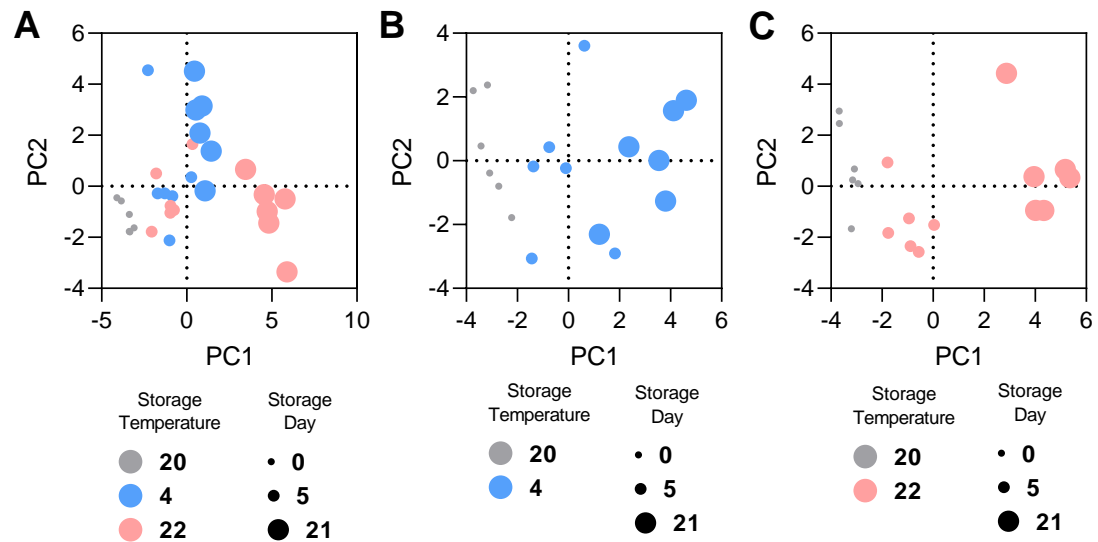




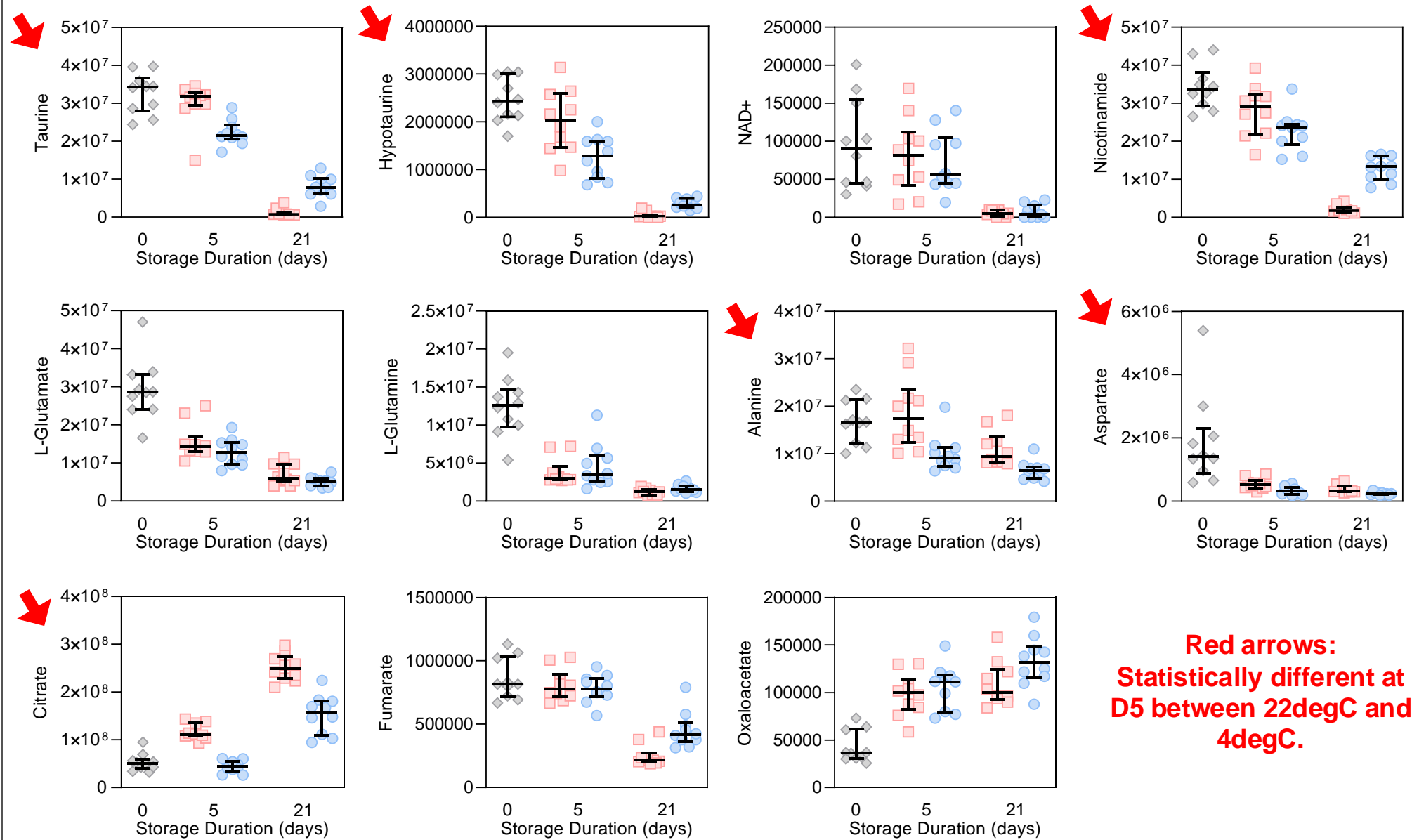
# Metabolic Profiling of Stored Platelet Products







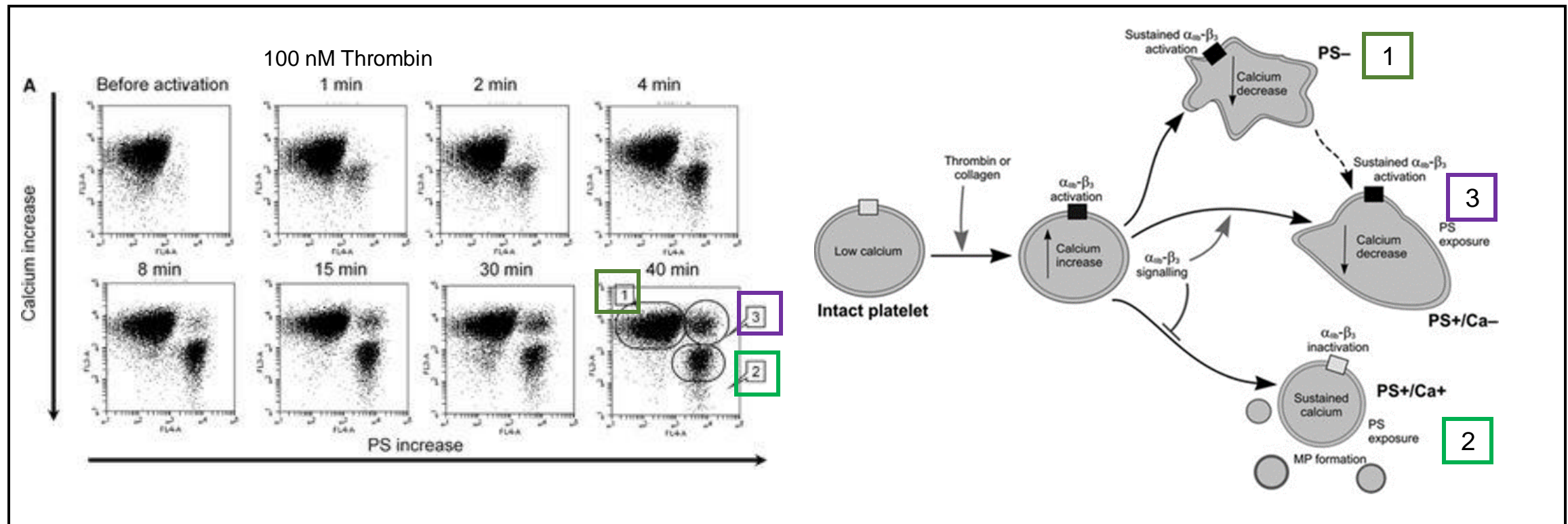
# Key Metabolites Differentiating Function

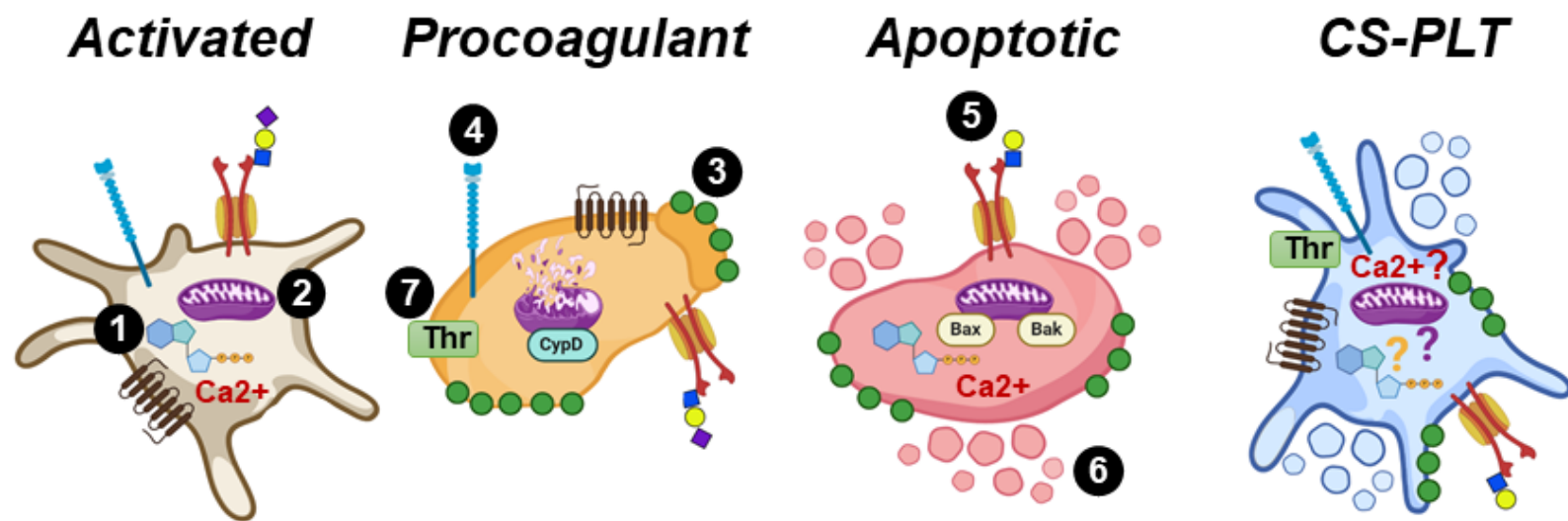


**Red arrows:**  
Statistically different at  
D5 between 22degC and  
4degC.

# CD41/61 (Integrin $\alpha$ IIb $\beta$ 3) & Platelet Phenotype

- Upregulated in response to cold storage by D5 (our data)
- Topolav et al. found high densities of platelets and storage with shaking led to a unique third population of activated platelets





	<i>Activated</i>	<i>Pro-coagulant</i>	<i>Aged/ Apoptotic</i>	<i>CS-PLT</i>
1. ATP-dependent	+	-	+	+?
2. Mitochondria	Intact	Disrupted	Intact	Intact?
Ca <sup>2+</sup> Responsive	+++	-	++	+?
3. PS Expression	-	+	+	+
4. CD62p Expression	+	+	-	+
5. Desialylated CD42b	-	-	+	+
6. Microparticle Production	-	-	+	+
7. Thrombin Generation	+/-	+++	+/-	+++
Adhesion	+++	-	+/-	++
Aggregation	+++	-	+/-	++
Coagulation	+	+++	+	+++
Cell Death	n/a	necrosis	apoptosis	?