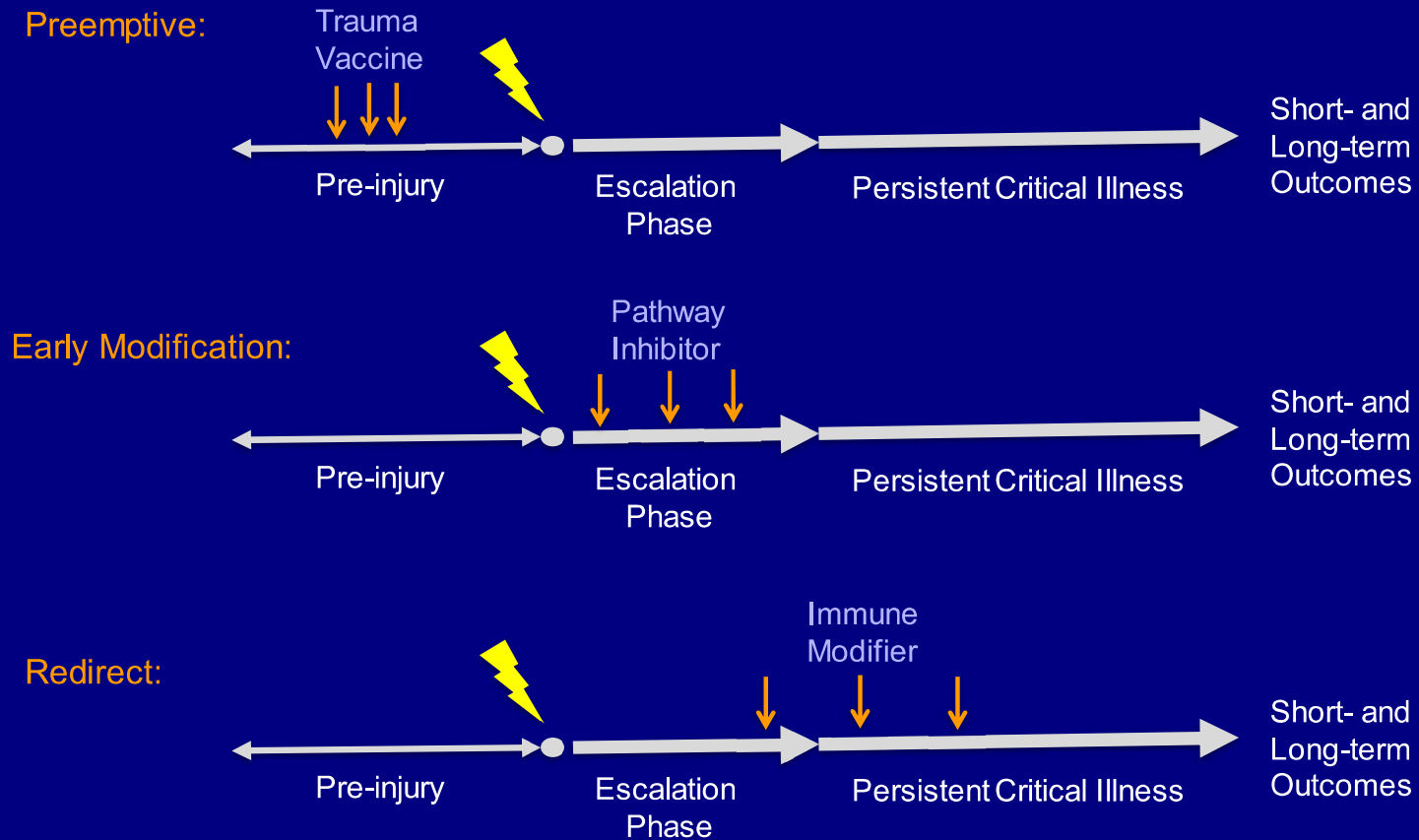


Opportunities for early interventions in trauma

Exploiting time zero



Lamparello, et al. JIM, 2019.

Reasons to perform multi-omics in trauma

- Gain insights on biologic processes driving outcomes
- Identify prognostic biomarkers
- Identify therapeutic targets
- Build representative models (e.g., blood atlas)

Measuring and characterizing the immune response

(Oversimplified)

- Flow cytometry
 - Assays for single mediators
- } Immuno-phenotyping 1.0

- Multi-plexing (multiple mediators, genes)
 - High dimensional cytometrics (e.g. Cytof)
- } Immuno-phenotyping 2.0

- √
- Single cell multi-omics
 - Multi-platform Multi-omics
- } Immuno-phenotyping 3.0
(2018-present)

Blood Plasma-based Cross platform Multi-omics



Junru Wu
Research Fellow
Department of Surgery
University of Pittsburgh

University of Pittsburgh:

Department of Surgery:

Dr. Jason L. Sperry*

Dr Matthew Neal*

Dr. Jillian Bonaroti

Dr. Hamed Moheimani

Dr. Shimena Li

Dr. Upendra Kar

Dr. Sultan Abdelhamid

Julia Conroy

Isabel Billiar

Jacob Soscia

Rob Voinchet

Dr. Yoram Vodovotz

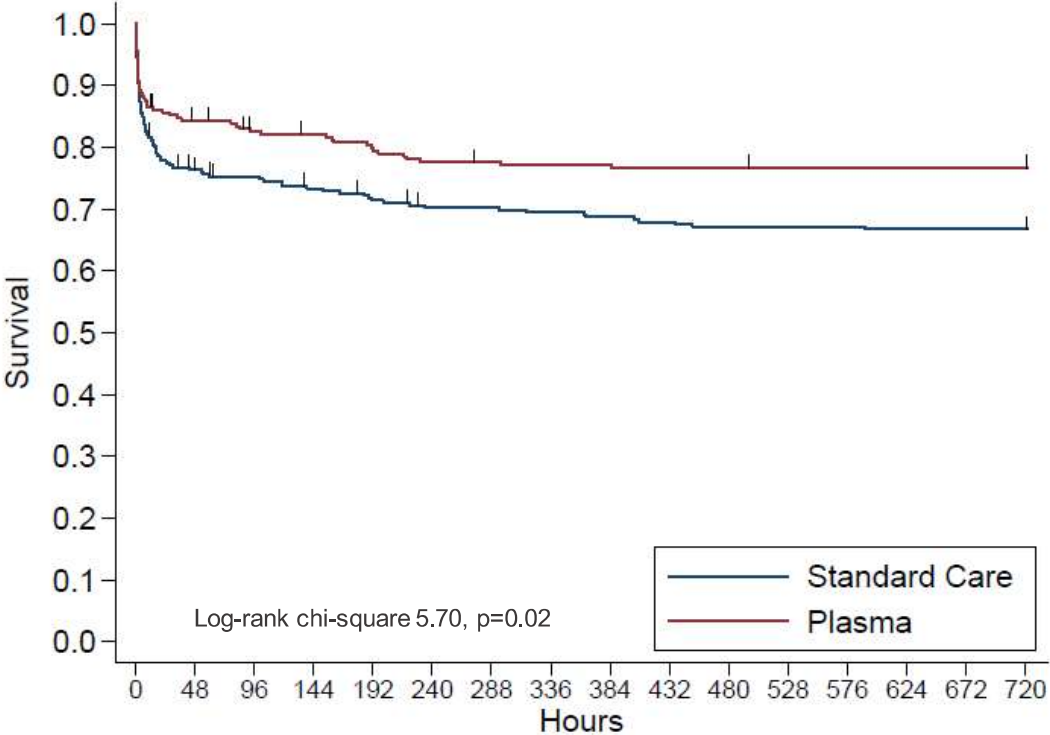
Dr. Rami A. Namas

Center for Systems Immunology:

Dr. Jisnu Das

- **PAMPer Study Group**
- **STAAMP Study Group**
- **TACTIC**

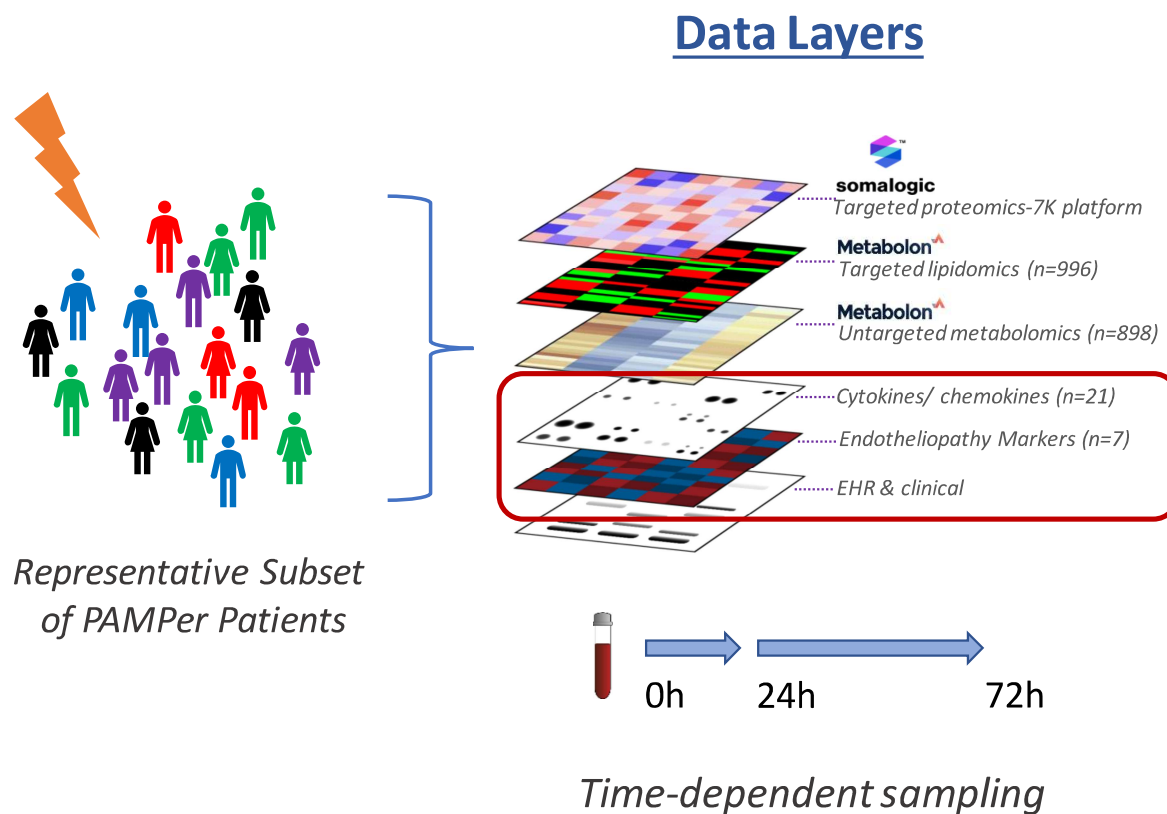
PAMPer: Pre-hospital thawed plasma (2 units) significantly reduced 30 day mortality



Number at risk							
Standard Care	271	194	181	179	173	172	172
Plasma	230	183	172	170	169	168	168

Multi-platform Multi-omics Program

Building Data Layers: Biology and Biomarkers



*Representative Subset
of PAMPer Patients*

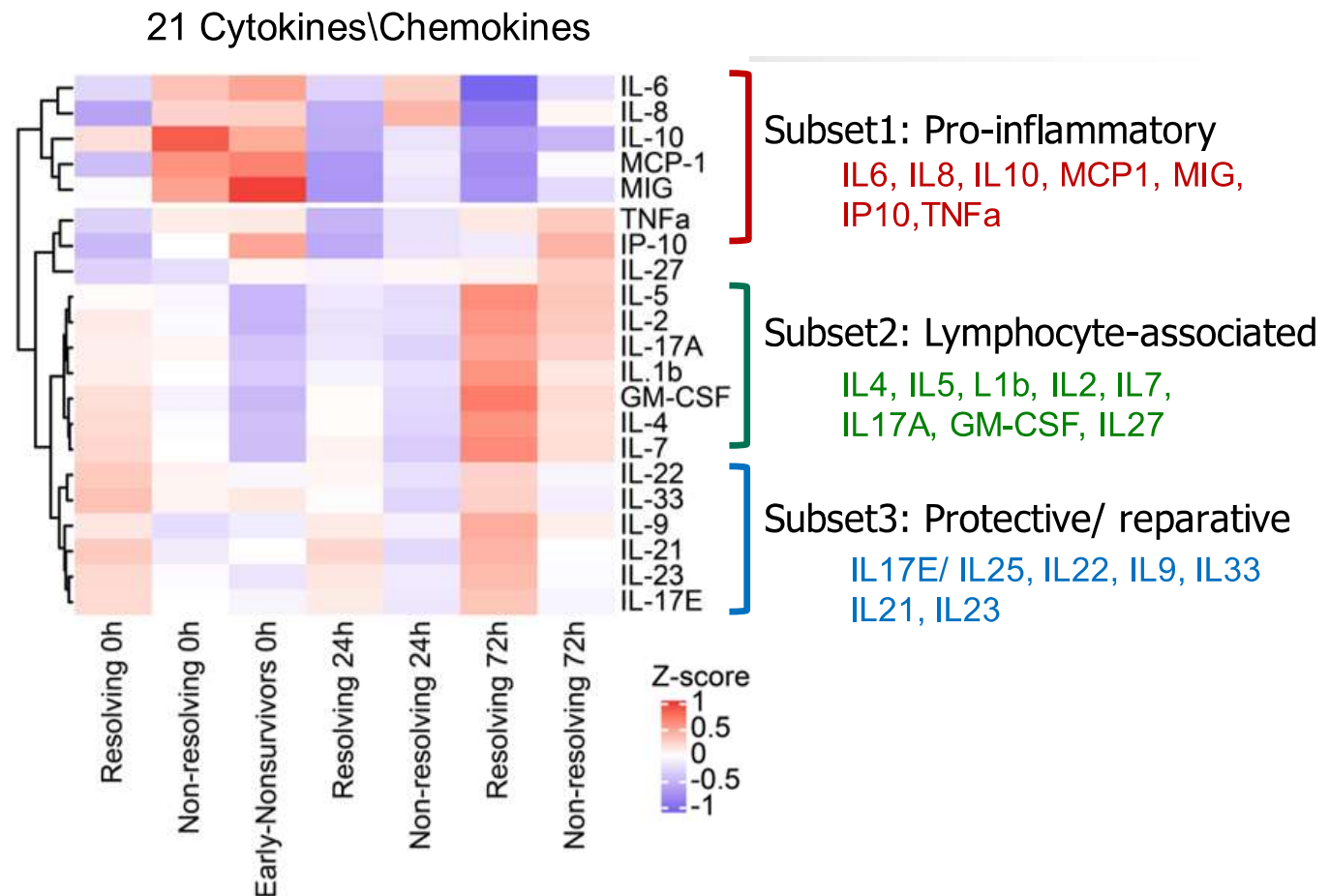
Time-dependent sampling

Major temporal pattern by outcome in the Cytokine Layer : Three cytokine modules



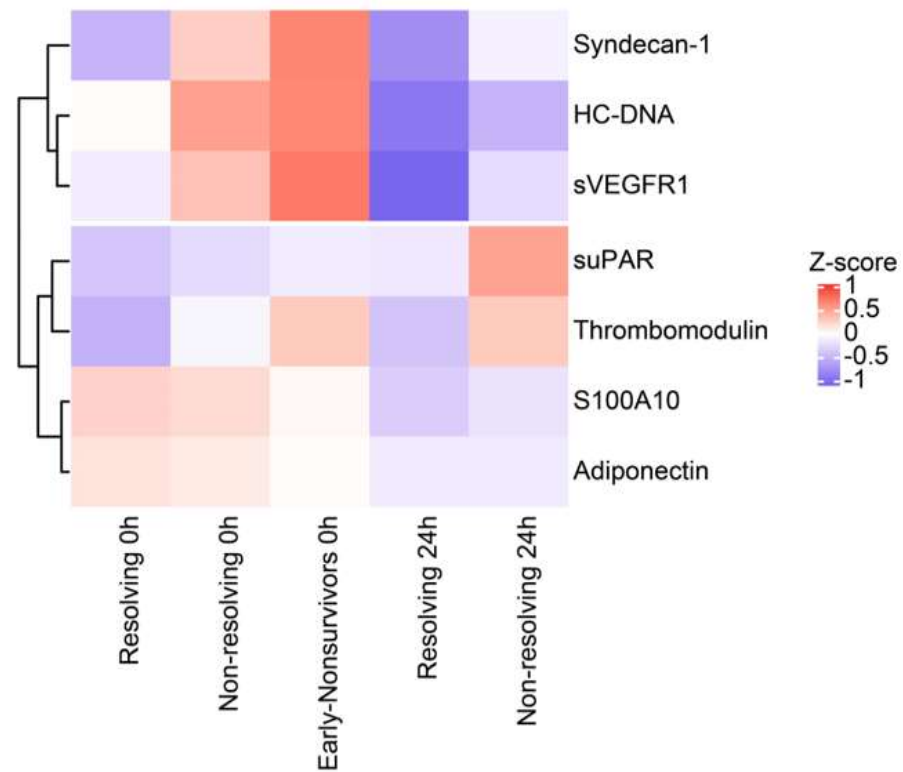
Definition of outcomes:

- Early-nonsurvivors (Died <72h)
- Non-resolving (ICU LOS \geq 7d OR died >72h)
- Resolving (ICU LOS <7d)



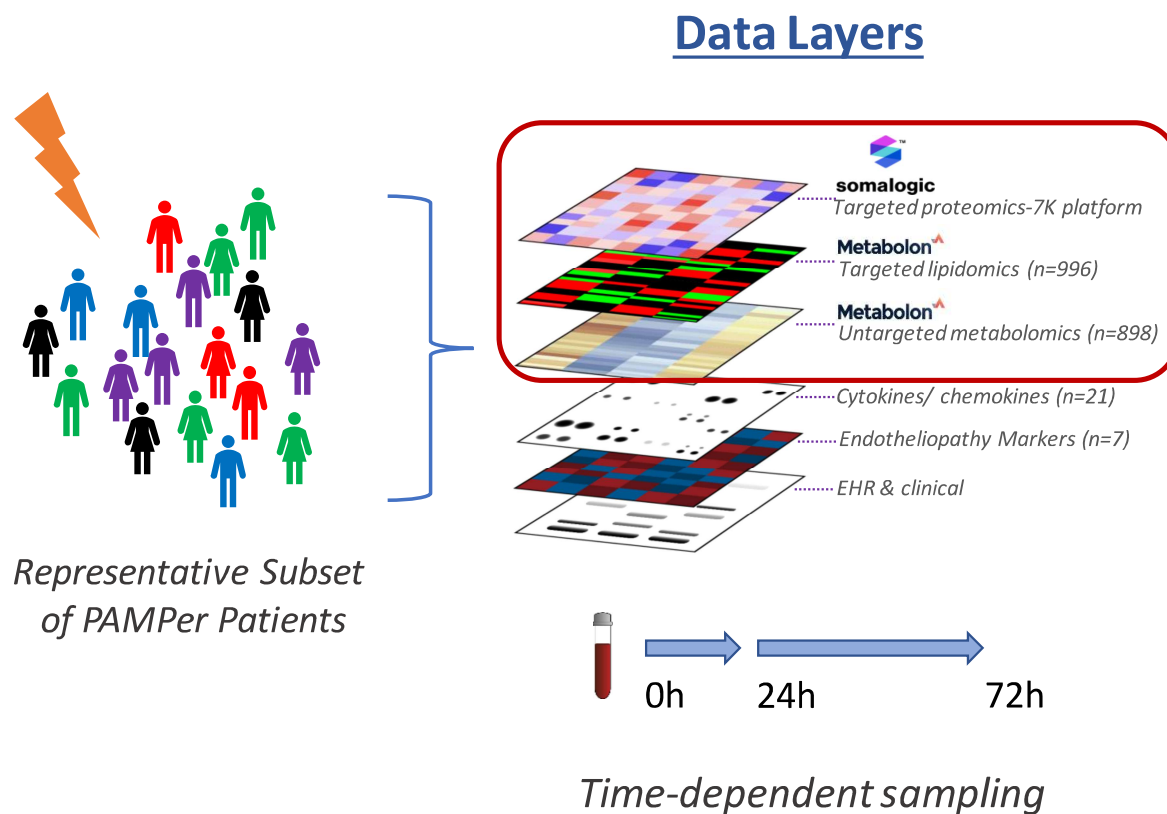
Major temporal patterns by outcome in the EC (Endotheliopathy) biomarkers Layer

7 EC Injury related biomarkers



Multi-platform Multi-omics Program

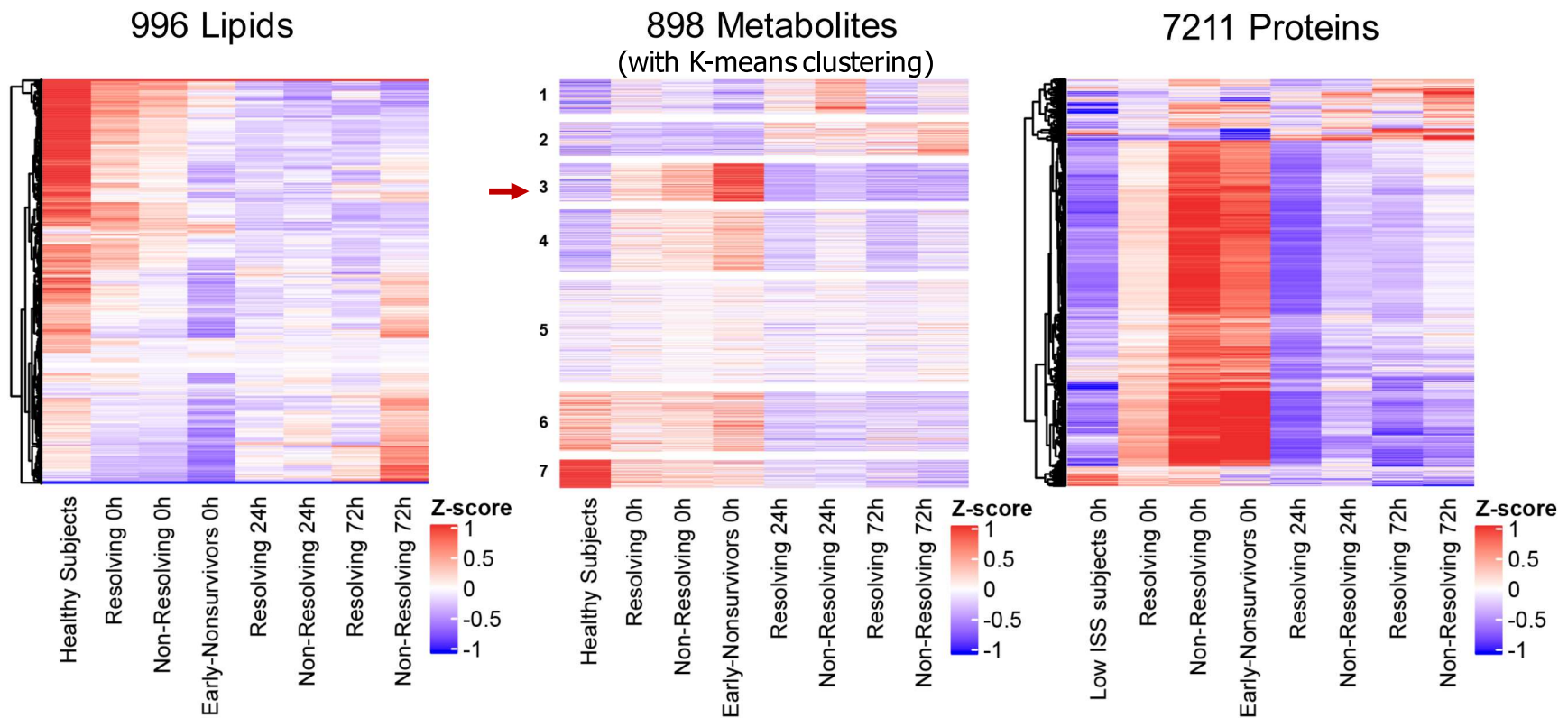
Building Data Layers: Biology and Biomarkers



*Representative Subset
of PAMPer Patients*

Time-dependent sampling

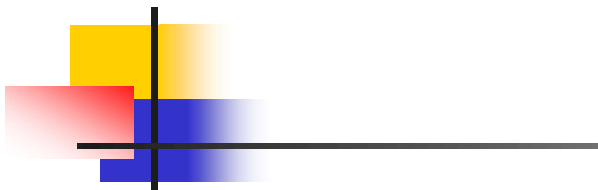
Global patterns: Lipidomics, metabolomics and proteomics



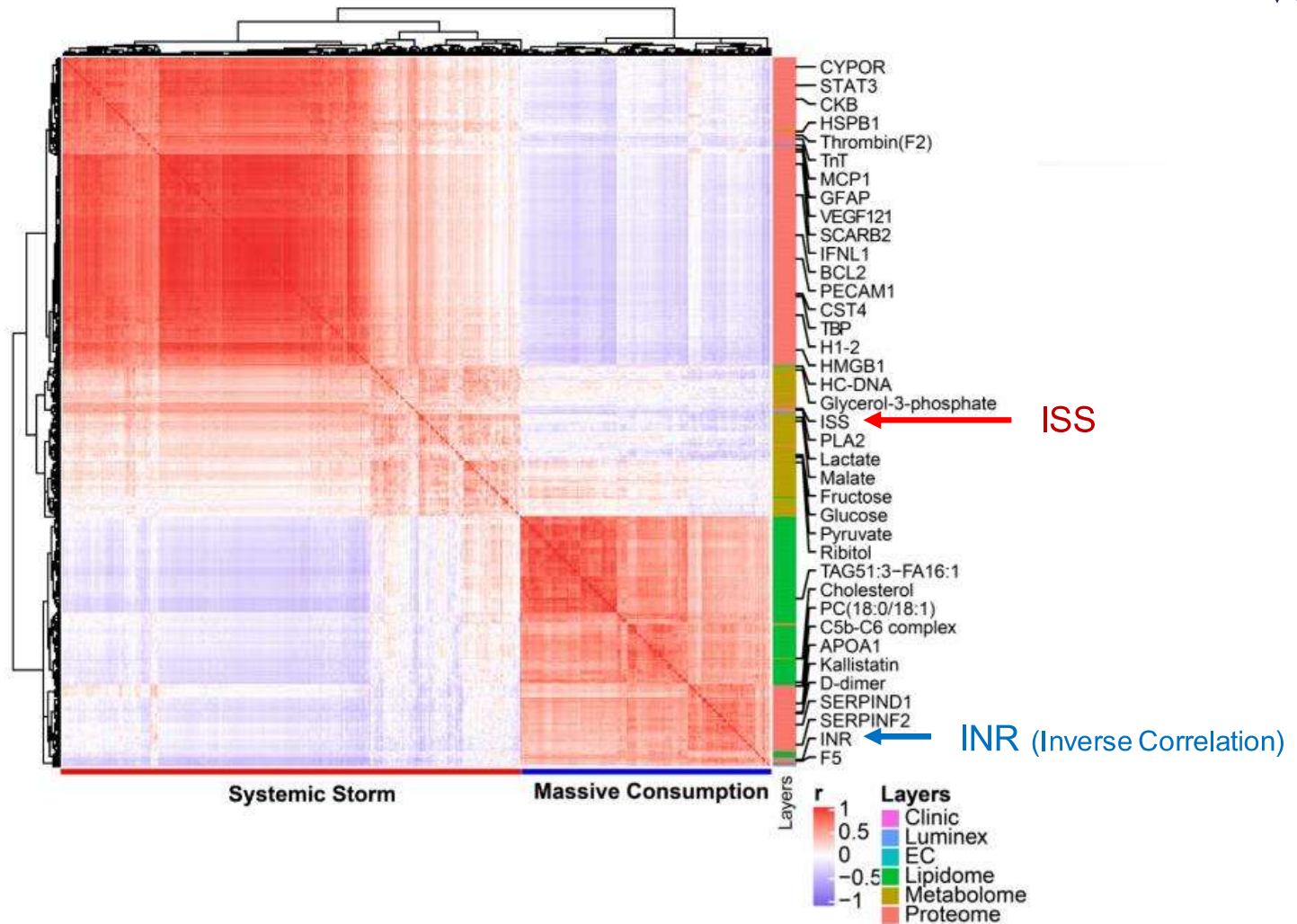
Integrated Analysis of Data Layers

Multi-omic data integration reveals two major patterns (time 0h):

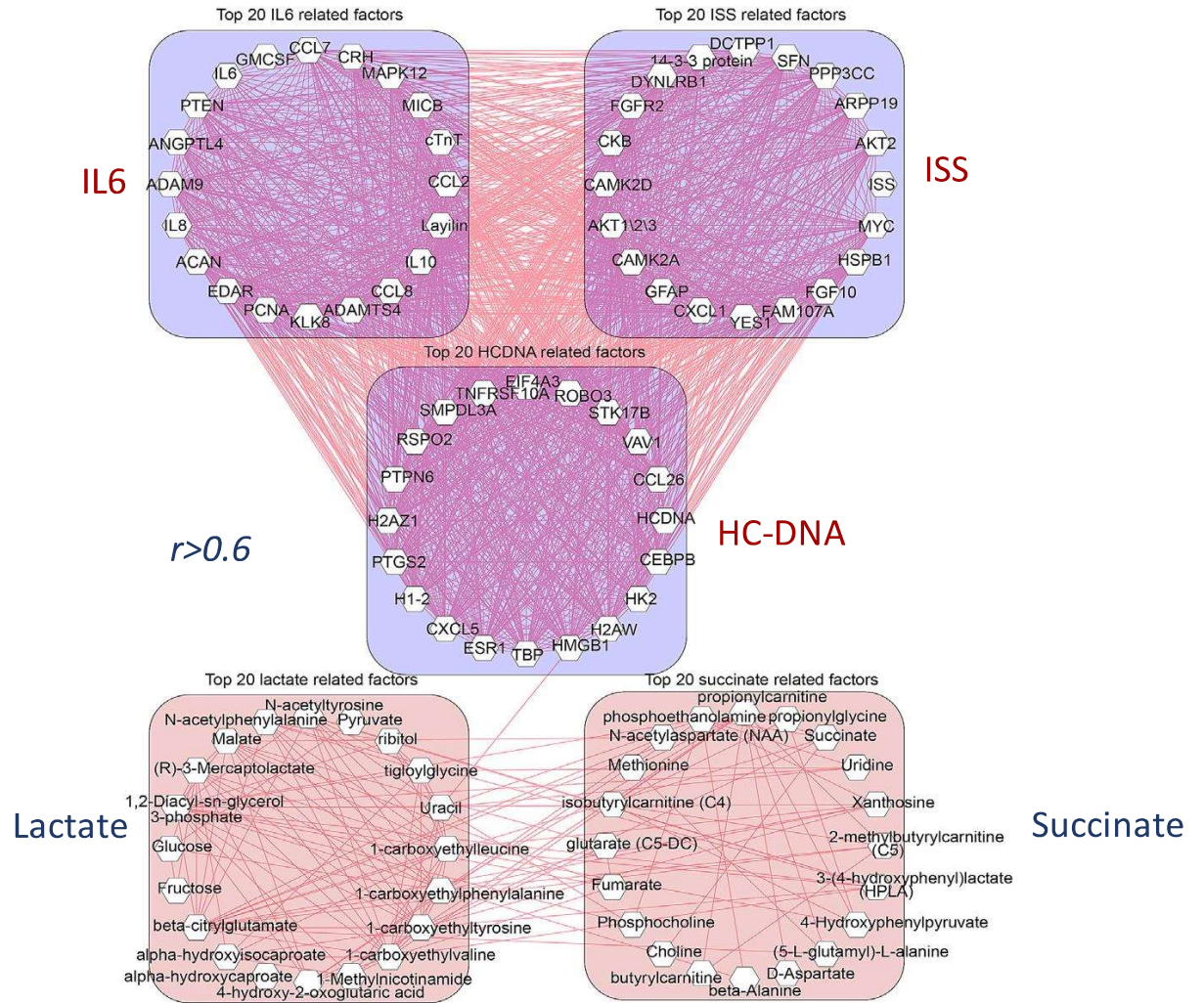
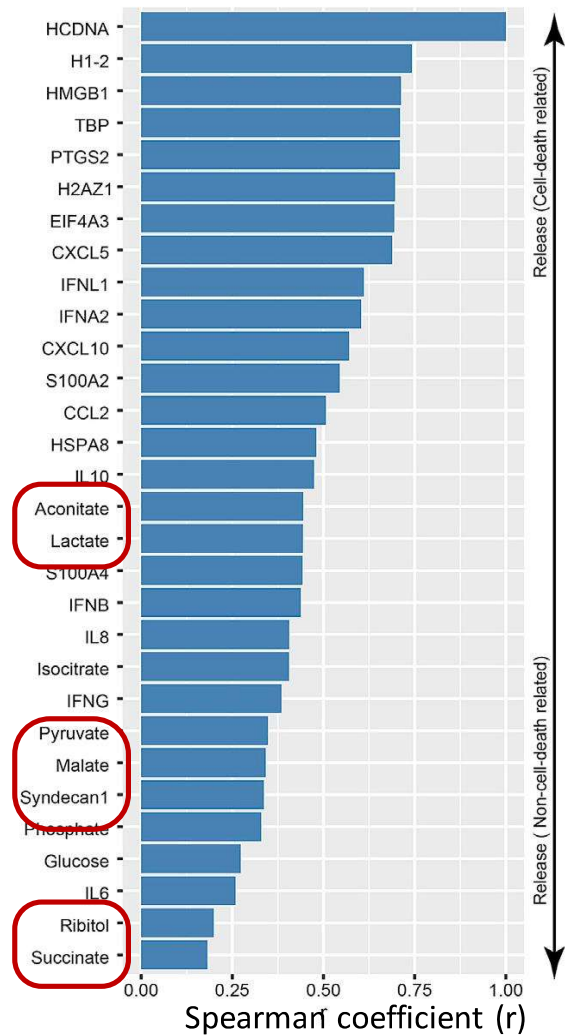
1. Systemic Storm $\uparrow\uparrow$
2. Massive Consumption $\downarrow\downarrow$



Top 200 features from six data layers

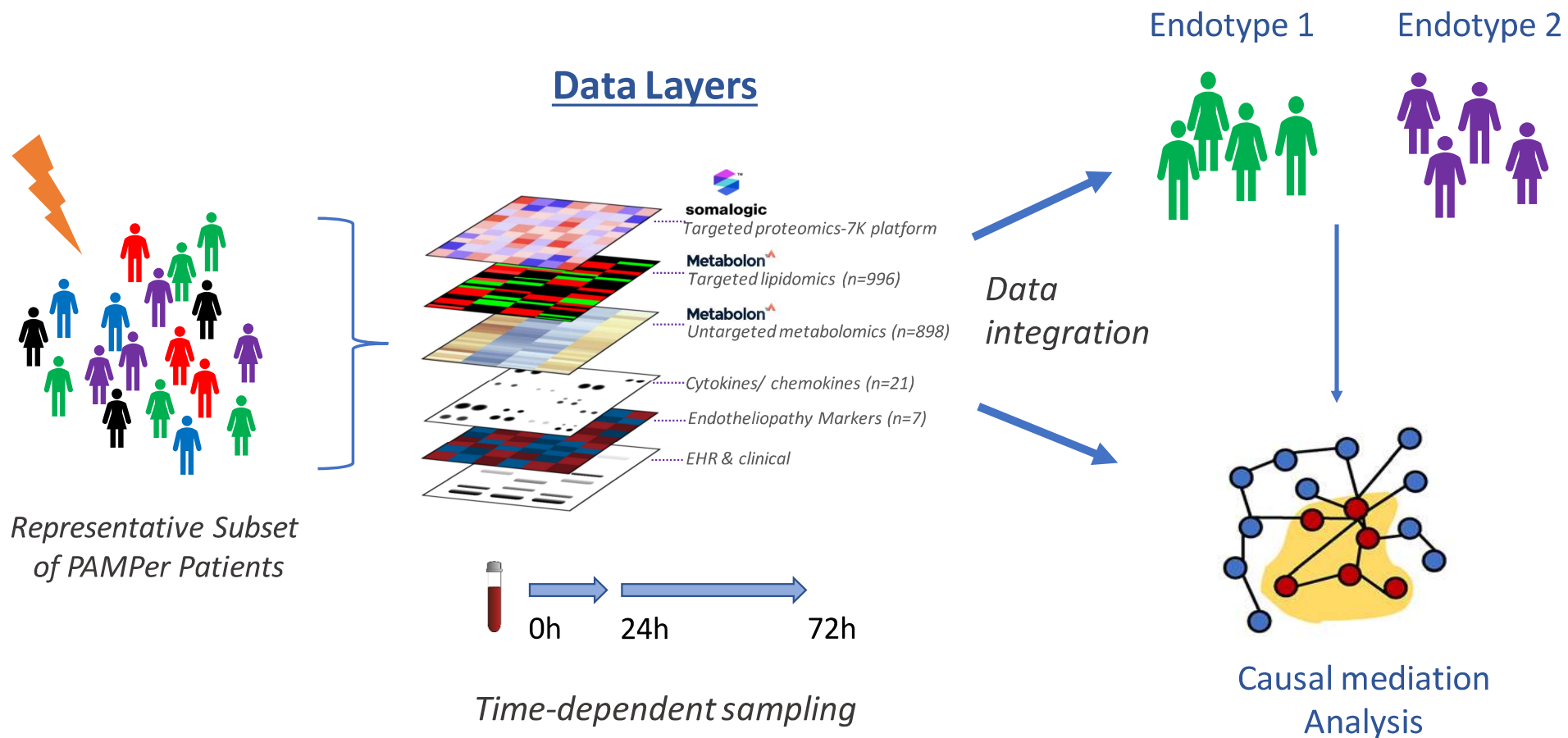


Two major release networks: Active and passive processes (time 0 h)

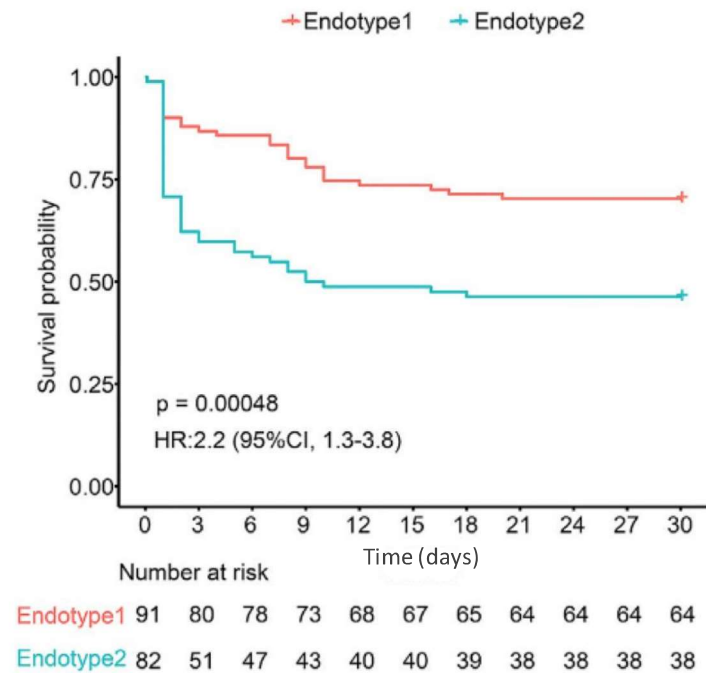
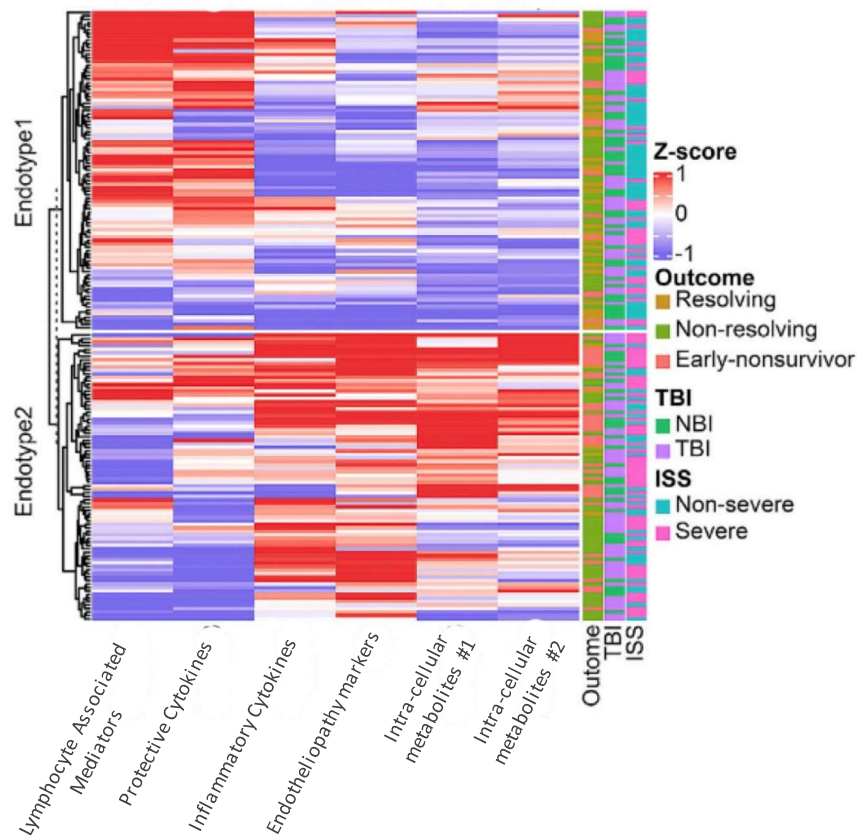


Multi-platform Multi-omics Program

Building Data Layers: Biology and Biomarkers

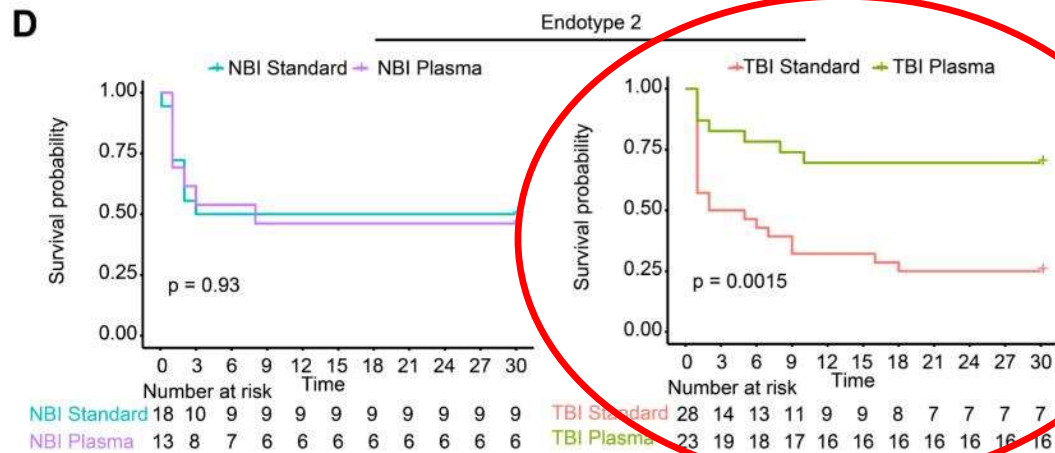
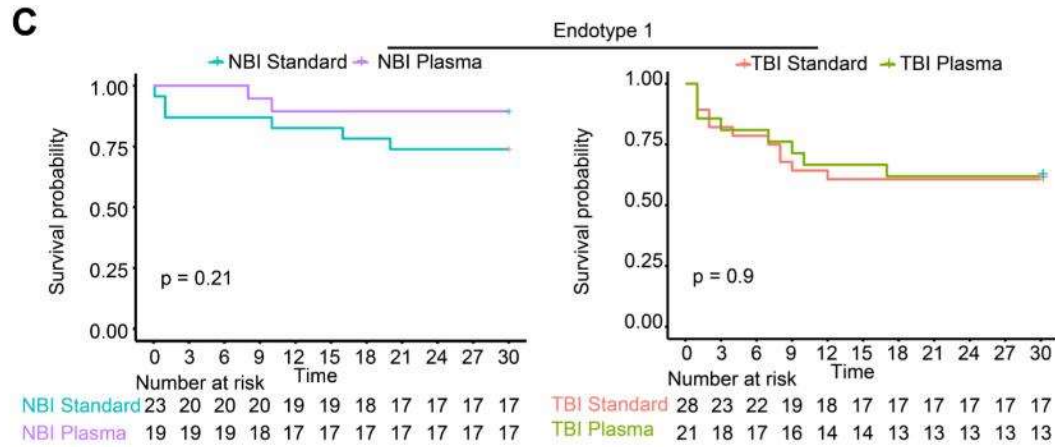


Clustering patients based on circulating levels of mediator types, endotheliopathy markers and intra-cellular metabolites identifies two distinct patient endotypes



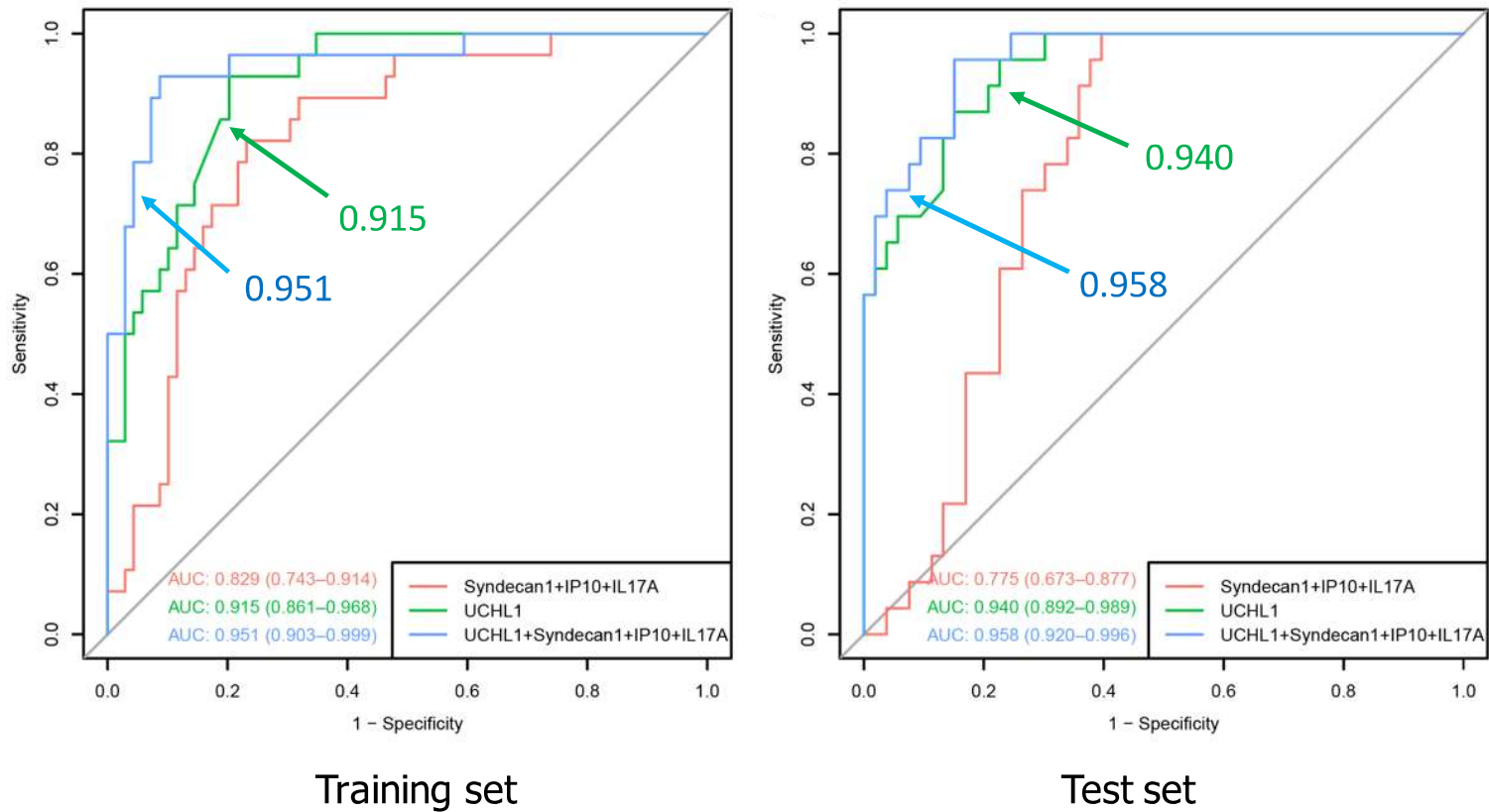
Pre-hospital Thawed Plasma only benefits (survival) the TBI & Endotype 2 subgroup

NBI=No Brain Injury
TBI=Traumatic Brain Injury

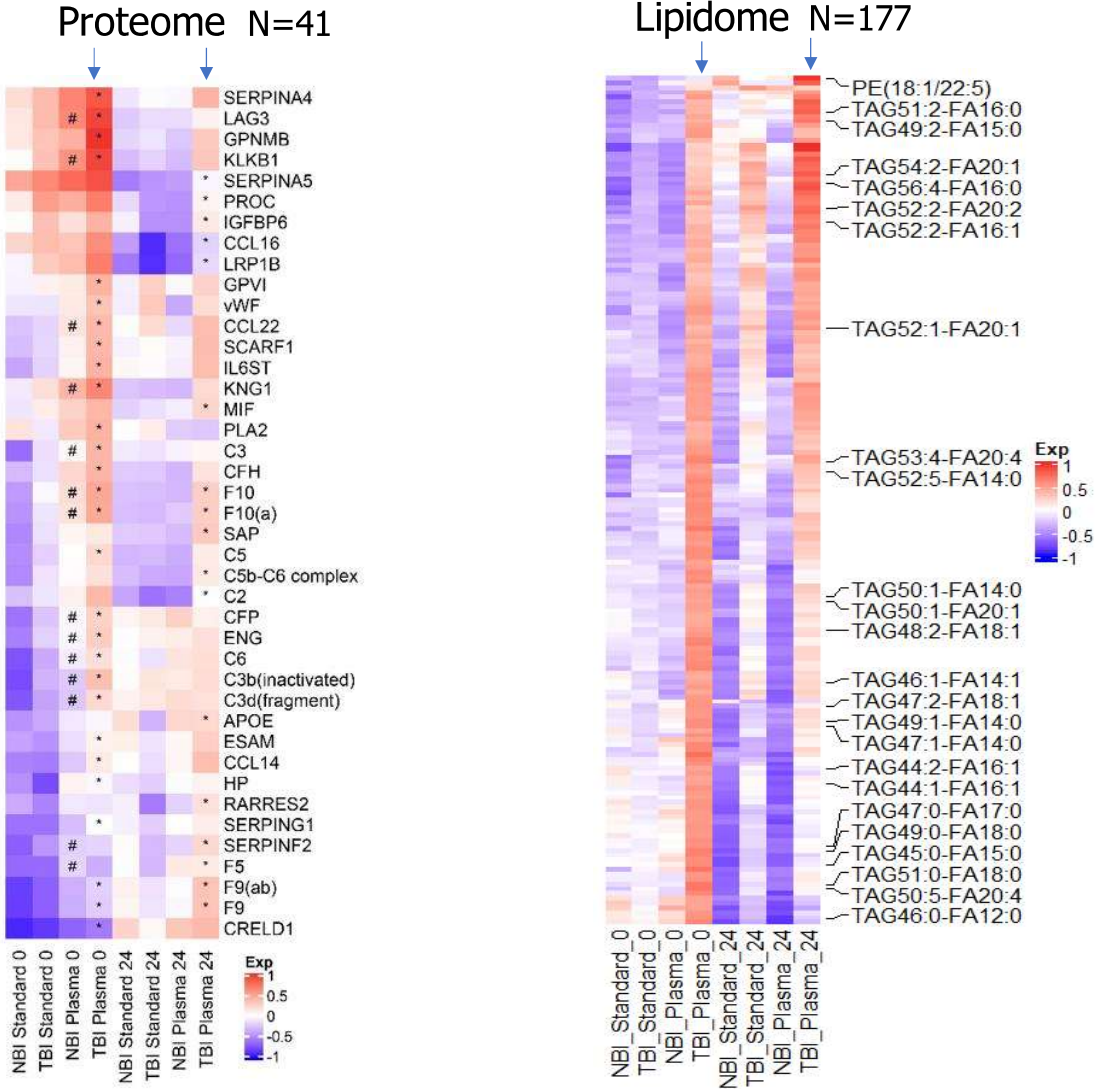


Unexpected Survivors
Plasma: 30%
Standard: 75%

Predictive models for classifying patients as TBI-E2 after trauma: Admission UCHL1 is highly predictive



Evidence that patients with TBI process thawed plasma differently



Wu et al. Cell Rep Med, 2021
 Wu et al. Res Sq, 2021

Hypothesis: Mediation analysis of the multi-omic database will yield insights into the subgroup-specific effects of early plasma

Patient subgroups used for the mediation analysis (n=149)

Endotype 1

Endotype 2

	E1-NBI STD (N=21)	E1-NBI TP (N=16)	E1-TBI STD (N=25)	E1-TBI TP (N=18)	E2-NBI STD (N=14)	E2-NBI TP (N=11)	E2-TBI STD (N=24)	E2-TBI TP (N=20)
Age	36 (± 43)	53 (± 26)	48 (± 41)	51 (± 45)	55 (± 29)	45 (± 25)	41 (± 33)	39 (± 28)
Gender (%M)	14 (66.7%)	13 (81.3%)	20 (80.0%)	13 (72.2%)	11 (78.6%)	6 (54.5%)	21 (87.5%)	13 (65.0%)
ISS	16 (± 12)	18 (± 6.5)	29 (± 10)	23 (± 17)	27 (± 13)	34 (± 26)	38 (± 14)	34 (± 19)
GCS	14 (± 3.0)	15 (± 8.3)	3.0 (± 3.0)	3.0 (± 11)	12 (± 10)	12 (± 12)	3.0 (± 0)	3.0 (± 2.5)
Shock Index	1.4 (± 0.40)	1.5 (± 0.09)	1.7 (± 0.53)	1.4 (± 0.19)	1.8 (± 0.74)	1.5 (± 0.70)	1.6 (± 0.24)	1.6 (± 0.42)
INR	1.4 (± 0.50)	1.2 (± 0.11)*	1.2 (± 0.31)	1.2 (± 0.12)	1.8 (± 0.78)	1.2 (± 0.29)*	1.5 (± 0.52)	1.3 (± 0.27)*
30d Mortality	5 (23.8%)	1 (6.25%)	10 (40.0%)	7 (38.9%)	7 (50.0%)	6 (54.5%)	19 (79.2%)	7 (35.0%)*

Dunn test was used to perform within subgroup comparison (STD vs. TP in the each of four subgroups).

*Adjusted P-value < 0.05. **Std: Standard care, TP: Thawed plasma**

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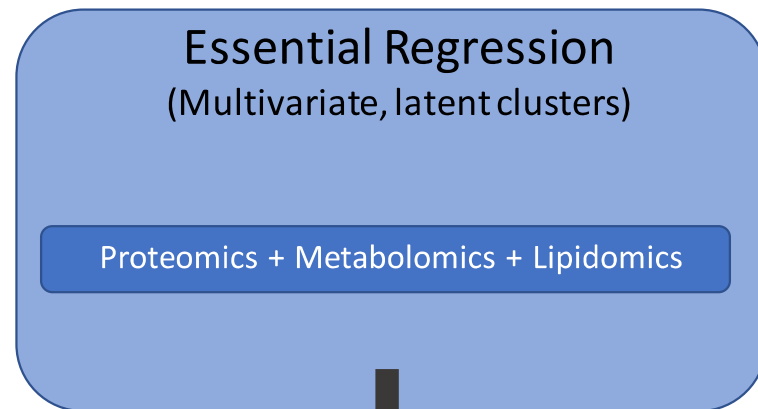
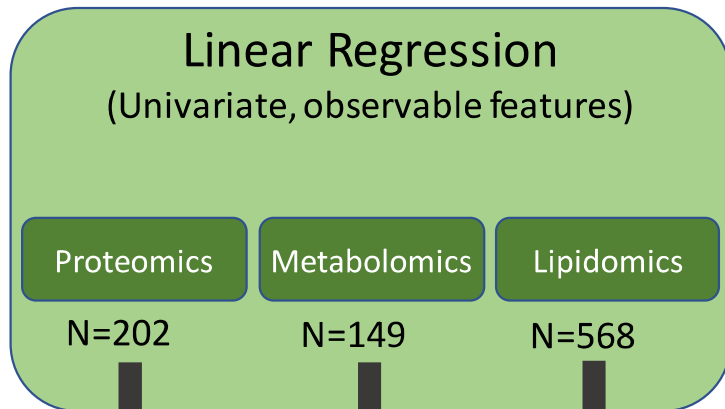
	Endotype 1				Endotype 2			
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Subset of PAMPer Patients (n=149)
Multi-omic dataset, Admission blood sample

Feature selection



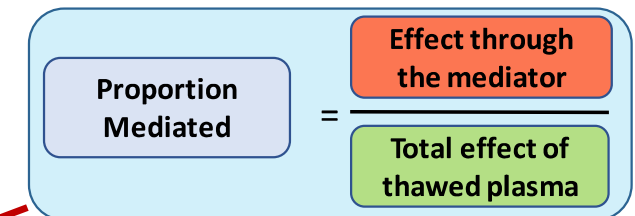
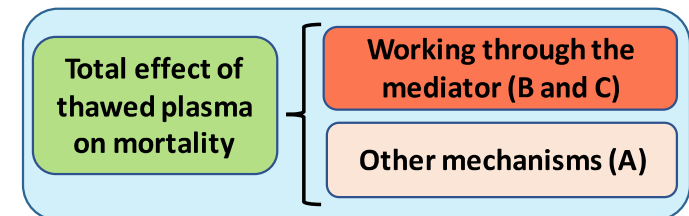
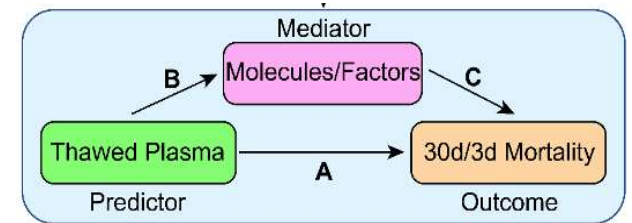
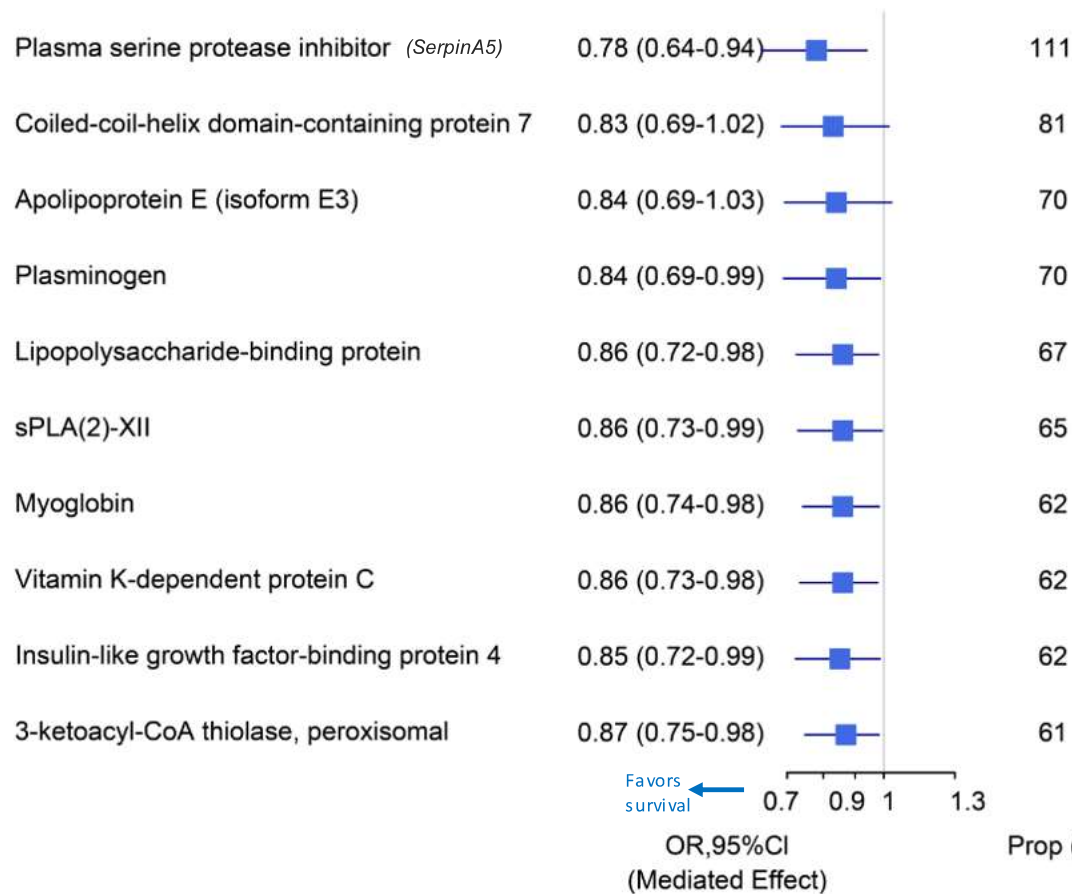
Mediation analyses on significant individual observable features

Mediation analyses on significant latent multi-omic clusters

Identify common features

Mediators of the effect of thawed plasma on survival in E2-TBI patients

*Mediation analysis identifies causal features by finding the features with the highest proportion mediated effect (proportion %)
(top ten features from the protein layer shown)*



Prop (%) ←

Features identified by linear regression + mediation analysis (proportion of effect >50%)

Proteins (n=16):

Coagulation/ fibrinolysis:

Serine protease inhibitor A5 (SERPINA5)
Carboxypeptidase B2 (CPB2)
Coagulation factor XI (F11)
Vitamin K-dependent protein C (PROC)
Plasminogen (PLG)

Lipid transport/ metabolism:

Apoprotein E3 (APOE3)
Soluble phospholipase A₂ Group XIIA (sPLA2-XII)
LPS binding protein (LBP)

Brain enriched proteins:

Neuropeptide S (NPS)
Cathepsin F (CTSF)

Other:

Insulin-like growth factor binding protein 4 (IGFBP4)
Myoglobin (MB)
Acetyl-Coenzyme A acyltransferase-1 (ACAA1)
Complement factor 3 (C3)
Ubiquitin conjugating enzyme E2 S (UBE2S)
Coiled coil-helix coiled coil-helix domain 7 (CHCHD7)

Lipids (n=41):

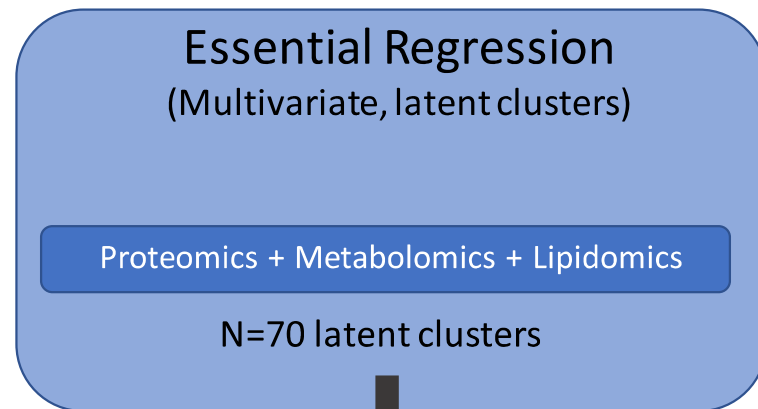
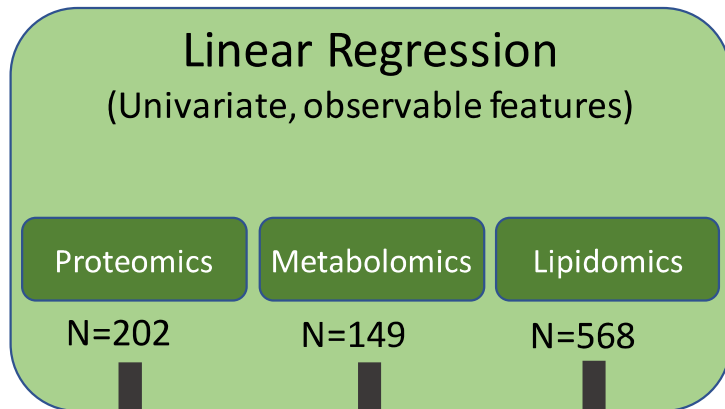
Phosphatidylethanolamines
Phosphatidylcholines
Lyso-phosphatidylcholines
Triacyl-and Diacylglycerides

Non-Lipid metabolites:

None

Subset of PAMPer Patients (n=149)
Multi-omic dataset, Admission blood sample

Feature selection



Mediation analyses on significant individual observable features

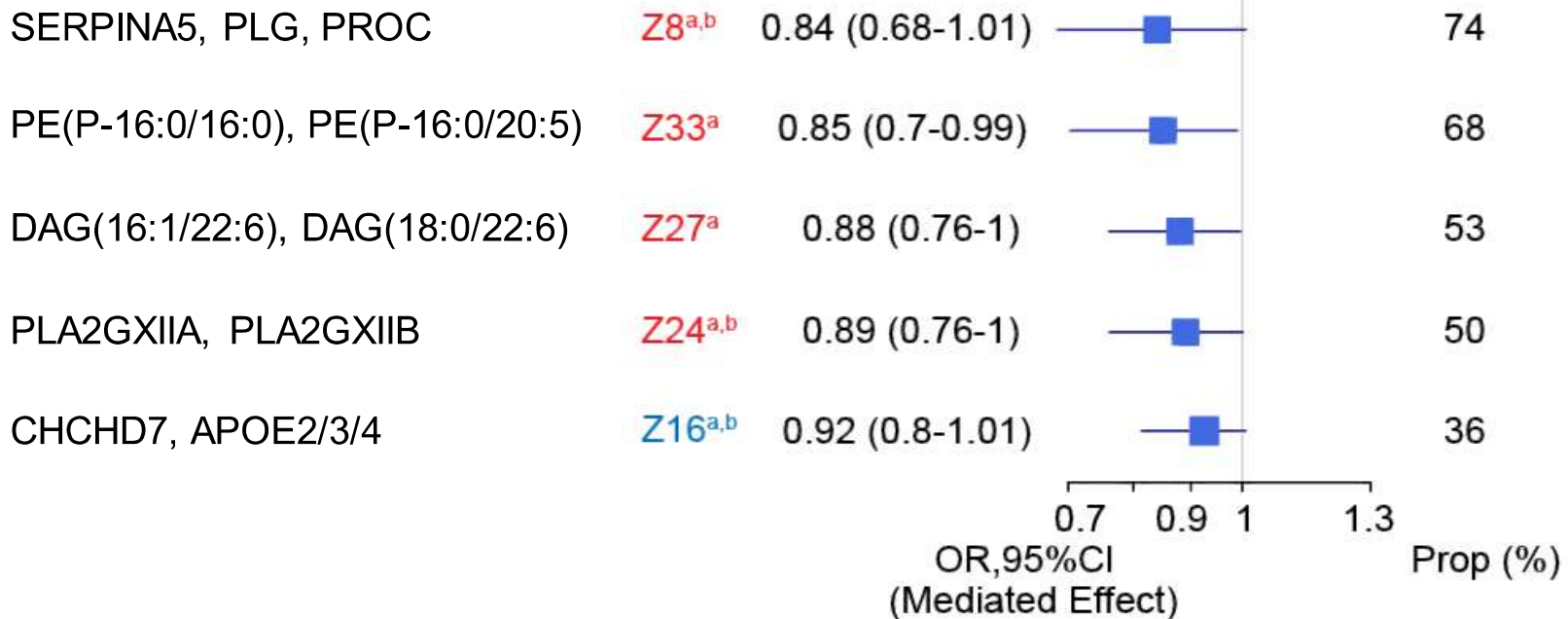
Mediation analyses on significant latent multi-omic clusters

Identify common features

Mediators of the effect of thawed plasma on survival in E2-TBI patients

Essential regression (multivariate latent factor regression analysis) + mediation analysis reveals 5 clusters of latent factors with proportion of effect >30%

Cluster-defining features

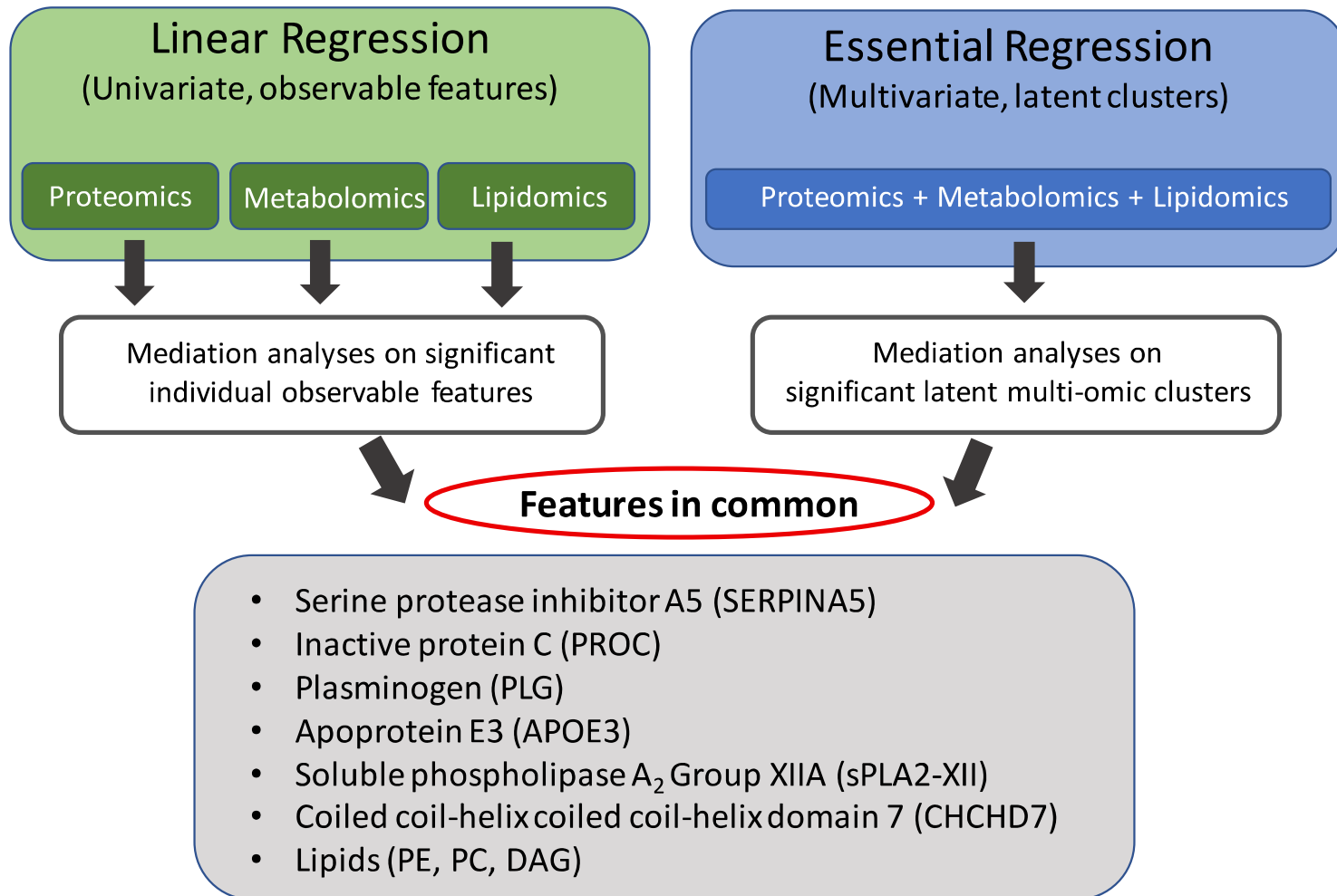


Red: Latent factors in relation to the outcome (3d Mortality)
 Blue: Latent factor in relation to the E2-TBI subgroup

a: Identified by the Least Squares method
 b: Identified by the Dantzig method

Subset of PAMPer Patients (n=149)

Multi-omic dataset, Admission blood sample



Potential biologic implications of causal mediators

Causal Mediators

Serine protease inhibitor A5 (SERPINA5, PCI, PAI3)
Carboxypeptidase B2 (CPB2)
Coagulation factor XI (F11)

Inactive protein C (PROC)
Plasminogen (PLG)

Apoprotein E3 (APOE3)
Soluble phospholipase A₂ Group XIA (sPLA2-XI)
Lipids (PE, PC, DAG)

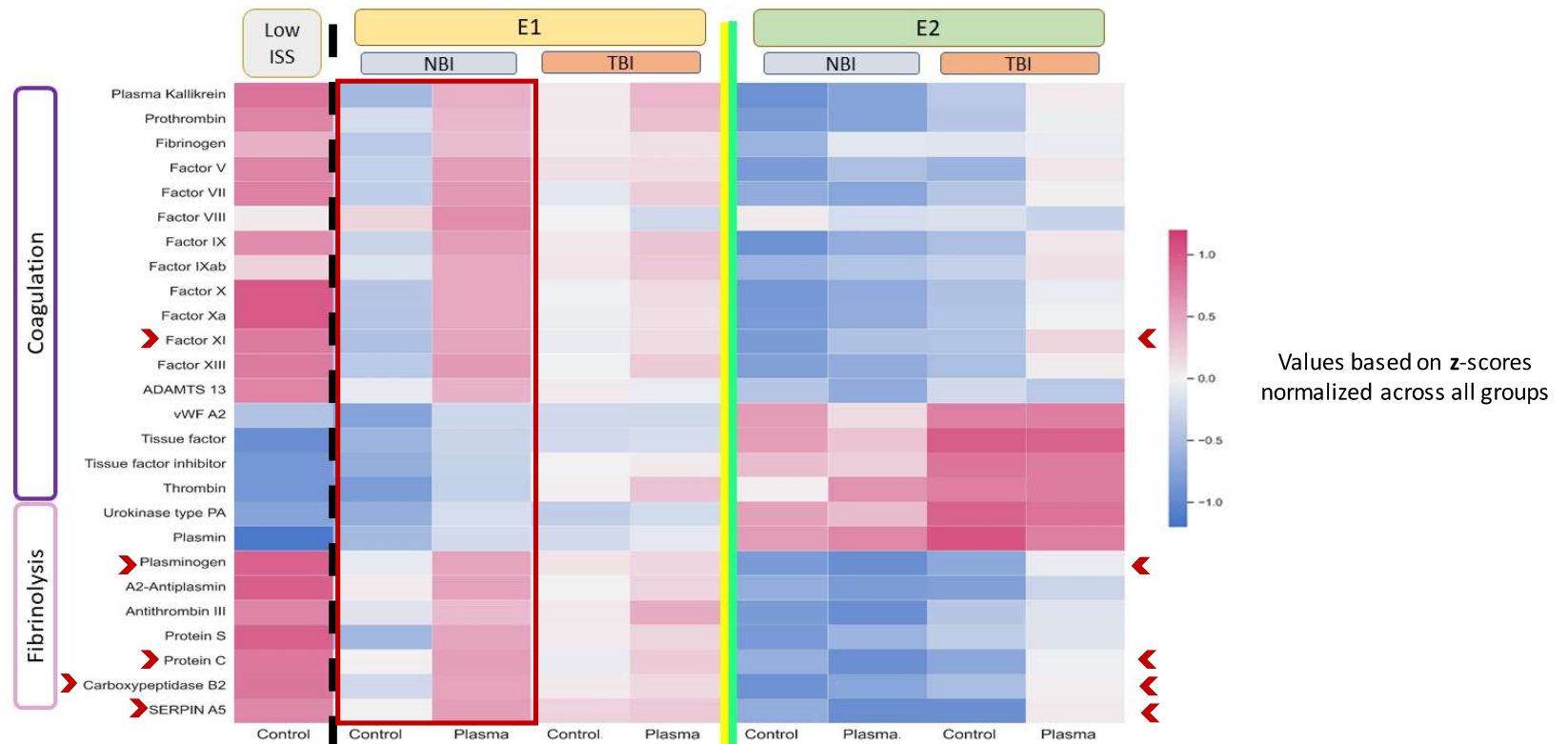
Biologic Processes

- Enhanced clotting
- Suppressed fibrinolysis

- Possible consequence of inhibitors

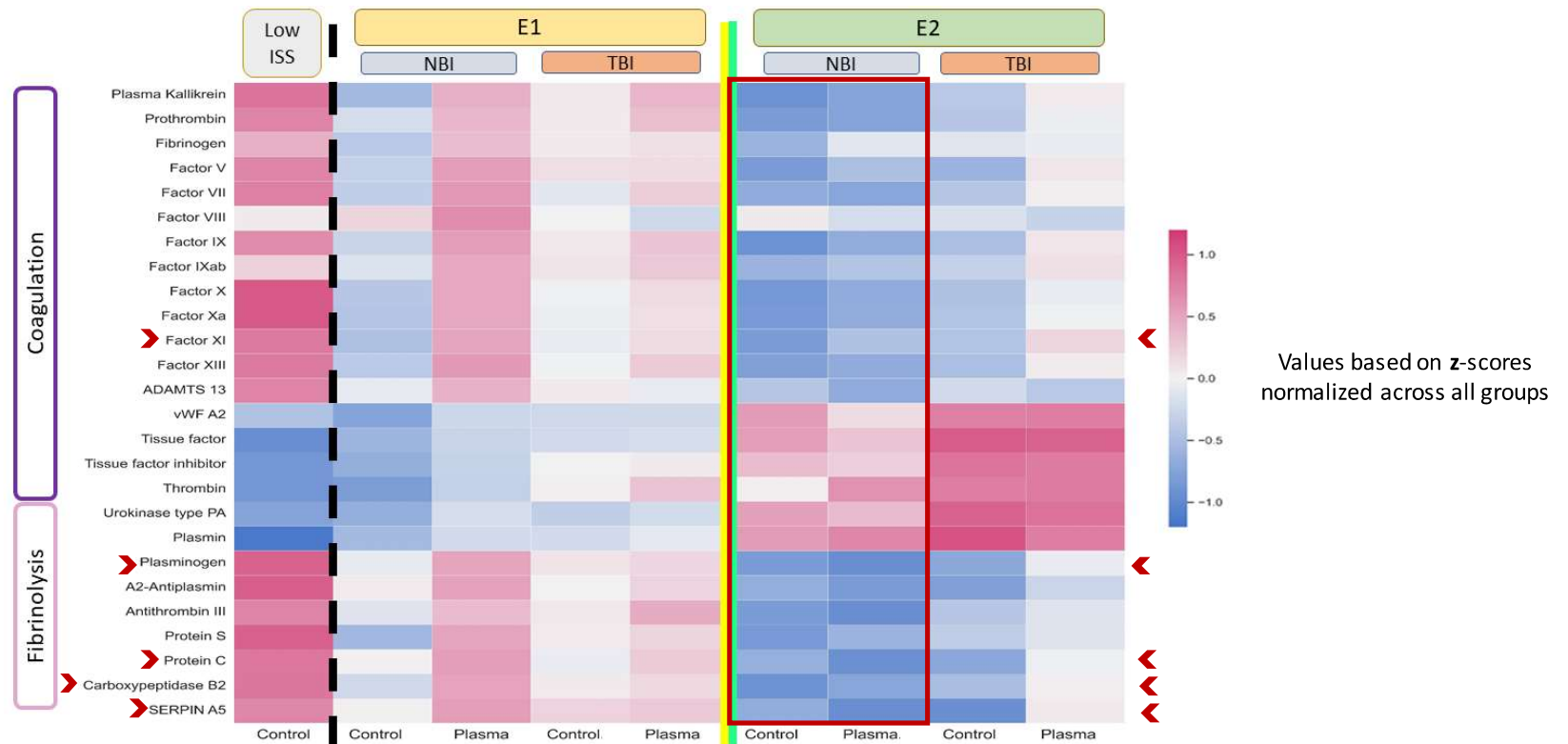
- Lipid transport
- Production of lipid mediators
- Brain protection

Heatmap showing the relative levels of proteins of the coagulation and fibrinolysis pathways across endotypes and treatment groups at admission



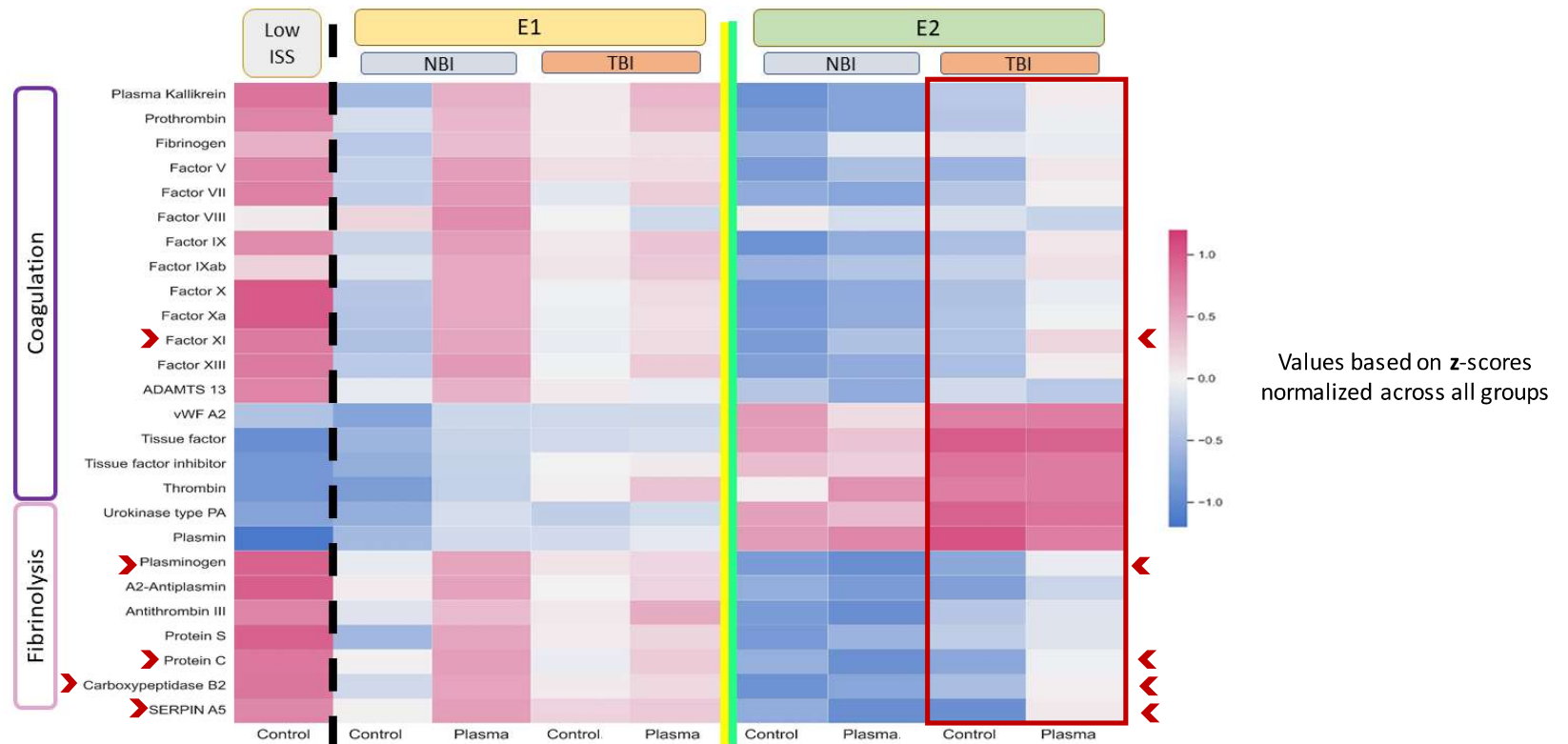
Low ISS: patients with no or minimal injuries

Heatmap showing the relative levels of proteins of the coagulation and fibrinolysis pathways across endotypes and treatment groups at admission



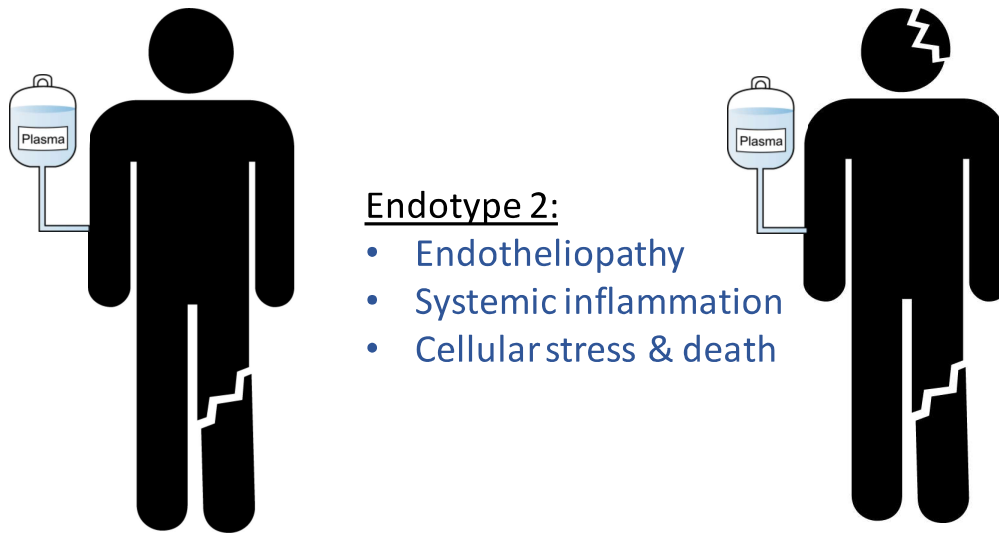
Low ISS: patients with no or minimal injuries

Heatmap showing the relative levels of proteins of the coagulation and fibrinolysis pathways across endotypes and treatment groups at admission



Low ISS: patients with no or minimal injuries

Summary/ Conclusions



- Endotype 2 trauma patients with TBI process early plasma differently than polytrauma patients without TBI
- Mediation analysis suggests improved clotting and actions of lipoproteins/ lipids as basis for plasma effect on mortality in E2-TBI patients
- **Inclusion of large-scale omics analysis in trauma trials should be considered to define treatment effects in patient subsets**

