



Soldier-on-a-Chip: Multi-Omics Investigation of *Staphylococcal Enterotoxin B* Exposures in Microphysiology Systems

Conor Jenkins¹, Gabrielle Rizzo¹, Allison Clay², Raquel Shortt², Priscilla Lee¹, Jennifer Horsmon¹, Dylan Fudge¹, Daniel Angelini¹, Elizabeth Dhumakupt¹, Tyler Goralski¹

¹U.S. Army Combat Capabilities Development Command Chemical Biological Center, Aberdeen Proving Ground, MD, ²Excet, A Precise Systems Company, Lexington Park, MD

Introduction

Microphysiology systems (MPS) are used to mimic human organ systems in order to collect more human-real data. Traditional experiments have relied on either cell cultures, which aren't physiologically accurate, or animal studies, which are very expensive. However, human-relevant data is important in understanding the human host's response to exposures of lethal chemicals (i.e. sarin, VX) and biological organisms (i.e. *Bacillus anthracis*, *Western equine encephalitis*). Understanding the molecular response to these threats will allow for further treatments and medical countermeasures to be developed.

In this project, a multi-organ system situated on a single chip was exposed to *Staphylococcal enterotoxin B* (SEB). SEB is a toxin produced by gram-positive *S. aureus* bacteria. It is mainly responsible for food poisoning but has the potential to be used as a biological threat agent. This MPS contained three organ systems – lung tissue, cardiac organoids, and skin tissue. These tissues are linked on-chip and share flow through micro-pumps, which is meant to mimic blood flow through a human system. The three tissues were harvested 24 hours after exposure to SEB and prepared for proteomic and metabolomic analysis via Orbitrap mass spectrometry.

Materials and Methods

The TissUse Chip 3+ system was seeded with cardiac organoids, MATTEK EpiAirway lung tissue, and MATTEK EpiDerm-FT skin tissue. The system equilibrated overnight under flow in an incubator. Three different concentrations of SEB were prepared (1 µg/mL, 10 µg/mL, and 100 µg/mL) and dosed onto the skin well of the chip. PBS in media was used as a vehicle control.

After 24 hours, all chip effluent, tissues, and organoids were collected from the multi-organ chip (MOC). The tissues and organoids were flash-frozen and then prepared for proteomic and metabolomic analysis [1]. Proteomic samples were analyzed on a Thermo Orbitrap Tribrid Eclipse mass spectrometer, and the data was analyzed via Proteome Discoverer (ver. 2.5.0.400). Metabolomic samples were analyzed on a Thermo Orbitrap Q Exactive Plus mass spectrometer, and the data was analyzed via Compound Discoverer (ver. 3.3.1.111).

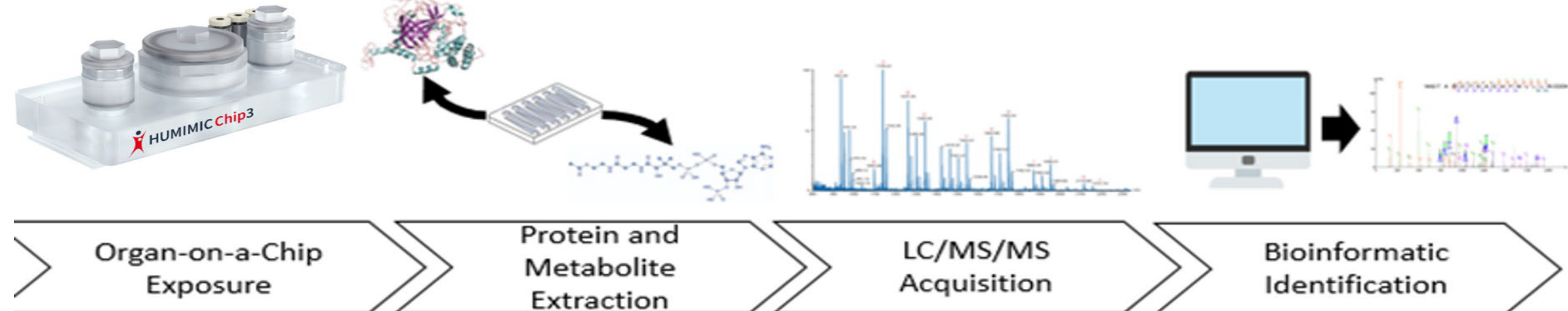


Figure 1. Multi-omic pipeline of organ-on-a-chip samples.



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Table 1. High confident proteins and significantly changing proteins identified from the chip tissues after SEB exposure.

Sample	High Confident Proteins	Significantly Changing Proteins
Cardiac	1334	303
Skin	2452	239
Lung	4338	340

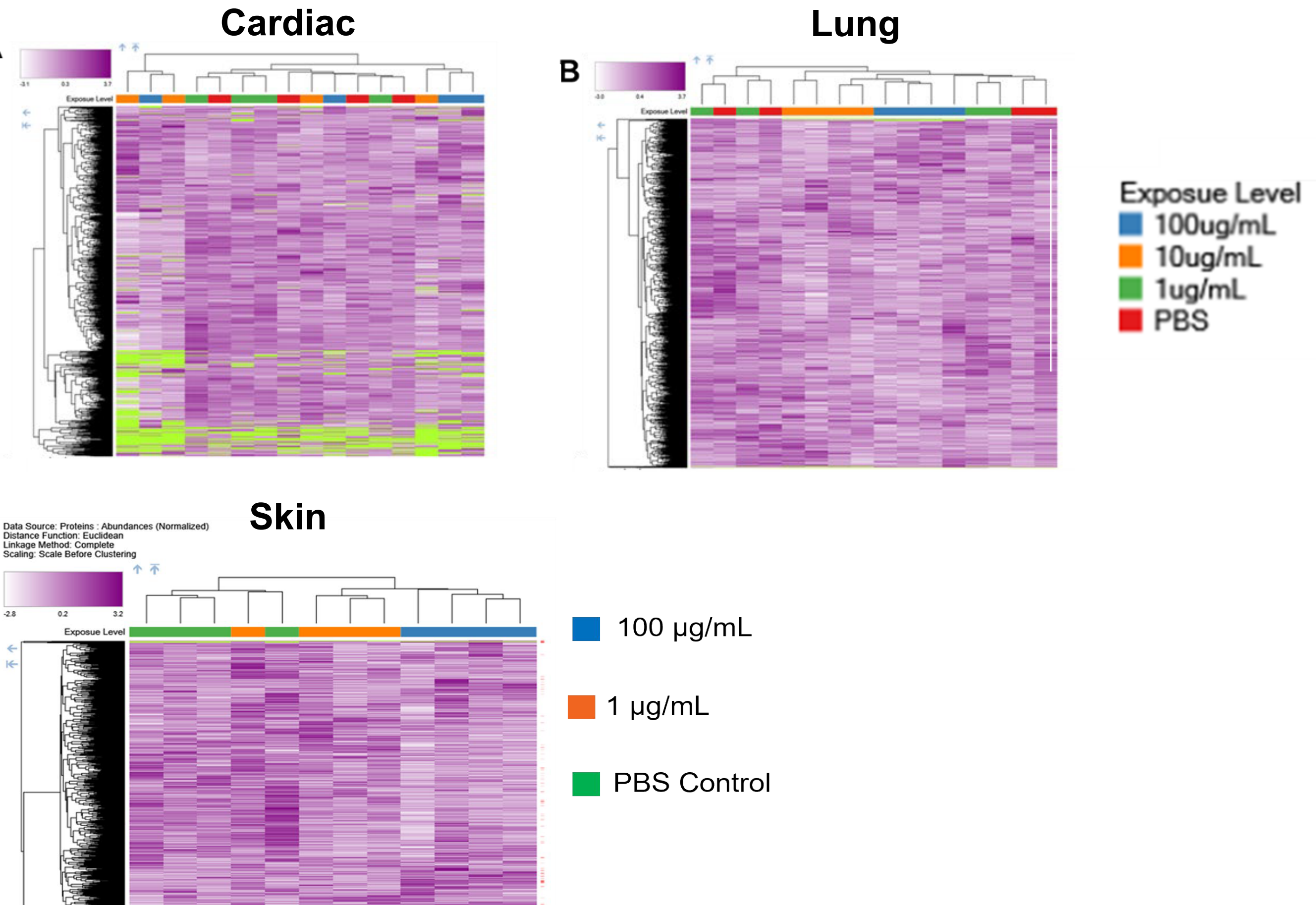
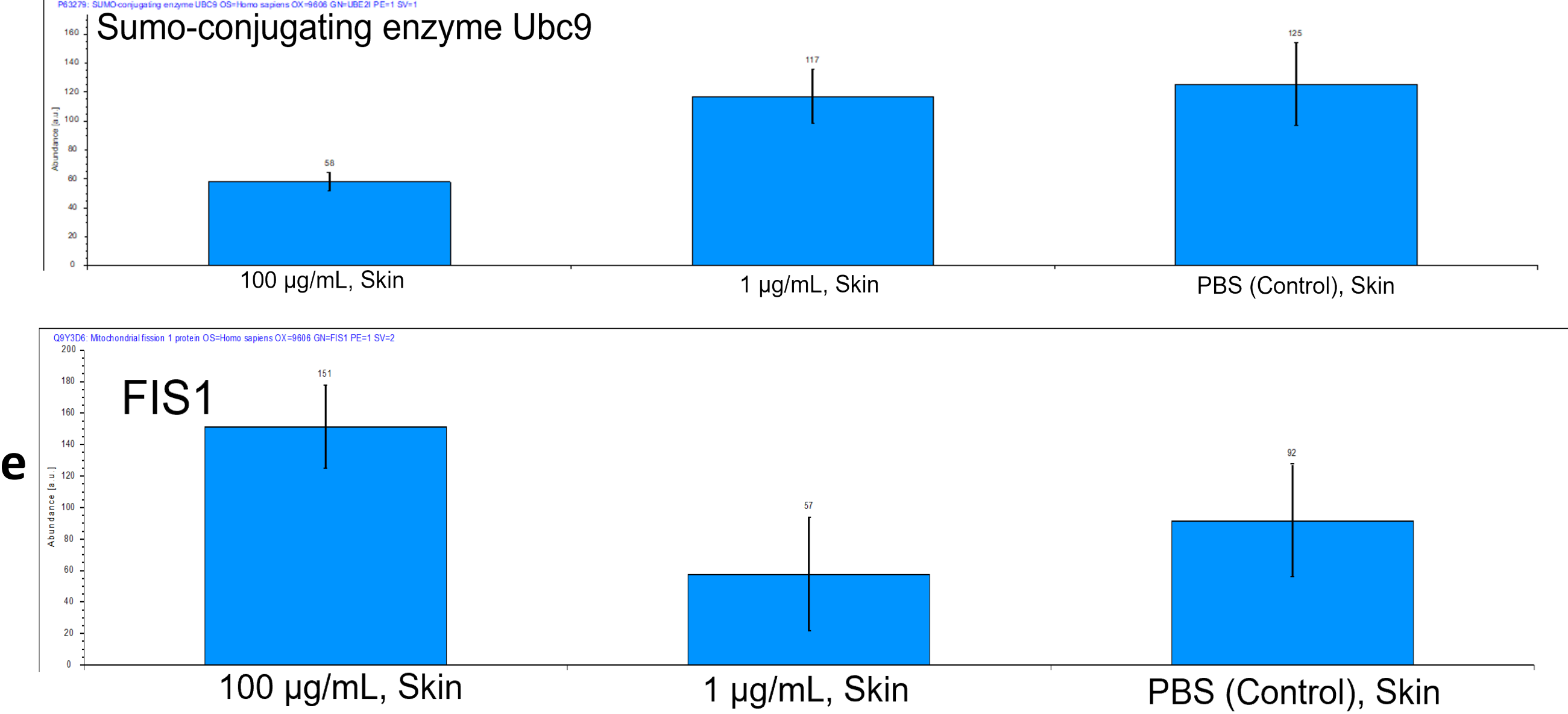


Figure 2. Cluster map of the proteomic expression values after SEB exposures. Color scaling was based on the Log2 Fold Change over untreated samples. The lung and skin tissues cluster better by exposure concentration than cardiac organoids. This is most likely due to low cell count in the cardiac organoid compartment in comparison to the lung and skin compartments.



Results

Table 2. Select pathways that are significantly disrupted due to SEB infection.

Pathway Name	Feature Match	P-value
RNA Transport	7/168	3.664 x 10 ⁻⁴
Valine, Leucine and Isoleucine Degradation	5/90	7.256 x 10 ⁻⁴
Beta-Alanine Metabolism	4/63	1.542 x 10 ⁻³
Necroptosis	6/172	2.419 x 10 ⁻³
PPAR Signaling Pathway	4/81	3.866 x 10 ⁻³
Mineral Absorption	4/87	4.991 x 10 ⁻³
Vasopressin-Regulated Water Reabsorption	3/46	5.725 x 10 ⁻³
Pantothenate and CoA Biosynthesis	3/47	6.081 x 10 ⁻³
Ribosome	5/153	7.318 x 10 ⁻³
Aminoacyl-tRNA biosynthesis	4/118	1.430 x 10 ⁻²
NOD-like Receptor Signaling Pathway	5/187	1.640 x 10 ⁻²
Arginine and Proline Metabolism	4/128	1.874 x 10 ⁻²
Valine, Leucine, and Isoleucine Biosynthesis	2/27	1.919 x 10 ⁻²
Dopaminergic Synapse	4/143	2.684 x 10 ⁻²
Renin Secretion	3/86	3.079 x 10 ⁻²
mRNA Surveillance Pathway	3/91	3.554 x 10 ⁻²
Adrenergic Signaling in Cardiomyocytes	4/159	3.749 x 10 ⁻²

Figure 3. Relative protein abundance graphs indicating protein expression from SEB exposed tissues compared to unexposed. **Top – Sumo-conjugating enzyme Ubc9 expression in skin tissues.**

Small ubiquitin-like modifier (SUMO)-conjugating enzyme Ubc9 is part of the SUMOylation process which modulates several downstream cellular processes, like transcription, translation, apoptosis, mitochondrial health, and stress response. Ubc9 specifically has shown to be essential in protein quality control in cardiomyocytes.

Bottom – FIS1 protein expression in skin tissues.

FIS1 is implicated in mitochondrial fusion and fission, which is a mechanism controlled by SUMOylation. Mitochondrial fission and fusion are critical in the maintenance of mitochondrial health, especially when the organelles are subjected to stressful conditions. The upregulation observed upon SEB exposure indicates that the cells are attempting to overcompensate for the lack of Ubc9.

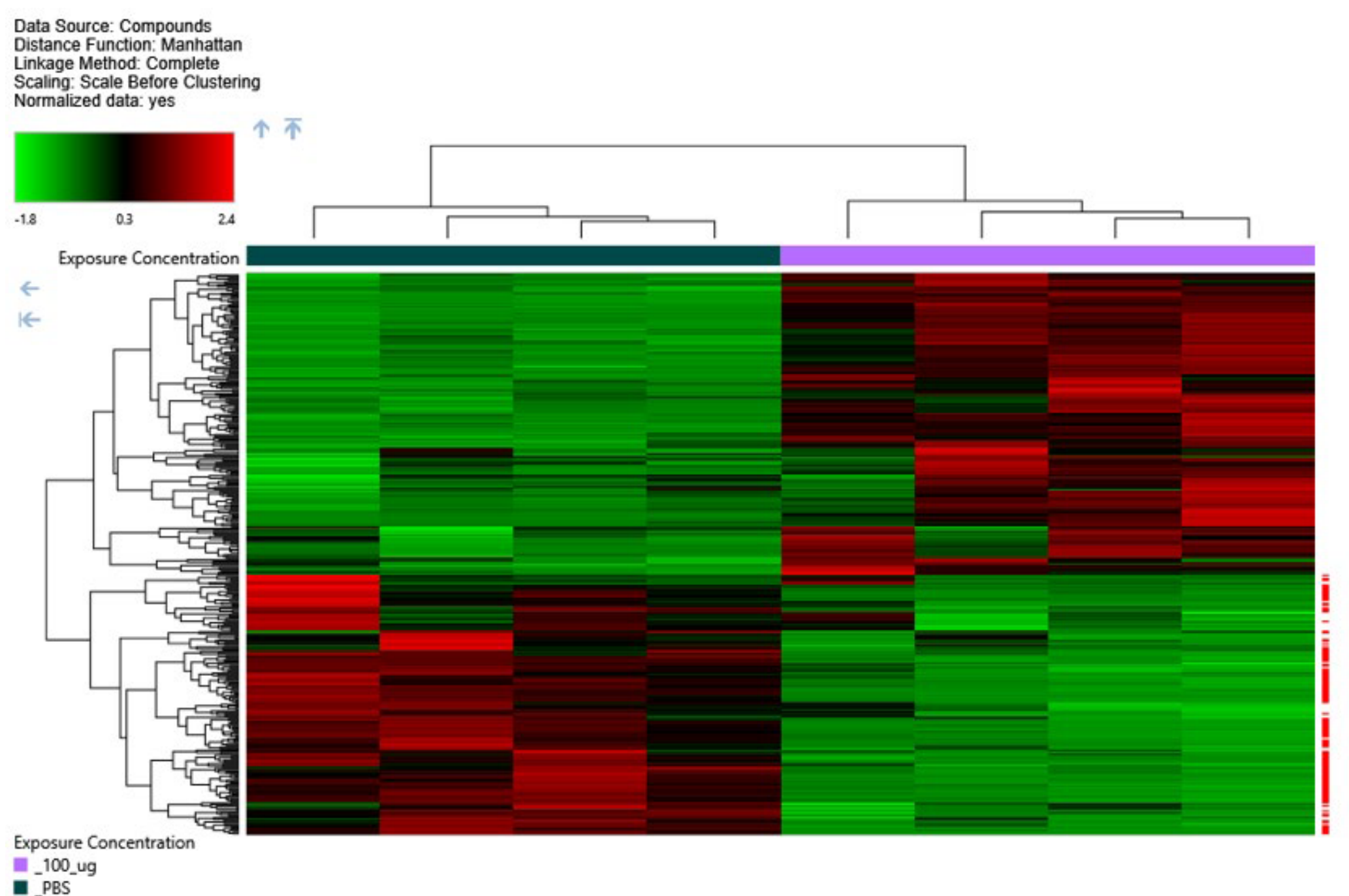


Figure 4. Heatmap of significantly changing metabolites from cardiac organoids exposed to SEB (purple) versus control (blue).

Conclusions

Microphysiological system, specifically a multi-organ chip, was used to infect three tissue systems with SEB. The 100 µg/mL dosage saw the most proteomic and metabolomic changes, but it is unknown if this same dose has the same effectiveness in animals or cell cultures. Several proteins in the skin showed changes in the SUMOylation process and further downstream processes, such as mitochondrial stress and damage. Interesting metabolic changes were observed in the cardiac organoids, including amino acid turnover and signatures of bacterial infections.

References

- Reyes DR, Esch MB, Ewart L, Nasiri R, Herland A, Sung K, Piergiovanni M, Lucchesi C, Shoemaker JT, Vukasinovic J et al. **From animal testing to in vitro systems: advancing standardization in microphysiological systems.** *Lab Chip* 2024, 24(5):1076-1087.
- Goralski TDP, Jenkins CC, Angelini DJ, Horsmon JR, Dhumakupt ES, Rizzo GM, Simmons BL, Liem AT, Roth PA, Karavis MA et al. **A novel approach to interrogating the effects of chemical warfare agent exposure using organ-on-a-chip technology and multiomic analysis.** *PLoS One* 2023, 18(2):e0280883.
- Gupta MK, Gulick J, Liu R, Wang X, Molkenin JD, Robbins J. **Sumo E2 enzyme UBC9 is required for efficient protein quality control in cardiomyocytes.** *Circ Res* 2014, 115(8):721-729
- Youle R.J. and Van der Blik, A.M. "Mitochondrial Fission, Fusion, and Stress." *Science*, 2012

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