



# U.S. ARMY COMBAT CAPABILITIES DEVELOPMENT COMMAND – CHEMICAL BIOLOGICAL CENTER

**Soldier-on-a-chip: interrogating the effects of  
chemical and biological threat agent exposure  
using a multi-organ microphysiological system**

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# WHY USE ORGANS-ON-A-CHIP?

## Problem



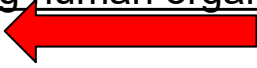
- Establishing a physiologically relevant biomimetic human model that will accurately, reliably, timely, and economically represent human organ-organ interactions. Historically this work has been performed in traditional tissue culture and animal models which can be time consuming, costly, and lack physiological accuracy and precision.

## Solution



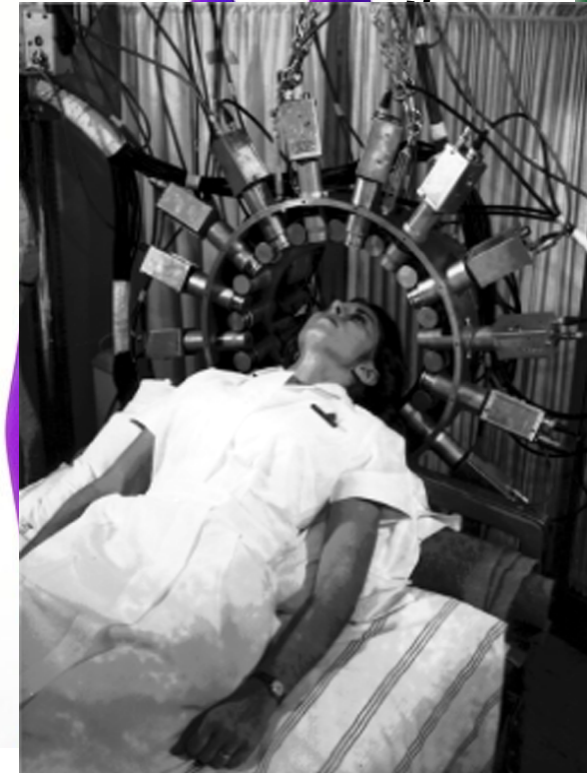
- Micro-physiological systems (MPS) technology offers a high throughput process that overcomes the drawbacks to both conventional *in vitro* and animal modeling by supplying cutting-edge Organs-on-a-chip (OOC) that imitate human tissue-tissue interfaces, chemical and mechanical microenvironments specific to living human organs.

## Goal



- Provide an ideal alternative and/or replacement to traditional tissue culture and animal models for a “human surrogate” toxicity and efficacy testing.

## MicroPhysiological Systems Human Surrogate





# FUNCTIONAL SYSTEMS FOR PREDICTIVE TOXICOLOGY AT THREAT AGENT SCIENCE

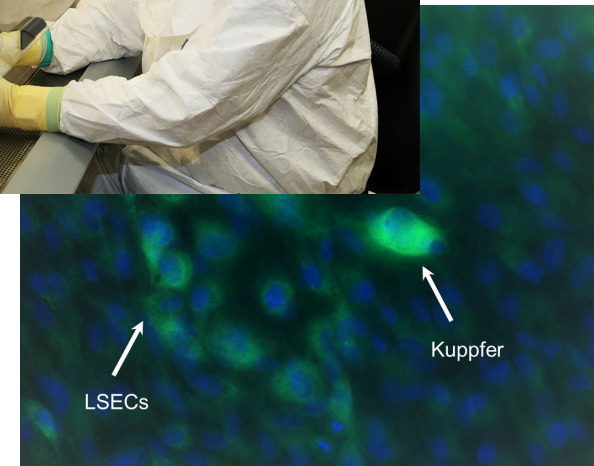
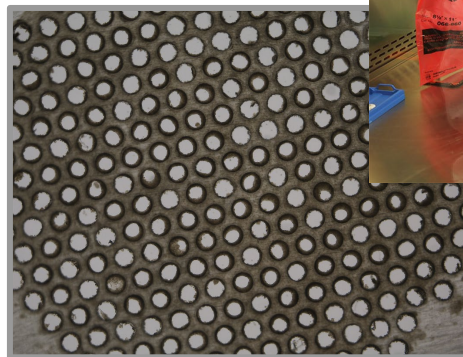
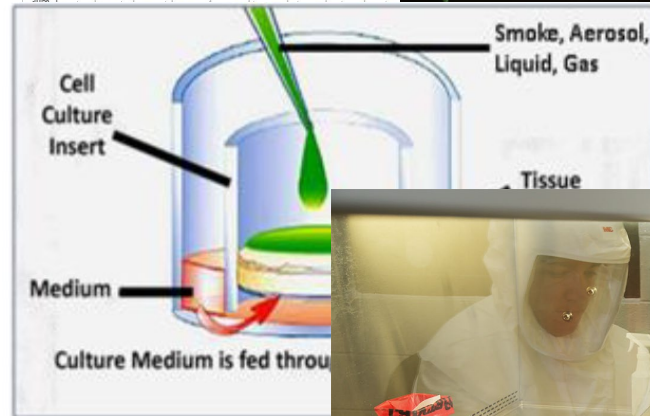
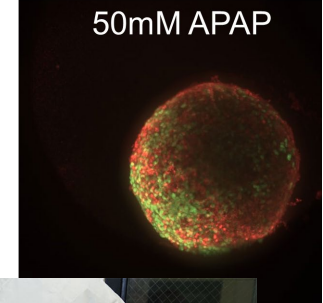
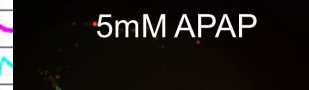
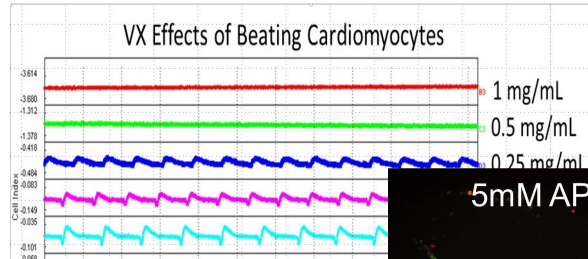


## • Operational

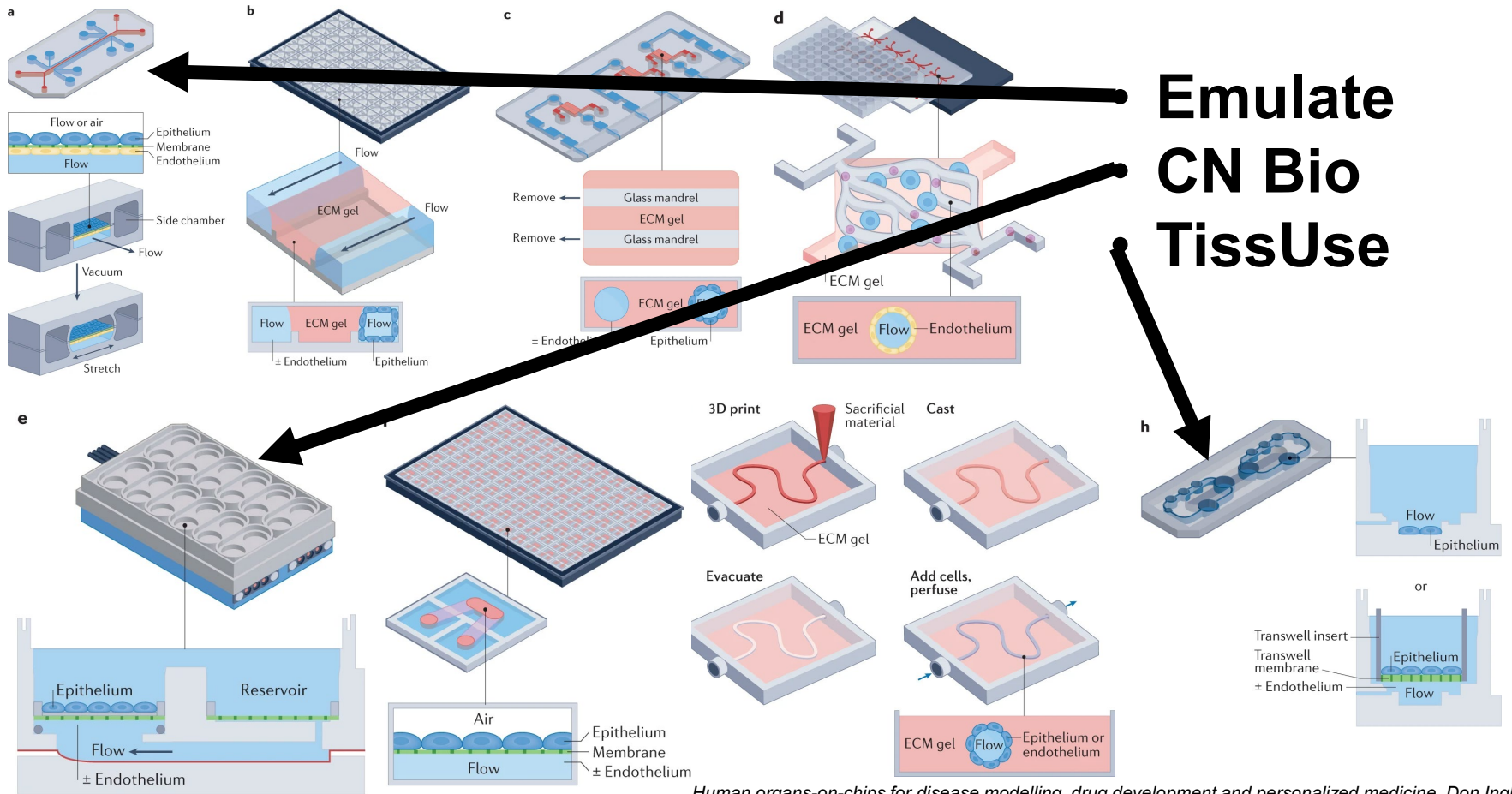
- Cardiac (RTCA, organoids)
- Liver (2D, 3D, MPS)
- CNS (2D)
- Lung (2D, 3D)
- Dermal (3D)
- Lung (MPS)
- Kidney (MPS)
- Intestinal (3D, MPS)

## • In Development

- CNS (3D organoids)
- BBB (MPS)



# CURRENT ORGAN-ON-A-CHIP DESIGNS







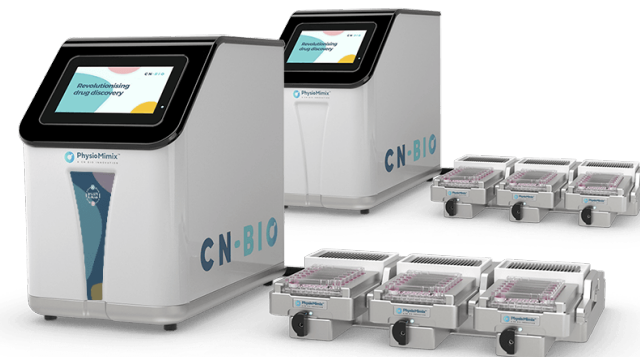
# CURRENT DEVCOM CBC SYSTEMS



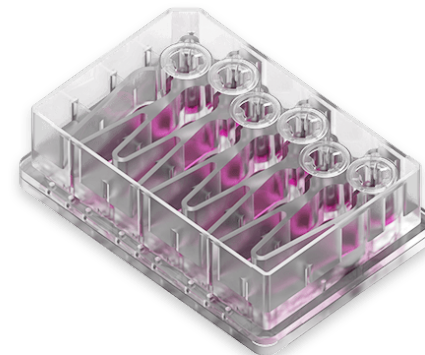
## Emulate



## CN Bio

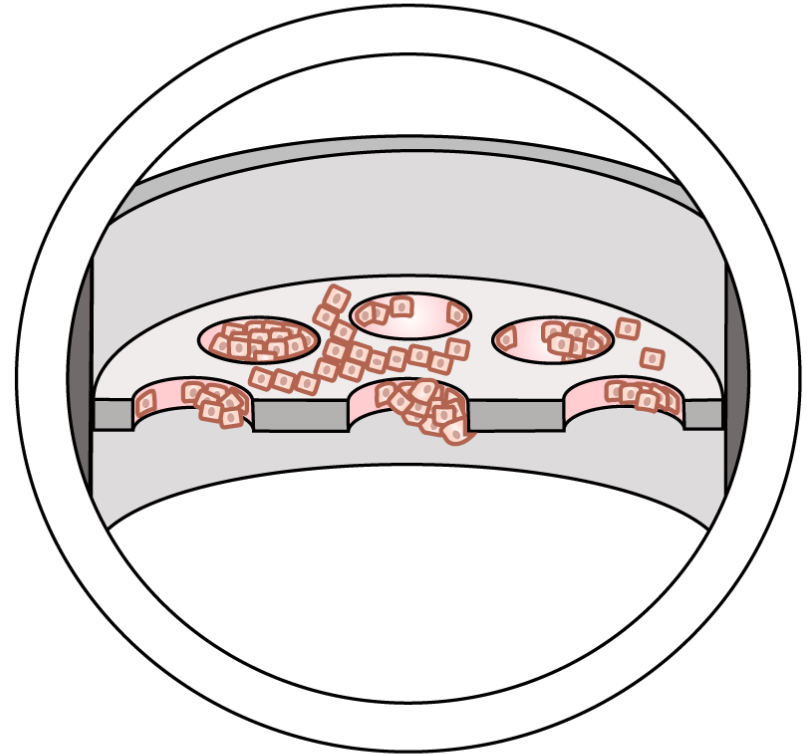
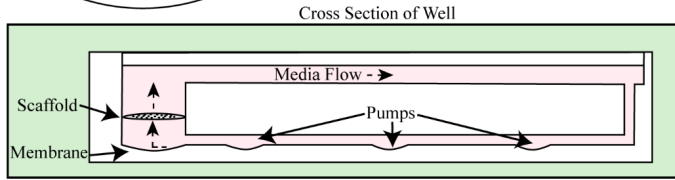
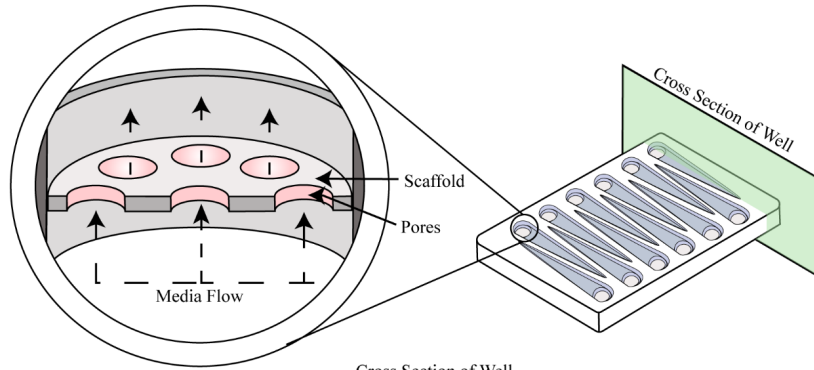


## TissUse





# CN BIO PHYSIOMIMIX

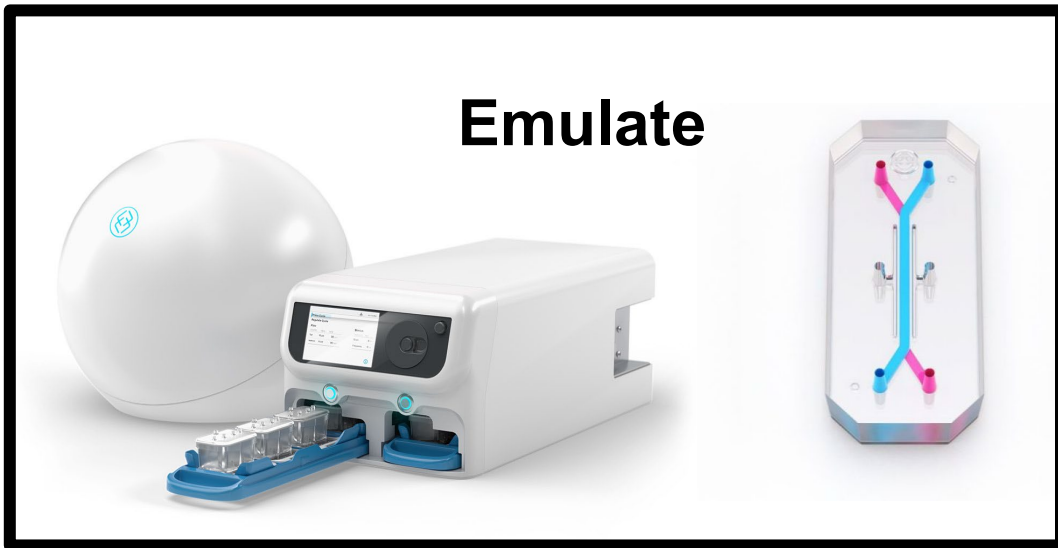




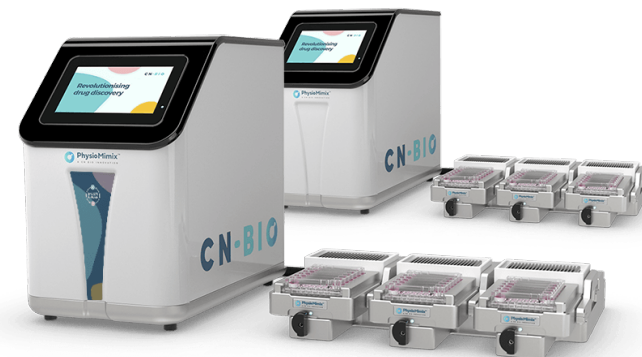
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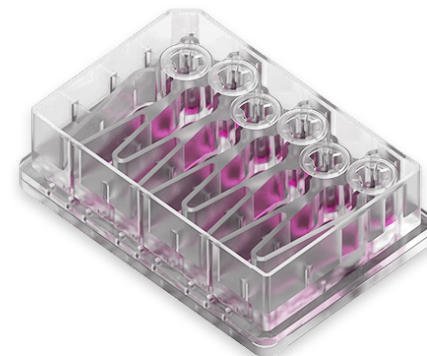
## Emulate



## CN Bio



## TissUse







# EMULATE CHIP OVERVIEW

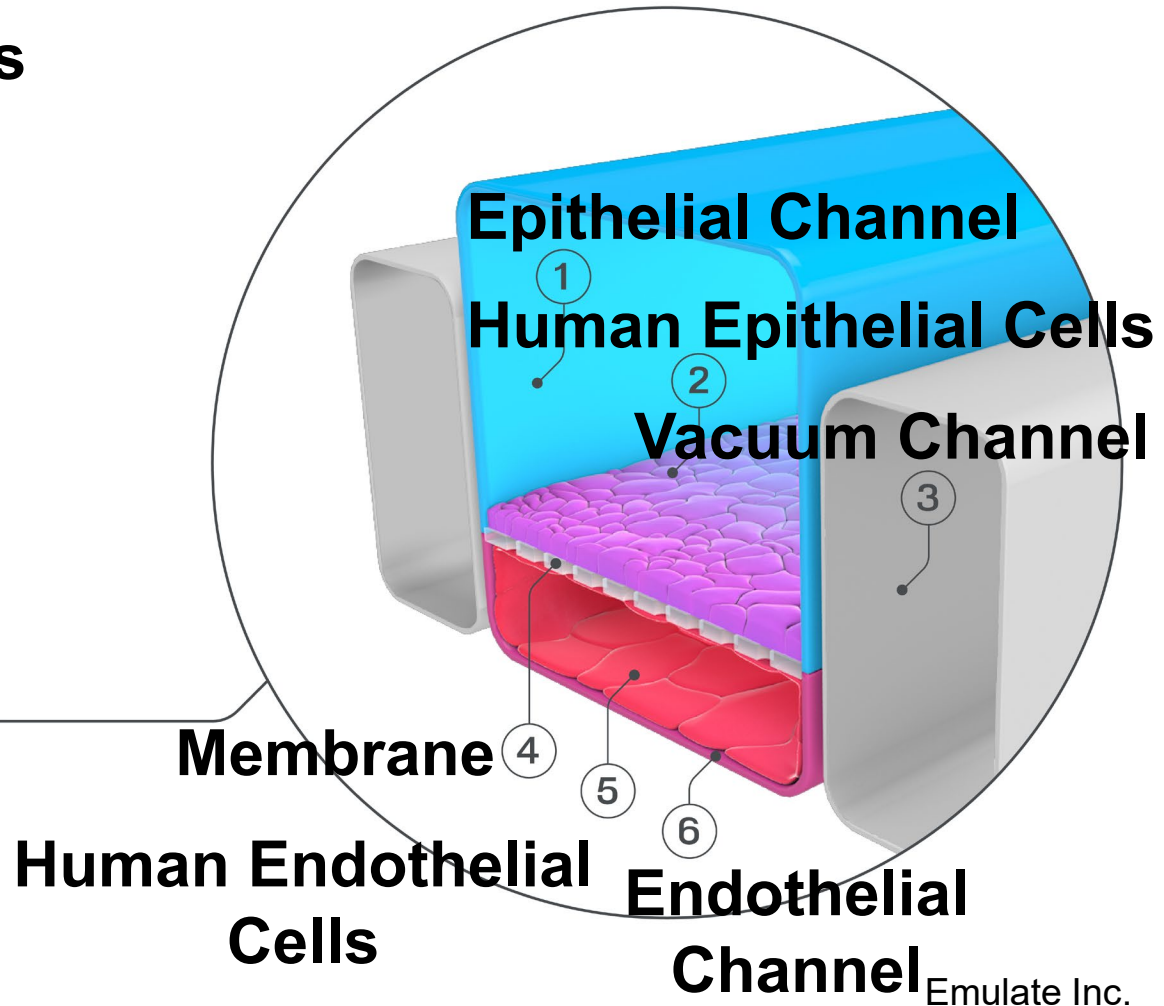
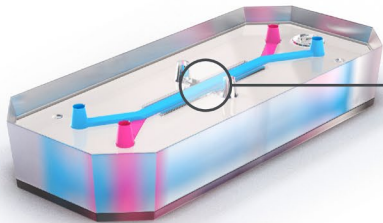


## Operational Tissues

- Liver
- Lung
- Kidney

## In Development

- Brain





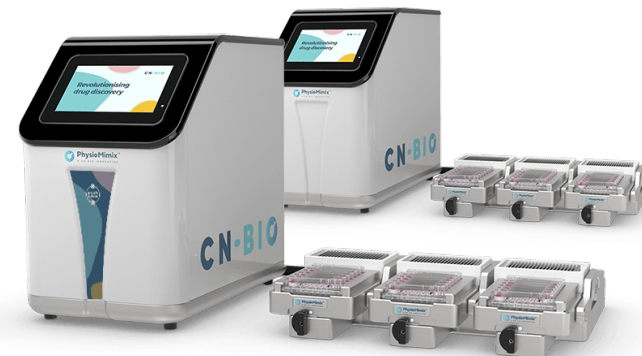
# CURRENT DEVCOM CBC SYSTEMS



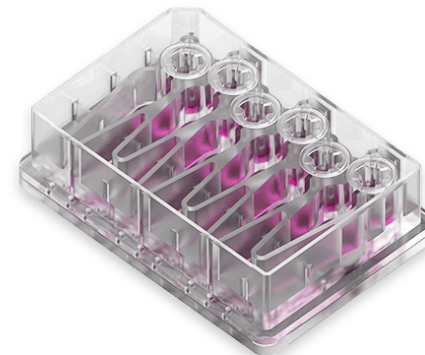
## Emulate



## CN Bio

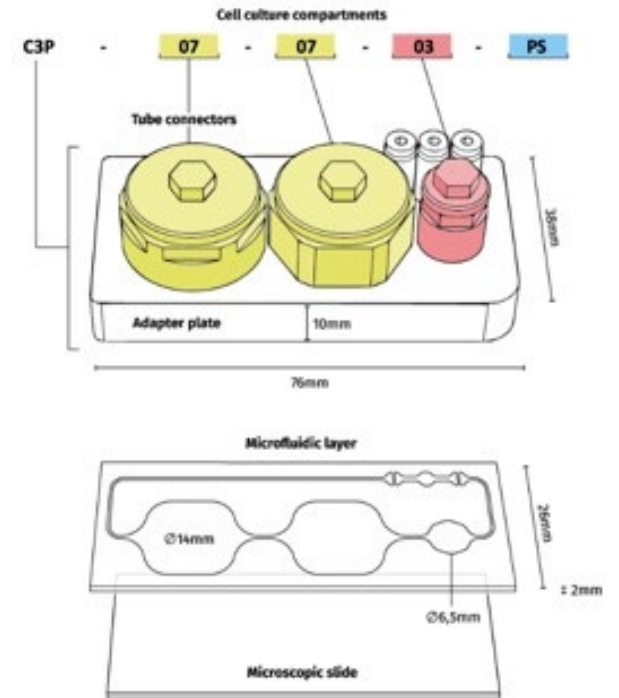


## TissUse

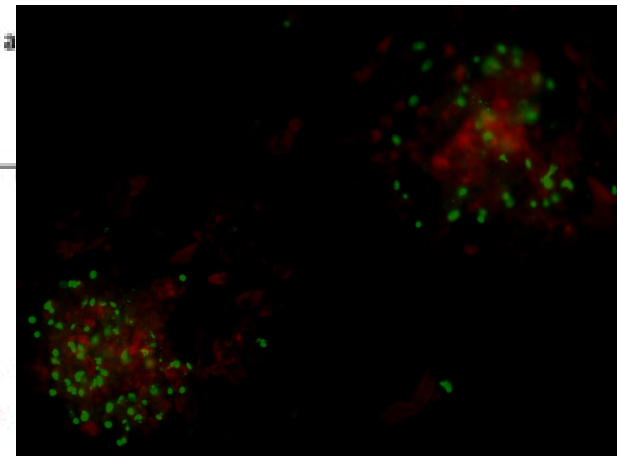
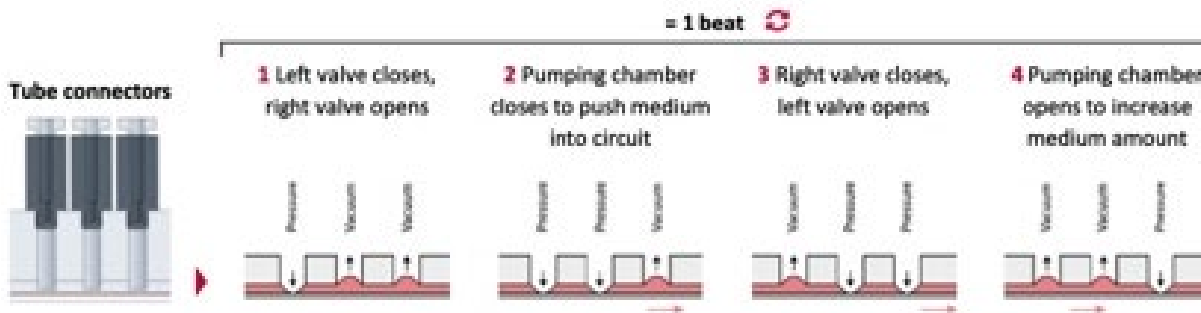




# TISSUE HUMIMMIC MOC SYSTEM



Each HUMIMMIC Chip3plus circuit contains three 500  $\mu\text{m}$  thick pump membranes, which are operated by a change of pressured air and vacuum. This leads to opening and closing the valves.





# MULTIOMIC ANALYSIS OF THREAT AGENTS USING MOC



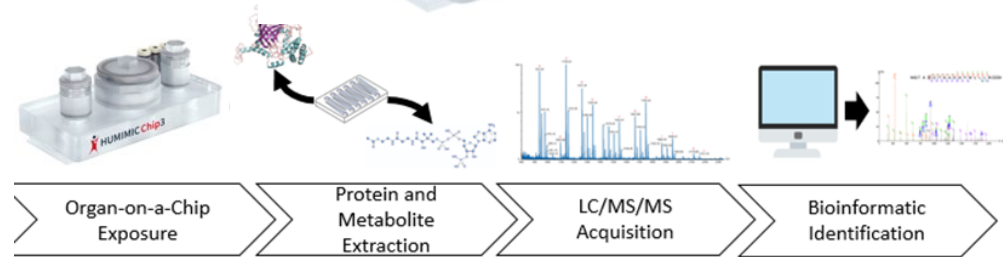
## TissUse Chip3+:

- Size of a standard microscope slide, suitable for iPSC-derived cells, primary cells, 3D tissues and cell lines. **3 organ systems on 1 chip.**
- Uses on-chip micro-pump enabling pulsatile flow.



## Current Efforts:

- Expose various tissue combinations to biological and chemical agents.
- Perform downstream analysis using the MOC technology.



## Analyses includes but is not limited to...

- **Proteomics/Transcriptomics, Metabolomics – Machine learning driven** feature detection, global metabolite system perturbation analysis, broad screening based on multiple chemometric profiles.
- Staining and imaging (both live and dead cell microscopy).
- ELISA and other immunoassays
- Electrophysiology
- And many more!



# CARFENTANIL EXPOSURE

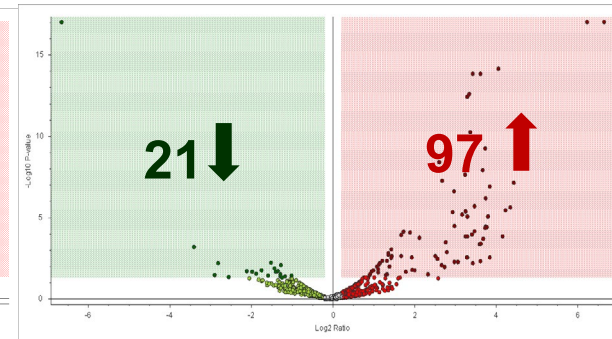
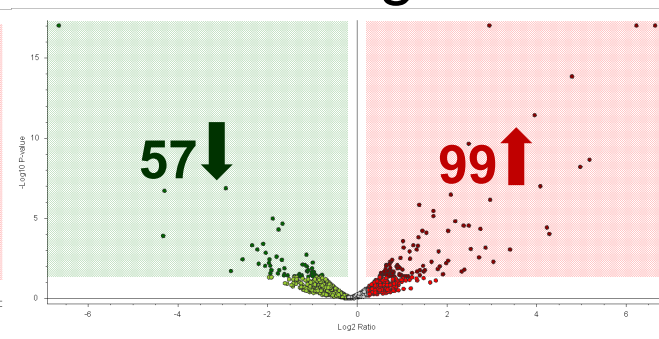
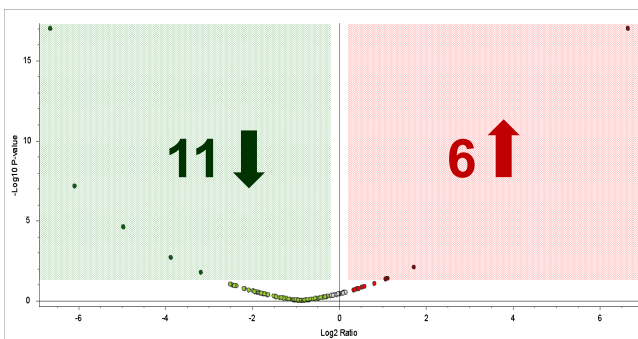


Sample	High Confident proteins Found	Significantly Changing Proteins
Cardiac	158	31
Skin	982	231
Lung	1580	299

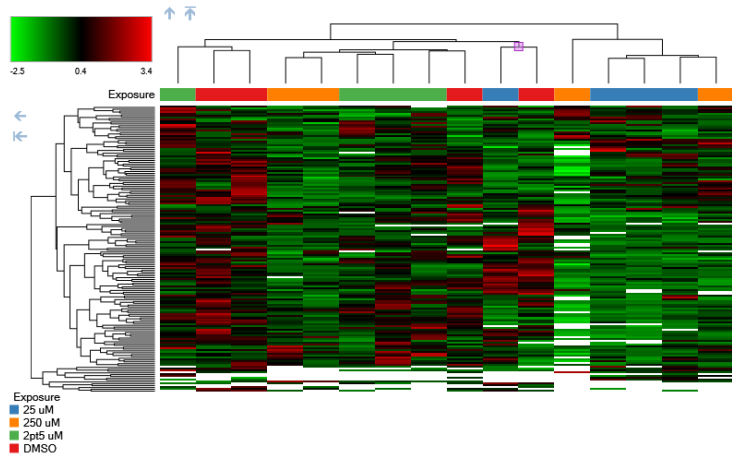
## Cardiac

## Lung

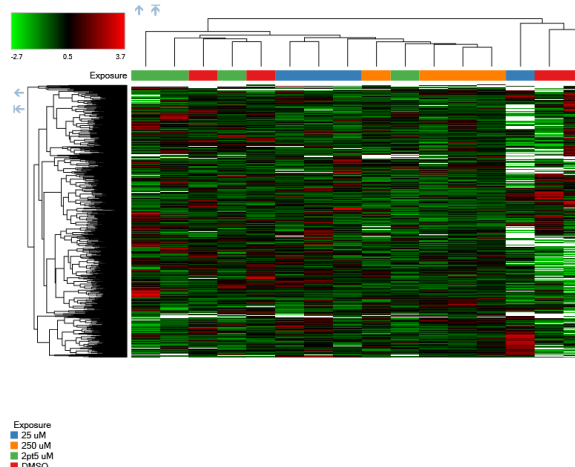
## Skin



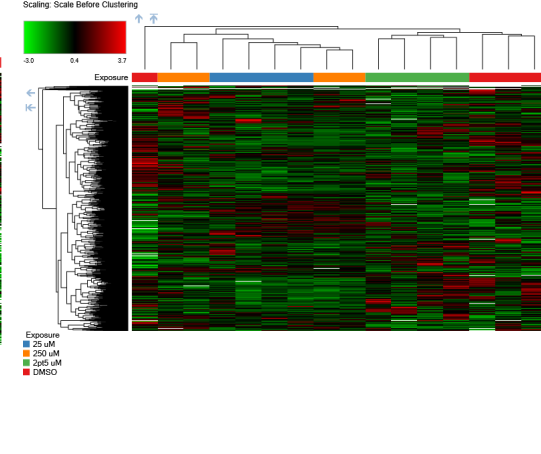
Data Source: Proteins : Abundances (Normalized)  
 Distance Function: Euclidean  
 Linkage Method: Complete  
 Scaling: Scale Before Clustering



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 Distance Function: Euclidean  
 Linkage Method: Complete  
 Scaling: Scale Before Clustering



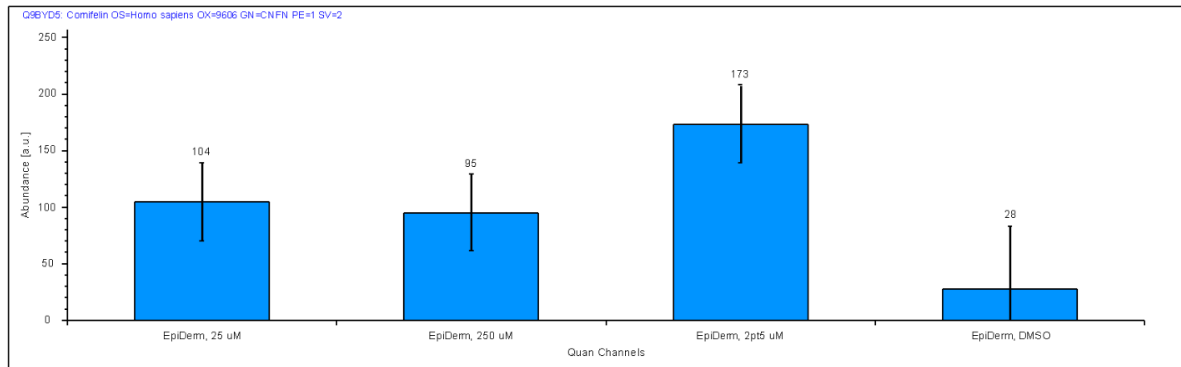
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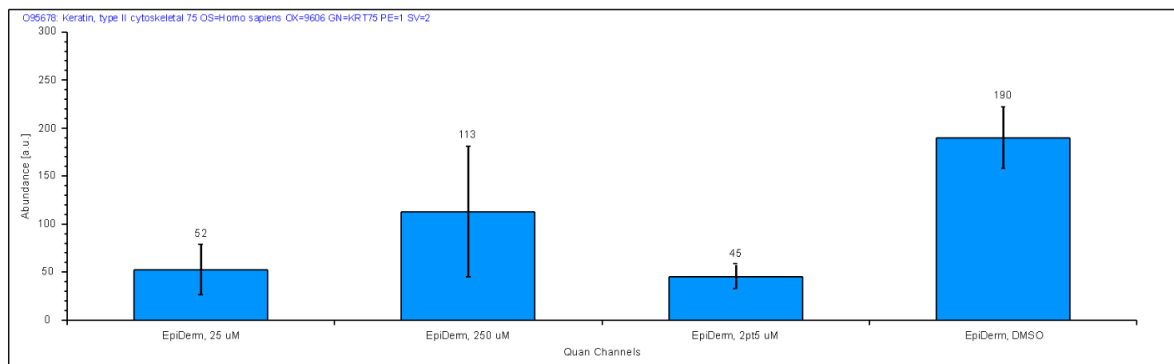




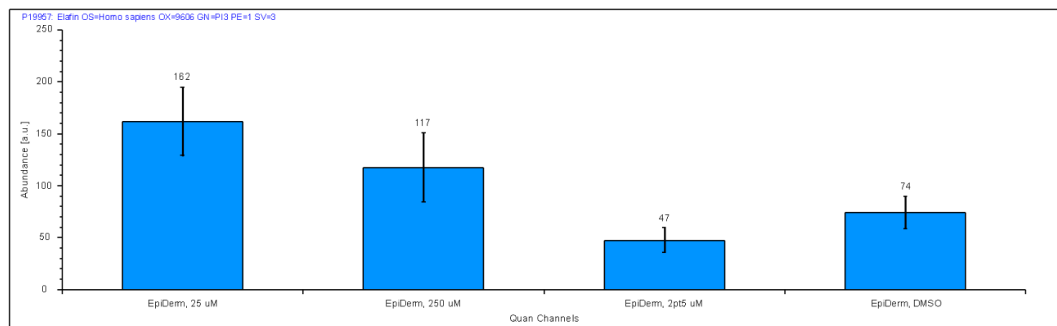
# CARFENTANIL SKIN: CELL STRUCTURE



**Cornifelin:** Part of the insoluble cornified cell envelope (CE) of stratified squamous epithelia.



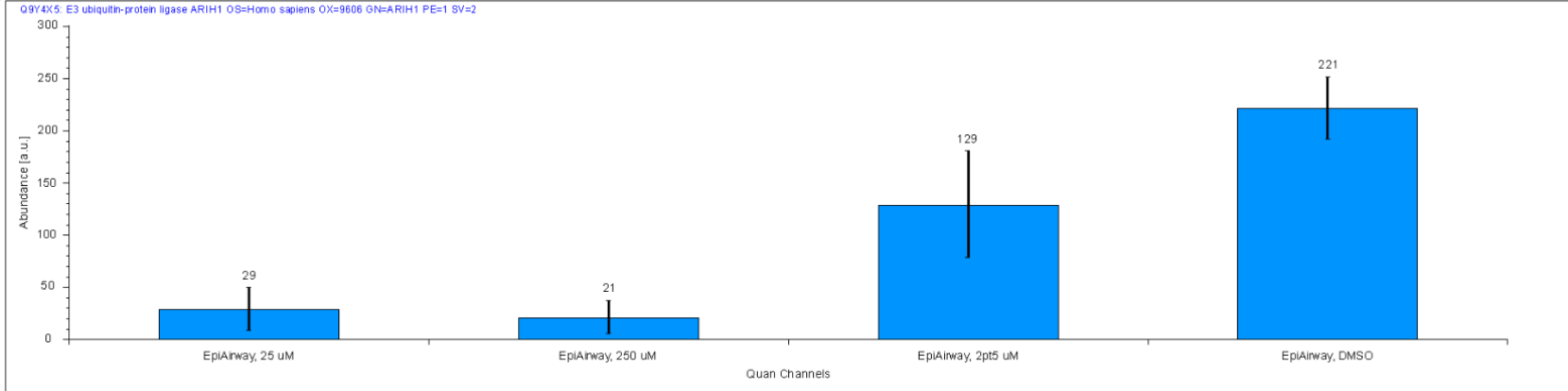
**Keratin:** It is the key structural material making up scales, hair, nails, feathers, horns, claws, hooves, and the outer layer of skin among vertebrates. Keratin also protects epithelial cells from damage or stress.



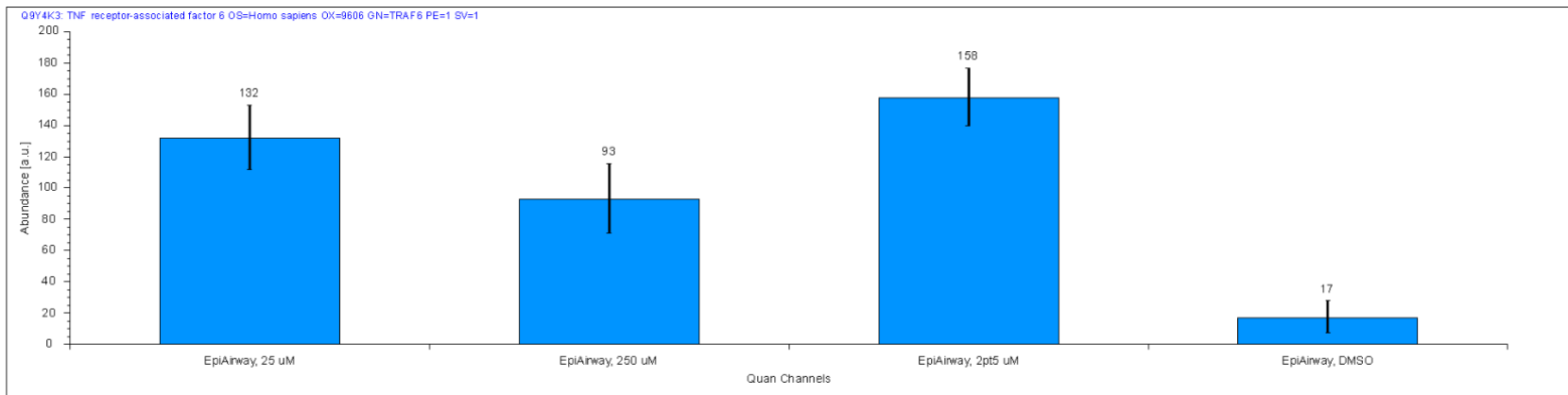
**Elafin:** Elastase inhibitor. Already being used as a biomarker for graft versus host disease, which is shown to be upregulated compared to those without. May function in TNF-alpha activation.



# CARFENTANIL LUNG: SIGNALING



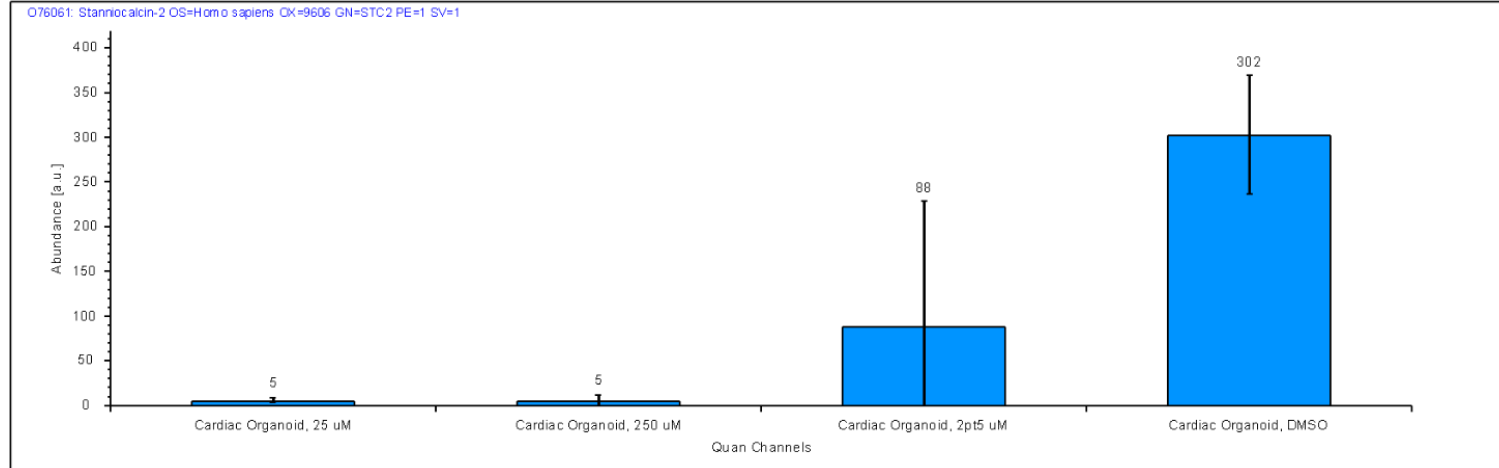
**E3 ubiquitin ligase:** E3s polyubiquitinate their substrate with Lys48-linked chains of ubiquitin, targeting the substrate for destruction by the proteasome. However, many other types of linkages are possible and alter a protein's activity, interactions, or localization. Ubiquitination by E3 ligases regulates diverse areas such as cell trafficking, DNA repair, and signaling.



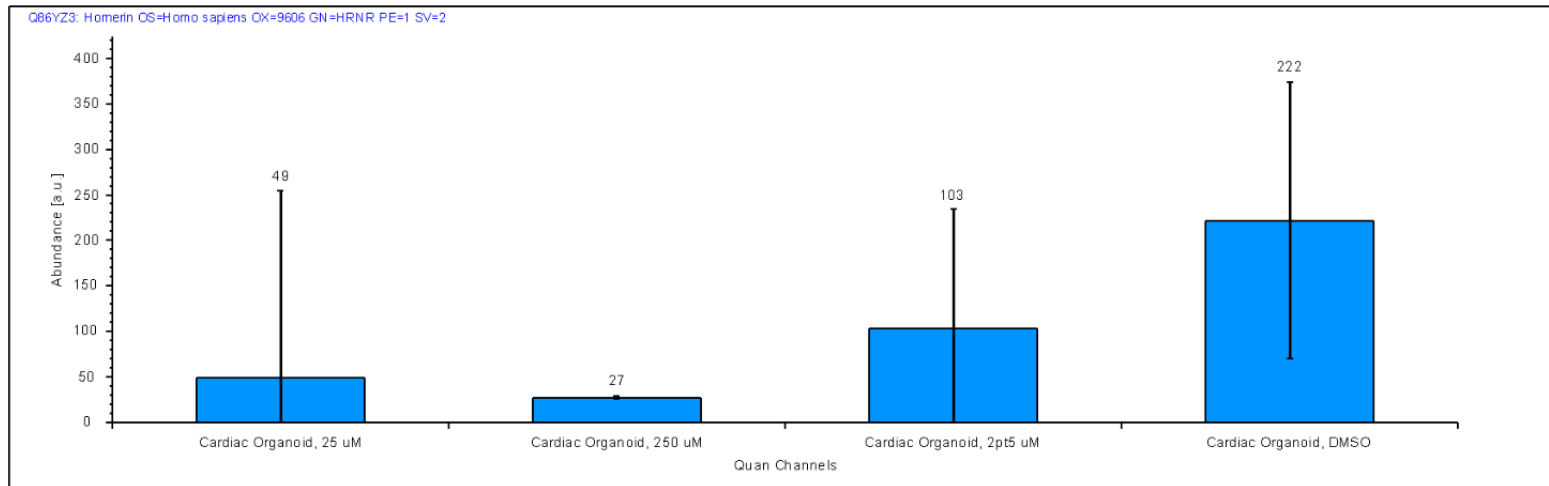
**TNF alpha receptor factor 6:** adaptor protein that mediates a wide array of protein-protein interactions via its TRAF domain and a RING finger domain that possesses non-conventional E3 ubiquitin ligase activity. Could be compensating for down regulation of E3.



# CARFENTANIL CARDIAC:CALCIUM FLUX



**Stanniocalcin:** family of hormones which regulate calcium and phosphate balance in the body.



**Homerin:** aids in coupling surface receptors to intracellular calcium release.



# SEB EXPOSURE

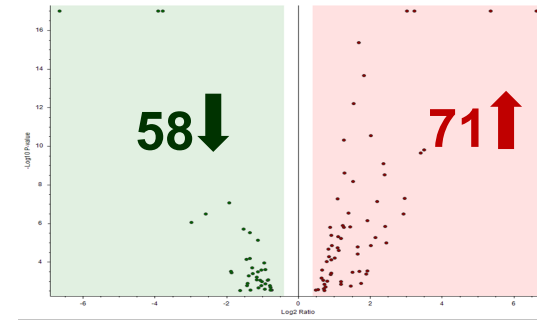
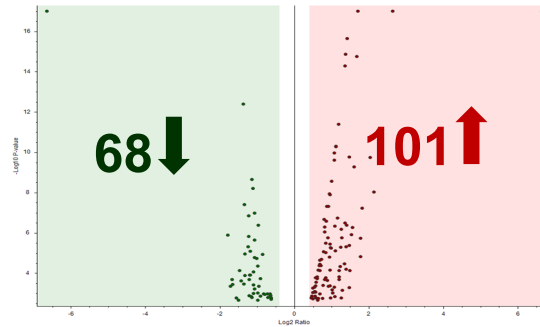
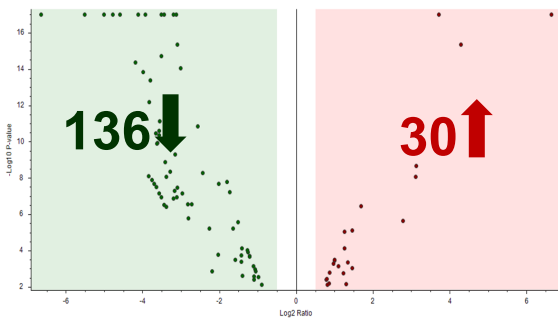


Sample	High Confident proteins Found	Significantly Changing Proteins
Lung	4338	340
Skin	2452	239
Cardiac	1334	303

## Cardiac

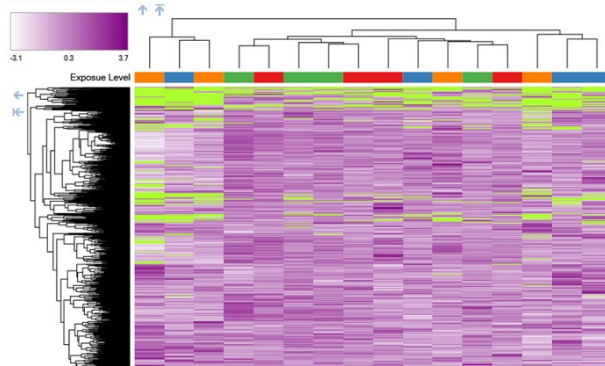
## Lung

## Skin

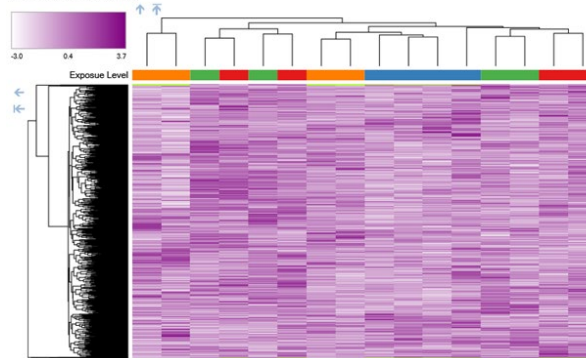


58 Down, 71 up Significantly in 100ug/mL Exposure

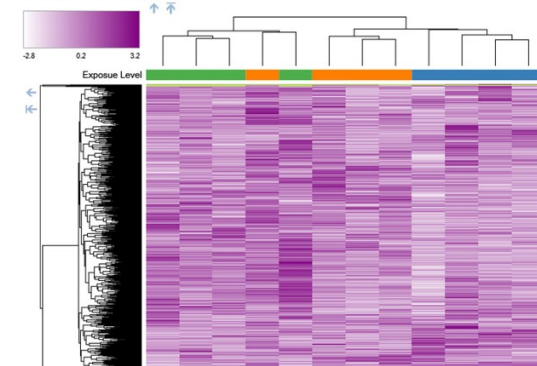
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 Distance Function: Euclidean  
 Linkage Method: Complete  
 Scaling: Scale Before Clustering



Data Source: Proteins - Abundances (Normalized)  
 Distance Function: Euclidean  
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Data Source: Proteins - Abundances (Normalized)  
 Distance Function: Euclidean  
 Linkage Method: Complete  
 Scaling: Scale Before Clustering



Exposure Level  
 100ug/mL  
 10ug/mL  
 1ug/mL  
 PBS

Exposure Level  
 100ug/mL  
 10ug/mL  
 1ug/mL  
 PBS

Exposure Level  
 100ug/mL  
 1ug/mL  
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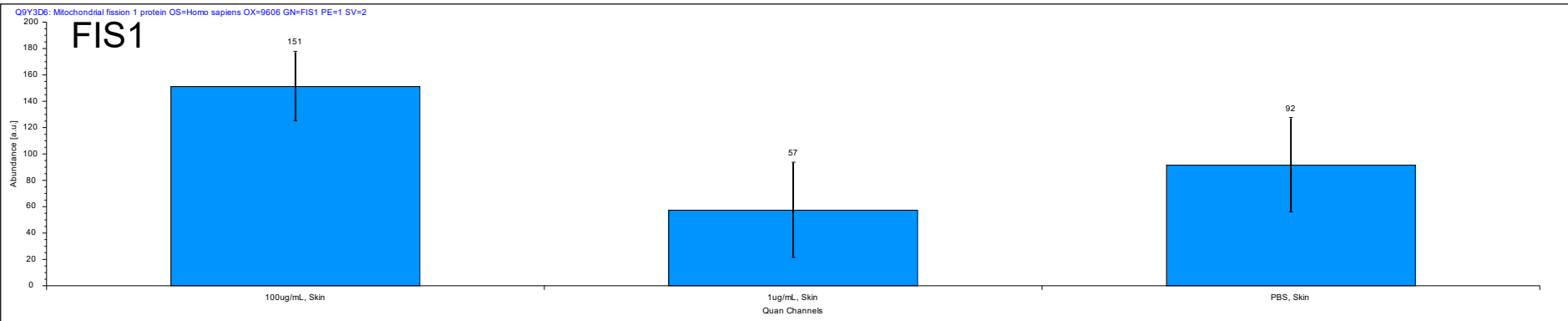




# FISSION AND FUSION



**Mitochondrial fission and fusion** play critical roles in maintaining functional mitochondria when cells experience metabolic or environmental stresses. Fusion helps mitigate stress by mixing the contents of partially damaged mitochondria as a form of complementation. Fission is needed to create new mitochondria, but it also contributes to quality control by enabling the removal of damaged mitochondria and can facilitate apoptosis during high levels of cellular stress. Disruptions in these processes affect normal development, and they have been implicated in neurodegenerative diseases, such as Parkinson's.



The adaptor protein FIS1 is involved recruiting Drp1 in peripheral division.

[Science](#). Author manuscript; available in PMC 2016 Feb 22.

Published in final edited form as:

[Science](#). 2012 Aug 31; 337(6098): 1062–1065.

doi: [10.1126/science.1219855](https://doi.org/10.1126/science.1219855)

PMCID: PMC4762028

NIHMSID: NIHMS757703

PMID: [22936770](https://pubmed.ncbi.nlm.nih.gov/22936770/)

## Mitochondrial Fission, Fusion, and Stress

[Richard J. Youle](#)<sup>1,\*</sup> and [Alexander M. van der Bliek](#)<sup>2,\*</sup>

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# MITOCHONDRIAL DYSFUNCTION



Skin

Accession	Description	Size	Overlap	Expected	Fold Enrichment	p-Value	-log <sub>2</sub> (p-Value)
GO:0042775	mitochondrial ATP synthesis coupled electron transport	41	3	0.4	7.88	0.00631	7.31
GO:0034642	mitochondrion migration along actin filament	1	1	0	100	0.00929	6.75
GO:0010821	regulation of mitochondrion organization	51	3	0.5	6.33	0.0115	6.44
GO:0046602	regulation of mitotic centrosome separation	2	1	0	53.8	0.0185	5.76
GO:0046604	positive regulation of mitotic centrosome separation	2	1	0	53.8	0.0185	5.76
GO:0007084	mitotic nuclear membrane reassembly	4	1	0	26.9	0.0366	4.77
GO:0101024	mitotic nuclear membrane organization	4	1	0	26.9	0.0366	4.77
GO:0006121	mitochondrial electron transport, succinate to ubiquinone	4	1	0	26.9	0.0366	4.77
GO:1901030	positive regulation of mitochondrial outer membrane permeabilization invol	5	1	0	21.5	0.0456	4.45
GO:0007064	mitotic sister chromatid cohesion	5	1	0	21.5	0.0456	4.45

Cardiac

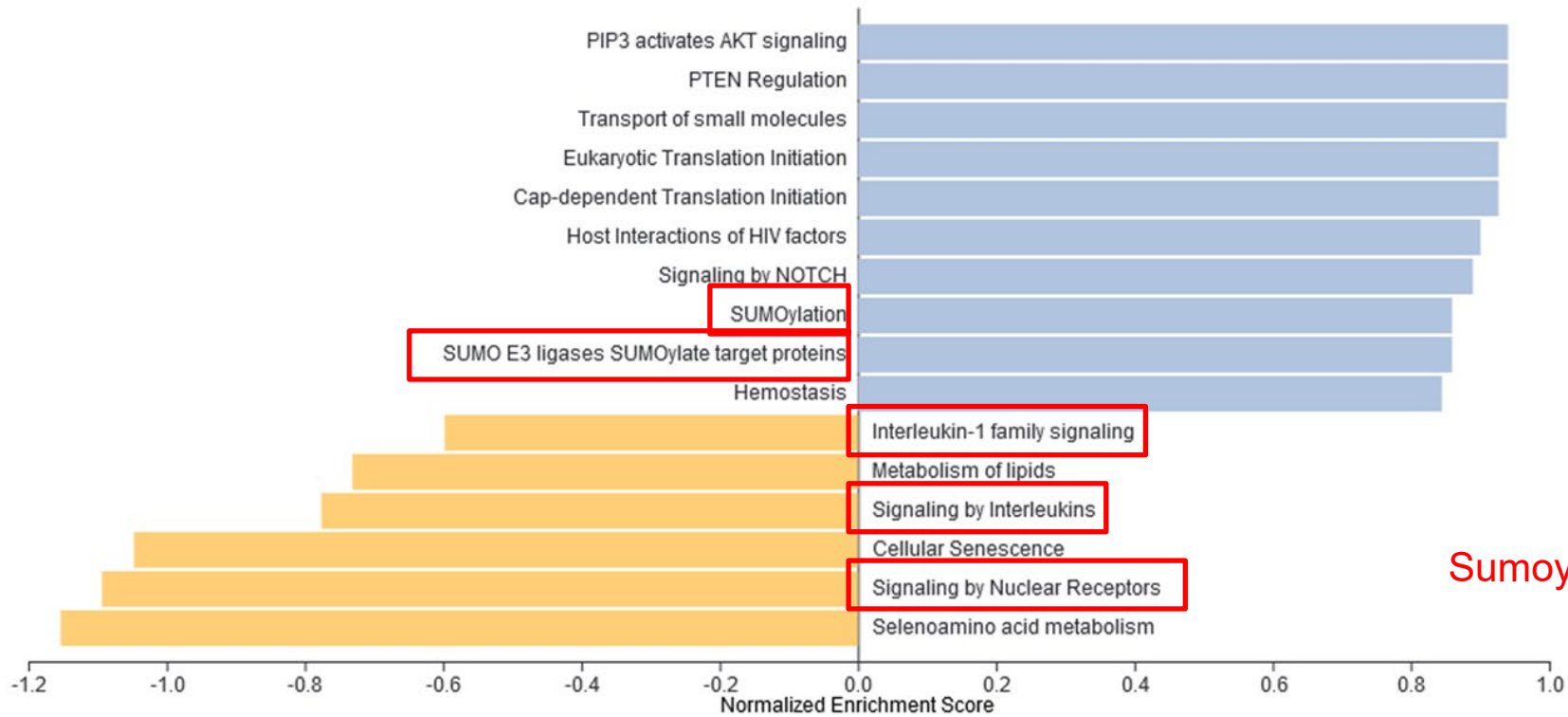
Accession	Description	Size	Overlap	Expected	Fold Enrichment	p-Value	-log <sub>2</sub> (p-Value)
GO:0010821	regulation of mitochondrion organization	33	6	1.3	4.7	0.0014	9.48
GO:0070584	mitochondrion morphogenesis	5	2	0.2	10.3	0.0137	6.18
GO:0051646	mitochondrion localization	6	2	0.2	8.62	0.0201	5.64
GO:0010822	positive regulation of mitochondrion organization	17	3	0.7	4.56	0.0258	5.27
GO:0090199	regulation of release of cytochrome c from mitochondrion	7	2	0.3	7.39	0.0274	5.19
GO:0035794	positive regulation of mitochondrial membrane permeabilization	8	2	0.3	6.47	0.0357	4.81
GO:1903146	regulation of autophagy of mitochondrion	8	2	0.3	6.47	0.0357	4.81
GO:0006264	mitochondrial DNA replication	1	1	0	25.9	0.0387	4.69
GO:0048312	intracellular distribution of mitochondria	1	1	0	25.9	0.0387	4.69
GO:0090149	mitochondrial membrane fission	1	1	0	25.9	0.0387	4.69
GO:0140141	mitochondrial potassium ion transmembrane transport	1	1	0	25.9	0.0387	4.69
GO:0003374	dynamin family protein polymerization involved in mitochondrial membrane fission	1	1	0	25.9	0.0387	4.69
GO:0062156	mitochondrial ATP-gated potassium channel activity	1	1	0	25.9	0.0387	4.69
GO:0006390	mitochondrial transcription	1	1	0	25.9	0.0387	4.69
GO:0048311	mitochondrion distribution	1	1	0	25.9	0.0387	4.69
GO:1905446	regulation of mitochondrial ATP synthesis coupled electron transport	1	1	0	25.9	0.0387	4.69
GO:1905448	positive regulation of mitochondrial ATP synthesis coupled electron transport	1	1	0	25.9	0.0387	4.69
GO:0007005	mitochondrion organization	86	7	3.3	2.1	0.047	4.41

Lung

Accession	Description	Size	Overlap	Expected	Fold Enrichment	p-Value	-log <sub>2</sub> (p-Value)
GO:0042775	mitochondrial ATP synthesis coupled electron transport	65	4	0.4	9.8	0.000721	10.44
GO:0006123	mitochondrial electron transport, cytochrome c to oxygen oxidoreductase activity	12	2	0.1	26.5	0.00246	8.67
GO:1903673	mitotic cleavage furrow formation	1	1	0	100	0.00628	7.32
GO:0045165	cell fate commitment	29	2	0.2	11	0.0141	6.14
GO:0002363	alpha-beta T cell lineage commitment	5	1	0	31.9	0.031	5.01
GO:0006390	mitochondrial transcription	6	1	0	26.5	0.0371	4.75
GO:0060795	cell fate commitment involved in formation of primary T cell lineage	6	1	0	26.5	0.0371	4.75
GO:1990456	mitochondrion-endoplasmic reticulum membrane tethering	6	1	0	26.5	0.0371	4.75
GO:0001711	endodermal cell fate commitment	6	1	0	26.5	0.0371	4.75
GO:0002360	T cell lineage commitment	7	1	0	22.8	0.0431	4.53
GO:0045039	protein insertion into mitochondrial inner membrane	7	1	0	22.8	0.0431	4.53



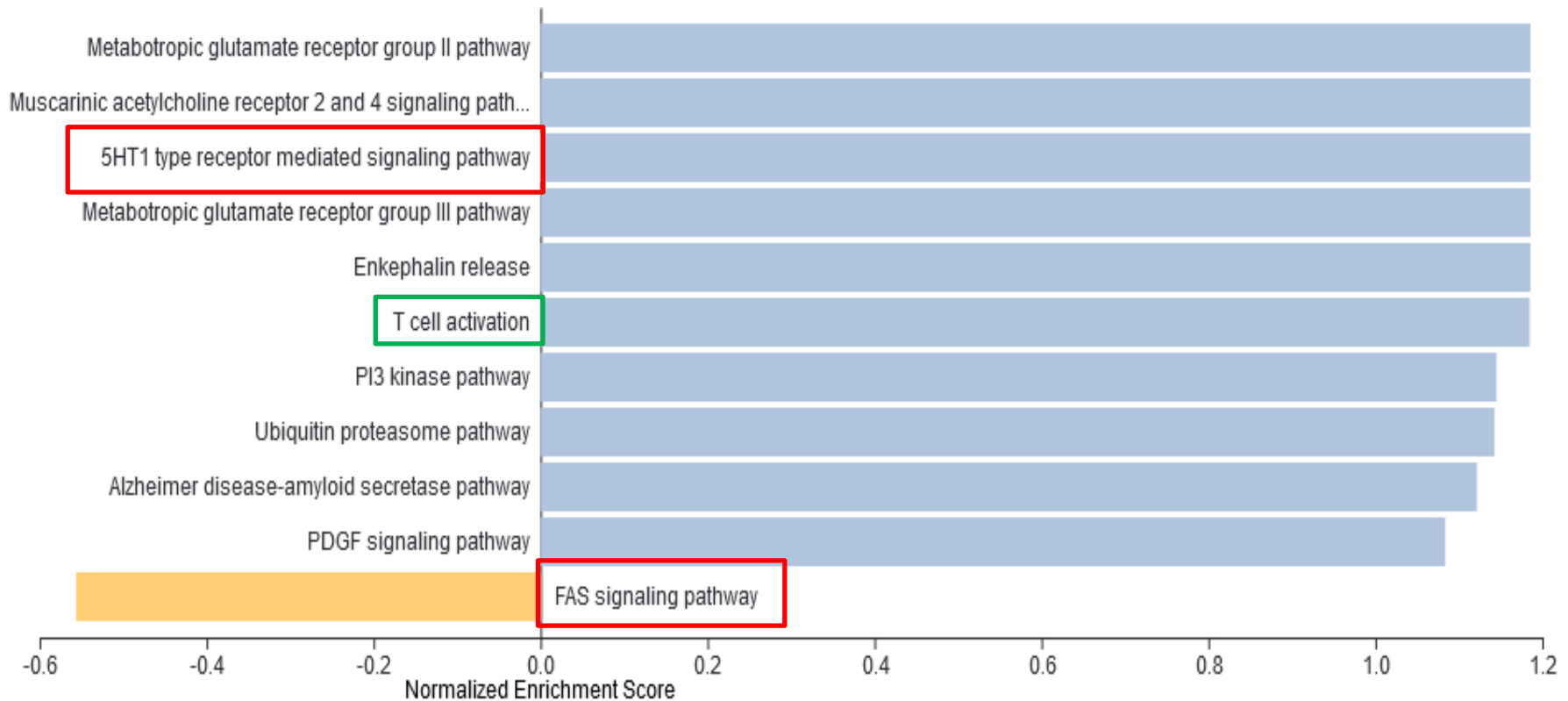
# TOP DYSFUNCTIONS: SKIN



Sumoylation



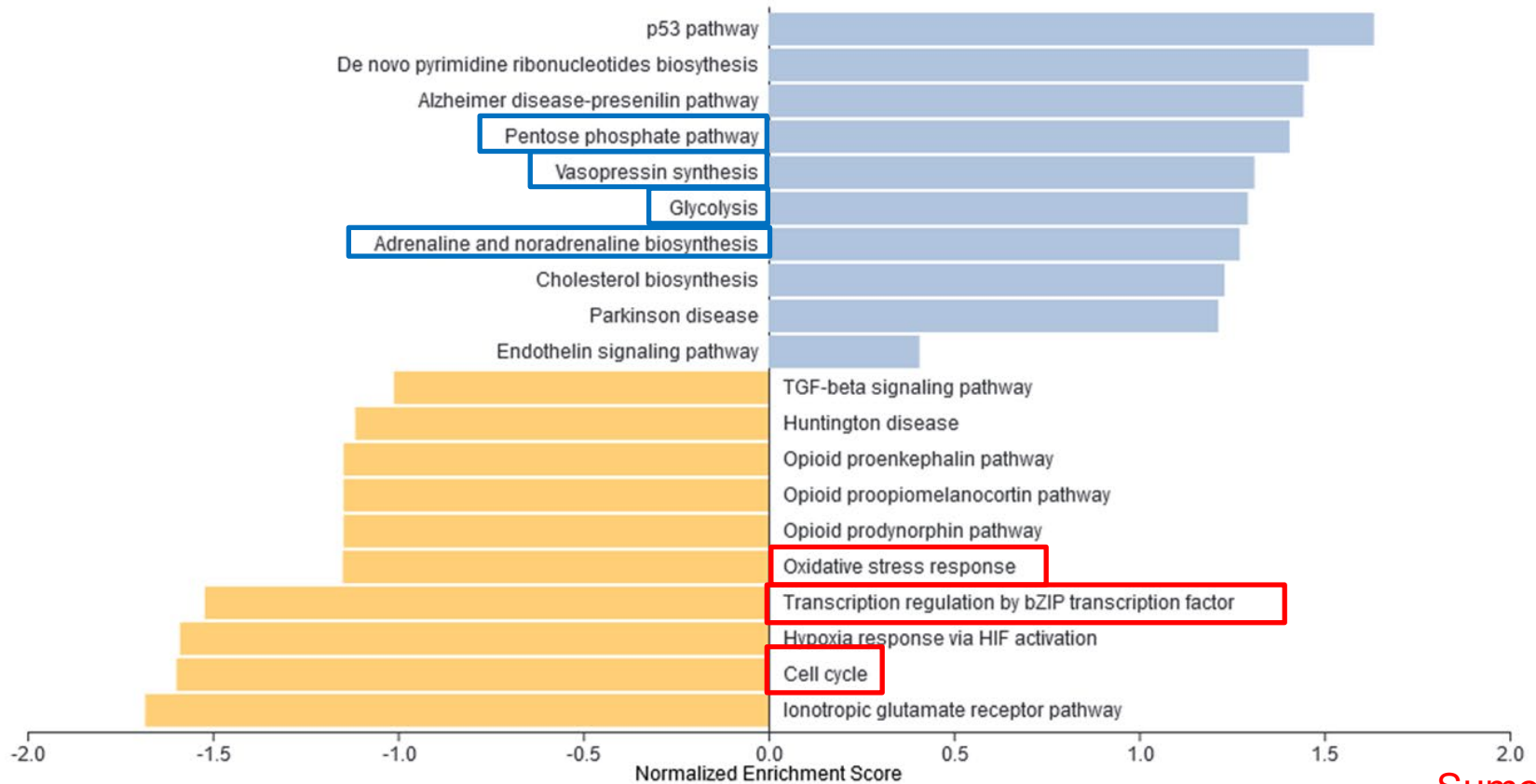
# TOP DYSFUNCTIONS: CARDIAC



Sumoylation  
MOA



# TOP DYSFUNCTIONS: LUNG

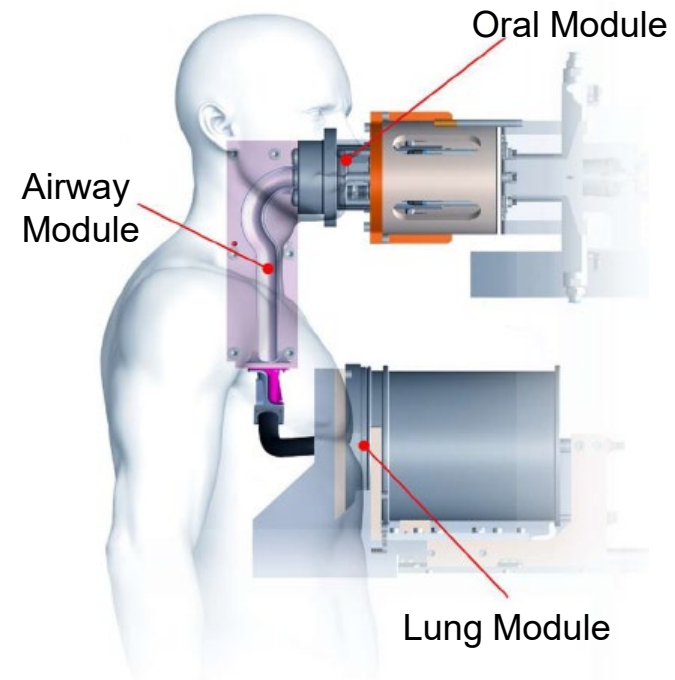
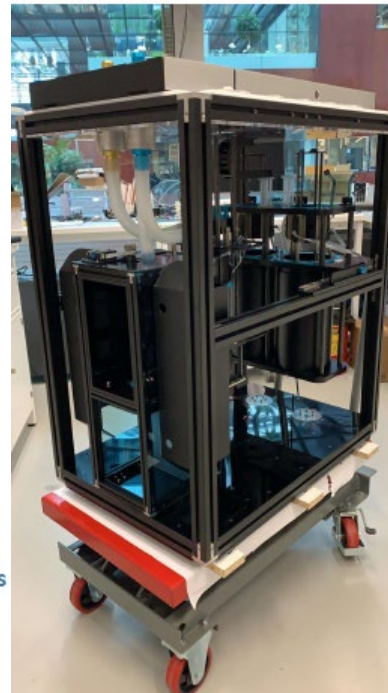
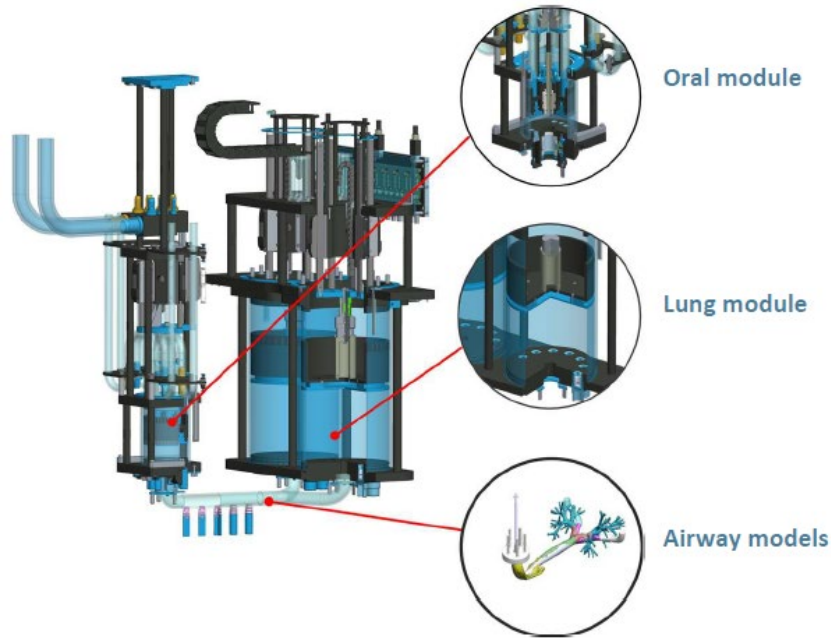


Sumoylation  
Biomarker





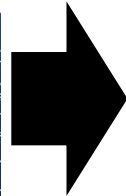
# Independent Holistic Air-Liquid Exposure System (InHALES)



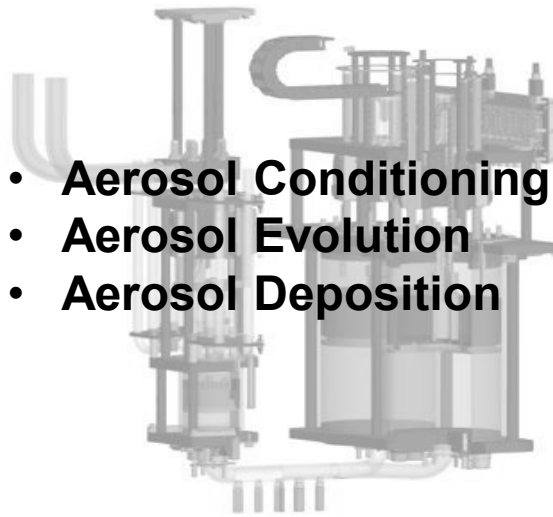


# InHALES

## Aerosol Source



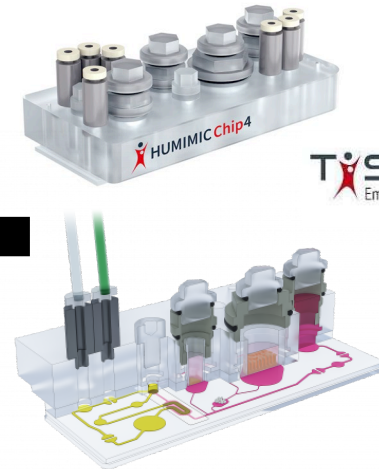
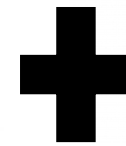
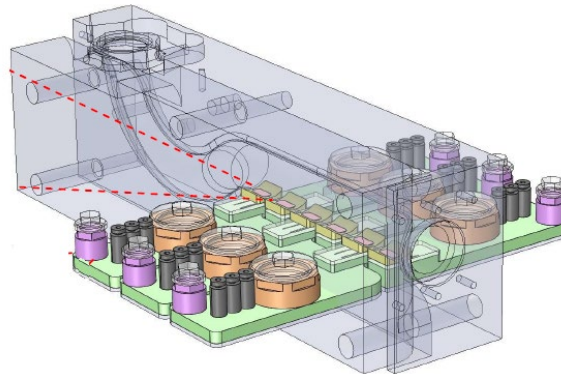
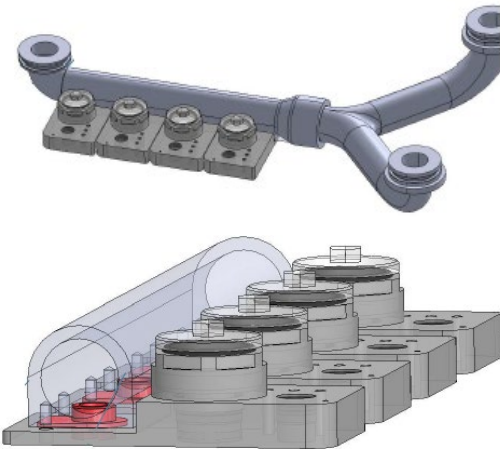
- Aerosol Conditioning
- Aerosol Evolution
- Aerosol Deposition



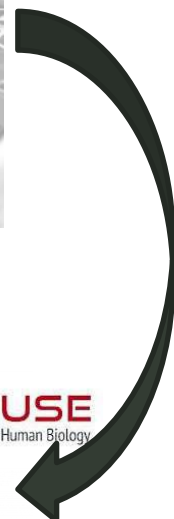
# Biology



- *In vivo* relevant doses and responses



**TISSUSE**  
Emulating Human Biology

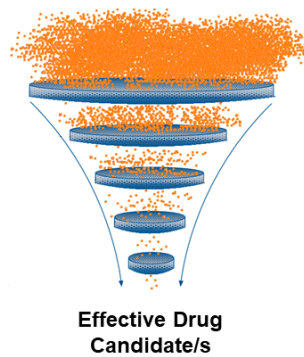
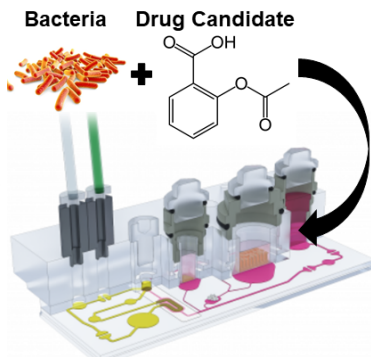
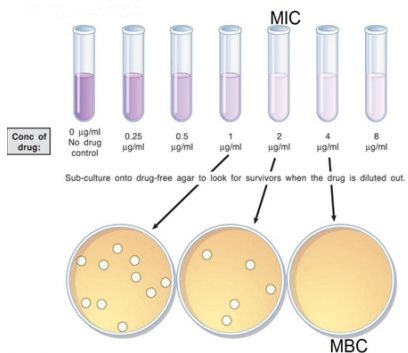




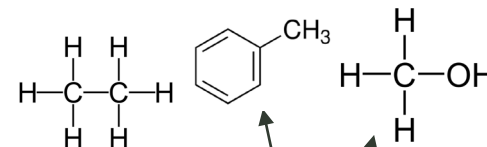
# Additional MOC Projects



## ADME MOC



## VOC Analysis

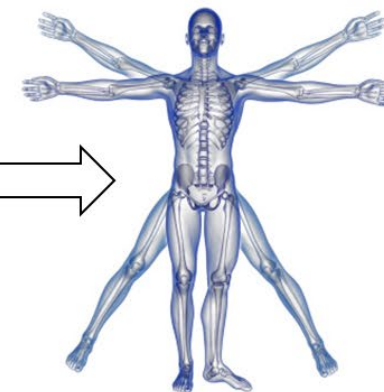
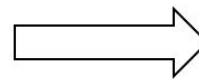
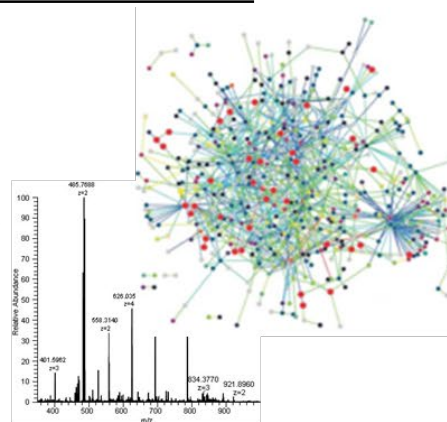
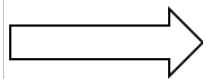


Minimum Inhibitory Concentration Assay

Organ-on-a-Chip Exposure

Characterization of Drug Candidate Efficacy

## Diagnostic Translation





# FUNCTIONAL SYSTEMS FOR PREDICTIVE TOXICOLOGY AT THREAT AGENT SCIENCE



## MPS Systems

### • Operational

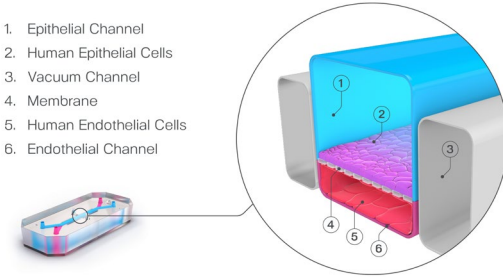
- Cardiac (RTCA, organoids)
- Liver (2D, 3D, MPS)
- CNS (2D)
- Blood-Brain-Barrier (TW)
- Lung (2D, 3D, MPS)
- Dermal (3D, MPS)
- Kidney (MPS)
- Intestinal (3D, MPS)

### • In Development

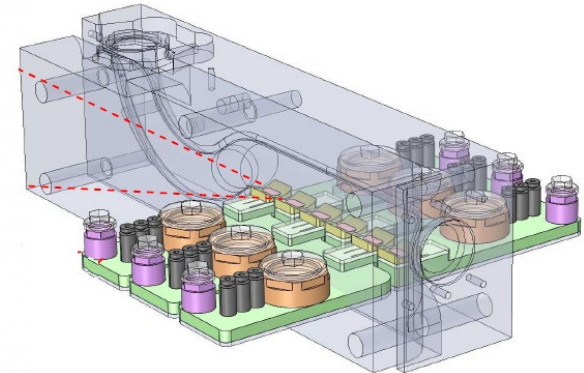
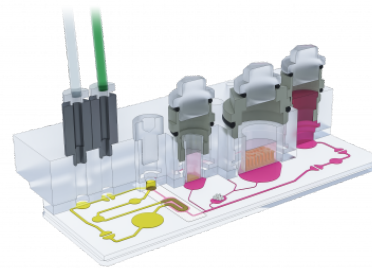
- CNS (3D organoids)
- BBB (MPS)
- Aerosol exposure

### Emulate

1. Epithelial Channel
2. Human Epithelial Cells
3. Vacuum Channel
4. Membrane
5. Human Endothelial Cells
6. Endothelial Channel



### TissUse



### CN Bio







**CBC TEAM**



## **Molecular Toxicology**

Dylan Fudge

Erin Gallagher

Tyler Goralski

Jen Horsmon

Priscilla Lee

Morgan Minyard

## **BioDefense**

Dan Angelini

Maria Arevalo

Liz Dhummakupt

Conor Jenkins

Amber Pugh

Todd Sickler

