SPATIAL INVESTIGATION OF STRESS AND INFLAMMATION IN THE HEALTHY AND SUSCEPTIBLE LUNG

Alessandro Venosa, PharmD, PhD

HEALTH
UNIVERSITY OF UTAH

Assistant Professor
Department of Pharmacology and Toxicology
Skaggs College of Pharmacy
University of Utah

September 18th, 2023

©UNIVERSITY OF UTAH HEALTH

LAB FOCUS: SUSCEPTIBILITY TO STRESS

* Lung epithelial dysfunction (mutations)

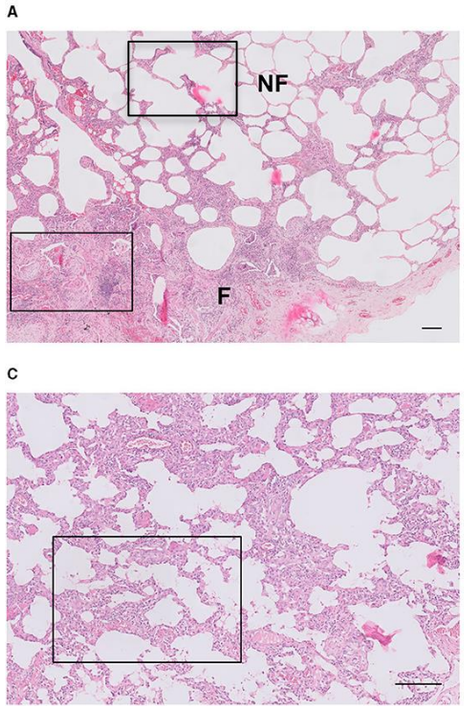
* Preexistent conditions (fibrosis) – NIEHS ONES Award

Aging

©UNIVERSITY OF UTAH HEALTH

PULMONARY FIBROSIS (PF)

- ✓ Rare and aggressive degenerative disease
- ✓ >200,000 Americans currently living with IPF
- ✓ >50,000 new diagnoses and an estimated 40,000 deaths → x2 since 2010
- ✓ Several challenges to advance approach to the disease
  - ➢ Disease of aging (66 years mean diagnosis)
  - ➢ Short patient survival (3-5 years from diagnosis)
  - ➢ Heterogeneity

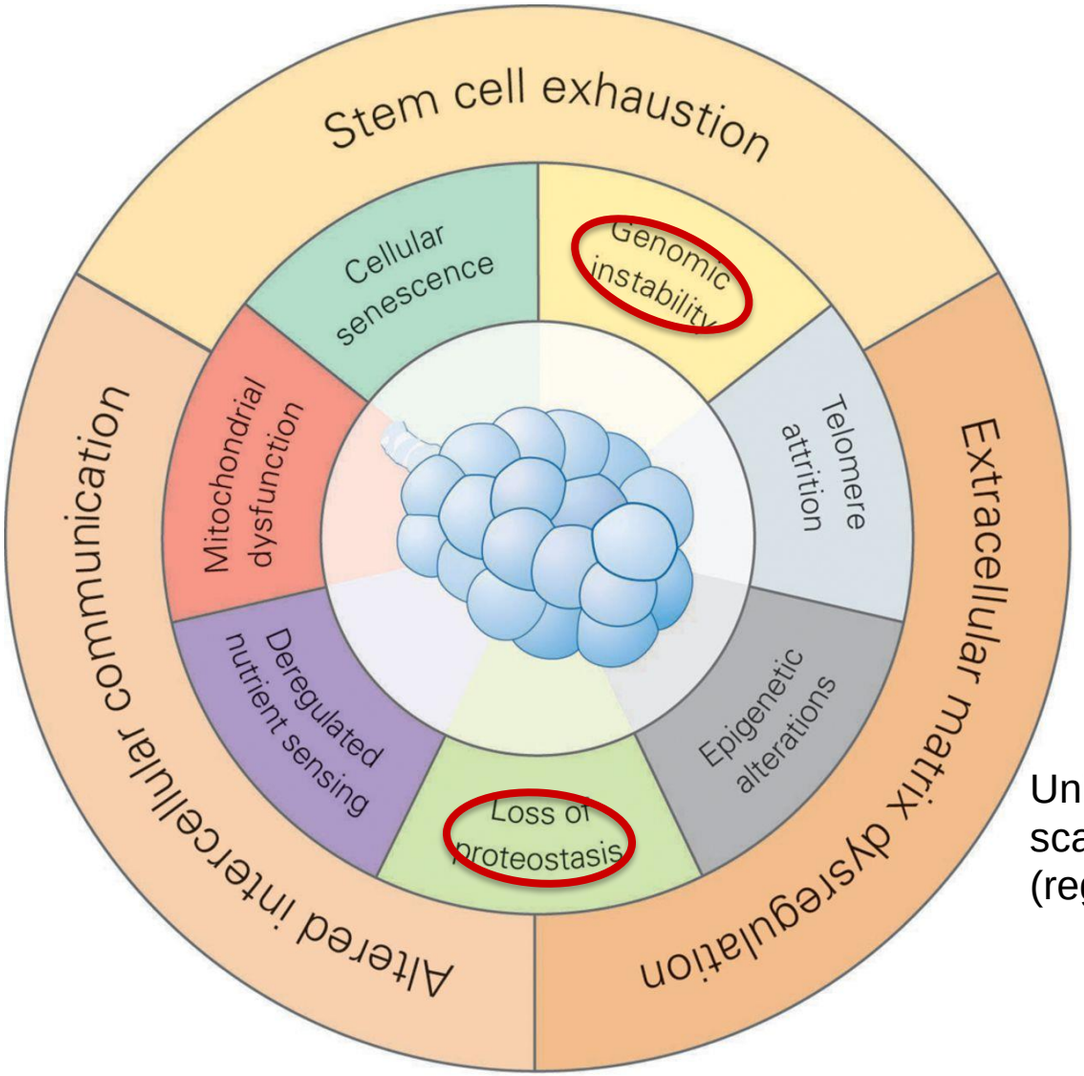


https://doi.org/10.3389/fmed.2021.736485

©UNIVERSITY OF UTAH HEALTH

HALLMARKS OF CANCER..AGING..FIBROSIS

Impaired wound repair capacity and epithelial-mesenchymal crosstalk + abnormal proliferation (bronchiolization)



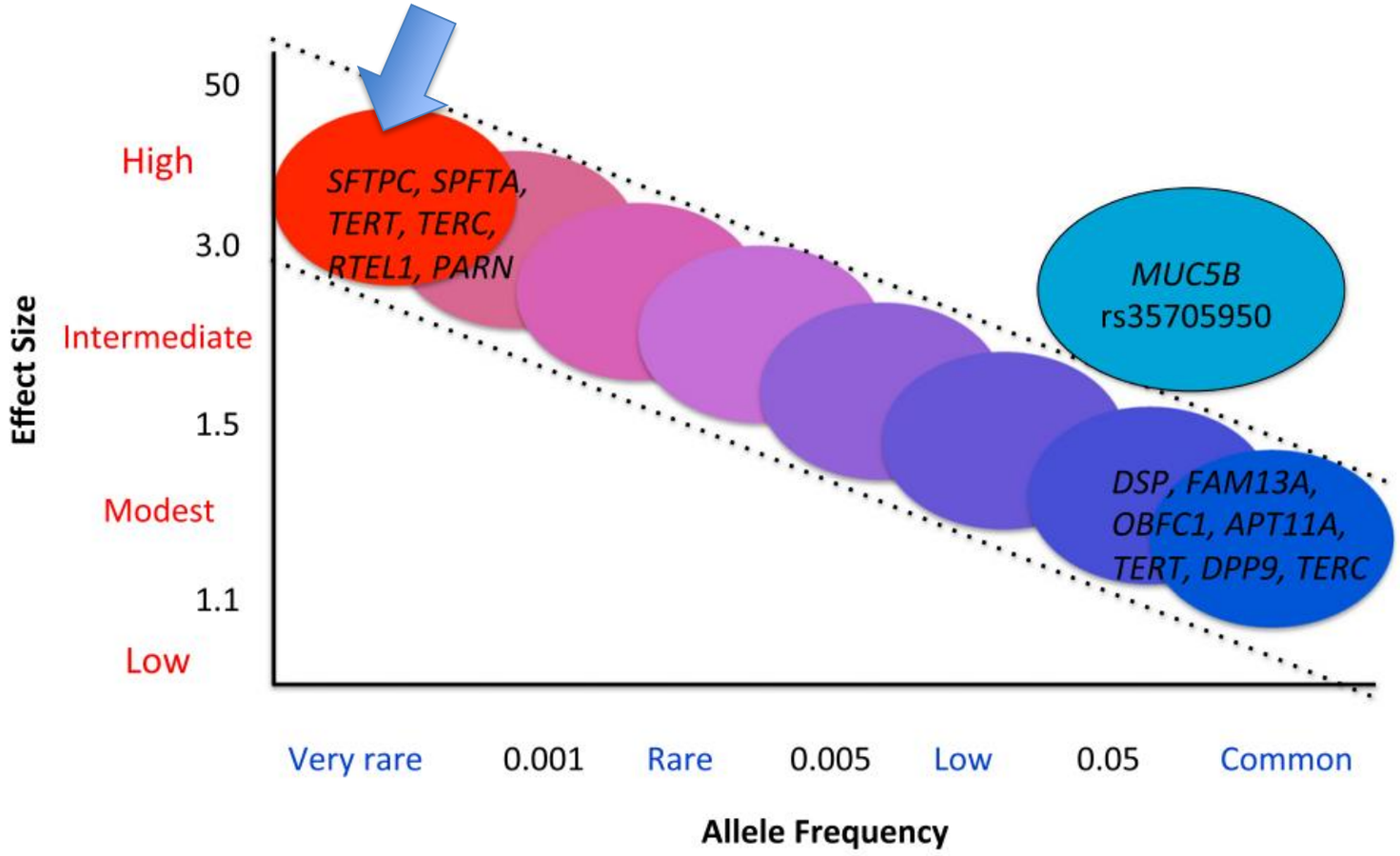
Heterogenous milieu from stressed parenchyma and activated immune cells

Unique matrisome within the scarred/remodeled tissue (regional differences?)

DOI: 10.1183/09031936.00186914

©UNIVERSITY OF UTAH HEALTH

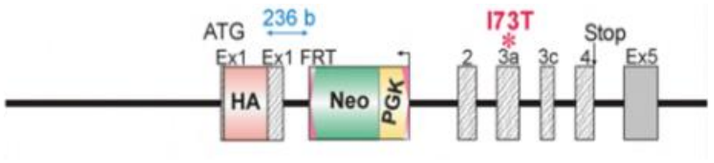
PARENCHYMAL MUTATIONS AND PULMONARY FIBROSIS

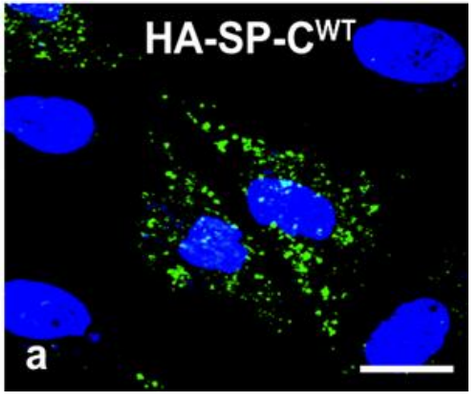


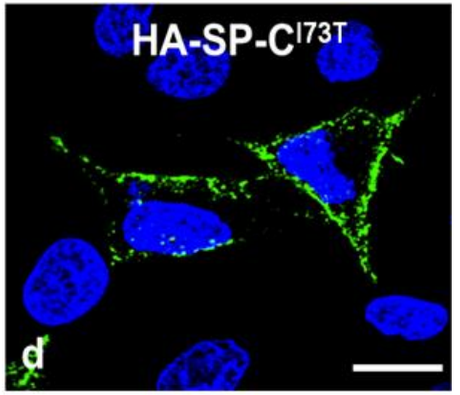
Epithelial-initiated injury

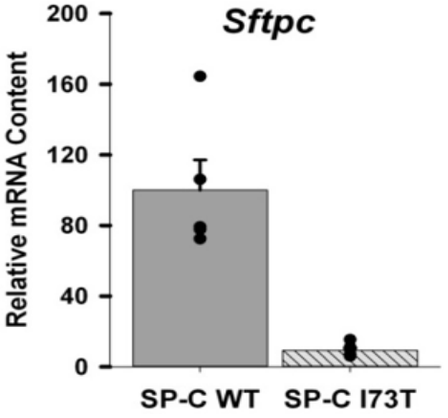
DOI: 10.1016/j.rppnen.2017.05.005

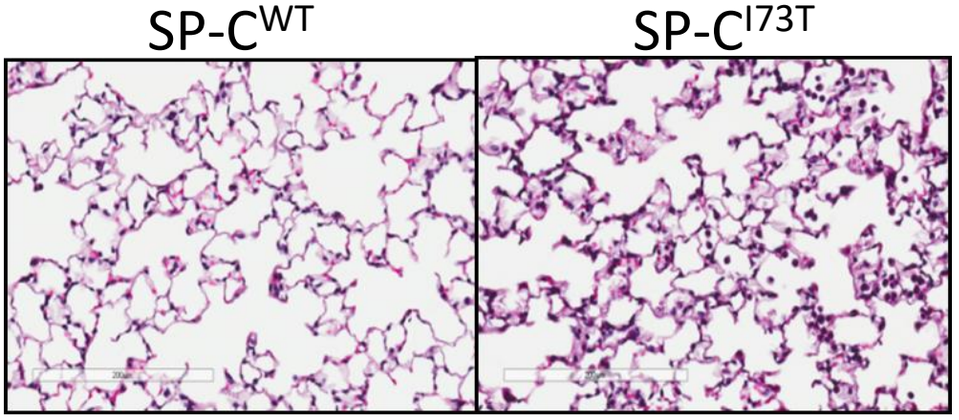
DEVELOPING A VIABLE SP-C MUTANT LINE – THE HYPOMORPH LINE









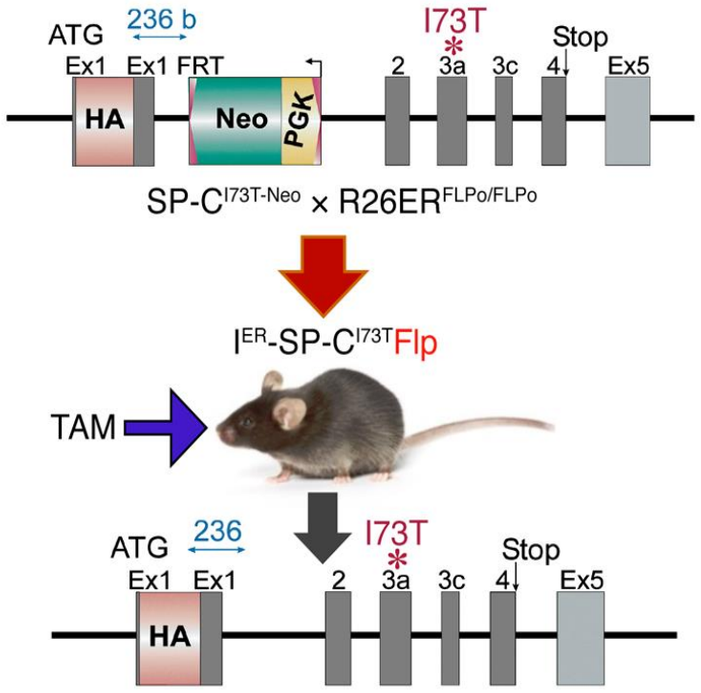


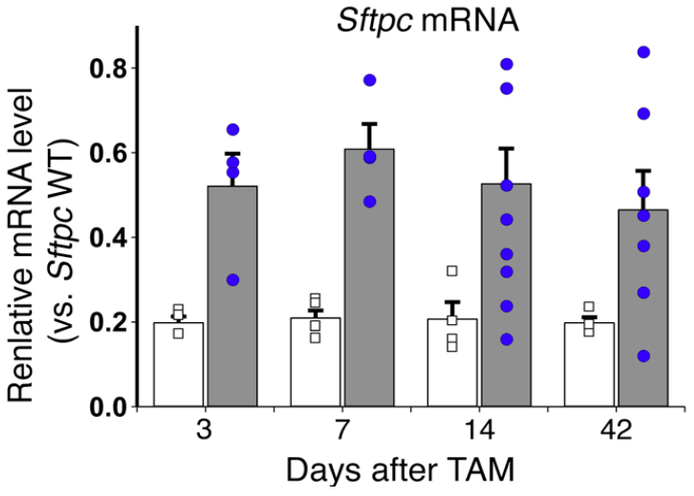
No overt lung phenotype

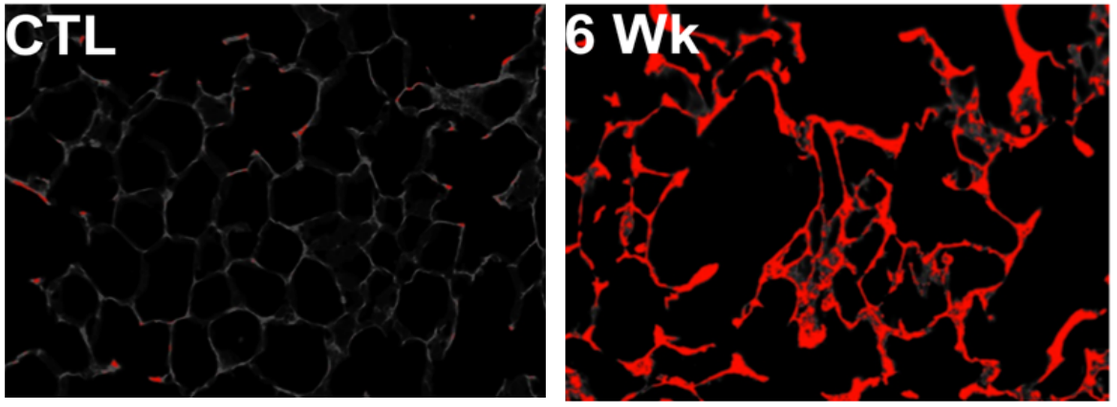
Nureki, Tomer, Venosa et al., 2018

©UNIVERSITY OF UTAH HEALTH

SP-C^{I73T}: A VIABLE MODEL OF REMODELING



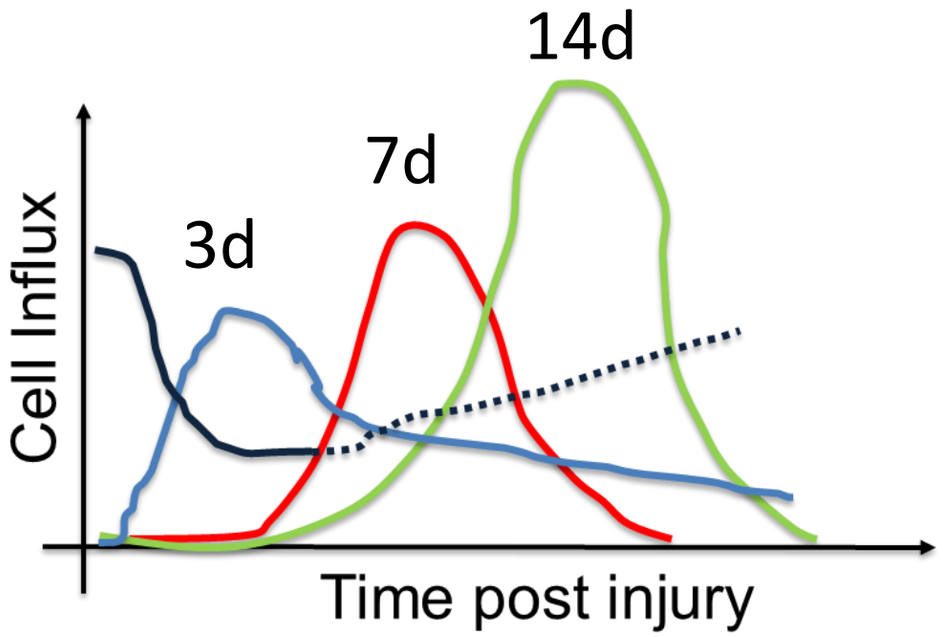




Nureki, Tomer, Venosa et al., 2018

©UNIVERSITY OF UTAH HEALTH

INFLAMMATORY CELL RECRUITMENT AT THE PEAK OF SP-C^{I73T} INDUCED FIBROSIS

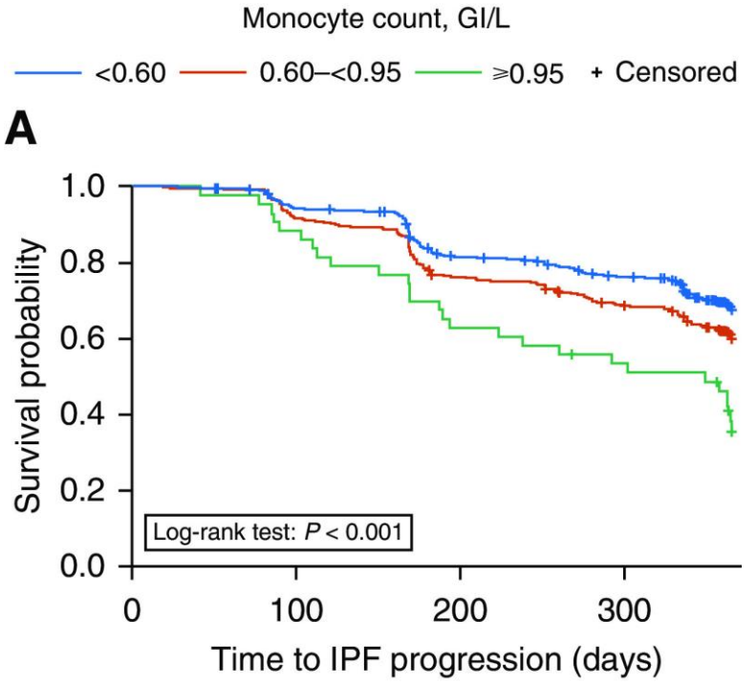


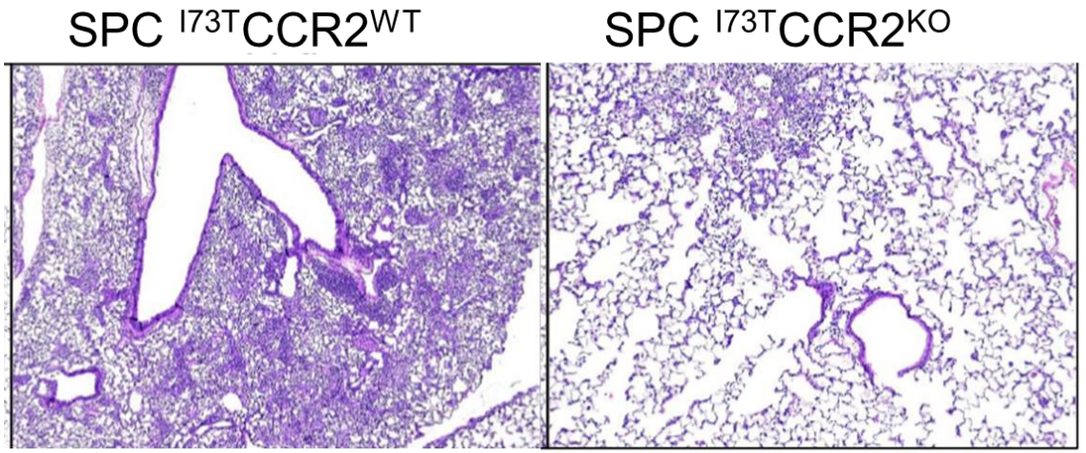
Dark Blue - Alv Macs
Dotted line – mono derived macs
Light blue – Mono
Red – Neu
Green - Eos

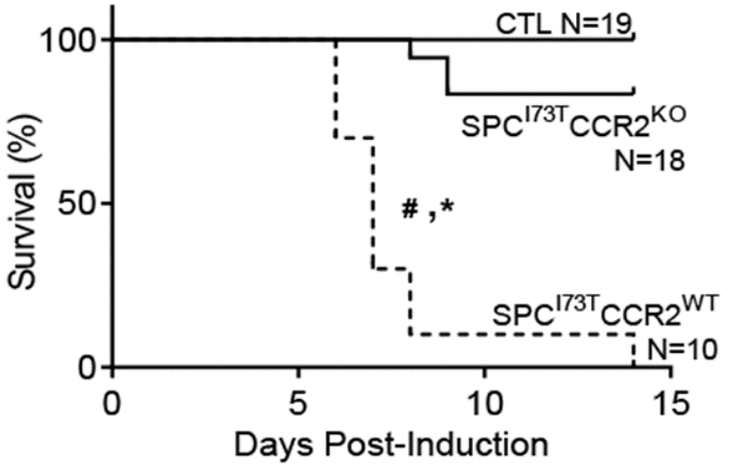
Venosa et al., 2019

©UNIVERSITY OF UTAH HEALTH

INFLAMMATORY MONOCYTES AND PF OUTCOME



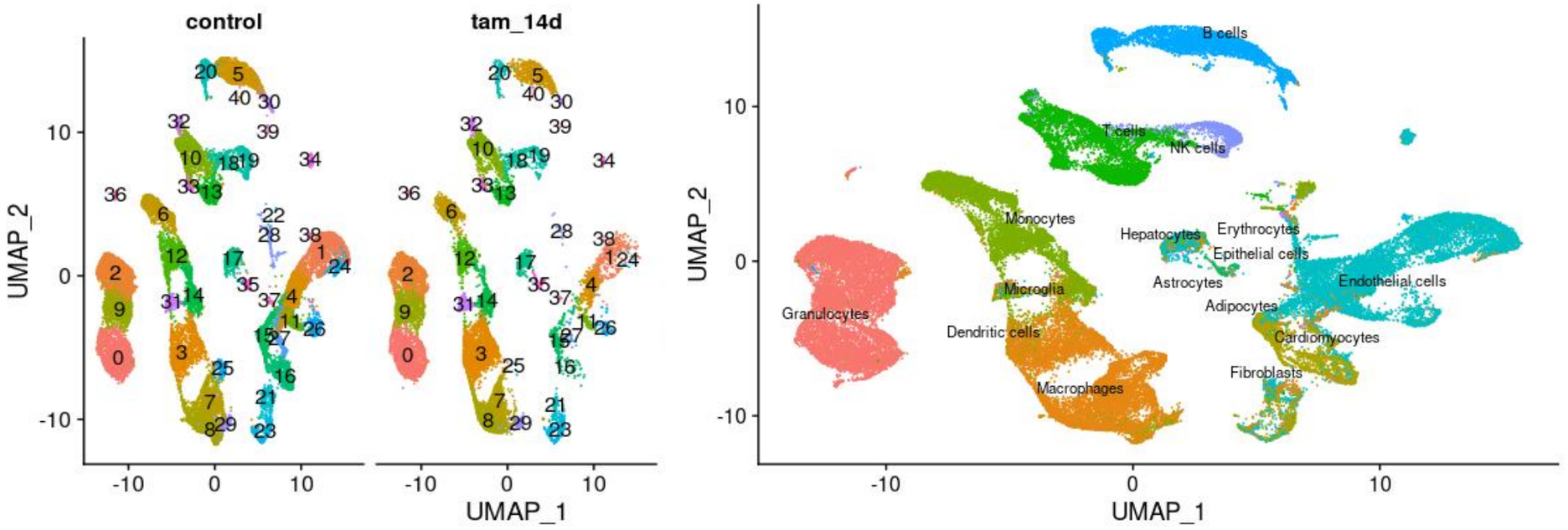




Venosa et al., 2021; Scott et al., 2019; Kreuter et al., 2021

©UNIVERSITY OF UTAH HEALTH

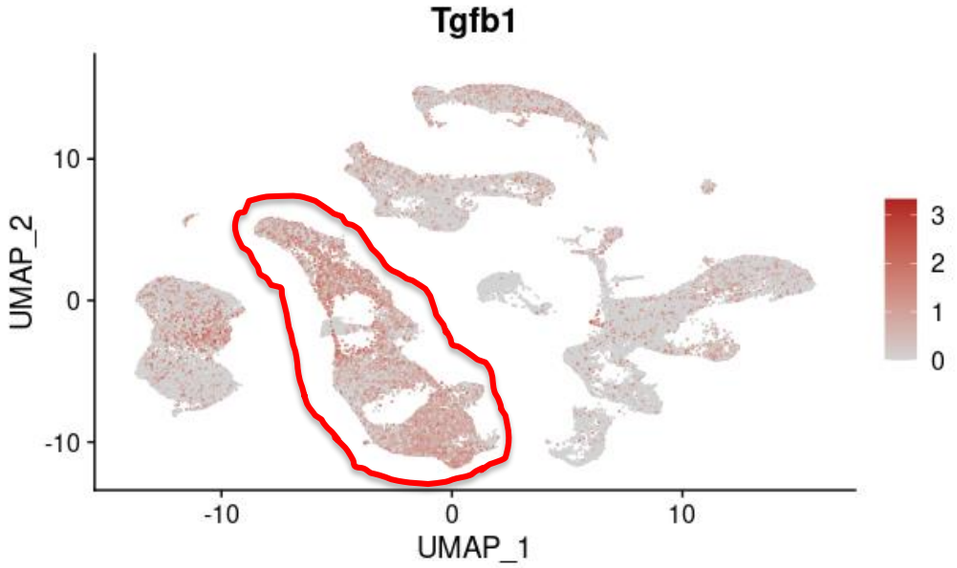
LUNG CELL BREAKDOWN DURING SP-C^{I73T} INDUCED INJURY

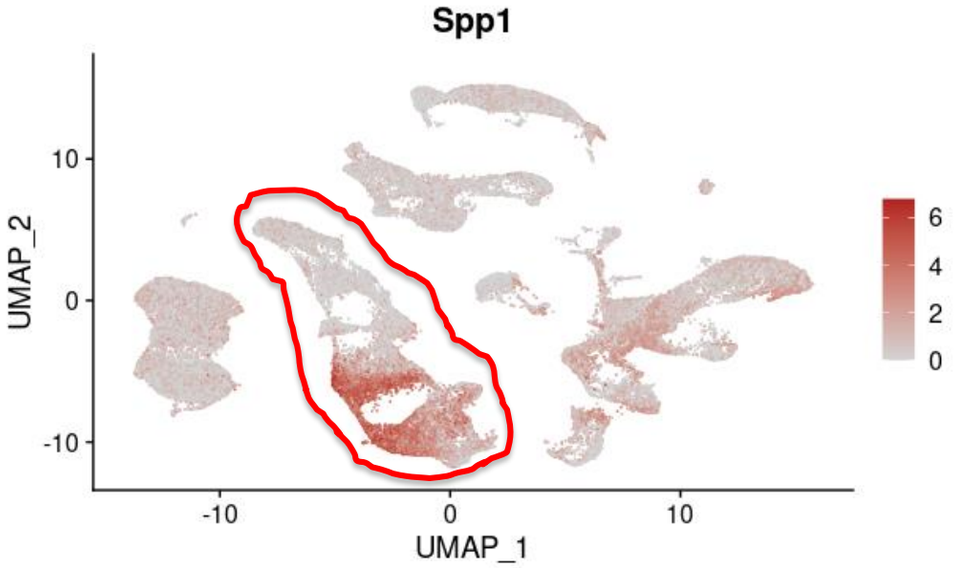


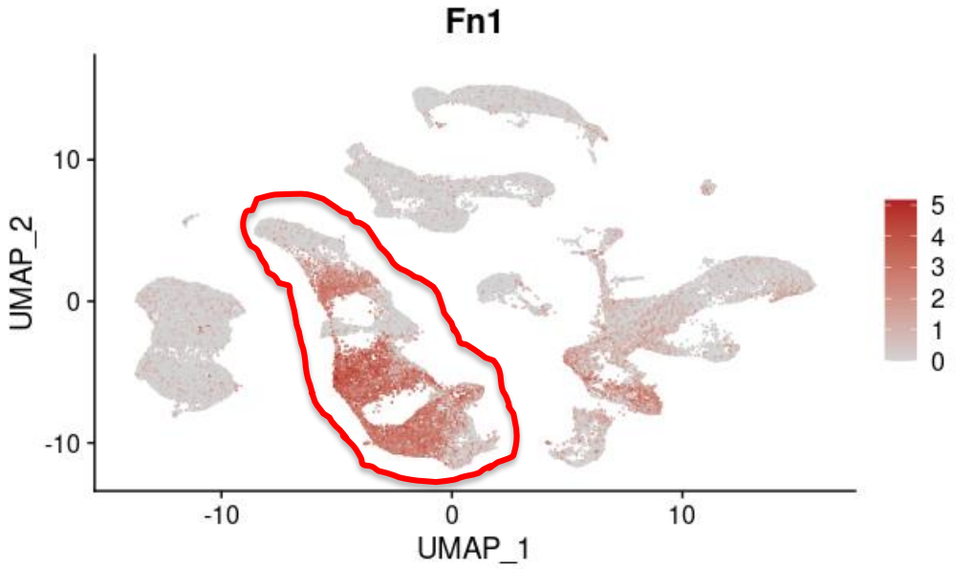
unpublished

©UNIVERSITY OF UTAH HEALTH

LUNG CELL BREAKDOWN DURING SP-C^{I73T} INDUCED INJURY



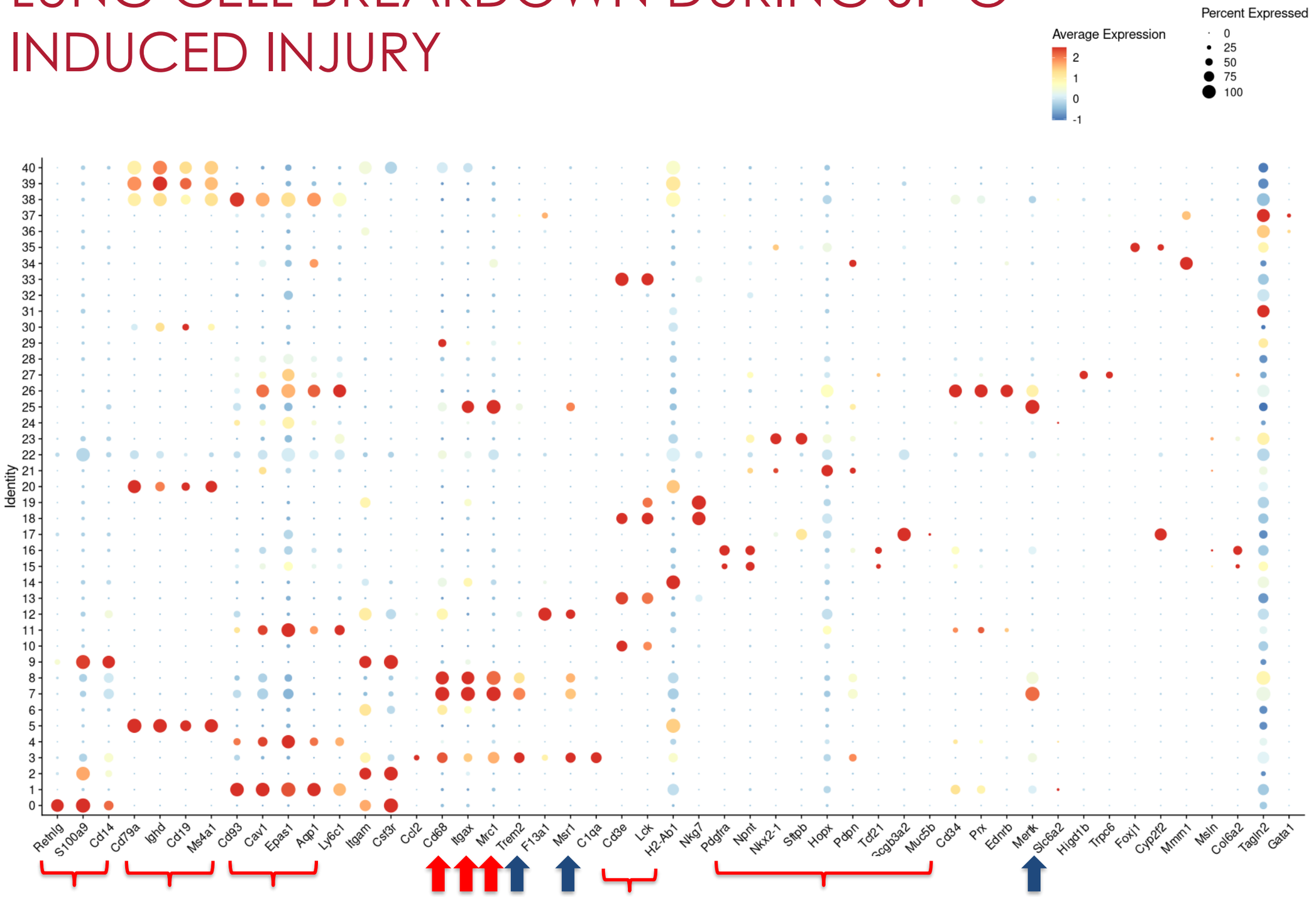




Unpublished data

©UNIVERSITY OF UTAH HEALTH

LUNG CELL BREAKDOWN DURING SP-C^{I73T} INDUCED INJURY



©UNIVERSITY OF UTAH HEALTH

HOW CAN WE TEASE OUT THE ROLE OF EACH INDIVIDUAL MACROPHAGE POPULATION?

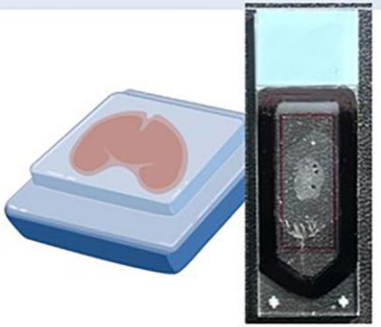
By signaling pathways – REACTOME/KEGG/GO, CellChat

By spatial localization

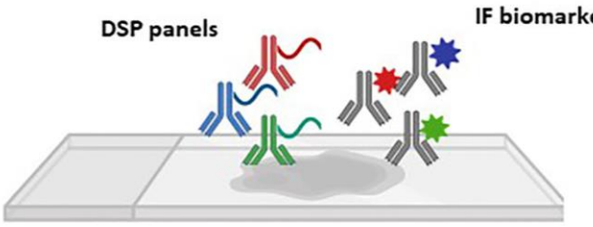
©UNIVERSITY OF UTAH HEALTH

SPATIAL ANALYSIS OF THE REMODELING LUNG

Retrieve FFPE samples and sectioning

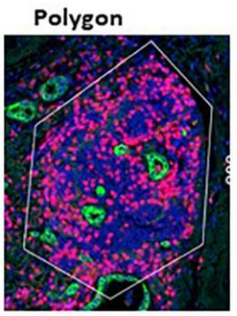


Staining with morphology biomarkers and DSP probes

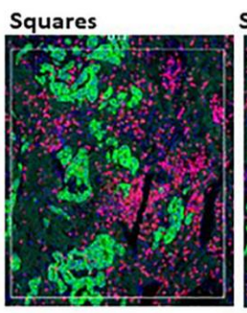


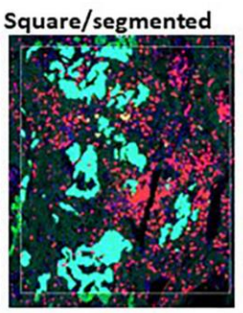
Manual or Automated in Leica autostainer

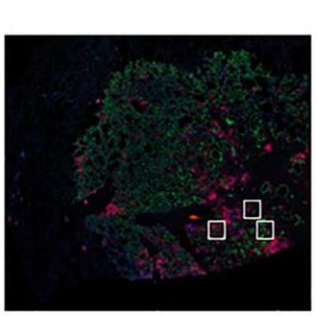
Scanning, Selection of ROI and probe collection in GeoMx DSP



ROI Selection



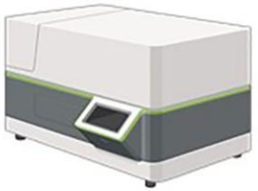




Imaging

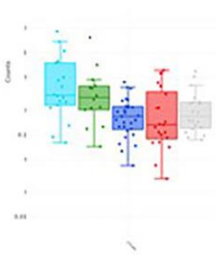
GeoMx DSP instrument

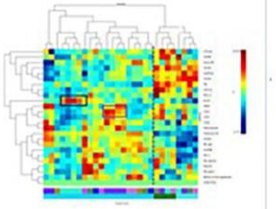
Hybridize ad count



nCounter instrument

Data visualization and analysis





GeoMx DSP instrument

©UNIVERSITY OF UTAH HEALTH

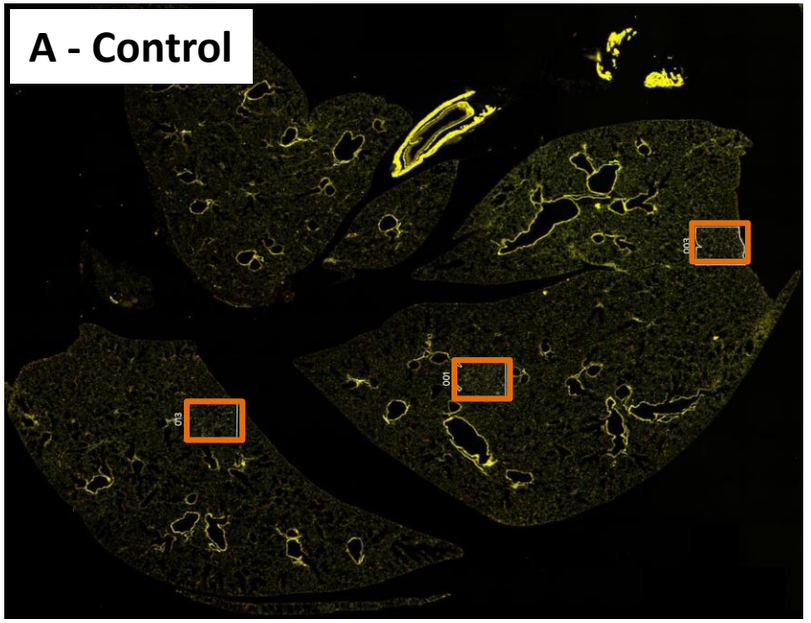
REGION SELECTION

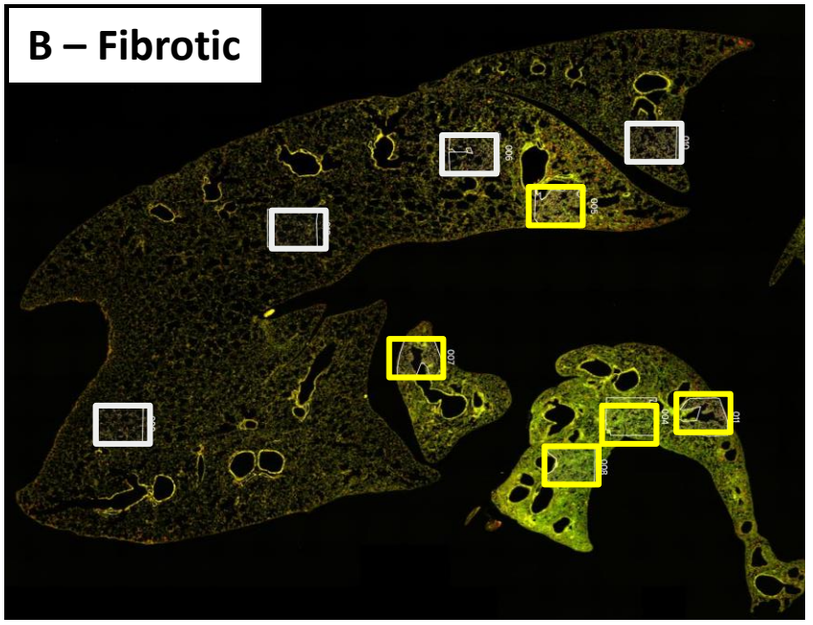
CD45 – yellow

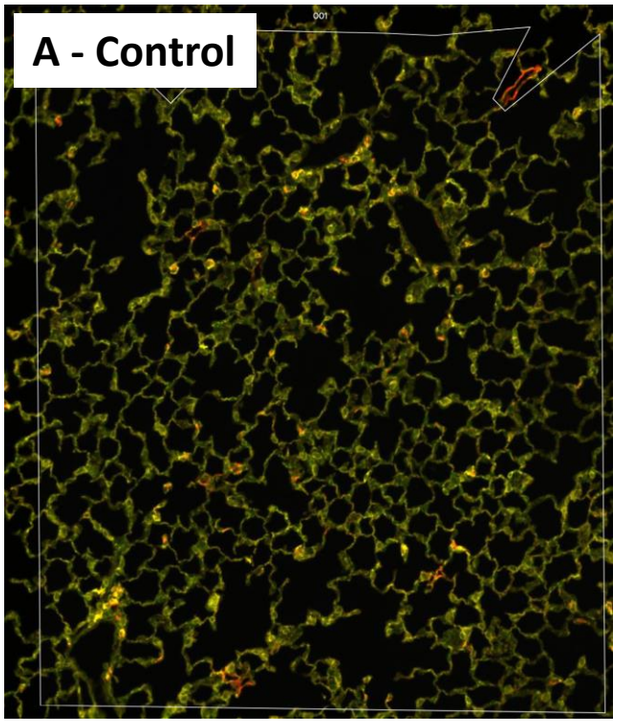
CD68 – red

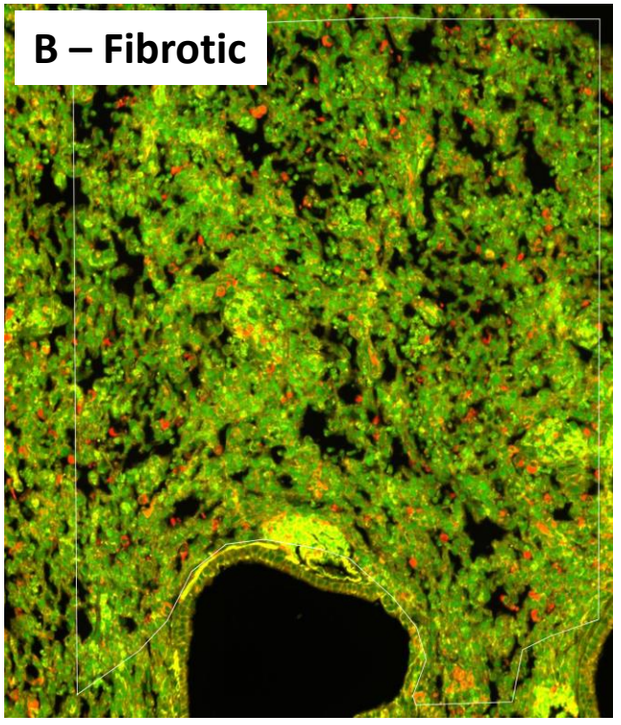
DNA – green

PanCK - cyan

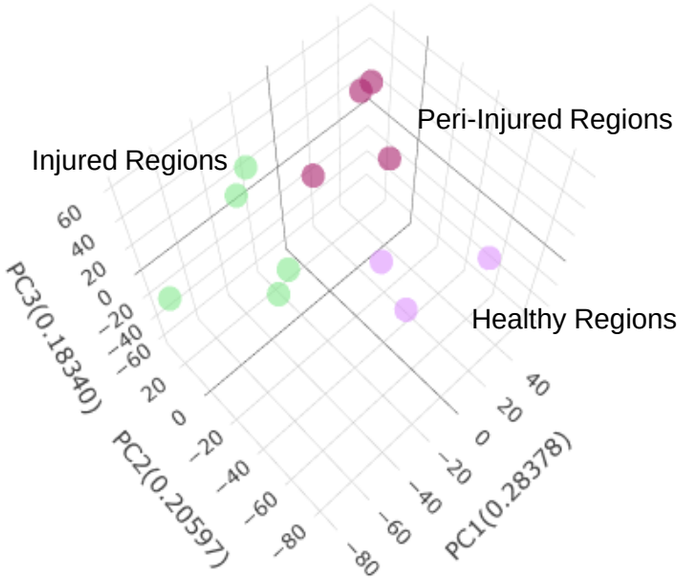


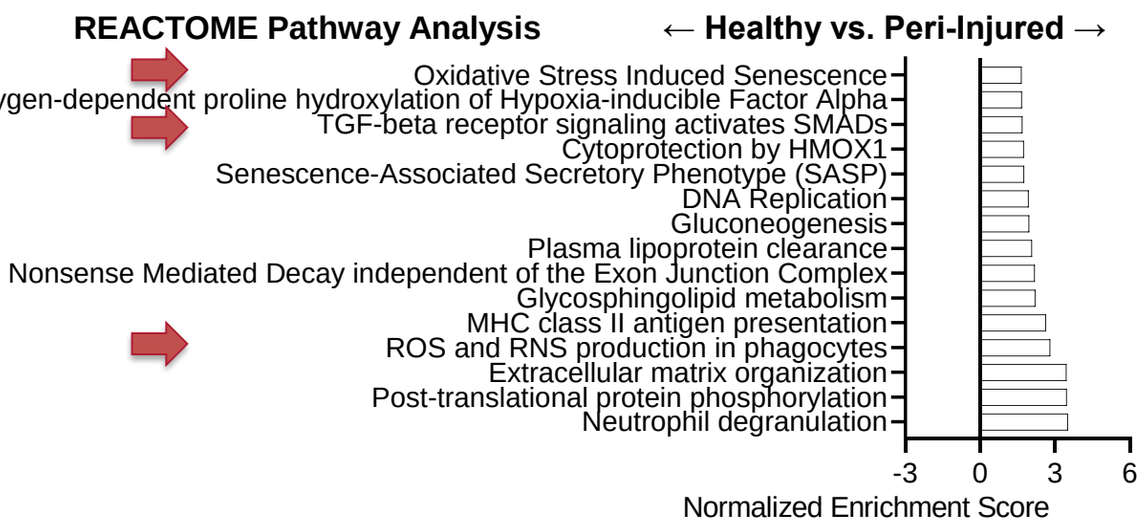


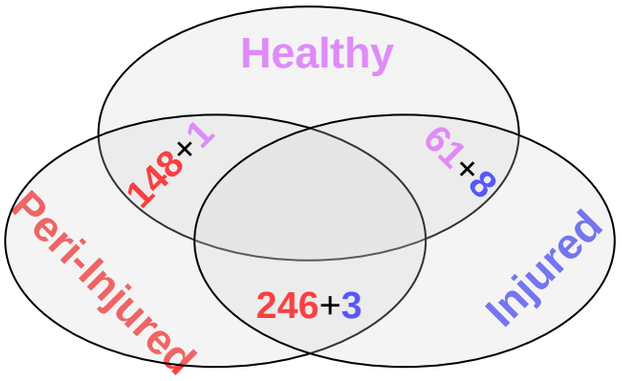


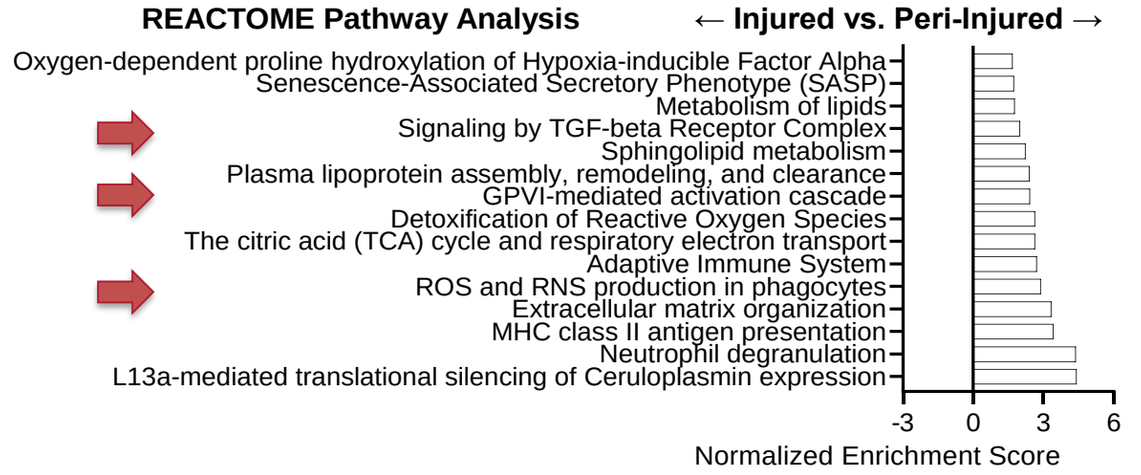


SP-C INDUCED STRESS DRIVES ACTIVATION OF THE PERI-INJURED REGIONS







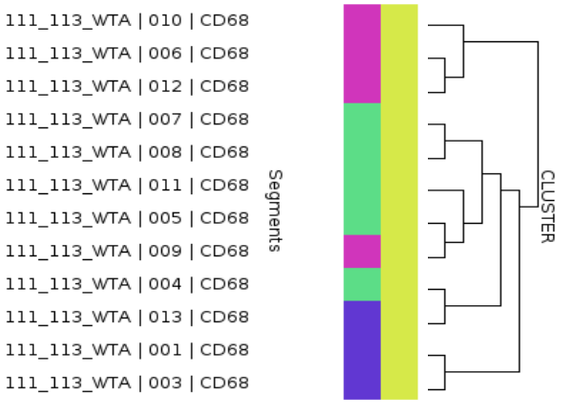


Unpublished data

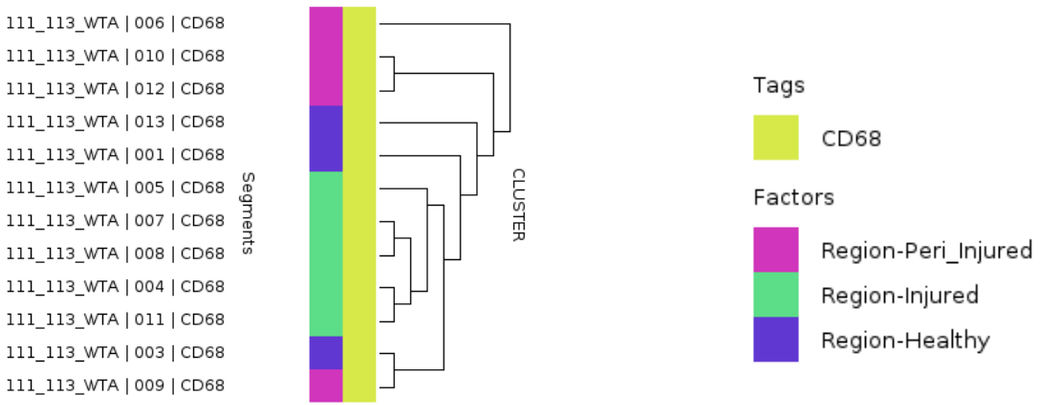
©UNIVERSITY OF UTAH HEALTH

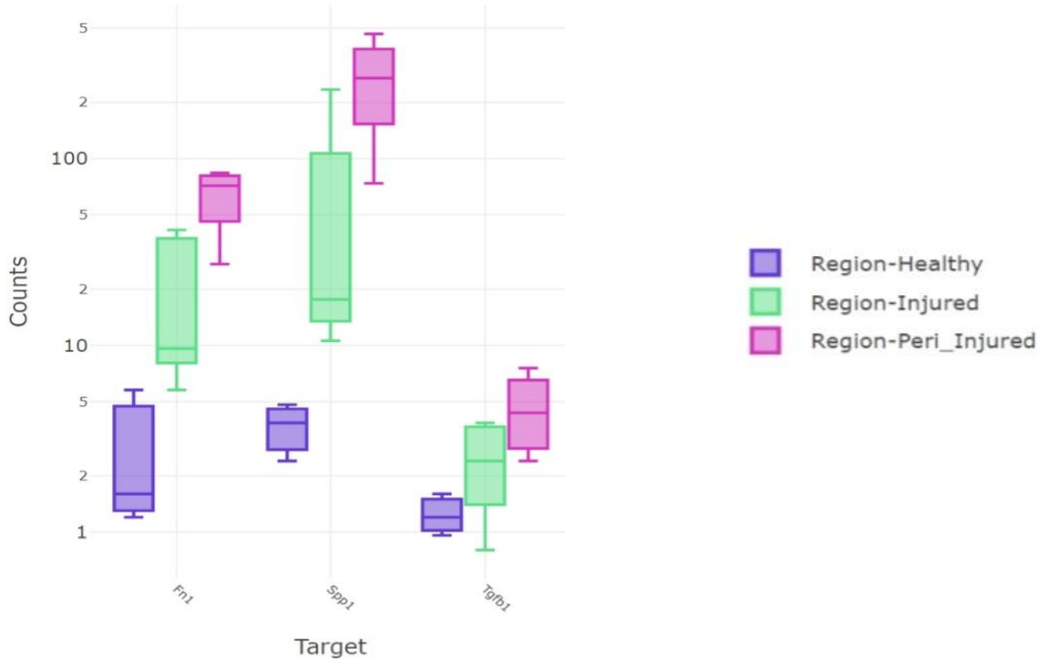
REGIONAL CLUSTERING IN THE SP-C LUNG – PROFIBROTIC SIGNALING

Degradation of the extracellular matrix



Assembly of collagen fibrils and other multimeric structures

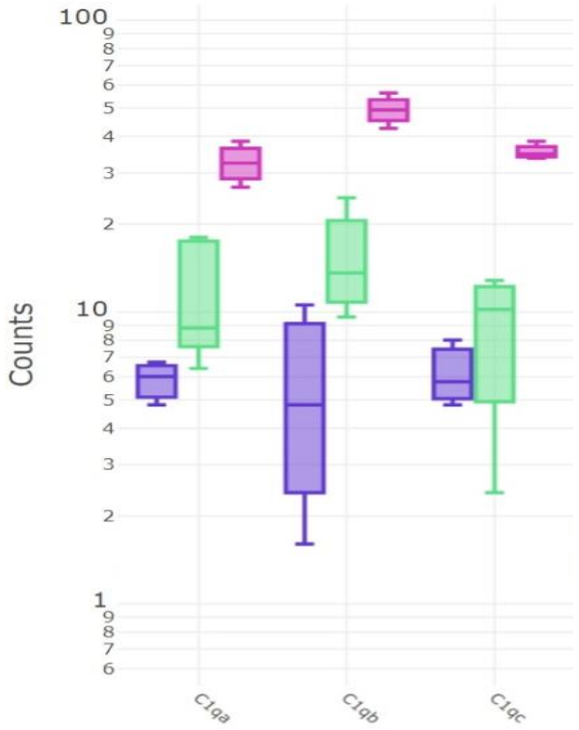


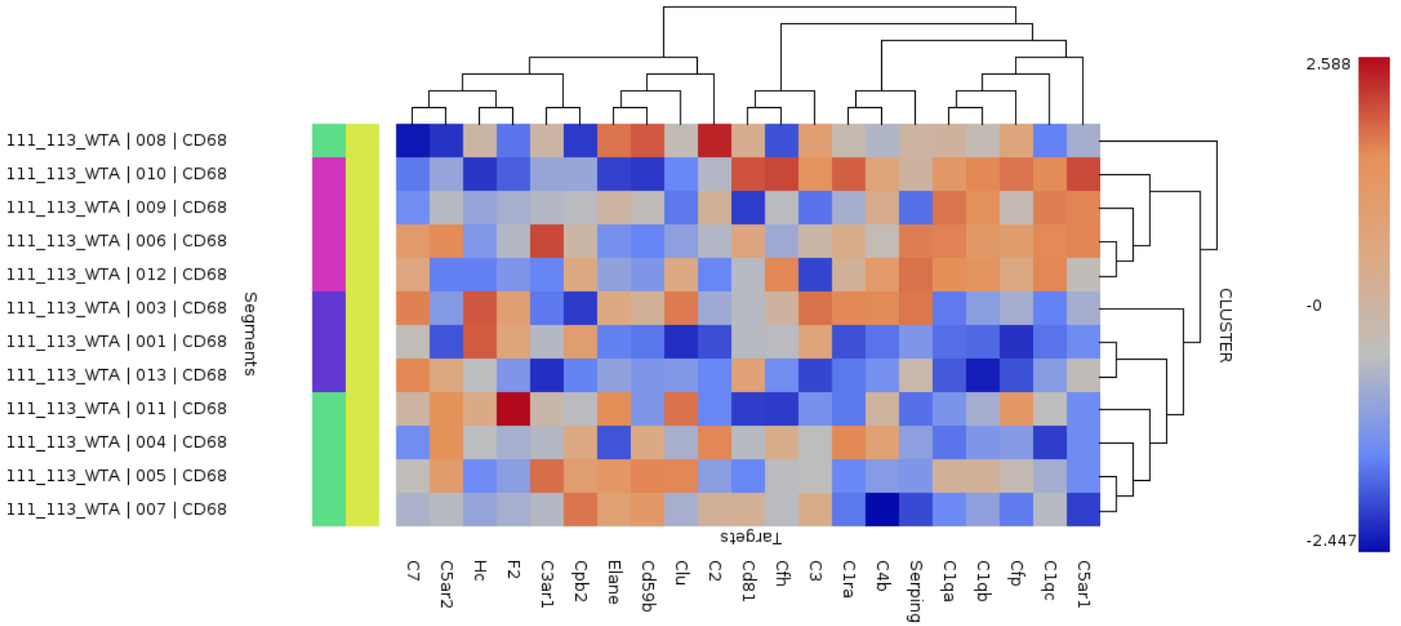


Unpublished data

©UNIVERSITY OF UTAH HEALTH

REGIONAL CLUSTERING IN THE SP-C LUNG – COMPLEMENT EXPRESSION





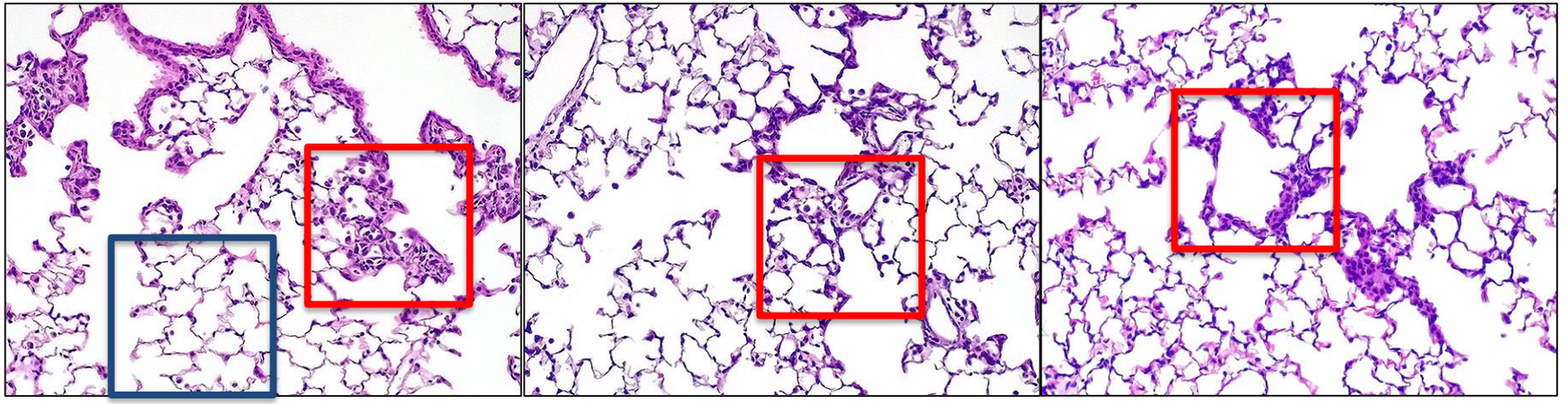
Unpublished data

©UNIVERSITY OF UTAH HEALTH

IN THE WORKS:
COMPARATIVE ANALYSIS OF MACROPHAGE ACTIVATION IN OZONE EXPOSURE

- Rapid onset of injury
- Transient
- Long term impact depends on dose, frequency of exposure, **individual susceptibility**

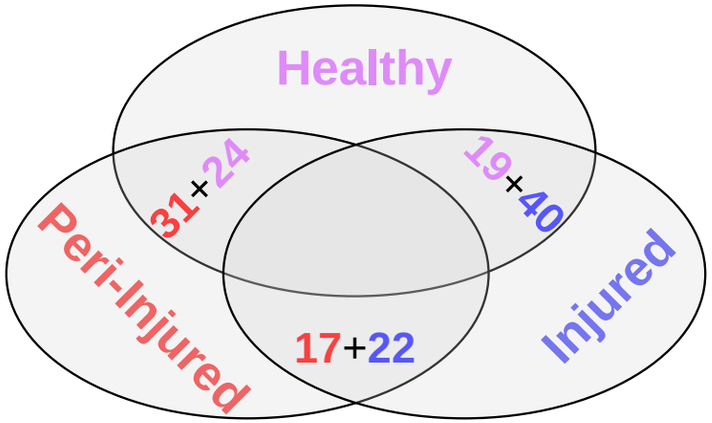
Single exposure – 0.8 ppm; 3h; 72h post exposure

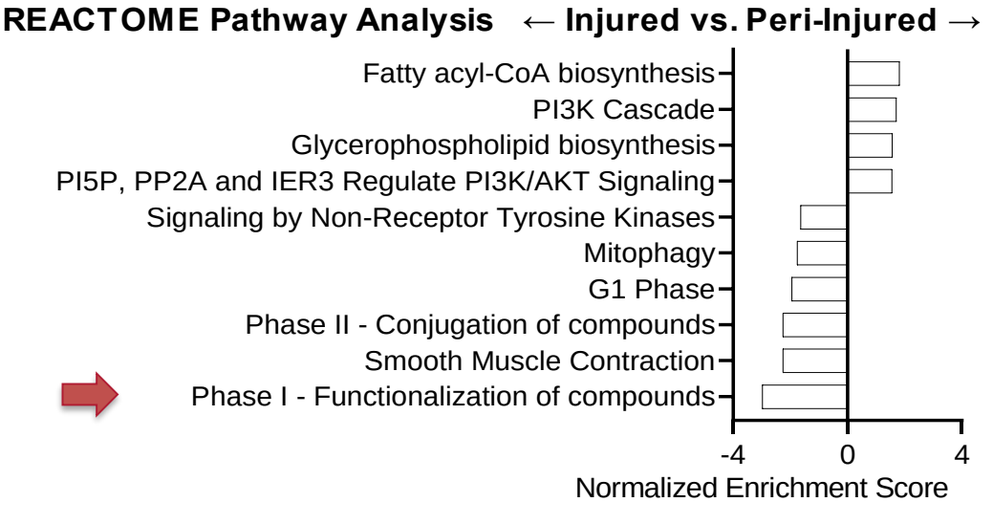


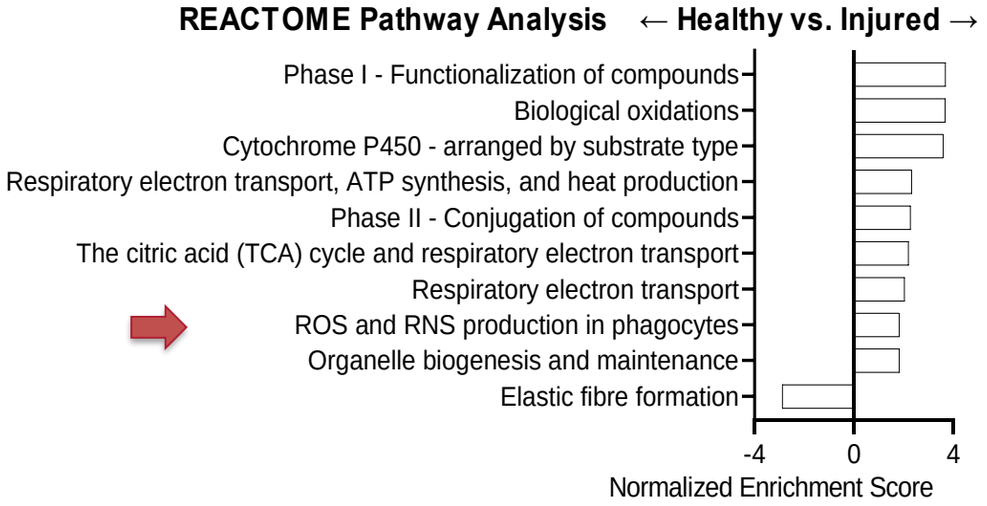
'Nguyen et al., 2021

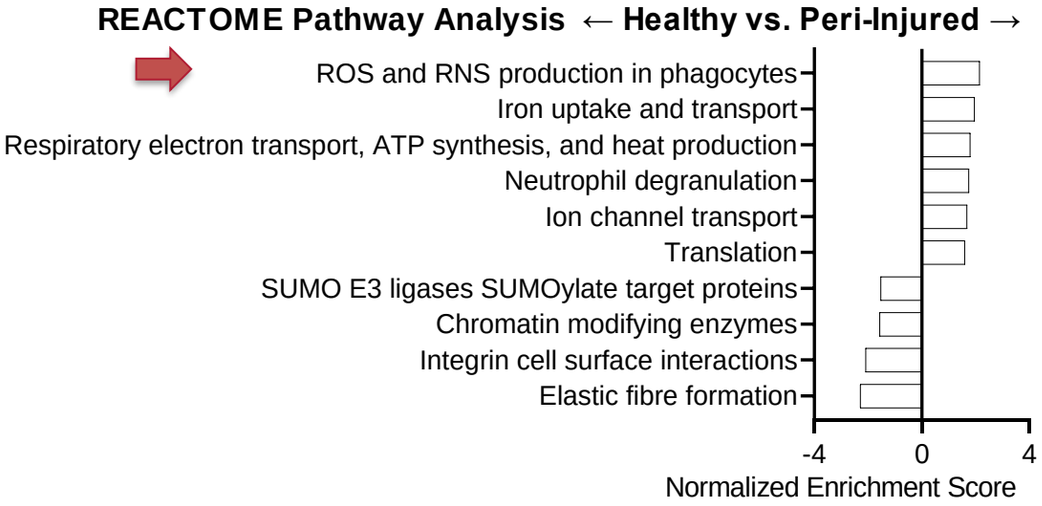
©UNIVERSITY OF UTAH HEALTH

OZONE DRIVES MULTIDIRECTIONAL MACROPHAGE ACTIVATION IN SP-C MUTANT MICE









Unpublished data

©UNIVERSITY OF UTAH HEALTH

TAKE HOME MESSAGE AND FUTURE DIRECTIONS

- SP-C^{I73T} induced injury – shows multicellular inflammatory cell infiltrate
- Spatial analysis of CD68^{+} cells show “unidirectional” macrophage activation (peri-injured)
- Comparative analysis with the air pollutant ozone, shows region-specific signatures in macrophages

- ❖ Use of predictive tools like CellChat/CellPhone to predict cell-cell communication
- ❖ Integration of spatial analysis with single cell sequencing
- ❖ Expansion of analysis to include O_3 exposure in the fibrotic lung

©UNIVERSITY OF UTAH HEALTH

ACKNOWLEDGEMENTS

Venosa Lab
Jenna Cheminant
Ujjwal Adhikari, MS
Robert Aguilar (PharmD student)

Former lab members/collaborators
Caleb Ham (PharmD student)
Adam Welker (PharmD student)
Philip Moos, PhD
Christopher Reilly, PhD
Cassandra Rice-Deering, PhD

U of Utah Cores

Data Consultant
David Scoville, PhD (Nanostring Technologies)
Jessica Noll, PhD
Sophie Cowman, PhD

U Penn/CHOP
Michael Beers, M.D.
Yaniv Tomer, BS
Christopher Massa MD, PhD

Funding:
- NIEHS ONES Award (R01)
- ALSAM Foundation Research Grant

©UNIVERSITY OF UTAH HEALTH

We are hiring! Alessandro.venosa@pharm.utah.edu

Questions?

©UNIVERSITY OF UTAH HEALTH