CLINICAL METAPROTEOMICS WORKFLOW TO STUDY HOST-MICROBIOME DYNAMICS

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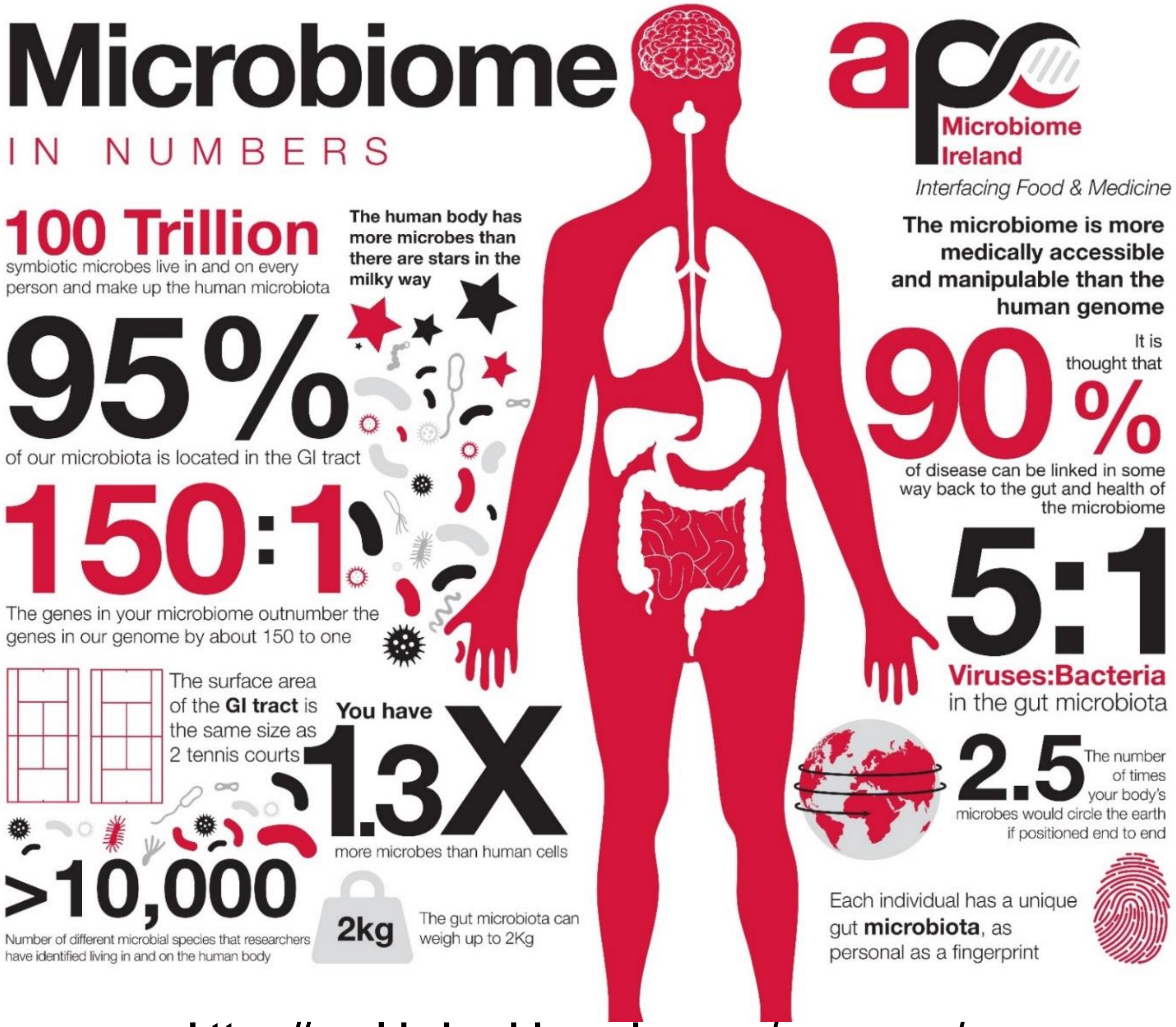
Bluesky: pratikomics
Threads: pratikomics

*Learn more at **galaxyp.org***

z.umn.edu/itcrgalaxyvideo

MICROBIOME RESEARCH



Microbiome

IN NUMBERS

apc Microbiome Ireland

Interfacing Food & Medicine

100 Trillion symbiotic microbes live in and on every person and make up the human microbiota

The human body has more microbes than there are stars in the milky way

95% of our microbiota is located in the GI tract

150:1 The genes in your microbiome outnumber the genes in our genome by about 150 to one

The surface area of the **GI tract** is the same size as 2 tennis courts

You have 1.3X more microbes than human cells

>10,000 Number of different microbial species that researchers have identified living in and on the human body

2kg The gut microbiota can weigh up to 2Kg

The microbiome is more medically accessible and manipulable than the human genome

It is thought that **90%** of disease can be linked in some way back to the gut and health of the microbiome

5:1 Viruses:Bacteria in the gut microbiota

2.5 The number of times your body's microbes would circle the earth if positioned end to end

Each individual has a unique gut **microbiota**, as personal as a fingerprint

https://worldmicrobiomeday.com/resources/

https://www.nature.com/articles/d41586-020-00193-3

MICROBIOME

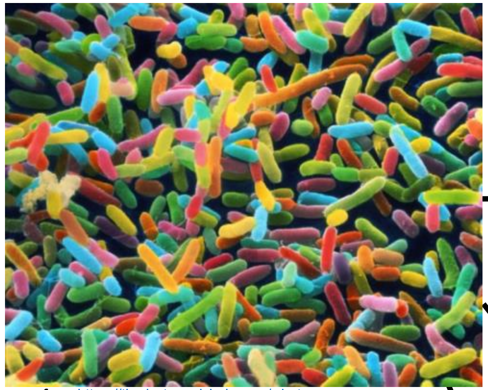
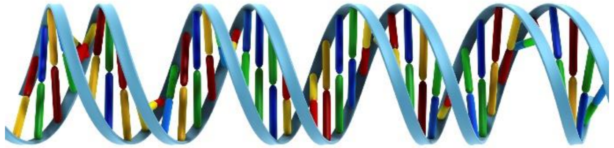


Image from https://thedoctorweighsin.com/what-everyone-should-know-about-the-infant-microbiome/

Potential to unravel the **mechanistic details of microbial interactions with host / environment** by analyzing the **functional dynamics of the microbiome.**



METAGENOMICS

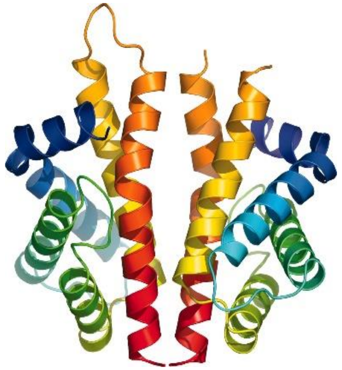
TAXONOMY

function

METATRANSCRIPTOMICS

TAXONOMY

function

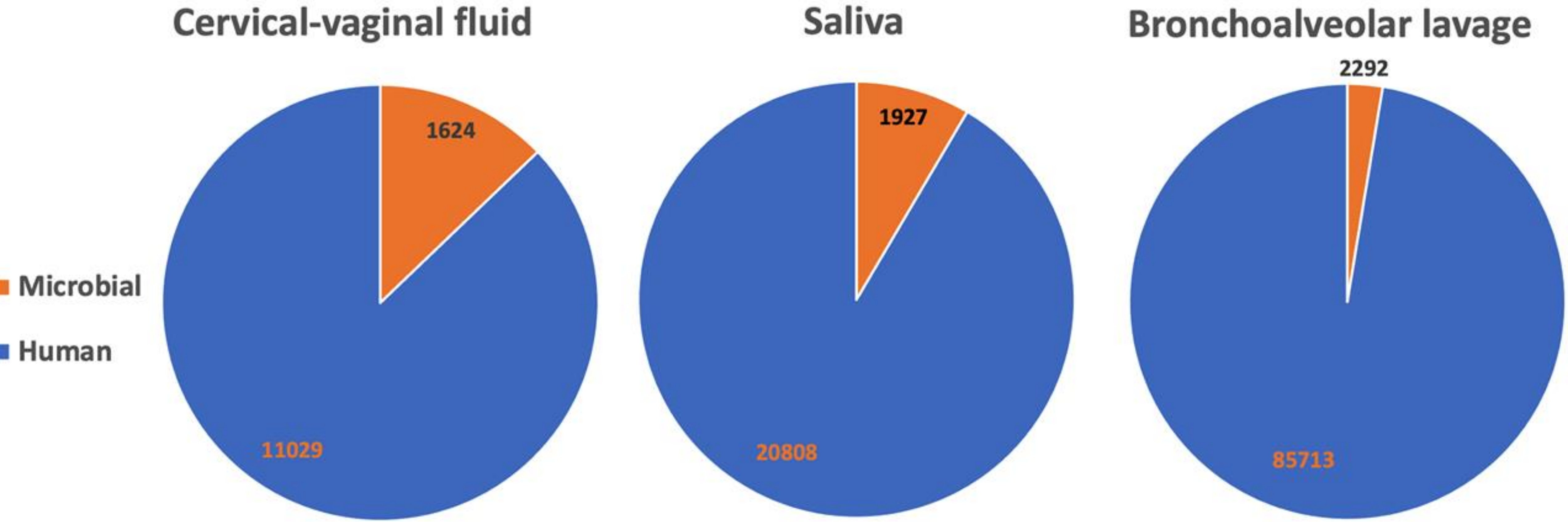


METAPROTEOMICS

TAXONOMY

FUNCTION

CLINICAL METAPROTEOMIC STUDIES DETECT LIMITED NUMBER OF MICROBIAL PEPTIDES

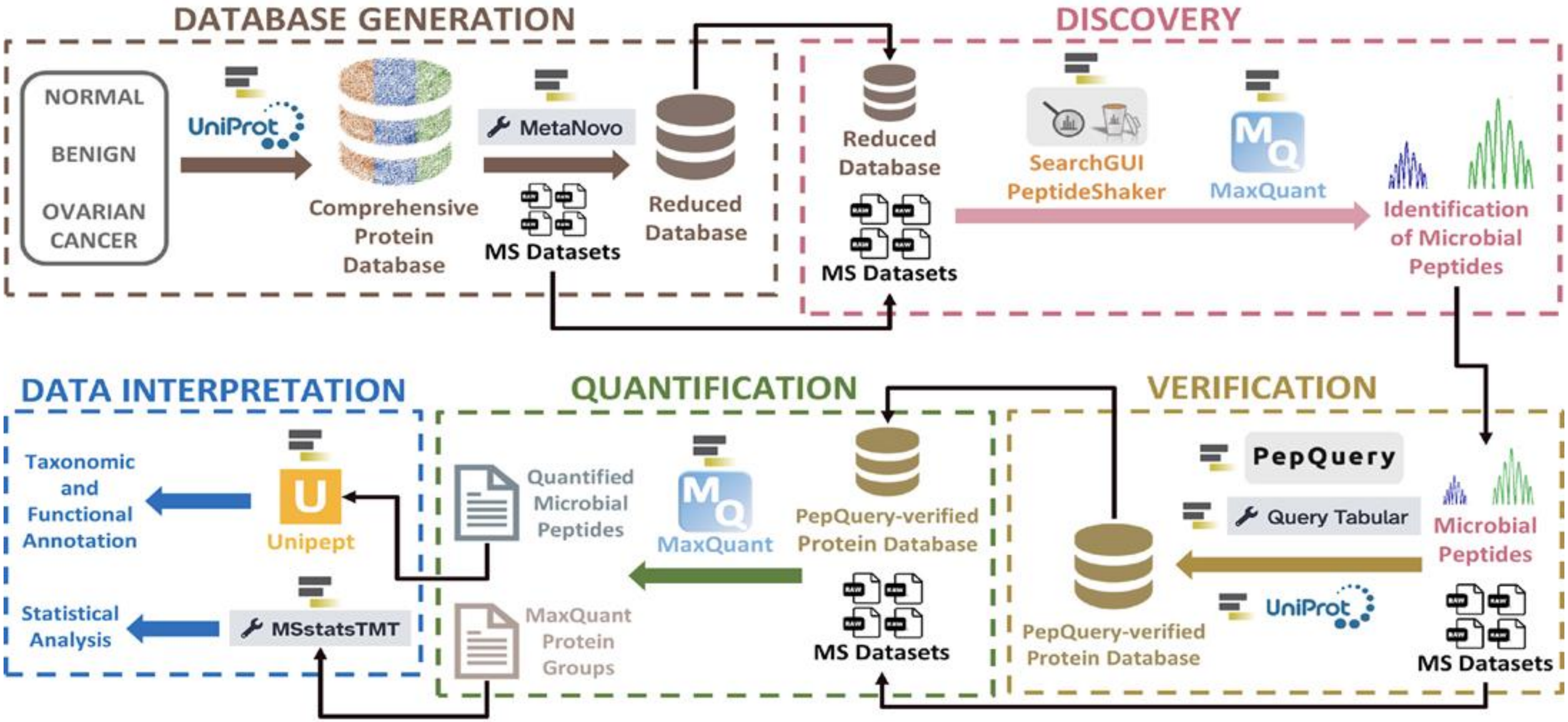


Afiuni-Zadeh *et al* (2018) Sci Rep . 8(1):10868.
doi: 10.1038/s41598-018-29092-4.

Jagtap *et al* (2012) Proteomics 12(7): 992–1001.
doi: 10.1002/pmic.201100503

Kruk *et al* (2024) mSystems
doi: 10.1128/msystems.00929-23

OVERVIEW OF CLINICAL METAPROTEOMICS WORKFLOW



Do K *et al* (2024). mSphere https://doi.org/10.1128/msphere.00793-23

The workflow modules, training data and documentation are available via the Galaxy Training Network.
https://training.galaxyproject.org/training-material/learning-pathways/clinical-metaproteomics.html

INVESTIGATING KEY HOST, MICROBIAL AND VARIANT PEPTIDES FOR DETECTION OF ORAL CANCER USING ADVANCED MULTI-OMICS METHODS.

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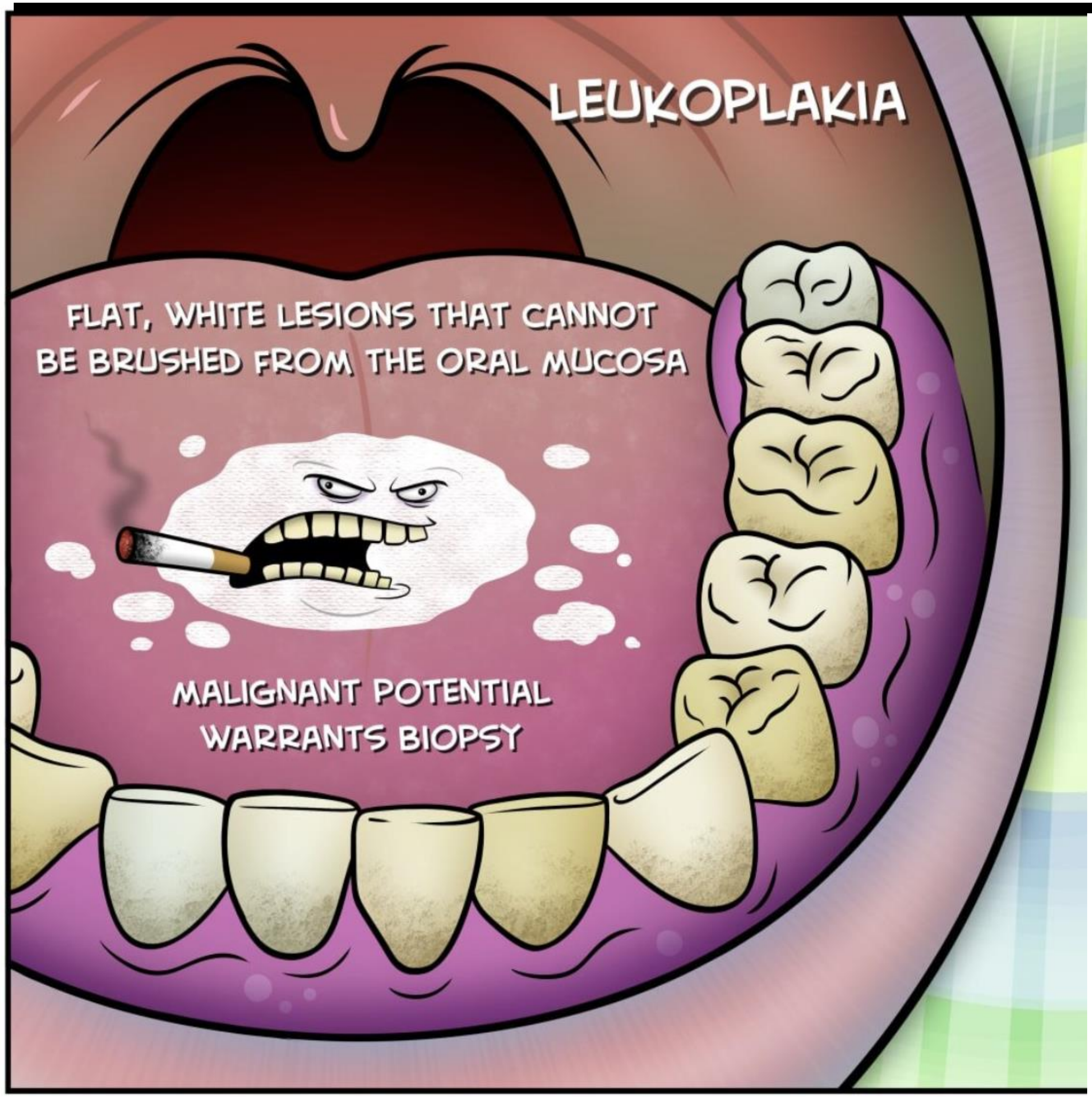
WORK NOT PUBLISHED YET

ORAL LEUKOPLAKIA: ORAL CANCER RISK



WWW.MEDCOMIC.COM

BACKGROUND

- PAINLESS, SLOW-GROWING LESION on MUCOUS MEMBRANES of ORAL CAVITY
- POTENTIAL PRECANCEROUS CONDITION

CAUSES

MOST COMMON

- HEAVY SMOKING
- CHEWING TOBACCO
- EXCESSIVE ALCOHOL USE
- POOR ORAL HEALTH
- LONG-TERM TRAUMA to ORAL CAVITY
- ADVANCED AGE
- HPV INFECTION

https://www.osmosis.org/answers/erythroplakia

EXPERIMENTAL WORKFLOW

ORAL LEUKOPLAKIA

PRE-TREATMENT

SIX SAMPLES

90-DAYS OF PIOGLITAZONE TREATMENT

SIX SAMPLES

ORAL RINSE SAMPLES

↓

Enrichment

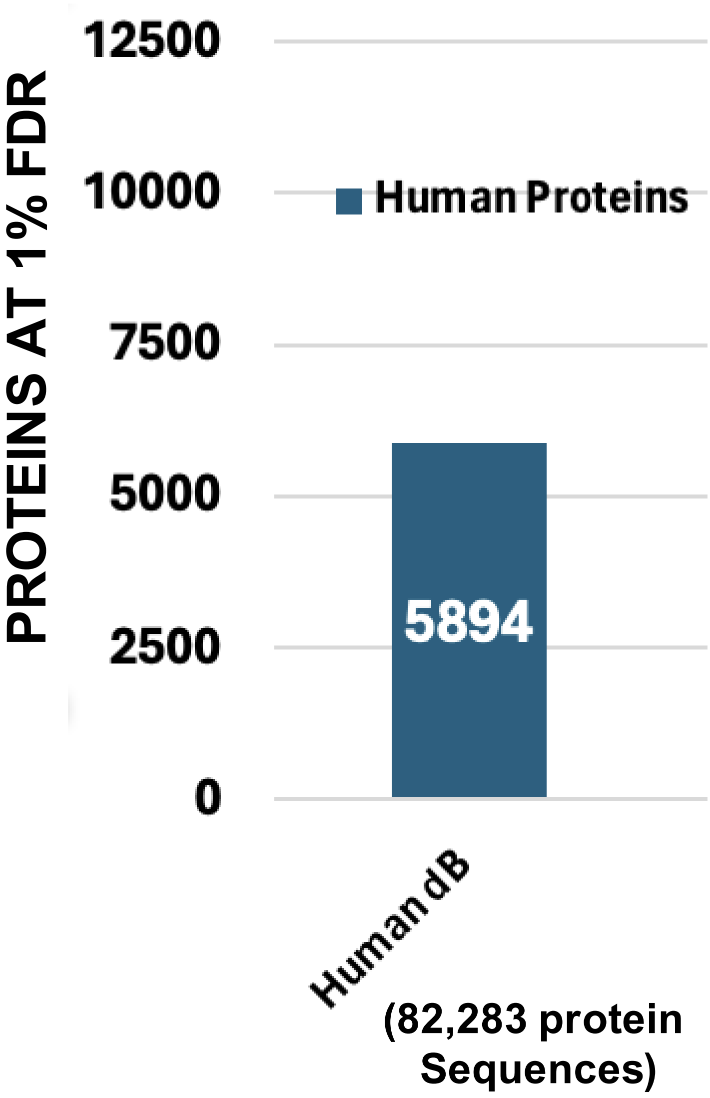
↓

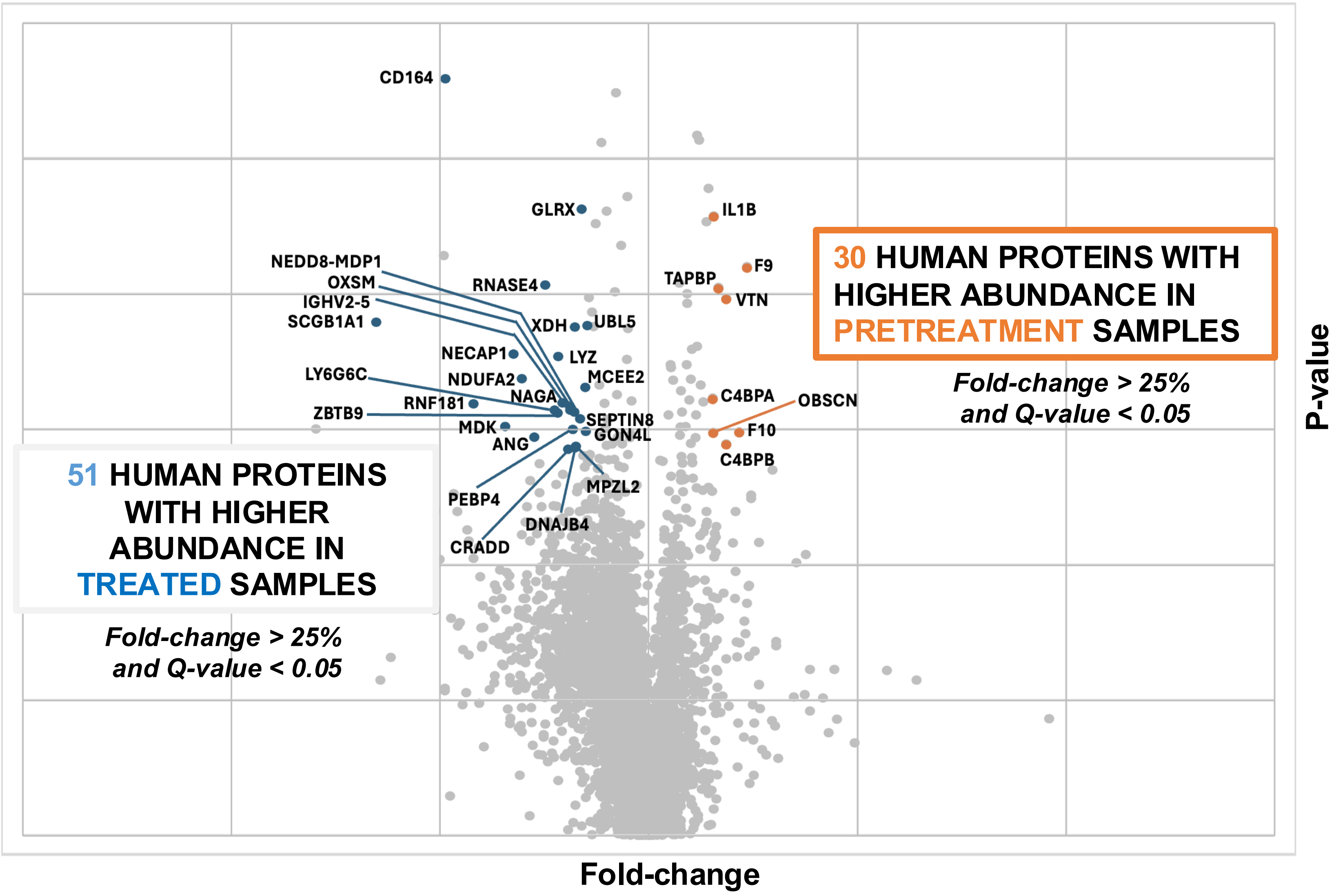
Mass spectrometry using DIA-PASEF

↓

Search against Human + Microbiome Protein sequences dB

PROTEINS DETECTED AND DIFFERENTIALLY ABUNDANT PROTEINS





51 HUMAN PROTEINS WITH HIGHER ABUNDANCE IN TREATED SAMPLES

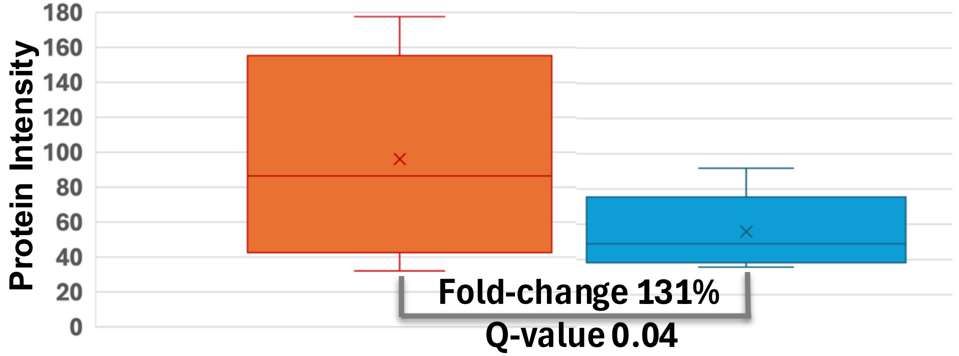
Fold-change > 25% and Q-value < 0.05

30 HUMAN PROTEINS WITH HIGHER ABUNDANCE IN PRETREATMENT SAMPLES

Fold-change > 25% and Q-value < 0.05

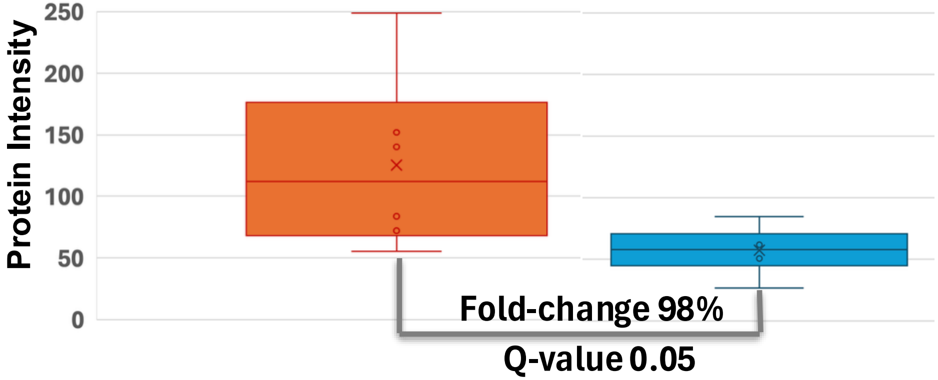
MMP1 & COAGULATION CASCADE: DOWNREGULATED AFTER TREATMENT

Interstitial collagenase MMP1 (17 peptides)



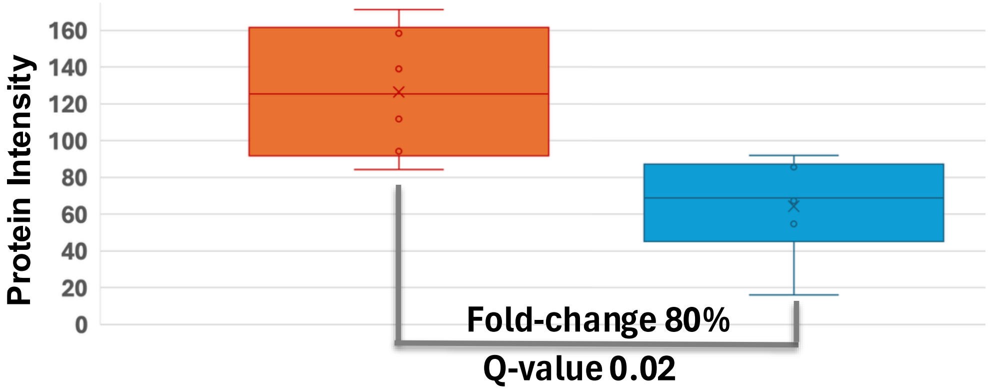
Fold-change 131%
Q-value 0.04

Vitamin K-dependent protein Z (3 peptides)



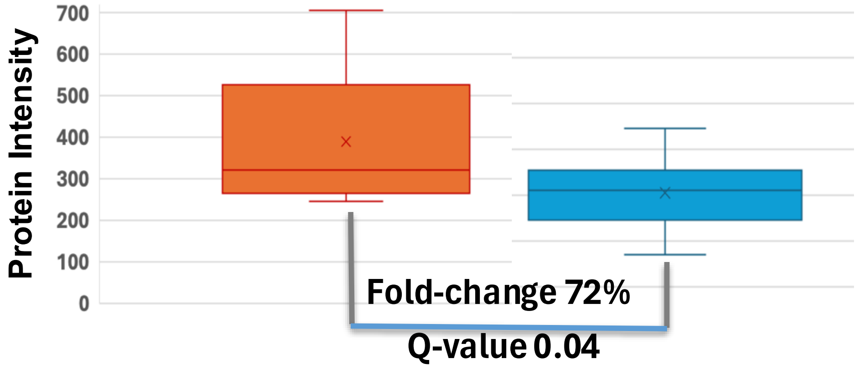
Fold-change 98%
Q-value 0.05

Coagulation factor IX (7 peptides)



Fold-change 80%
Q-value 0.02

Coagulation factor X (6 peptides)

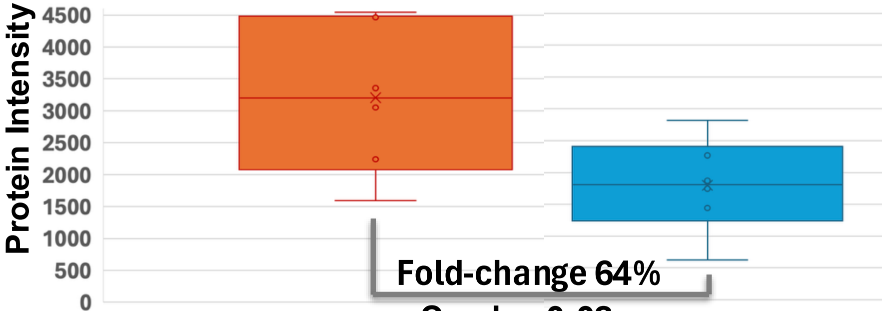


Fold-change 72%
Q-value 0.04

Coagulation Cascade

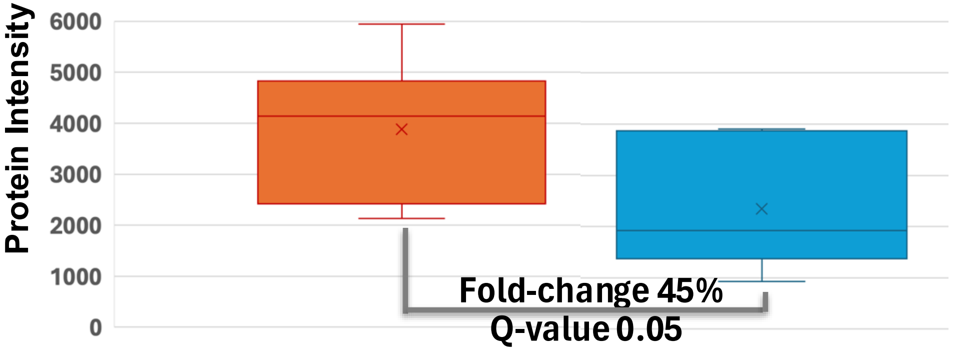
COMPLEMENT CASCADE: DOWNREGULATED AFTER TREATMENT

VITRONECTIN (12 peptides)



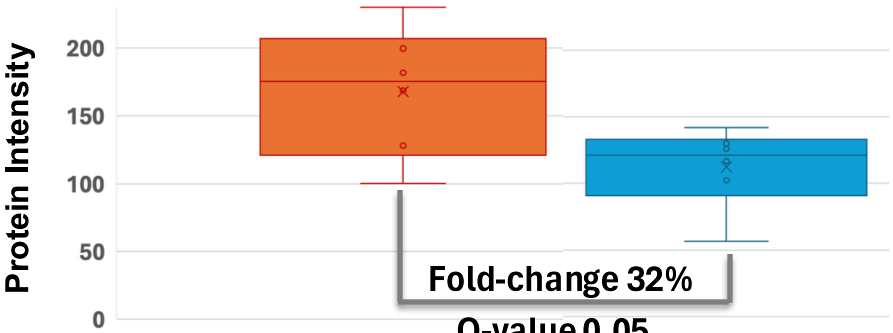
Fold-change 64%
Q-value 0.03

C4b-binding protein alpha chain (18 peptides)



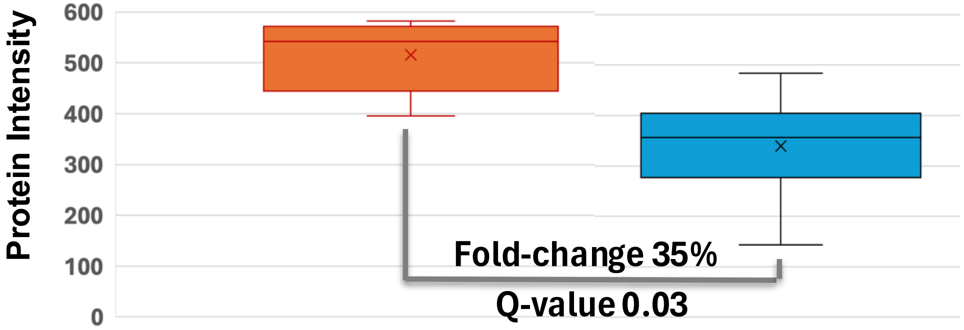
Fold-change 45%
Q-value 0.05

Carboxypeptidase B2 (9 peptides)



Fold-change 32%
Q-value 0.05

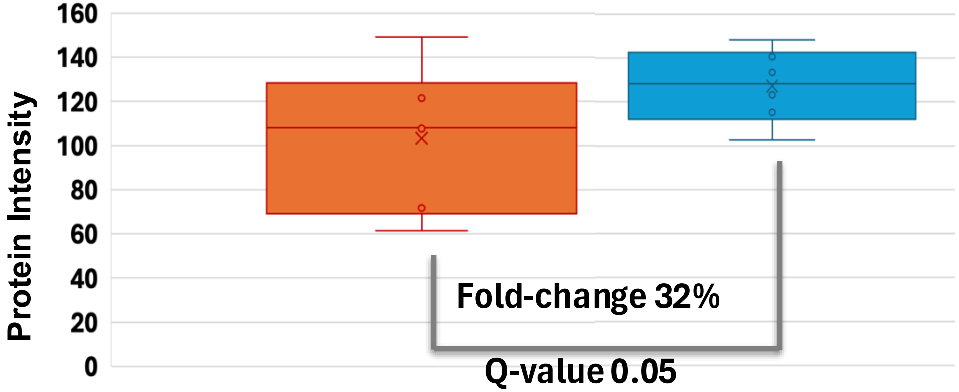
Complement component C8 beta chain (18 peptides)



Fold-change 35%
Q-value 0.03

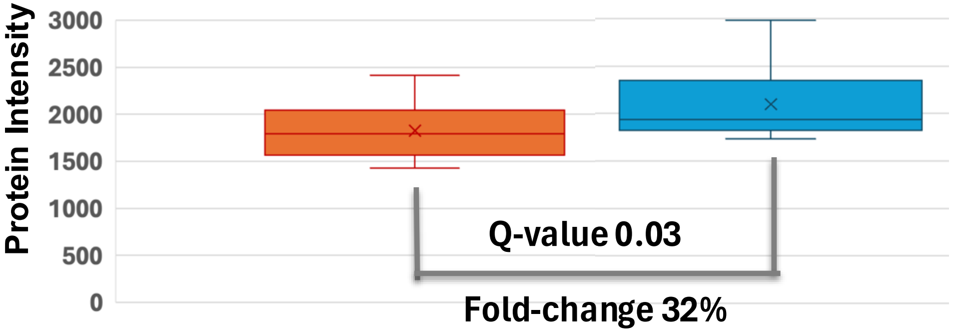
Regulation of Complement cascade

APOPTOSIS: UPREGULATED AFTER TREATMENT

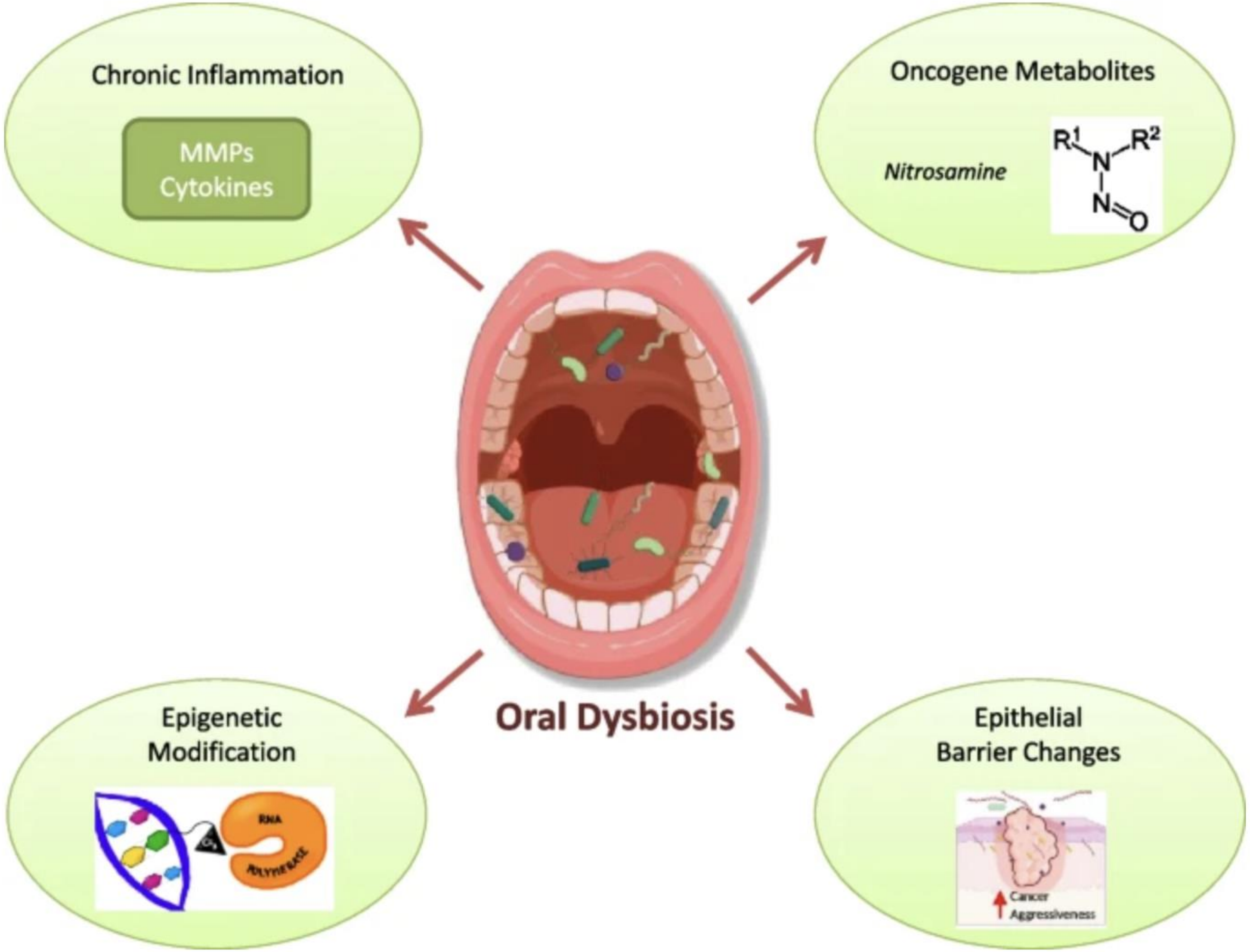

DESMOGLEIN-2 (16 peptides)

- Component of intercellular desmosome junctions mediating cell-cell adhesion.
- Involved in apoptotic pathway.
- Prognostic marker in renal cancer, pancreatic cancer, lung cancer, head and neck cancer, colorectal cancer and cervical cancer.

- An adaptor protein that is composed of two protein-protein interaction domains
- Functions as key mediator in apoptosis and inflammation via the activation of caspases.
- Prognostic marker in renal cancer

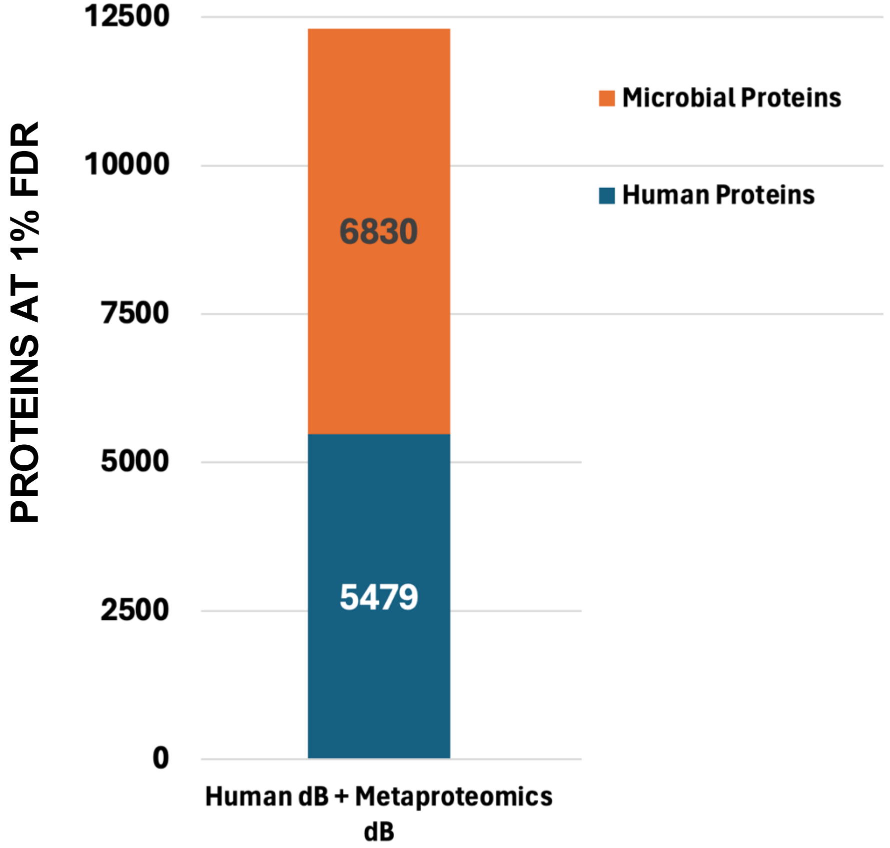

Apoptosis-associated speck-like protein containing a CARD (13 peptides)

ROLE OF BACTERIA IN ORAL CANCER DEVELOPMENT

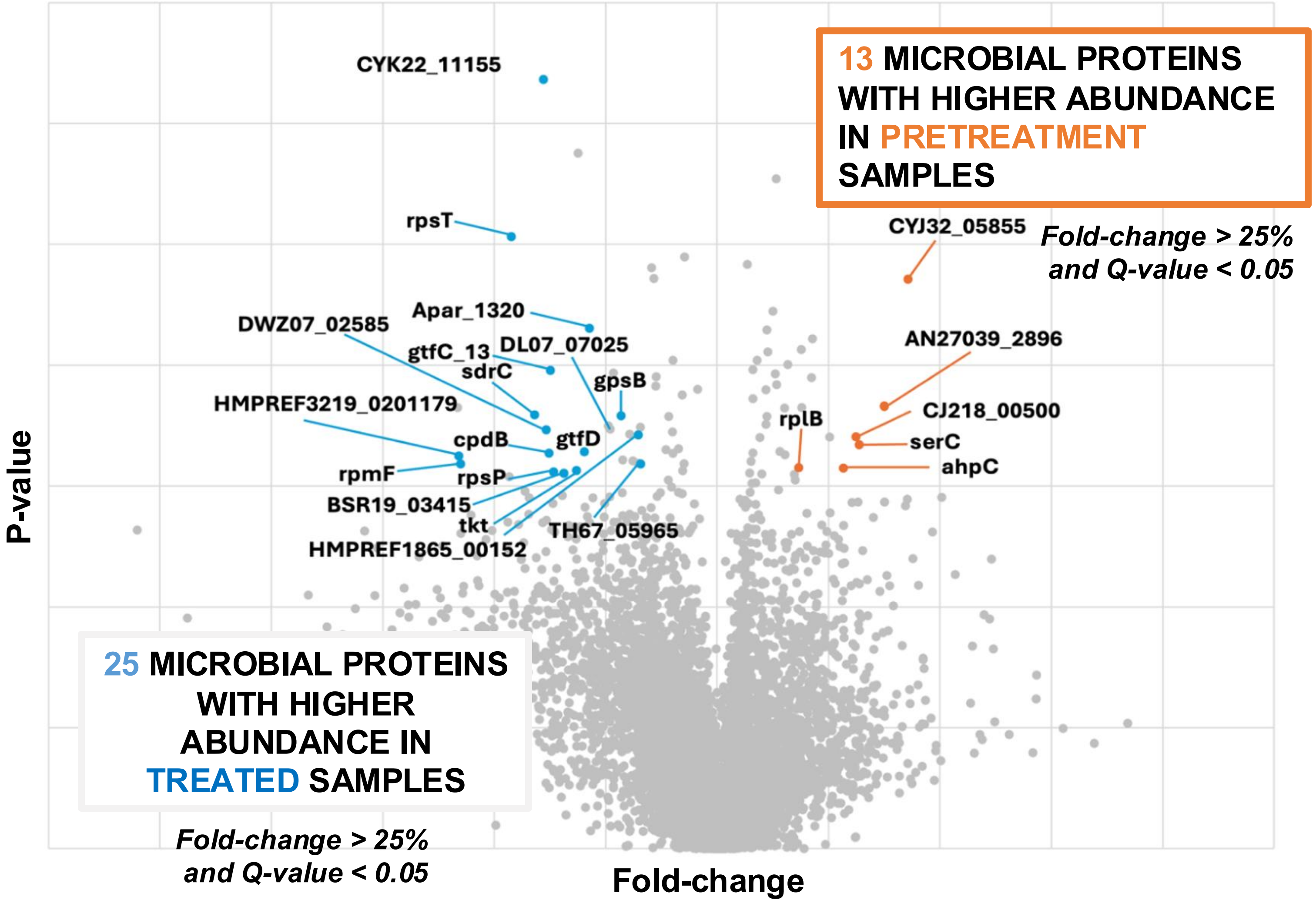


Asili, P., et al. J Gastrointest Canc (2023). https://doi.org/10.1007/s12029-022-00901-4

MICROBIAL DATABASE SEARCH RESULTS



(198,609 protein sequences)



13 MICROBIAL PROTEINS WITH HIGHER ABUNDANCE IN PRETREATMENT SAMPLES

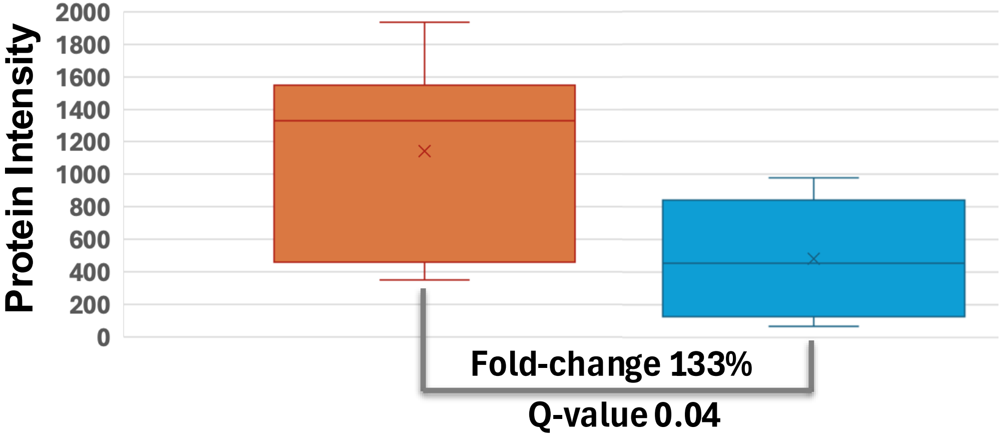
Fold-change > 25% and Q-value < 0.05

25 MICROBIAL PROTEINS WITH HIGHER ABUNDANCE IN TREATED SAMPLES

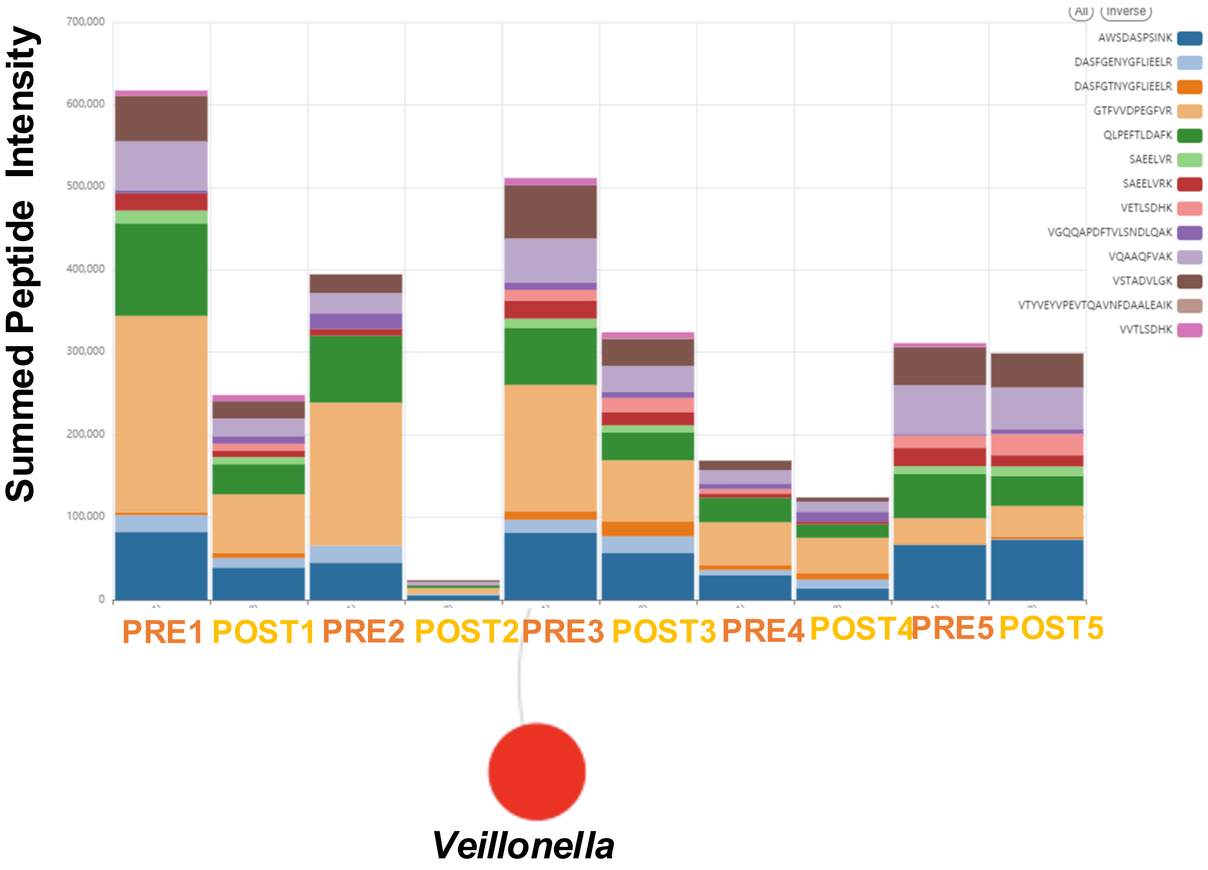
Fold-change > 25% and Q-value < 0.05

MICROBIAL PROTEINS DOWNREGULATED AFTER TREATMENT

Alkyl hydroperoxide reductase C (11 peptides)



- Responsible for the detoxification of reactive oxygen species.
- Survival under environmental stresses or during infection.



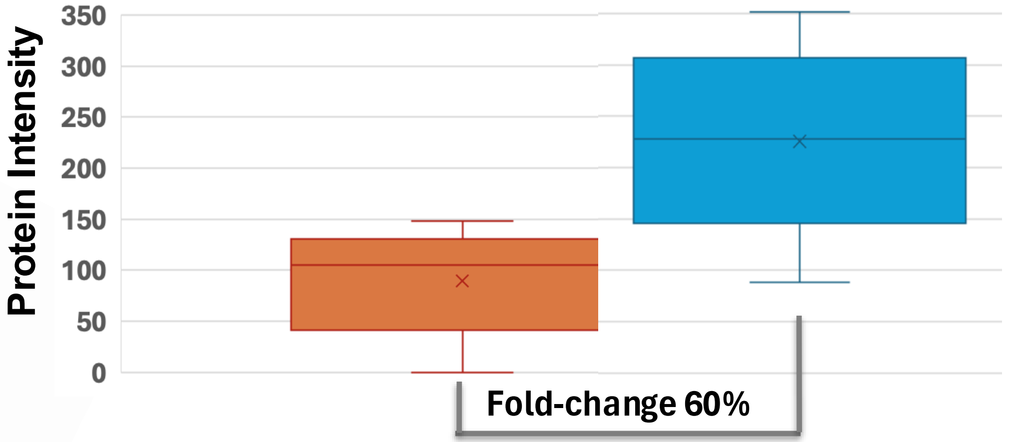
In this study, the protein was expressed by *Veillonella* genus.

MetaLab and MetaX analysis by Kai Cheng, Qing Wu (Daniel Figeys Lab, University of Ottawa)

MICROBIAL PROTEINS UPREGULATED AFTER TREATMENT

- **Glucosyltransferase that catalyzes the transfer of glucosyl residues to dextran polymer.**
- **Involved in biofilm formation.**

Dextransucrase (61 peptides)

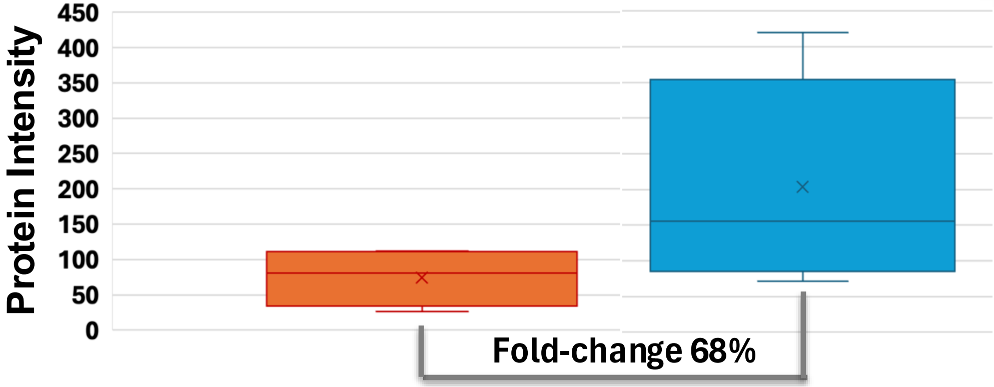


Fold-change 60%

Q-value 0.02

Streptococcus salivarius

serine-type D-Ala-D-Ala carboxypeptidase (21 peptides)



Fold-change 68%

Q-value 0.03

- **Involved in bacterial cell wall synthesis by mediating peptidoglycan cross-linking.**

PEPTIDES FOR TARGETED ANALYSIS

	AFTER TREATMENT	
HUMAN PROTEINS	UP-REGULATED	DOWN-REGULATED
	Peptides (for targeted analysis)	Peptides (for targeted analysis)
Desmoglein-2	16 (3)	
Apoptosis-associated speck-like protein containing a CARD	13 (6)	
Chitinase-3-like protein 2	14 (5)	
Cathepsin D	18 (10)	
Lymphocyte antigen 6 complex locus protein G6c	2 (2)	
Lysozyme C	8 (7)	
Alpha-N-acetylgalactosaminidase	5 (3)	
Receptor-type tyrosine-protein phosphatase S	21 (6)	
Interstitial collagenase MMP1		17 (1)
Coagulation factor IX		7 (2)
Coagulation factor X		6 (2)
Vitronectin		12 (4)
C4b-binding protein alpha chain		18 (4)
Carboxypeptidase B2		9 (3)
Complement component C8 beta chain		6 (4)
MICROBIAL PROTEINS		
Dextransucrase	61 (3)	
Serine-type D-Ala-D-Ala carboxypeptidase	21 (2)	
Glutamate--ammonia ligase	7 (2)	
Alkyl hydroperoxide reductase C		11 (5)

ORAL CANCER DATASET: CONCLUSIONS AND FUTURE WORK

- **Several human, microbial proteins were detected to be differentially abundant in pretreatment and treated samples.**
- **Pathways such as coagulation and complement cascade were downregulated and apoptotic pathways were upregulated after treatment.**
- **Microbial functions associated with glucosyltransferase activity were upregulated and oxidative stress functions were downregulated after treatment.**
- **Peptides associated with differentially abundant human and microbial proteins will be used for targeted analysis.**

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http://galaxyp.org/contact/

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QUESTIONS?