

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

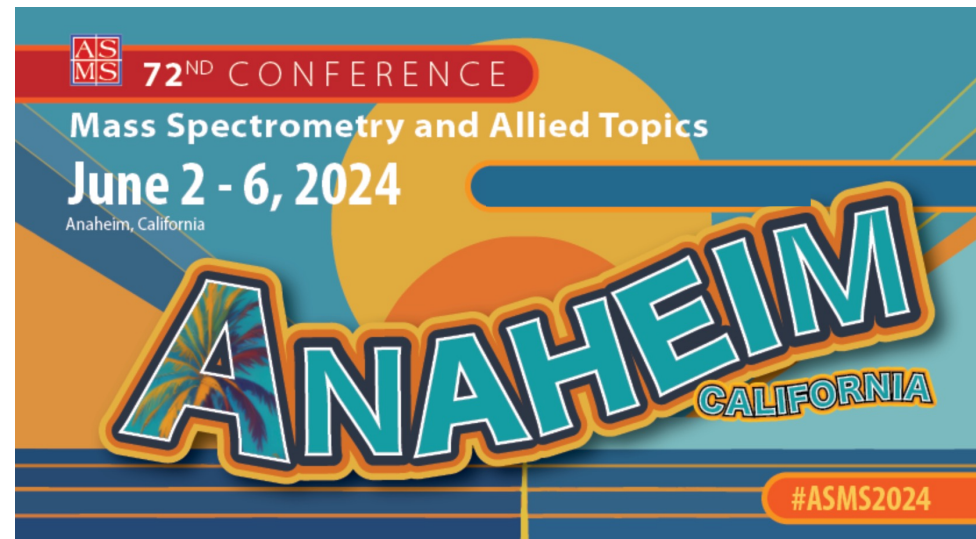
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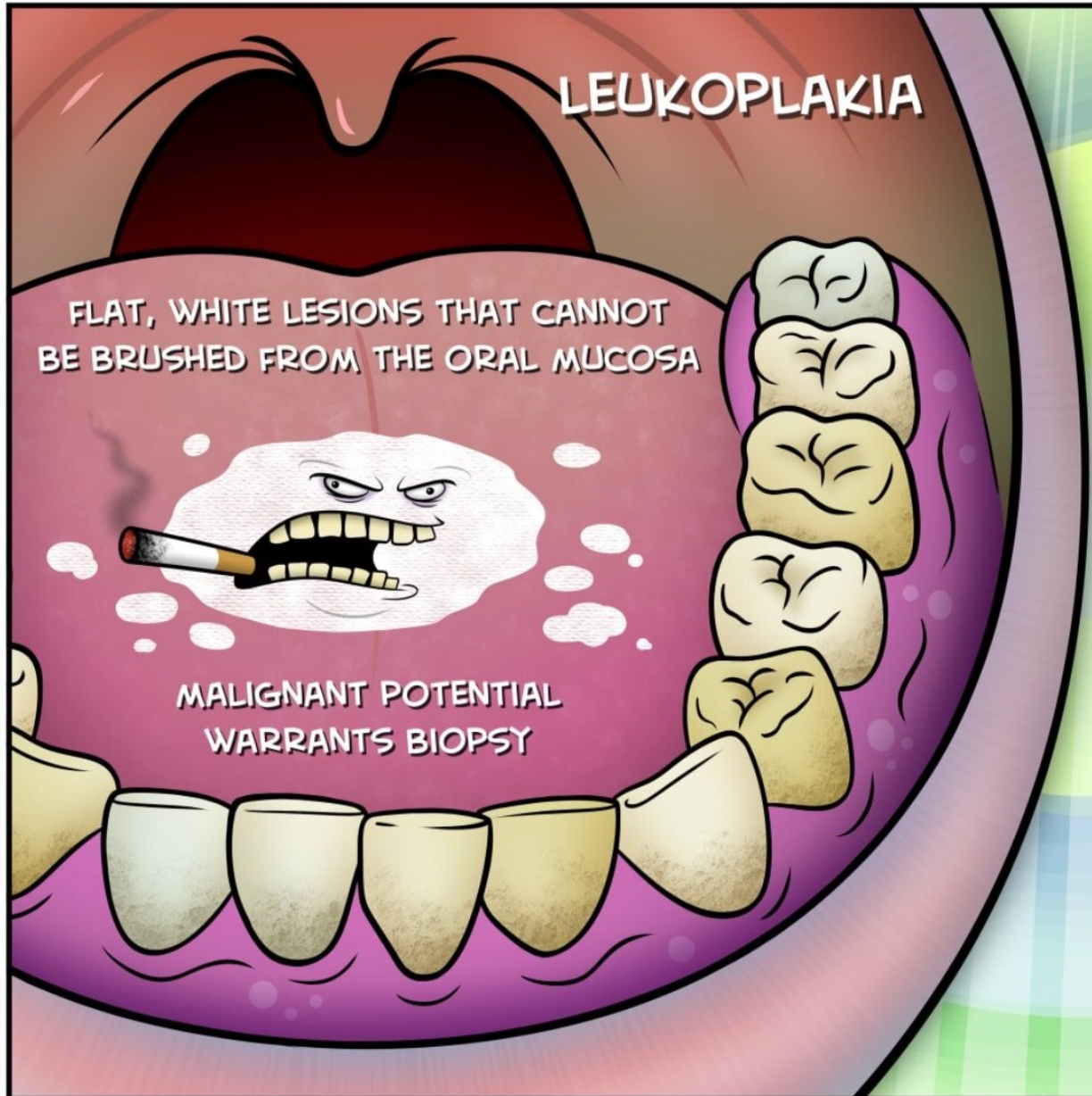


# **CONFLICT OF INTEREST DISCLOSURE**

***THE AUTHORS DECLARE NO COMPETING FINANCIAL INTEREST***



# ORAL LEUKOPLAKIA: ORAL CANCER RISK



## BACKGROUND

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

## CAUSES

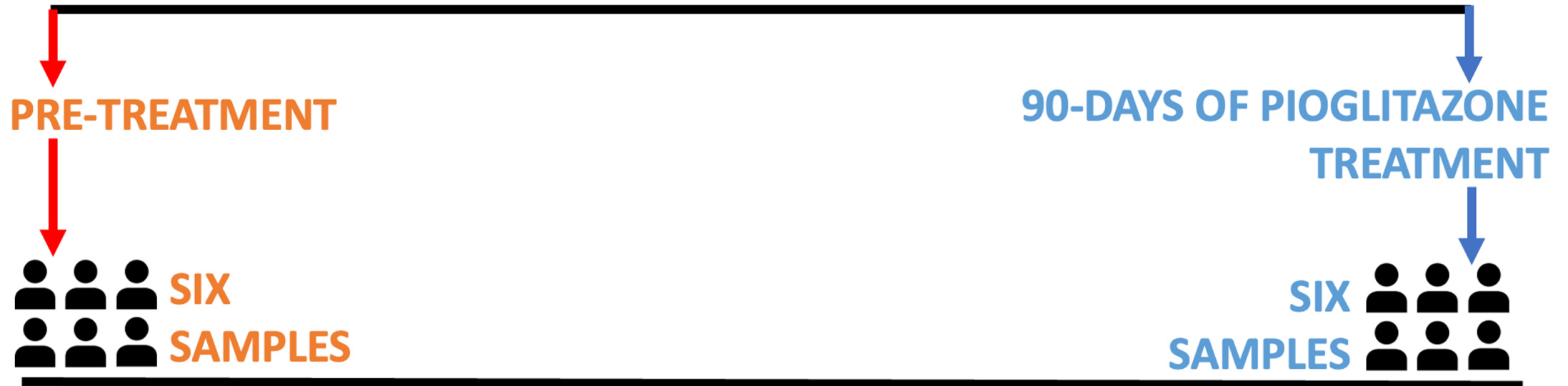


- \* HEAVY SMOKING
- \* CHEWING TOBACCO
- \* EXCESSIVE ALCOHOL USE
- \* POOR ORAL HEALTH
- \* LONG-TERM TRAUMA to ORAL CAVITY
- \* ADVANCED AGE
- \* HPV INFECTION



# EXPERIMENTAL WORKFLOW

## ORAL LEUKOPLAKIA



## ORAL RINSE SAMPLES

Enrichment

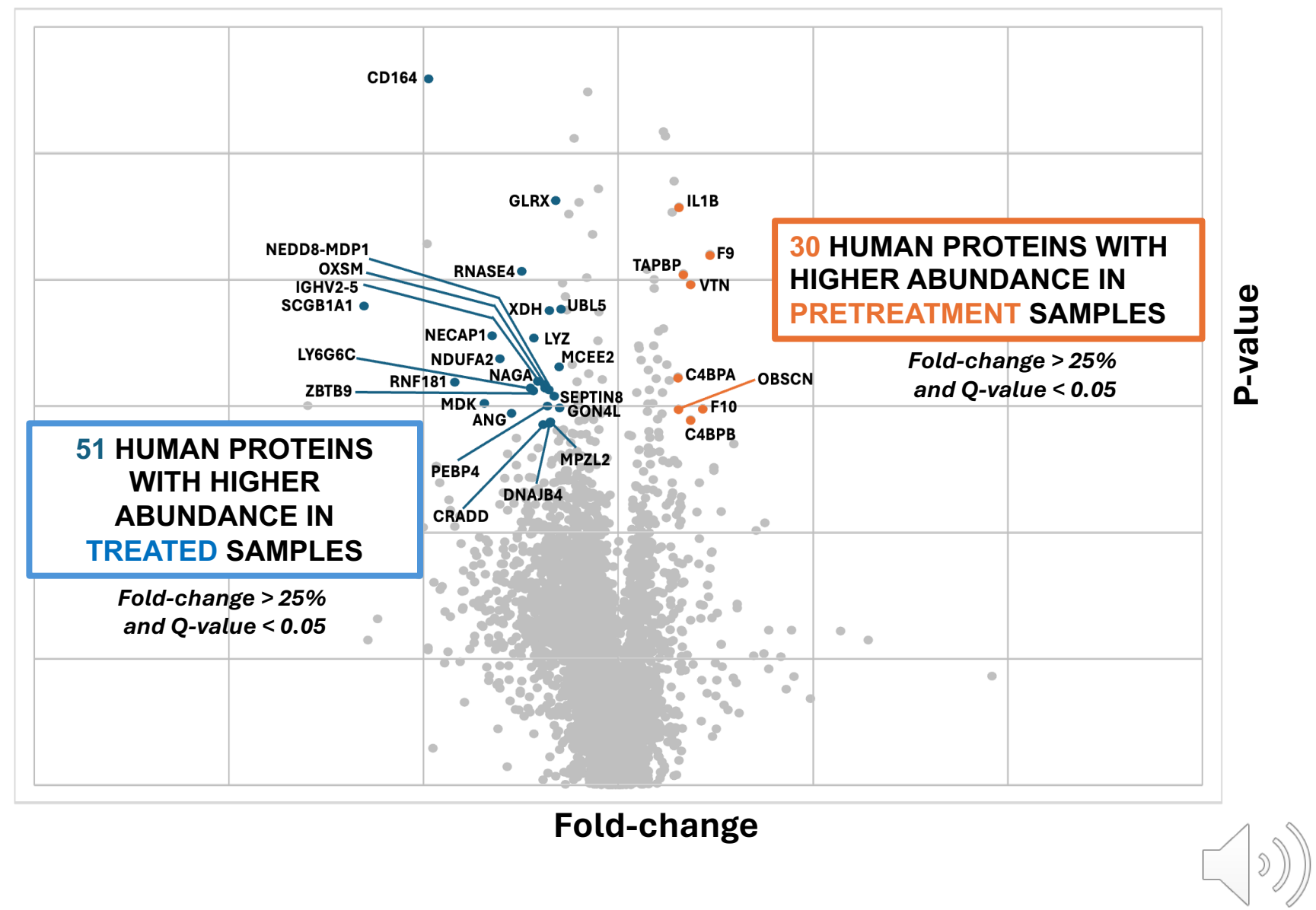
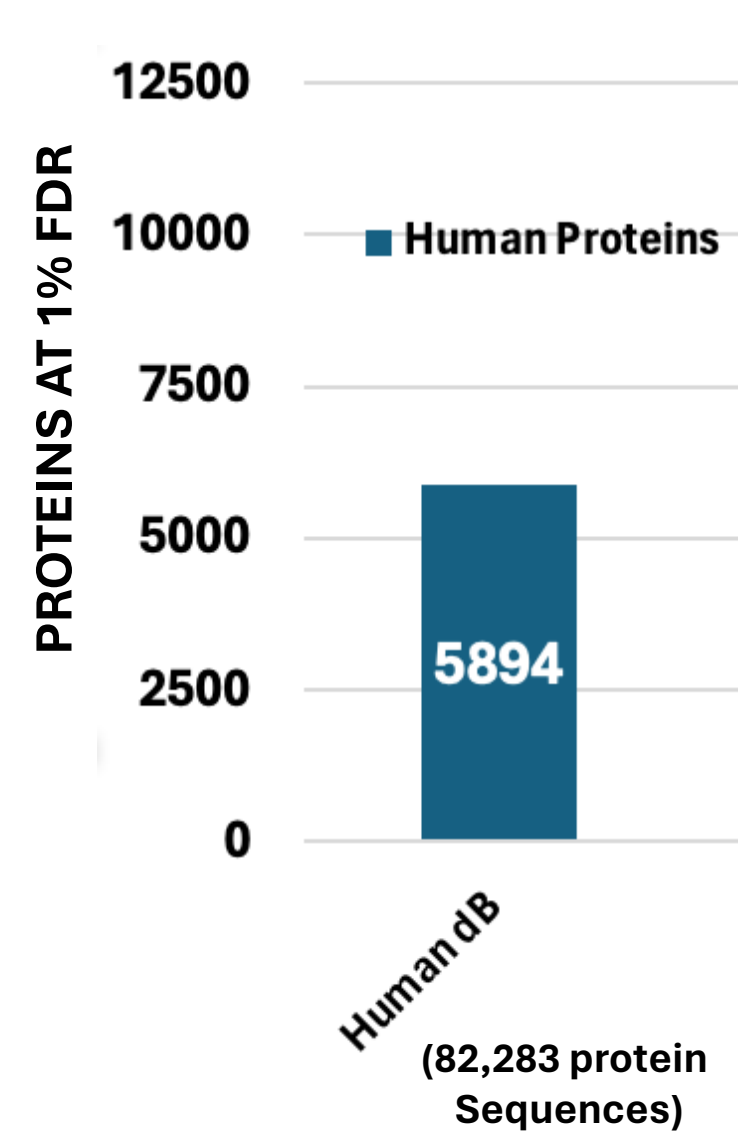
Mass spectrometry using DIA-PASEF

Search against Human + Microbiome + Novel Proteoforms

PO

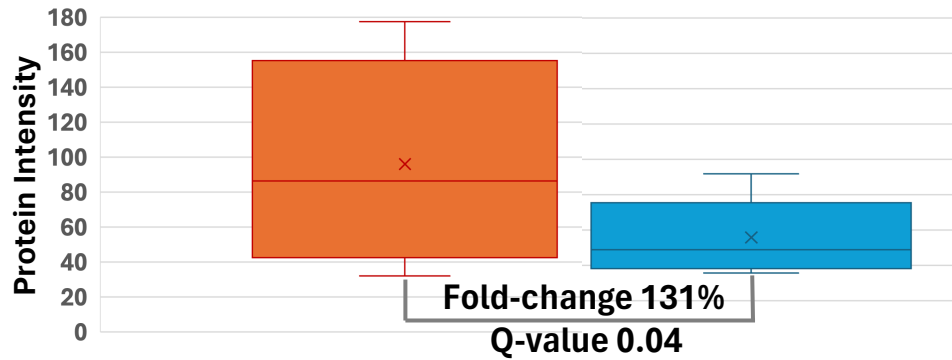


# PROTEINS DETECTED AND DIFFERENTIALLY ABUNDANT PROTEINS

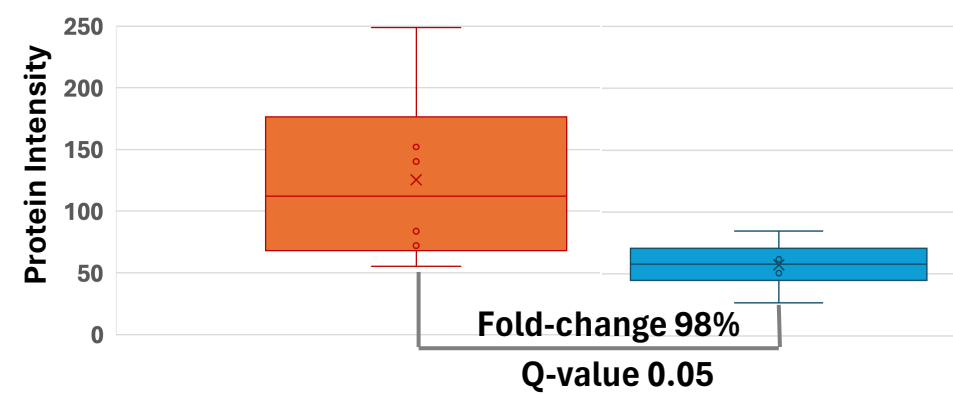


# MMP1 & COAGULATION CASCADE: DOWNREGULATED AFTER TREATMENT

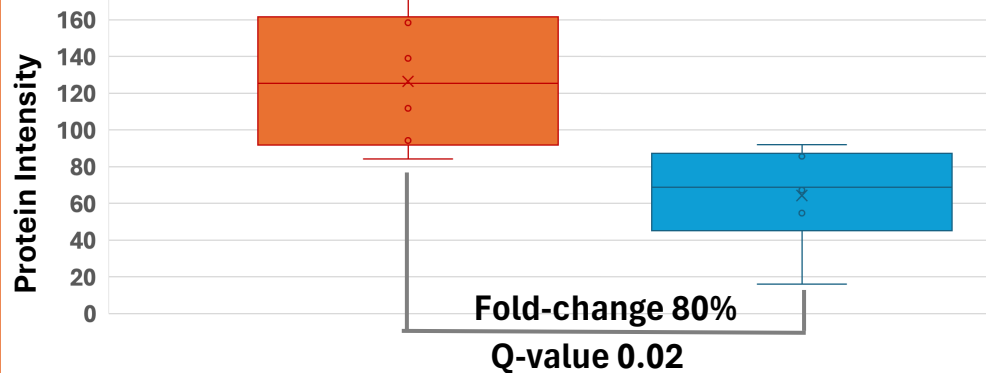
## Interstitial collagenase MMP1 (17 peptides)



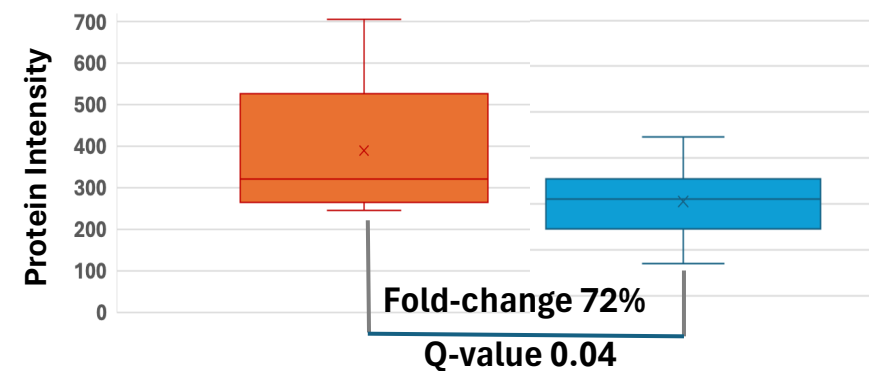
## Vitamin K-dependent protein Z (3 peptides)



## Coagulation factor IX (7 peptides)



## Coagulation factor X (6 peptides)

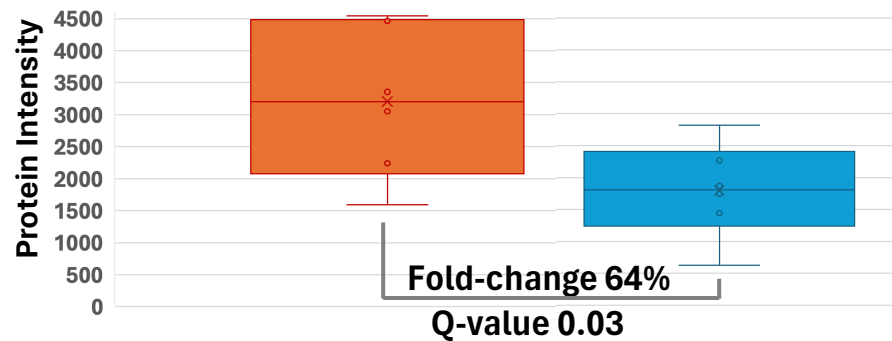


# Coagulation Cascade

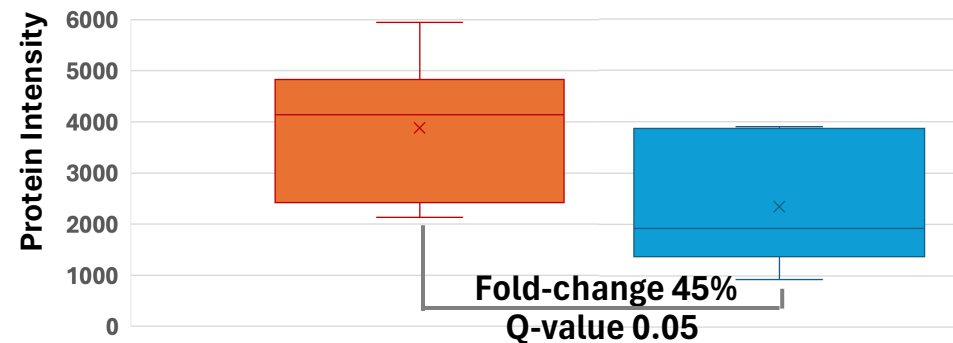


# COMPLEMENT CASCADE: DOWNREGULATED AFTER TREATMENT

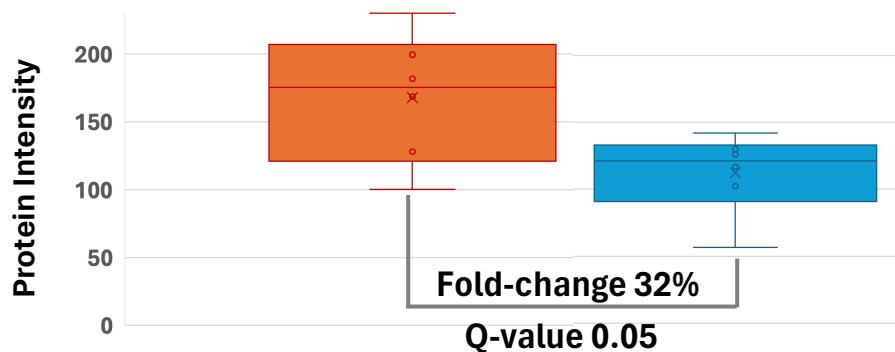
## VITRONECTIN (12 peptides)



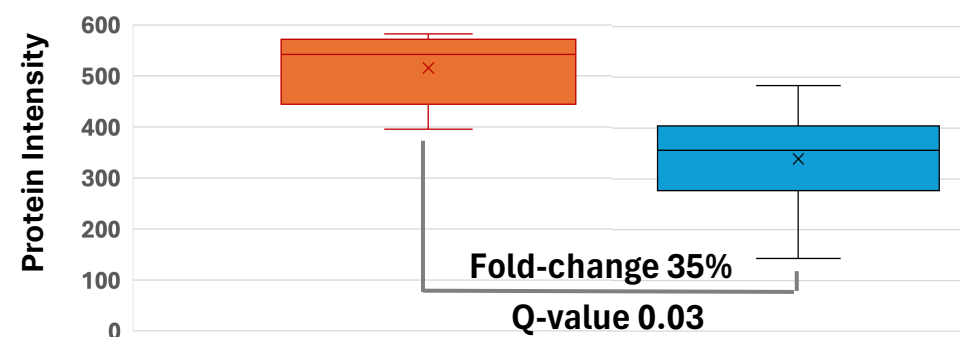
## C4b-binding protein alpha chain (18 peptides)



## Carboxypeptidase B2 (9 peptides)



## Complement component C8 beta chain (18 peptides)

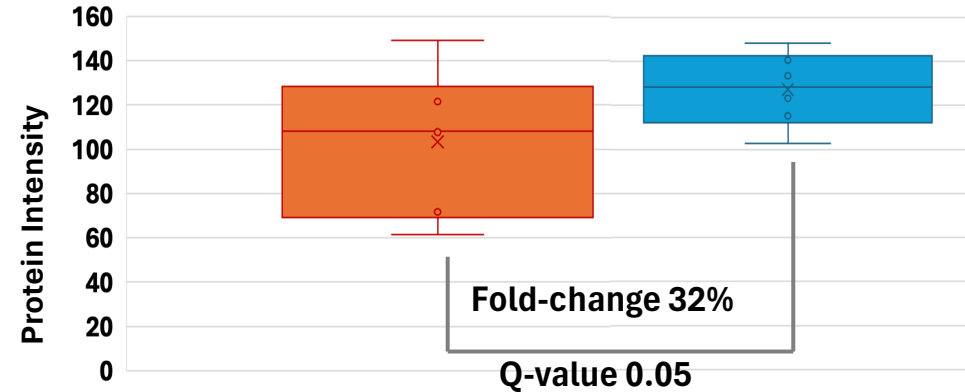


# Regulation of Complement cascade



# APOPTOSIS: UPREGULATED AFTER TREATMENT

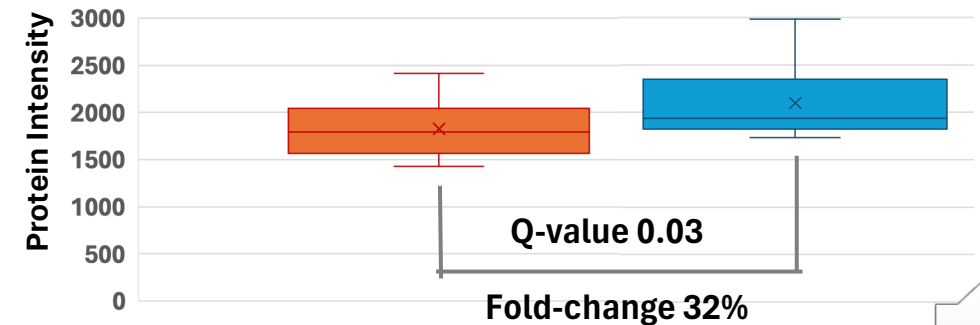
DESMOGLEIN-2 (16 peptides)



- 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)





# CANCER RELEVANCE OF DIFFERENTIALLY ABUNDANT PROTEINS

HIGHER ABUNDANCE IN  
TREATED SAMPLES

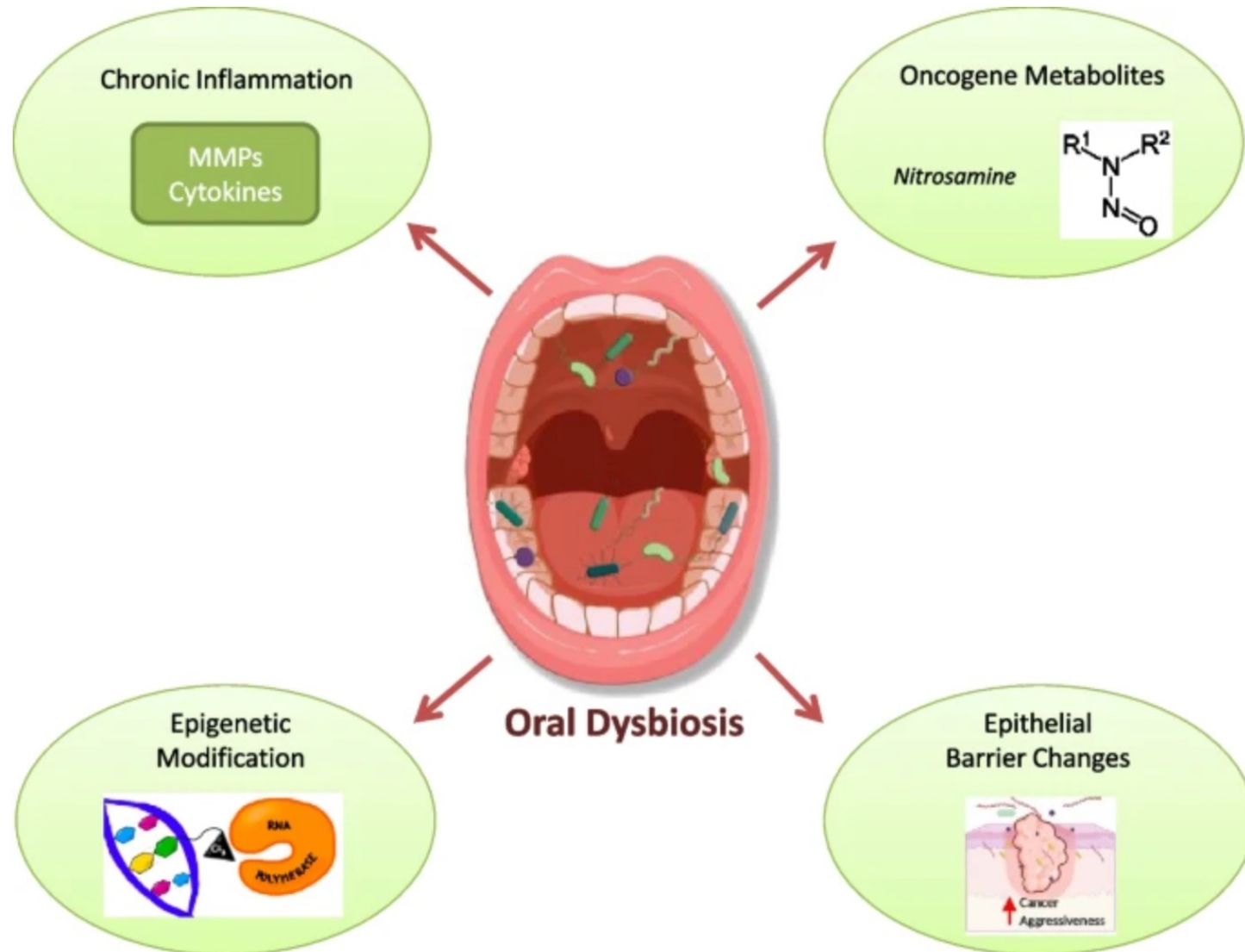
HIGHER ABUNDANCE IN  
PRE-TREATMENT SAMPLES

PROTEIN DESCRIPTION	RENAL	STOMACH	HEAD & NECK	PANCREATIC	ENDOMETRIAL	BREAST	UROTHELIAL	PROSTATE	LIVER	LUNG	COLORECTAL	CERVICAL	GLIOMA	THYROID	ORAL
Cathepsin D	FAVORABLE					HIGHER ABUNDANCE					UNFAVORABLE	HIGHER ABUNDANCE			
Lysozyme C		HIGHER ABUNDANCE	FAVORABLE												HIGHER ABUNDANCE
Golgi reassembly-stacking protein 2	UNFAVORABLE		UNFAVORABLE												
Receptor-type tyrosine-protein phosphatase S	UNFAVORABLE			FAVORABLE											HIGHER ABUNDANCE
Sialate O-acetyltransferase	FAVORABLE	HIGHER ABUNDANCE			FAVORABLE										
Plexin-B3						UNFAVORABLE									
Glycerol-3-phosphate phosphatase	FAVORABLE						FAVORABLE								
Cystatin-D						HIGHER ABUNDANCE									
Serine hydroxymethyltransferase, cytosolic	FAVORABLE								FAVORABLE						
Di-N-acetylchitobiase															
Desmoglein-2	FAVORABLE		UNFAVORABLE	UNFAVORABLE						UNFAVORABLE	FAVORABLE	UNFAVORABLE			
Chitinase-3-like protein 2	UNFAVORABLE		FAVORABLE										UNFAVORABLE		HIGHER ABUNDANCE
Apoptosis-associated speck-like protein containing a CARD	UNFAVORABLE														UNFAVORABLE
Xanthine dehydrogenase/oxidase							FAVORABLE								
Carboxypeptidase B2									FAVORABLE						HIGHER ABUNDANCE
C4b-binding protein alpha chain				HIGHER ABUNDANCE	FAVORABLE				FAVORABLE						
Vitronectin	UNFAVORABLE								FAVORABLE						HIGHER ABUNDANCE
Ferritin light chain	UNFAVORABLE														HIGHER ABUNDANCE
Cytochrome P450 4F3									HIGHER ABUNDANCE						HIGHER ABUNDANCE
Complement component C8 beta chain									FAVORABLE						
Cytochrome c oxidase subunit 4 isoform 1, mitochondrial	FAVORABLE														
Mitochondrial adenyl nucleotide antiporter SLC25A24											FAVORABLE				
Coagulation factor X	UNFAVORABLE			FAVORABLE					HIGHER ABUNDANCE						
Interstitial collagenase	UNFAVORABLE		HIGHER ABUNDANCE						UNFAVORABLE			UNFAVORABLE			HIGHER ABUNDANCE
Coagulation factor IX									FAVORABLE						
Signal peptidase complex catalytic subunit SEC11			UNFAVORABLE		FAVORABLE									FAVORABLE	

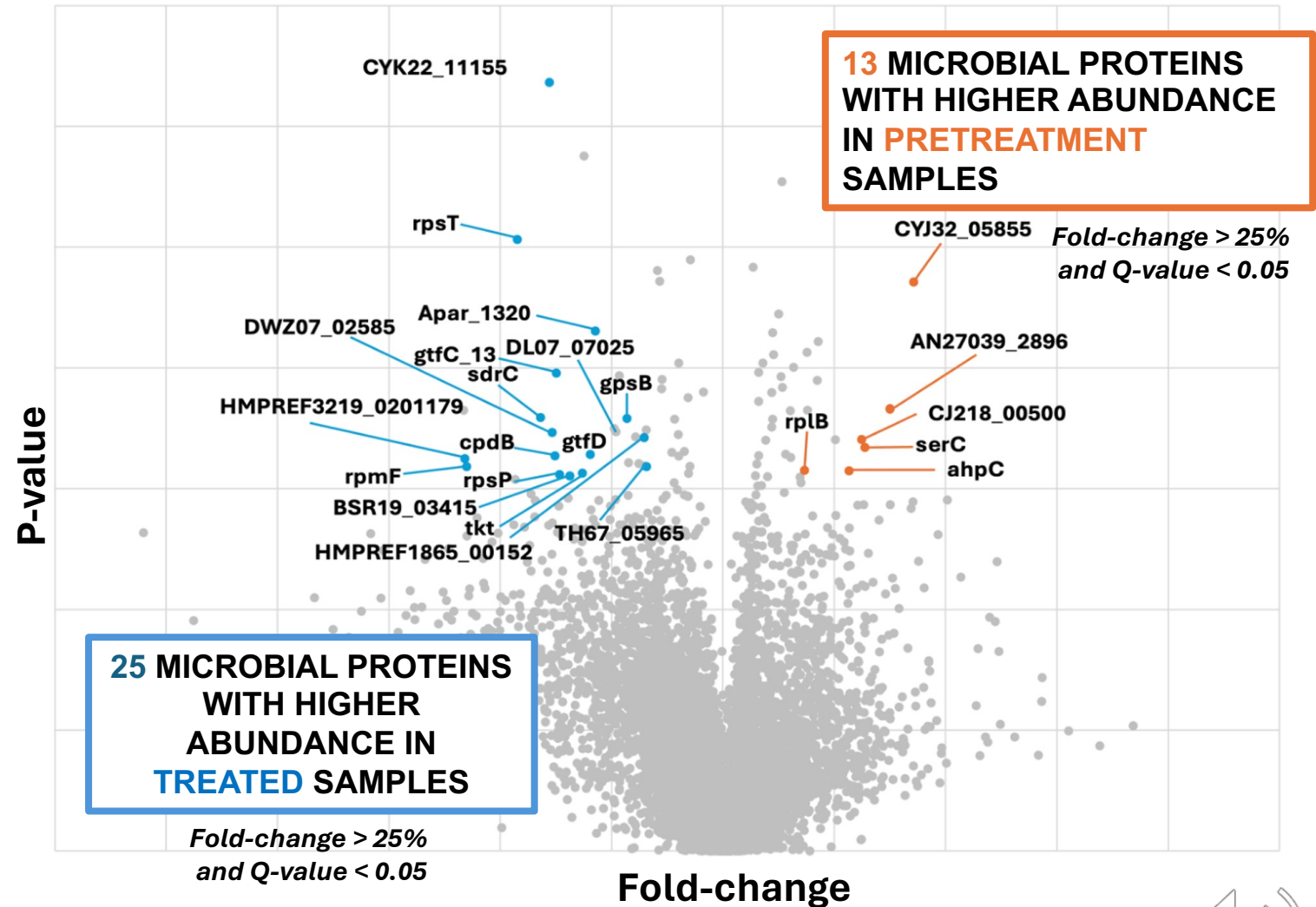
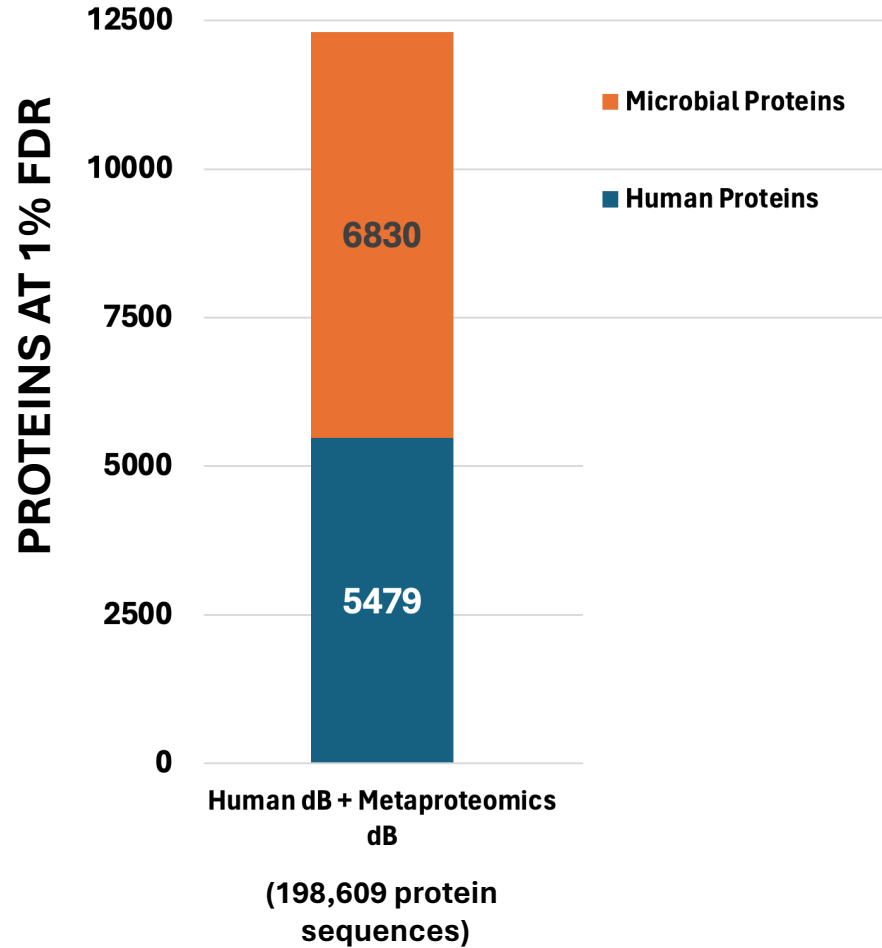
- FAVORABLE PROGNOSIS VALUE
- UNFAVORABLE PROGNOSIS VALUE
- HIGHER ABUNDANCE



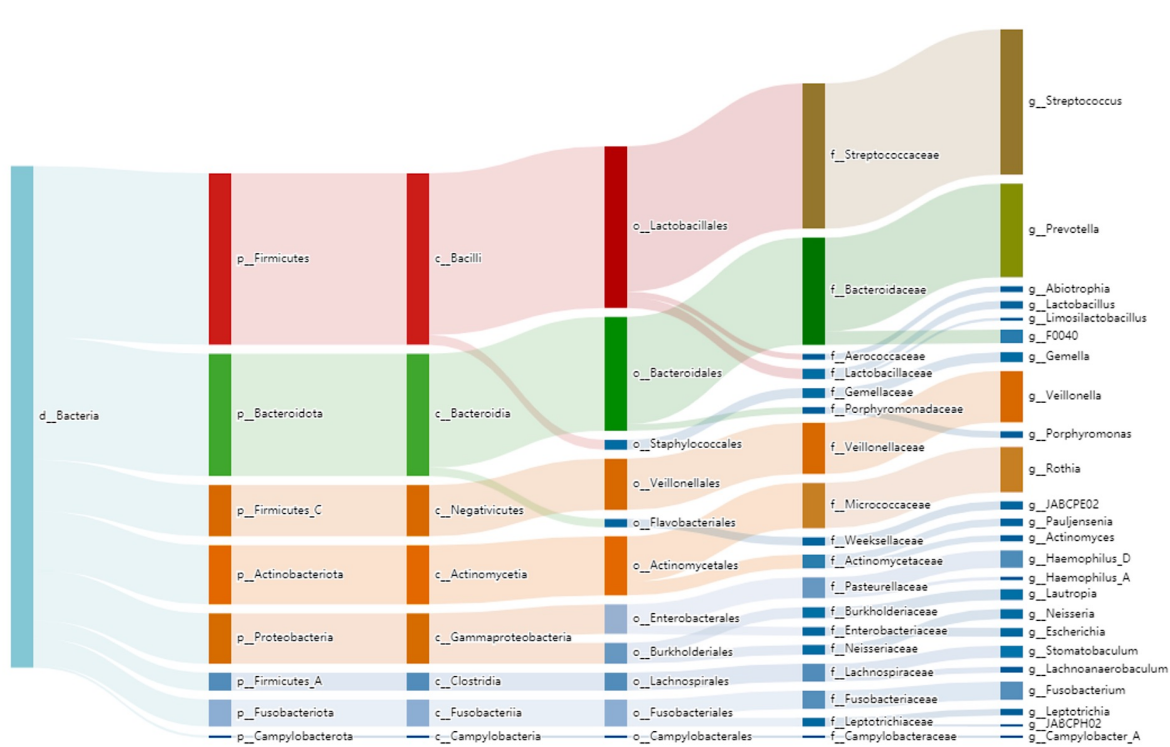
# ROLE OF BACTERIA IN ORAL CANCER DEVELOPMENT



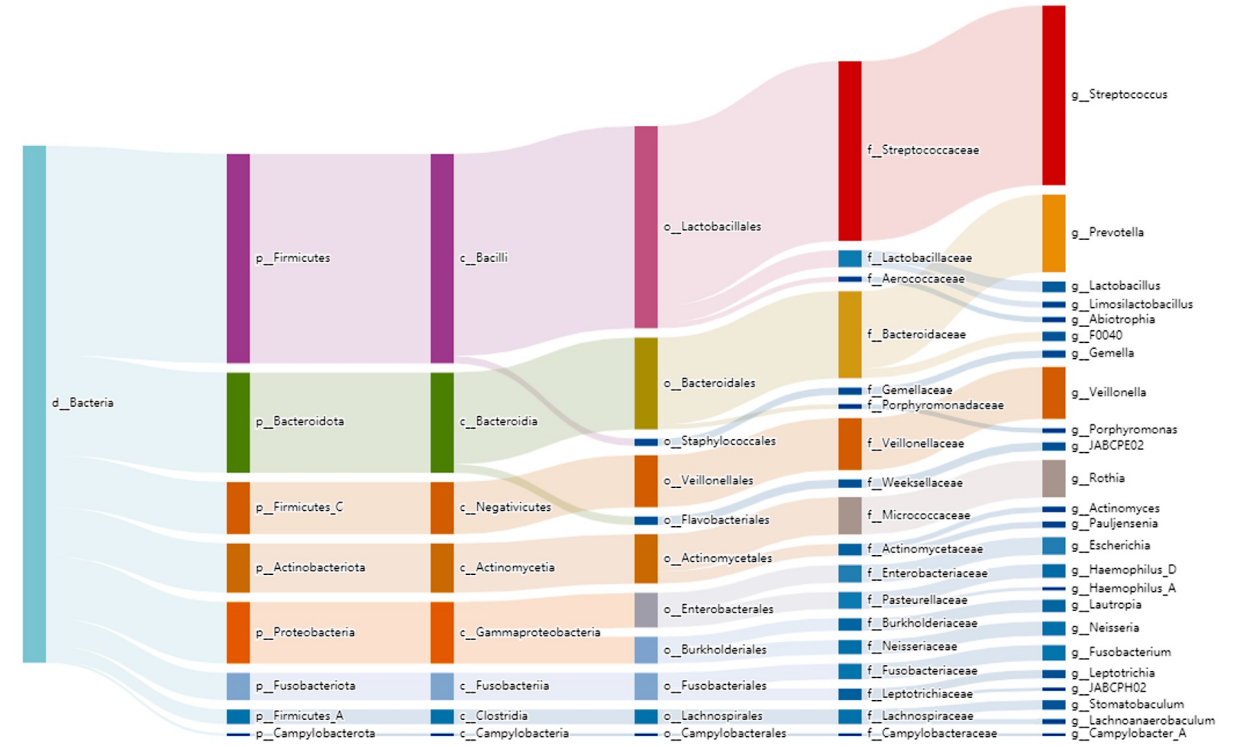
# MICROBIAL DATABASE SEARCH RESULTS



# MICROBIAL TAXONOMY OUTPUTS



Genera detected in pretreated samples



Genera detected in treatment samples



MetaLab and

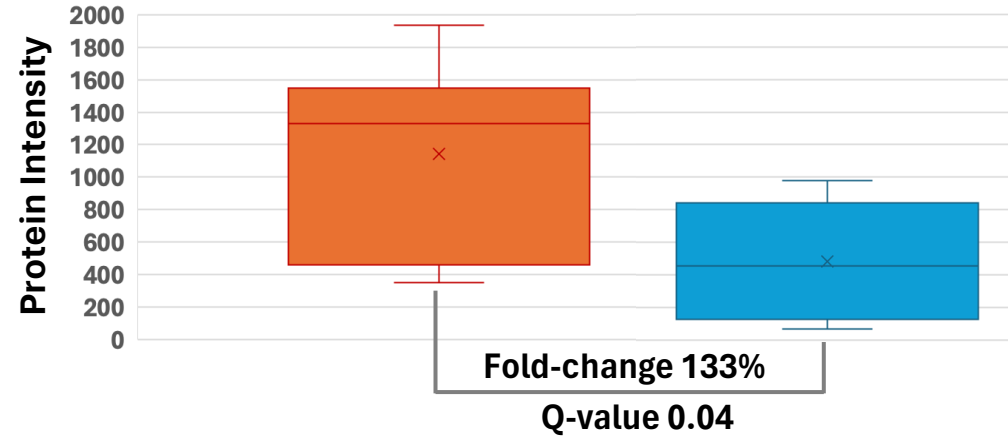


analysis by Kai Cheng, Qing Wu (Daniel Figey's Lab, University of Ottawa)

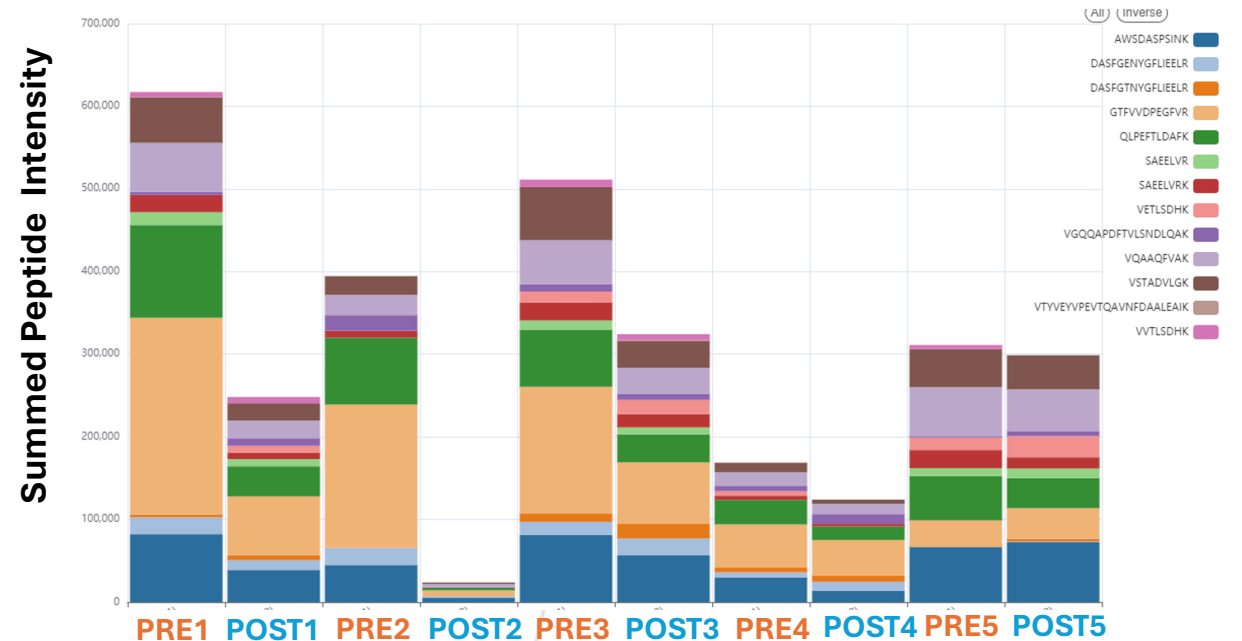


# MICROBIAL PROTEINS DOWNREGULATED AFTER TREATMENT

## Alkyl hydroperoxide reductase C (11 peptides)



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



*Veillonella*

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



MetaLab

and



analysis by Kai Cheng, Qing Wu (Daniel Figey's Lab, University of Ottawa)

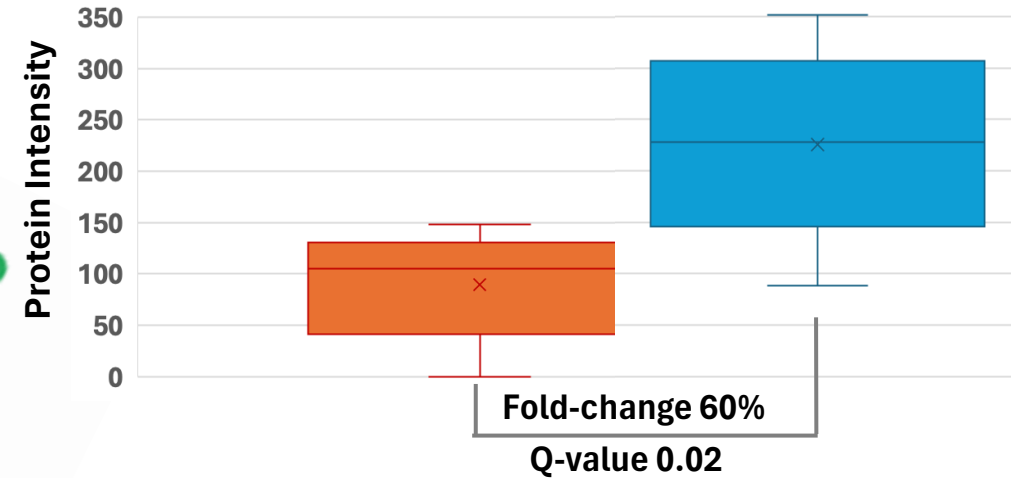


# MICROBIAL PROTEINS UPREGULATED AFTER TREATMENT

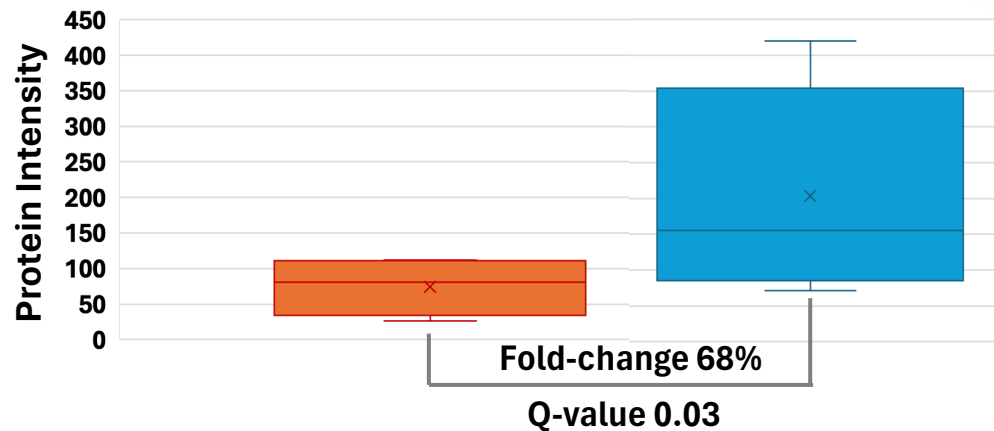
- Glucosyltransferase that catalyzes the transfer of glucosyl residues to dextran polymer.
- Involved in biofilm formation.

*Streptococcus salivarius*

Dextranucrase (61 peptides)



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



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

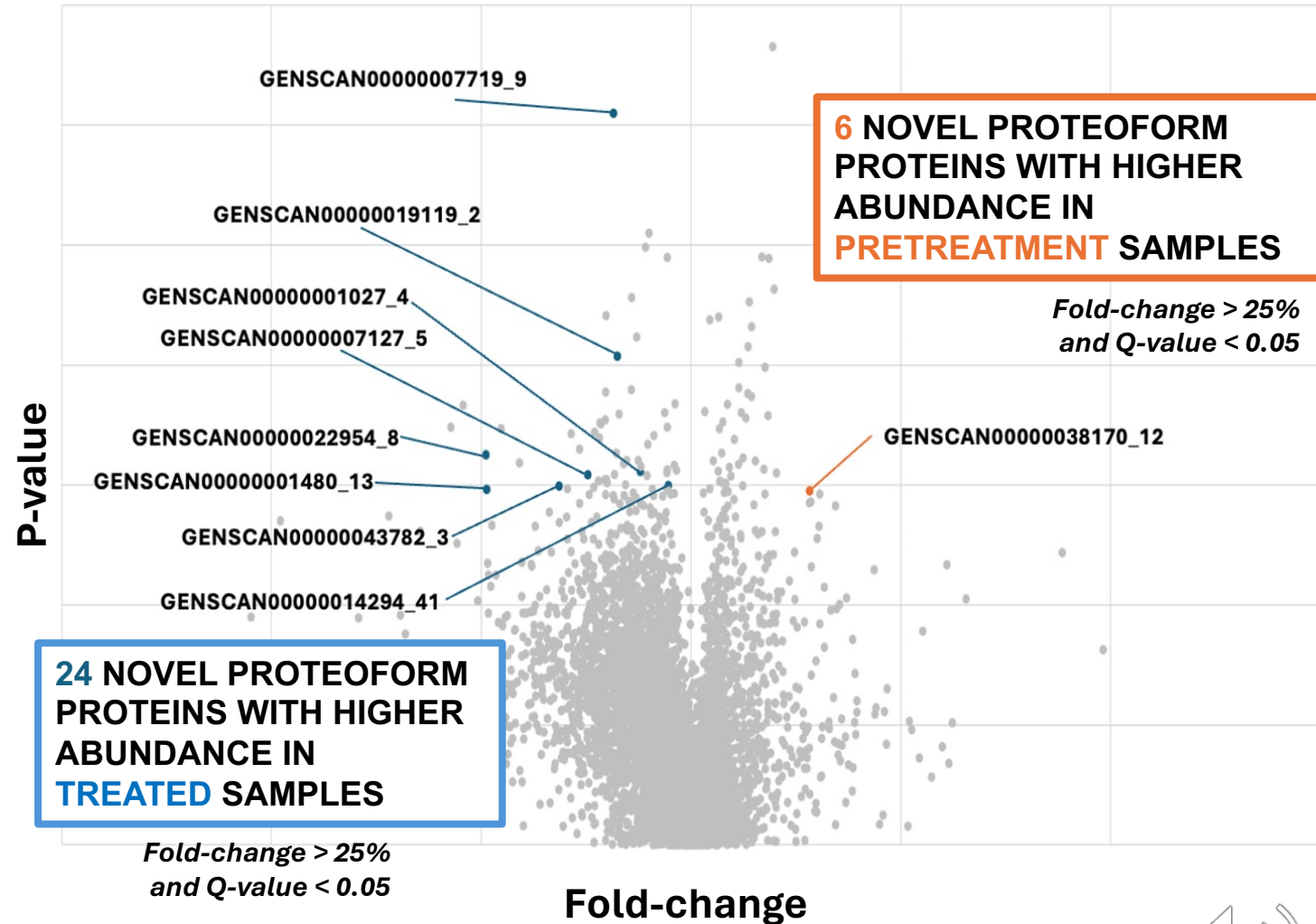
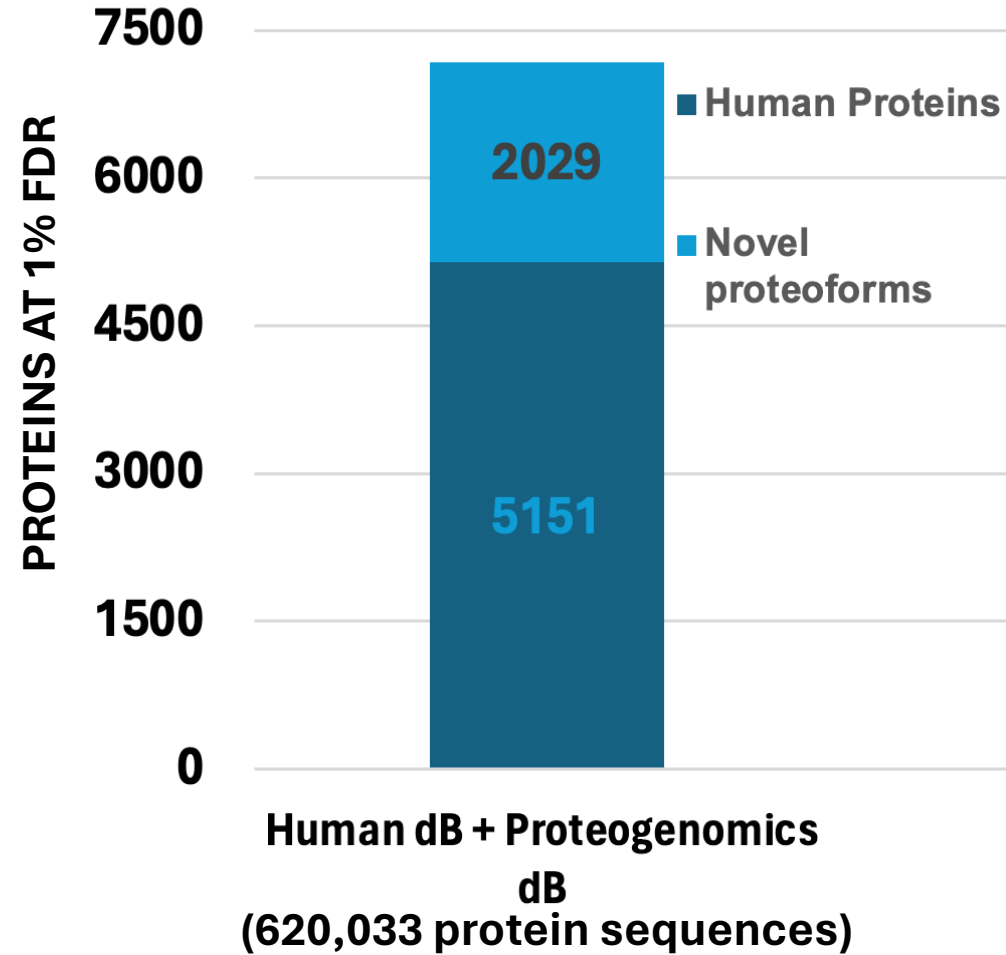


# DIFFERENTIALLY ABUNDANT MICROBIAL PROTEINS

PROTEIN DESCRIPTIONS	UNIPROTIDS	% CHANGE	QVALUE	# UNIQUE PEPTIDES
Alkyl hydroperoxide reductase C	E4LFM1	133.47	0.04	11
Dextranucrase	E9DP35, J7SIV6, J7TRB0	-62.27	0.02	61
Serine-type D-Ala-D-Ala carboxypeptidase	A0A2A5QD93	-67.91	0.03	21
Serine protease	E3H076	-44.89	0.05	4
Uncharacterized protein	A0A2S7ZRS7	-46.03	0.01	5
RND transporter, HAE1 family	E1L728	-37.59	0.03	5
Ribosomal subunit protein S16	A5VKN7;C8P927	-64.94	0.03	3
Glutamate--ammonia ligase	Q5M2N1	-58.15	0.04	7
Elongation factor Ts	A0A0X8K315	-46.57	0.03	8



# PROTEOGENOMICS DATABASE SEARCH RESULTS





# KEY HOST, MICROBIAL AND VARIANT PEPTIDES FOR ORAL CANCER DETECTION

	Higher Abundance in Treated Samples	Higher Abundance in pretreatment Samples
HUMAN PEPTIDES	281	184
MICROBIAL PEPTIDES	108	17
NOVEL PROTEOFORM PEPTIDES	59	21

Detected peptides will be prioritized to generate a biomarker panel which can be later assessed for:

- a) risk stratification in oral leukoplakia and
- b) deciphering mechanisms for host-microorganism interaction in oral carcinogenesis.



# **CONCLUSIONS AND FUTURE WORK**

- **Several human, microbial and variant 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.**
- **Multiple proteins and peptides associated with novel proteoforms were differentially abundant after treatment.**
- **Peptides associated with differentially abundant human, microbial and variant proteins will be used for targeted analysis.**



# ACKNOWLEDGEMENTS



MASONIC CANCER CENTER

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- **Sebastian Vaca**
- **Matthew Willets**
- **Jon Lenz**



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