Metaproteomic Approach to Detect Key Host and Microbial Peptides from Oral Leukoplakia samples



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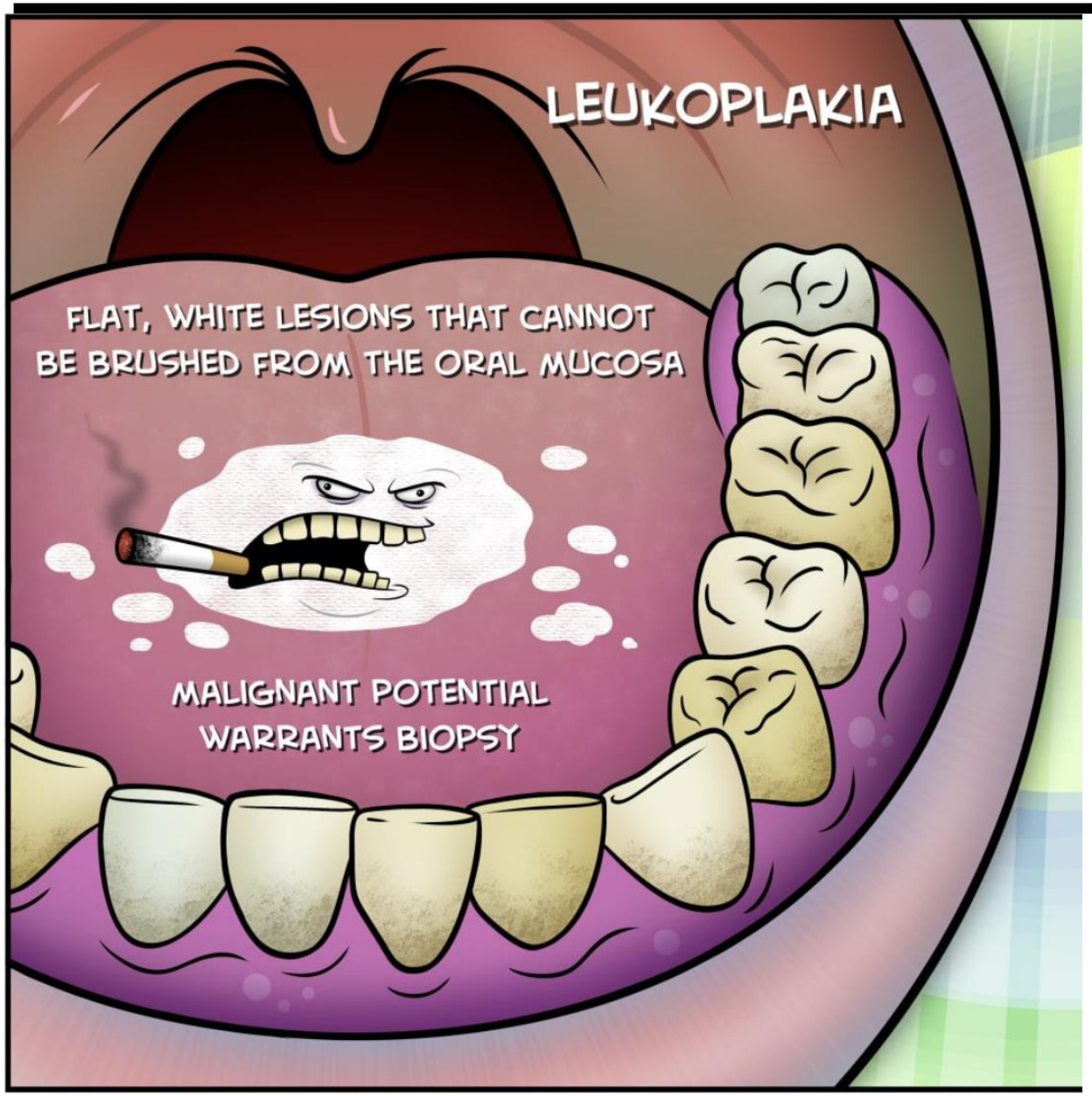
ORAL LEUKOPLAKIA: ORAL CANCER RISK



BACKGROUND

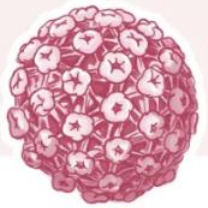
* PAINLESS, SLOW-GROWING LESION on MUCOUS MEMBRANES of ORAL CAVITY
* POTENTIAL PRECANCEROUS CONDITION

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

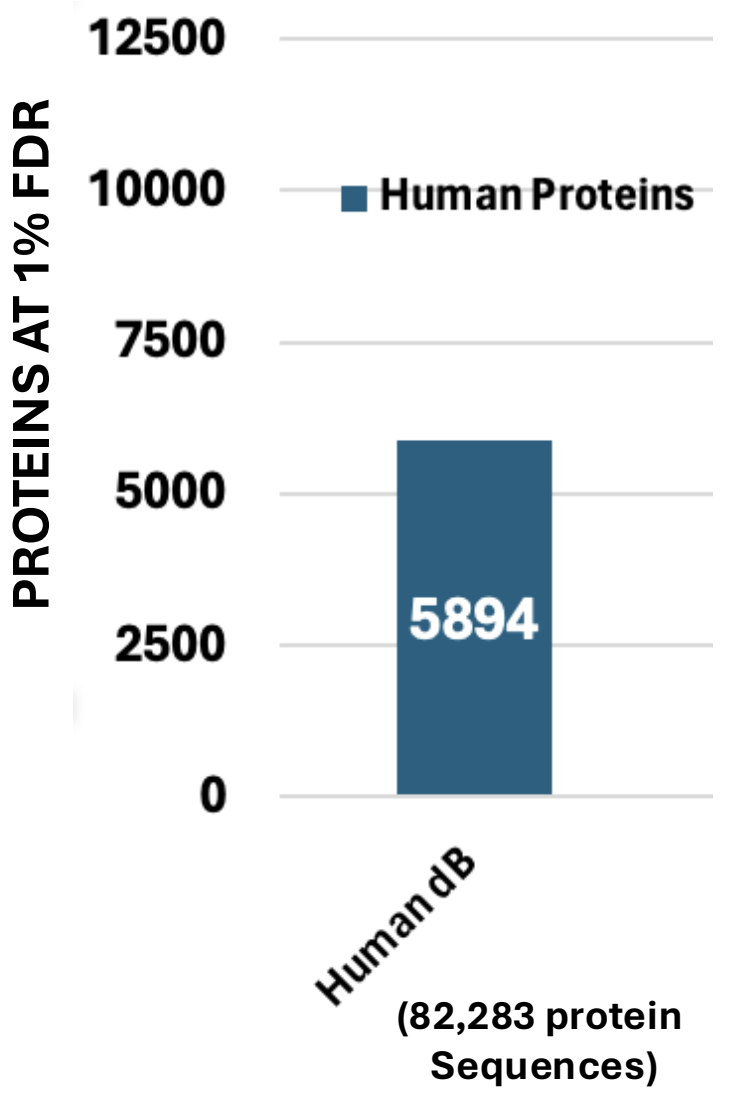
Mass spectrometry using DIA-PASEF

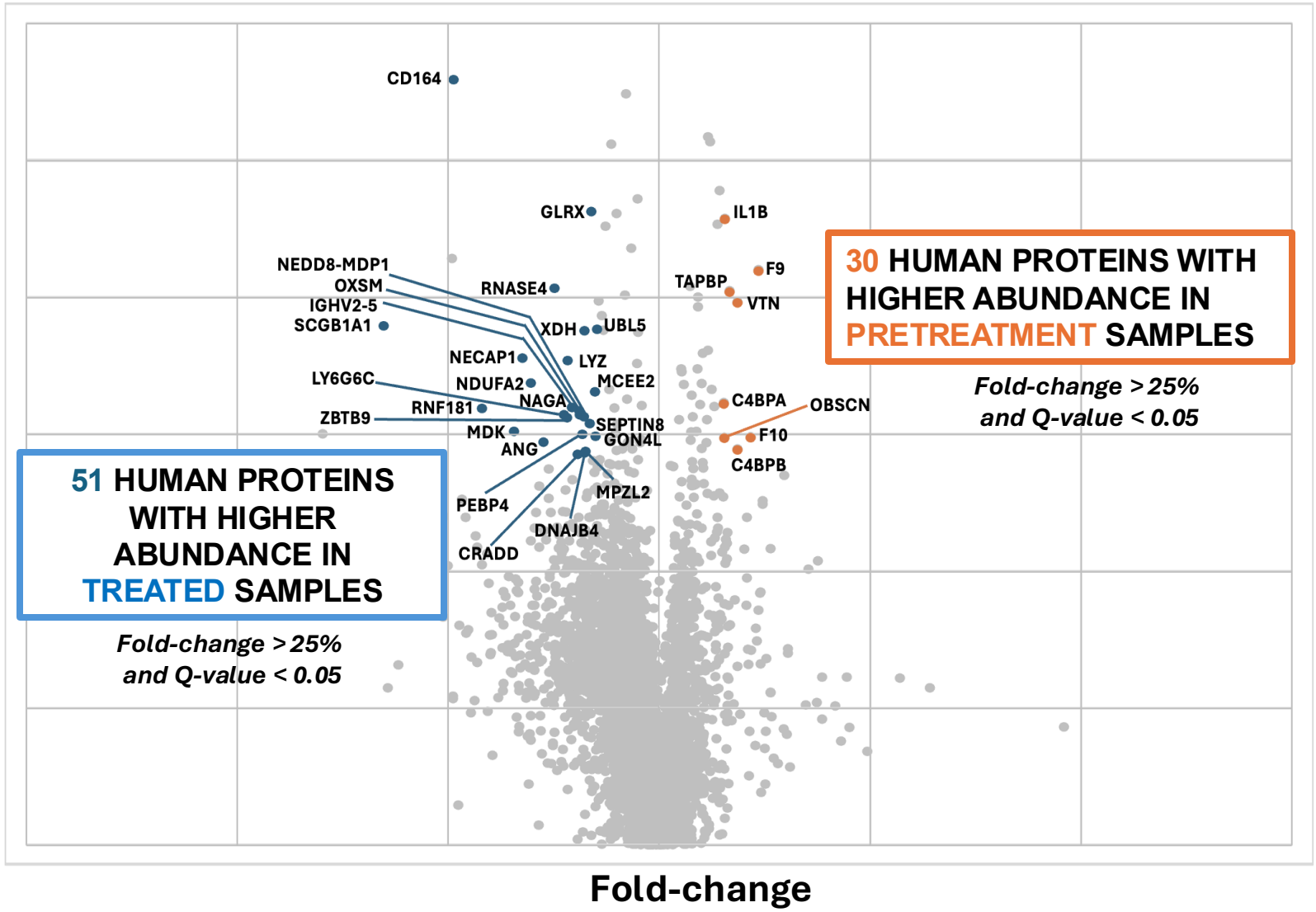
Search against Human + Microbiome Protein sequences dB

PO

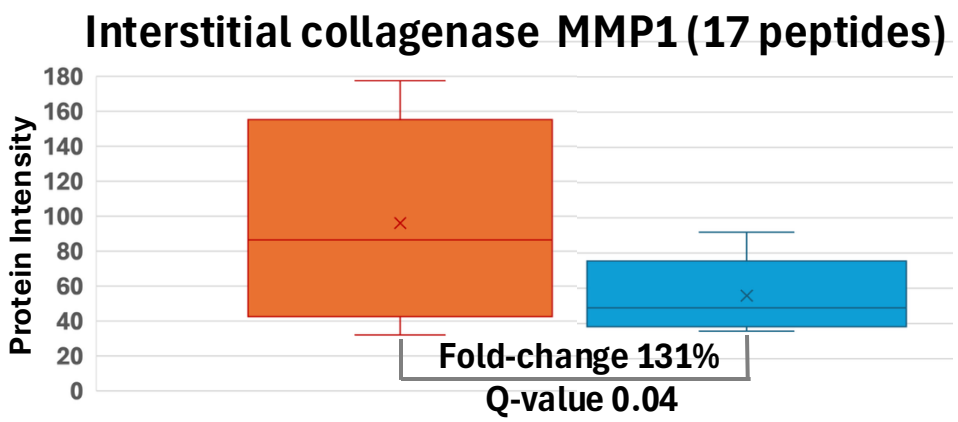
BRUKER

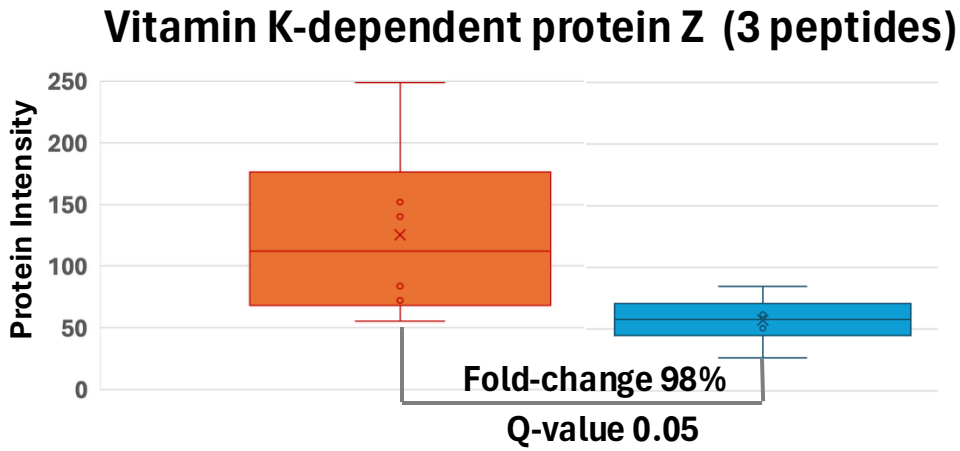
PROTEINS DETECTED AND DIFFERENTIALLY ABUNDANT PROTEINS

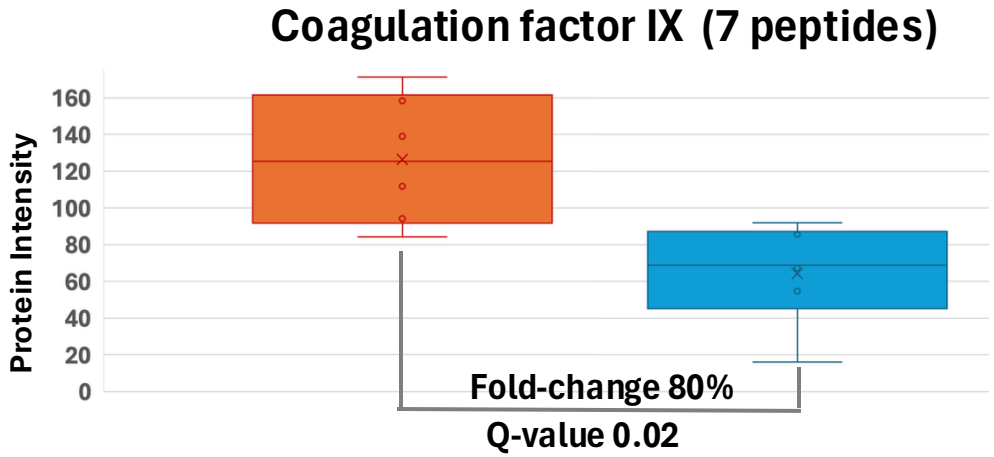


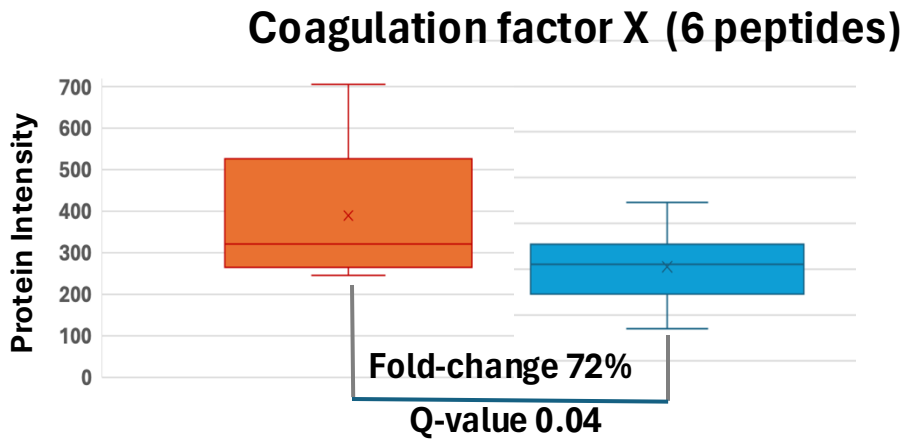


MMP1 & COAGULATION CASCADE: DOWNREGULATED AFTER TREATMENT



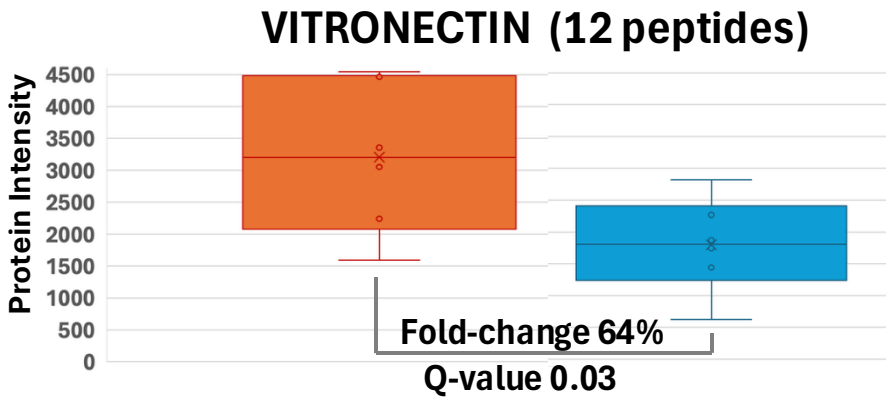


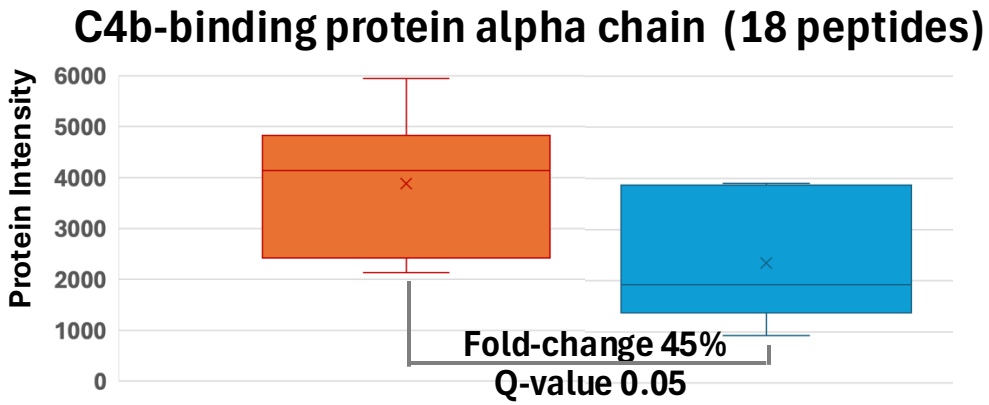


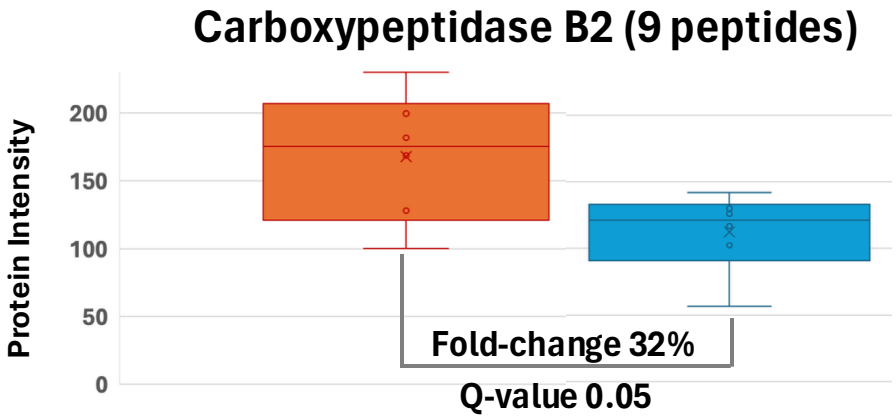


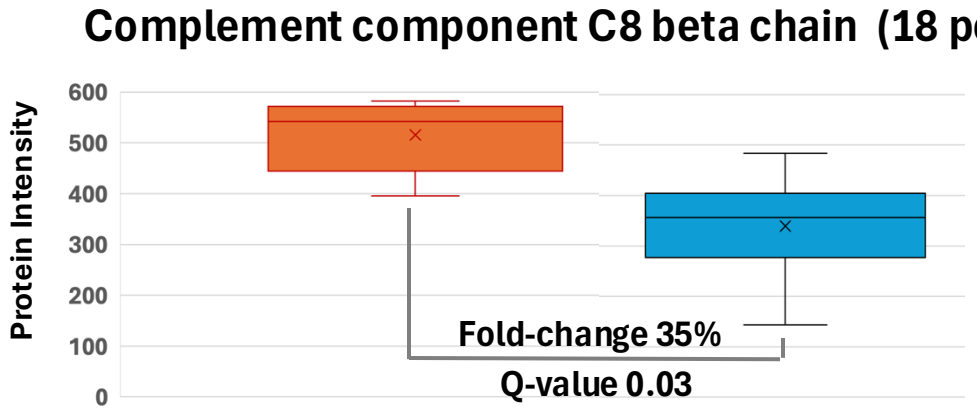
Coagulation Cascade

COMPLEMENT CASCADE: DOWNREGULATED AFTER TREATMENT



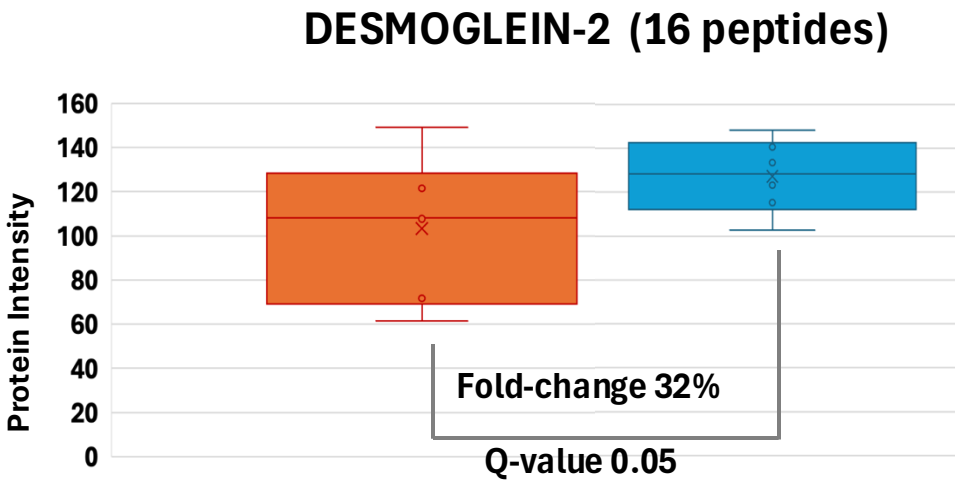






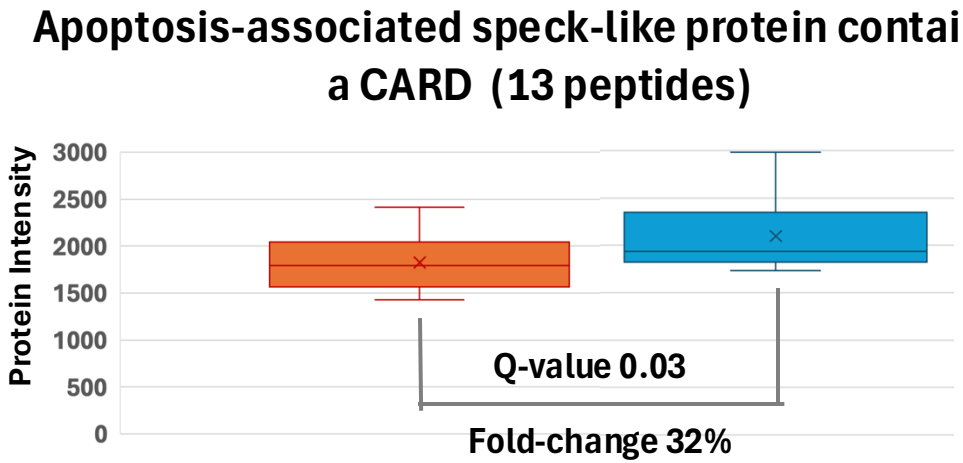
Regulation of Complement cascade

APOPTOSIS: UPREGULATED AFTER TREATMENT

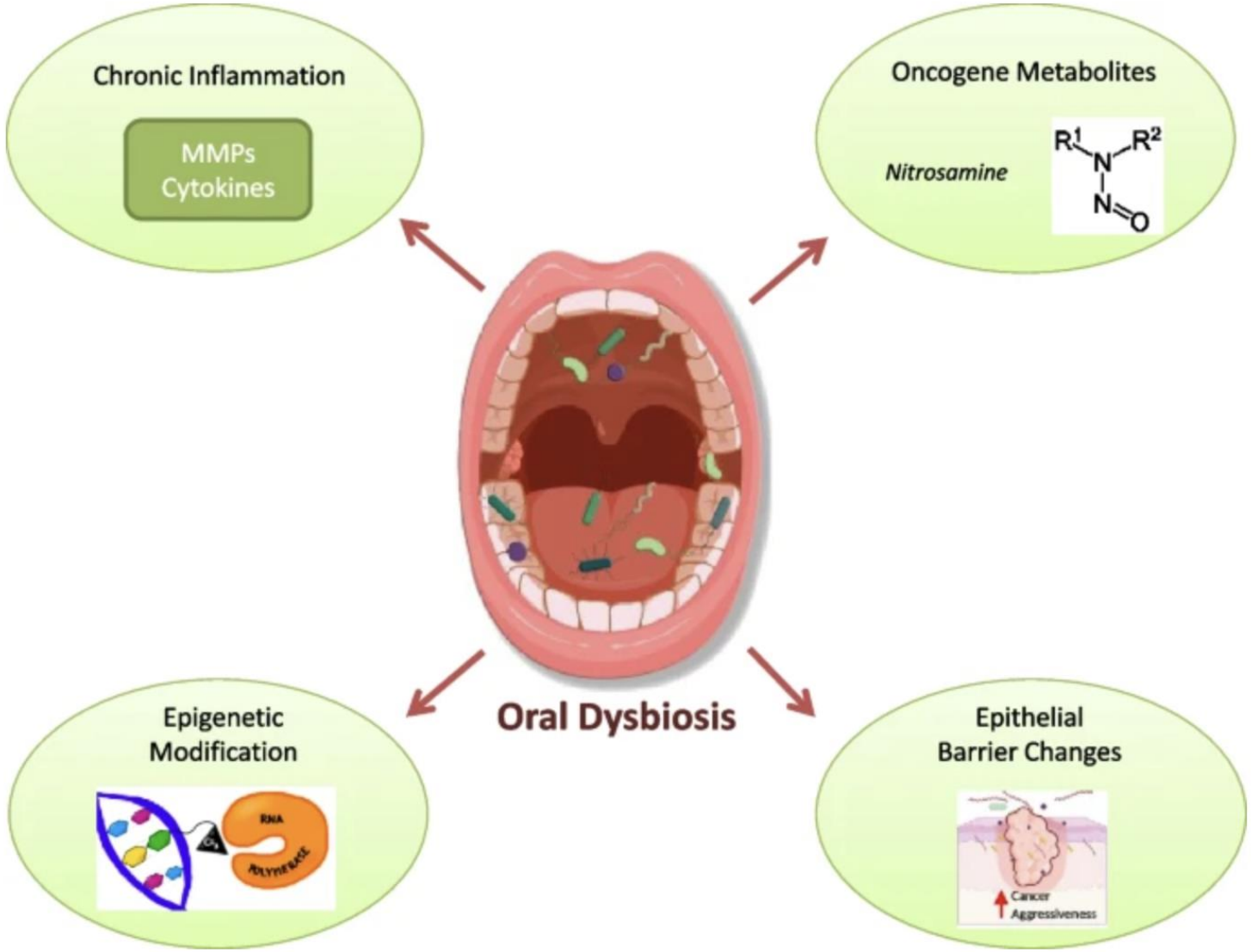


- Component of intercellular desmosome junctions mediating cell-cell adhesion.
- Involved in apoptotic pathway.
- Prognostic marker in renal cancer, pancreatic cancer, lung cancer, head and neck cancer, colorectal cancer and cervical cancer.

- 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

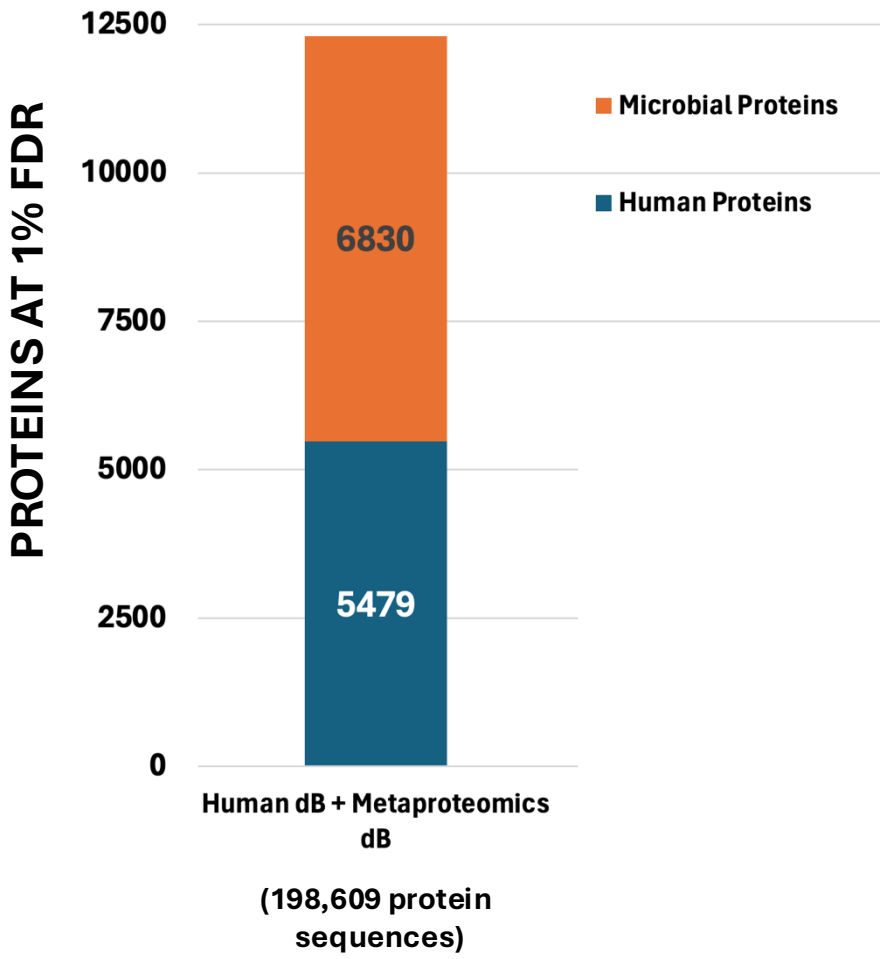


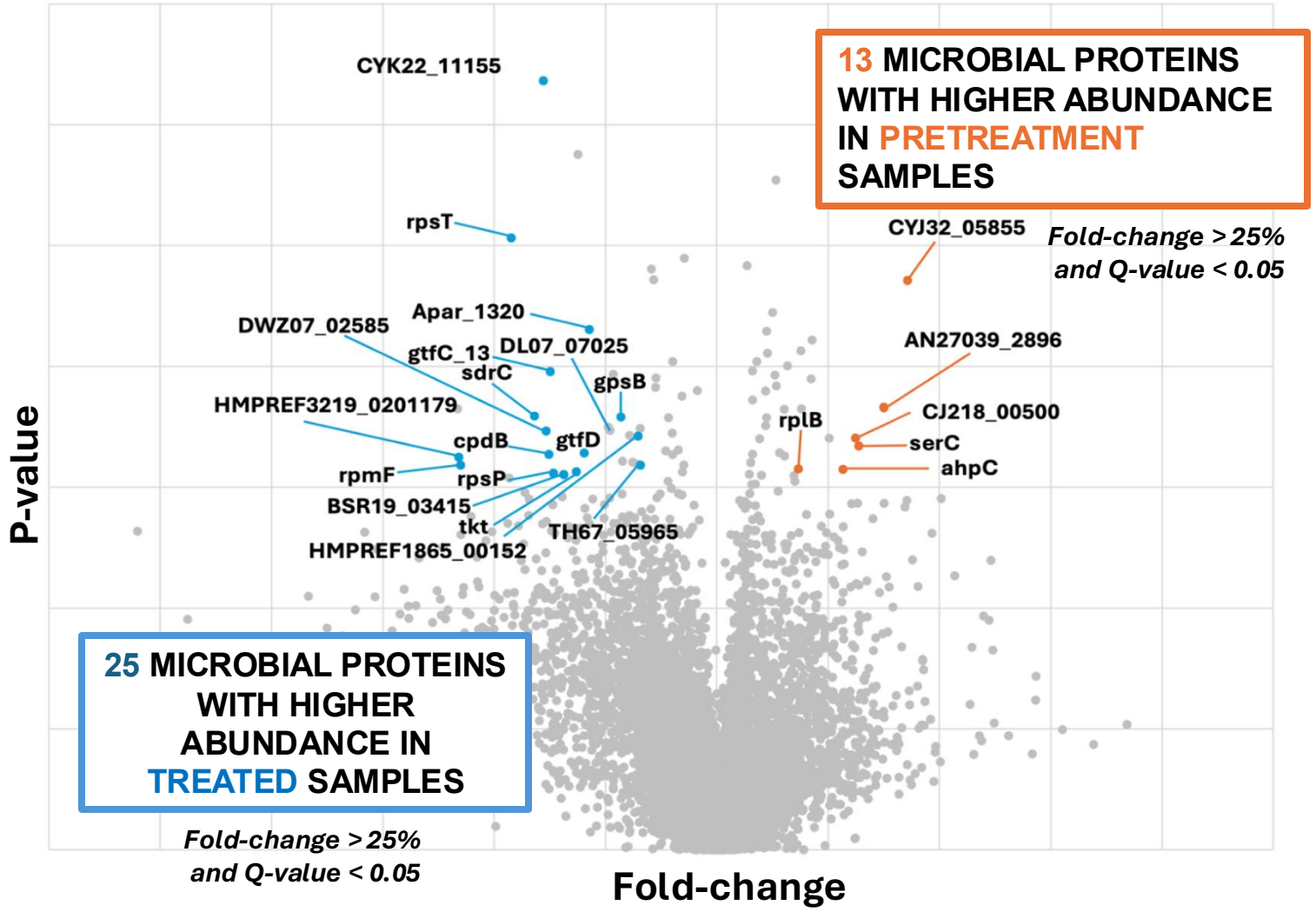
ROLE OF BACTERIA IN ORAL CANCER DEVELOPMENT



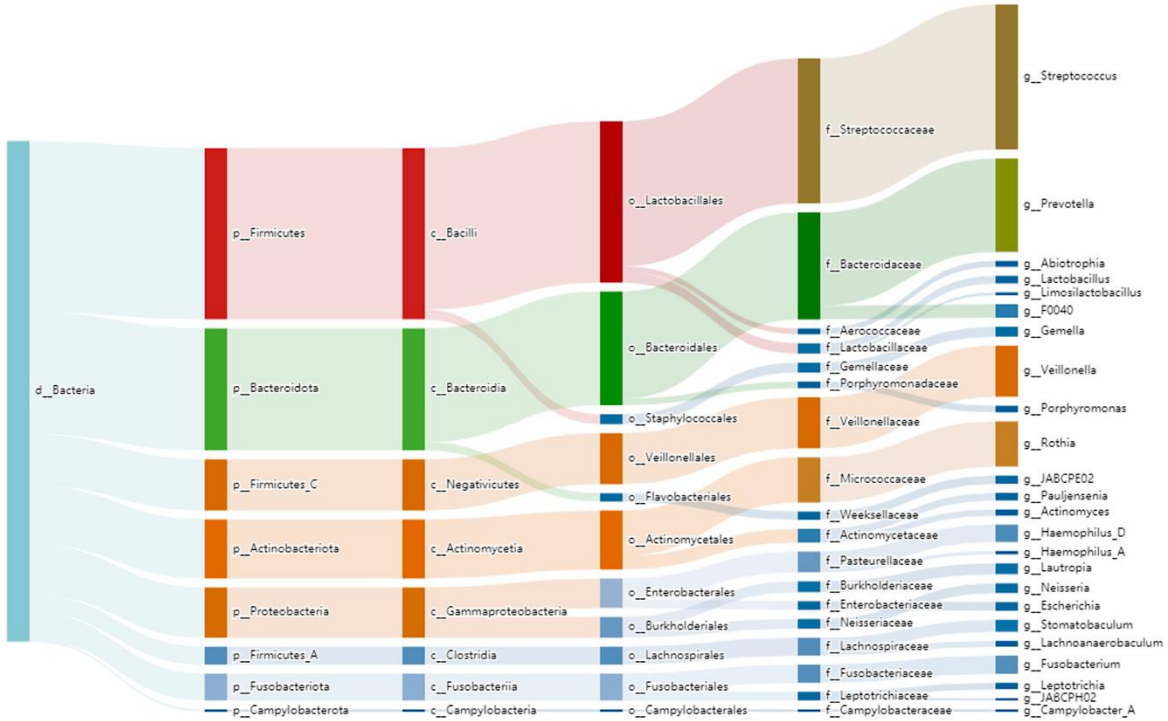
Asili, P., et al. J Gastrointest Canc (2023). https://doi.org/10.1007/s12029-022-00901-4

MICROBIAL DATABASE SEARCH RESULTS

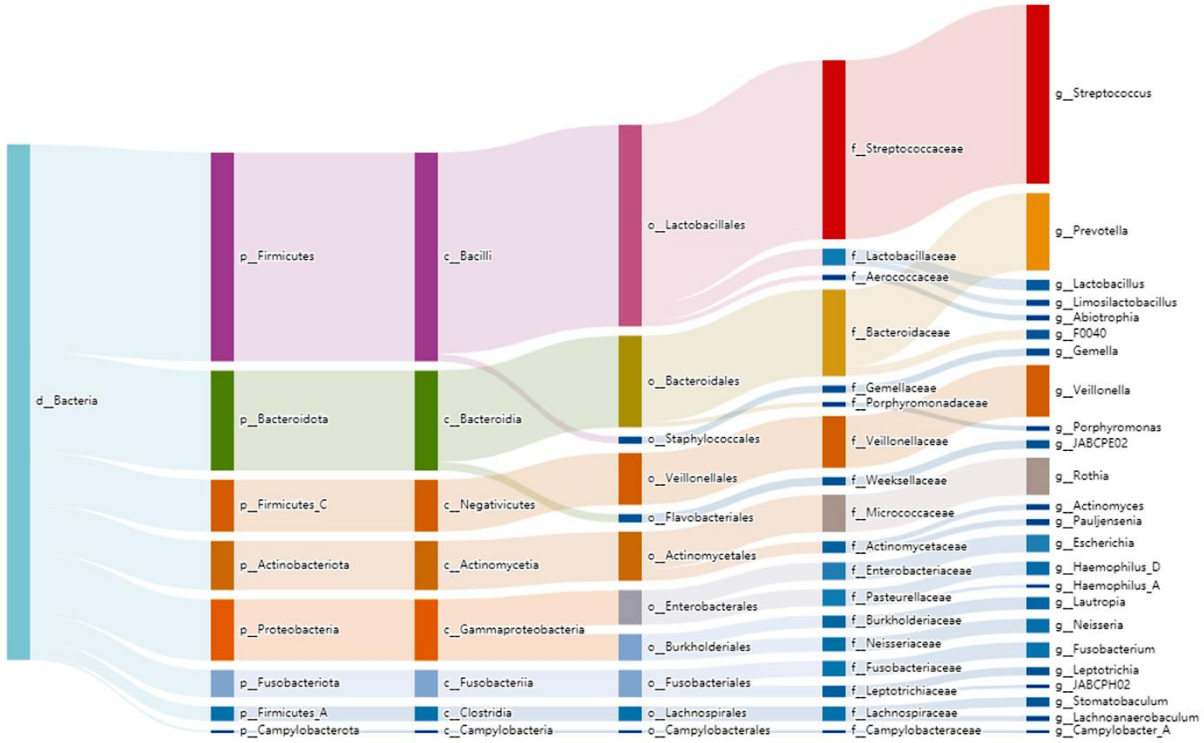




MICROBIAL TAXONOMY OUTPUTS



Genera detected in pretreated samples

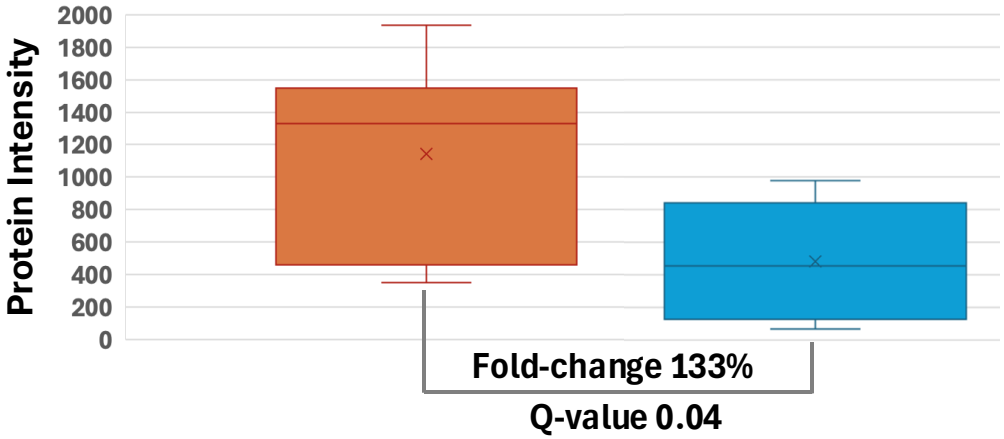


Genera detected in treatment samples

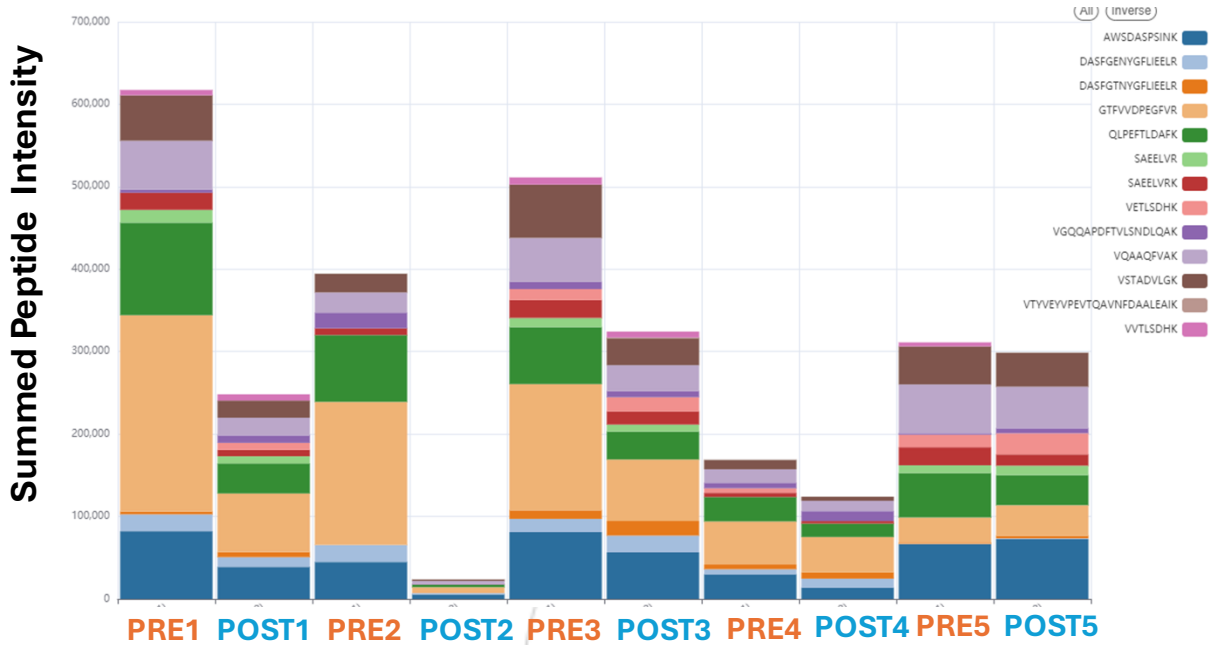
MetaLab *and* MetaX *analysis by Kai Cheng, Qing Wu (Daniel Figeys Lab, University of Ottawa)*

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.



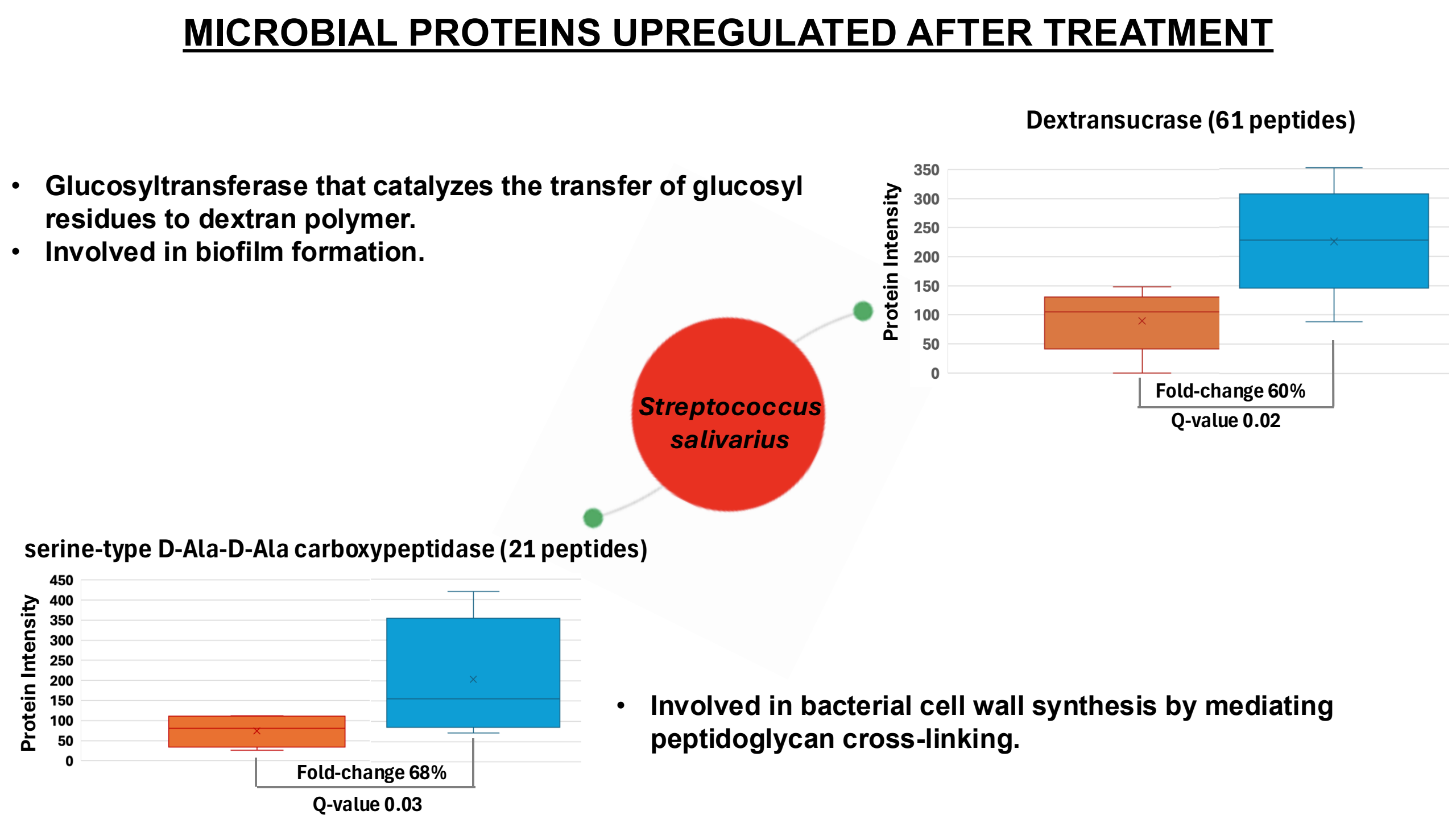
In this study, the protein was expressed by *Veillonella* genus.

Veillonella

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)

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

- 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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Leiden (2024)
Luxembourg (2021)
Oslo (2025)
Avignon (2023)
Leipzig (2018)
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QUESTIONS?