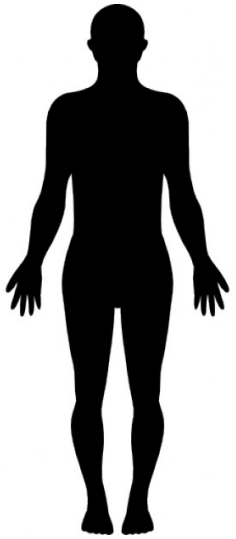
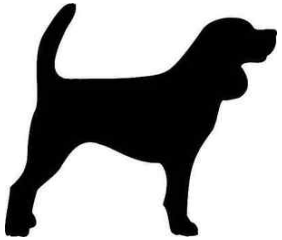
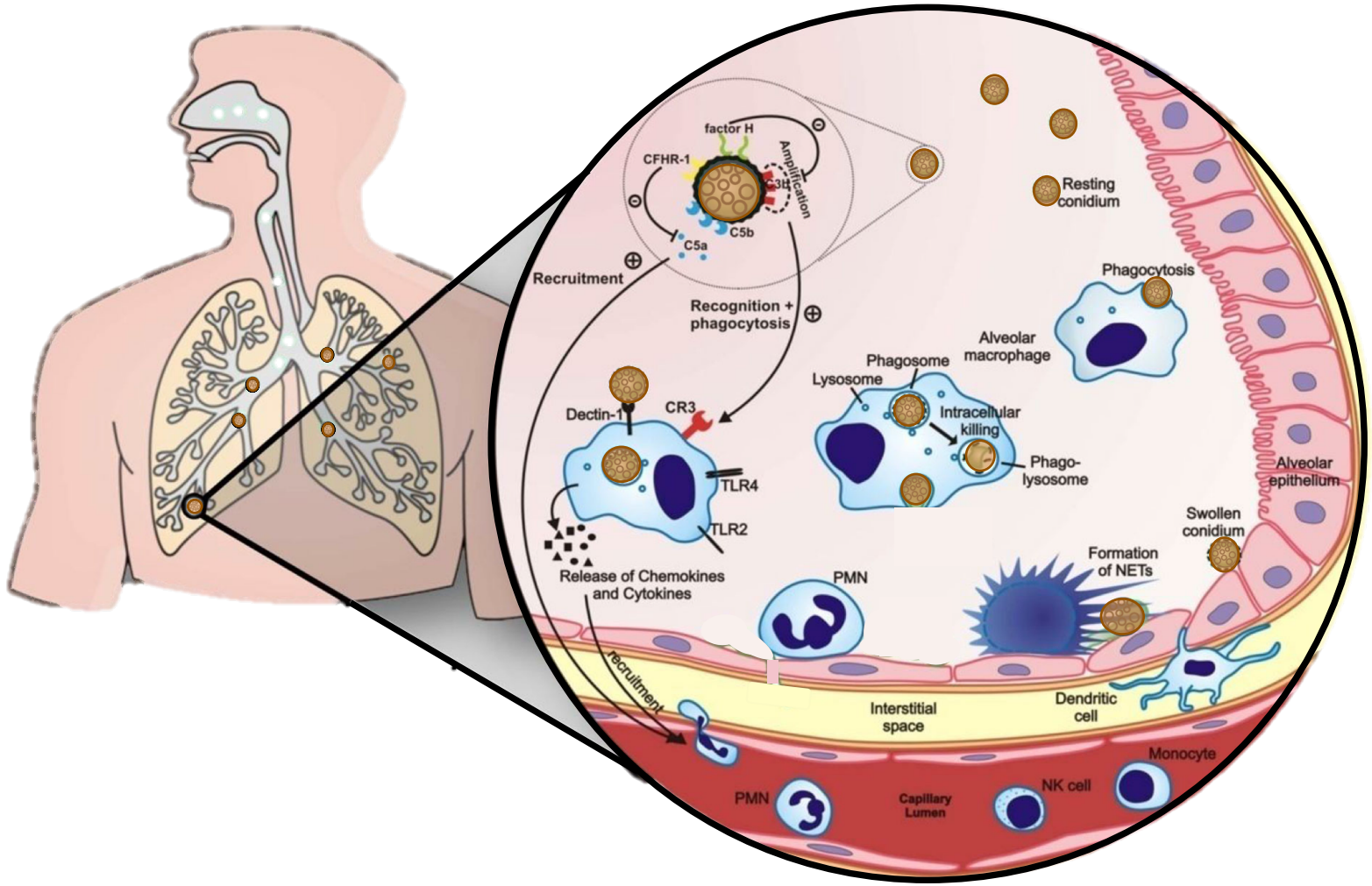
Host-pathogen Protein Profiling of Coccidioidomycosis In Multiple Host Species



Natalie Mitchell
Microbiology PhD Candidate
Douglas Lake Lab

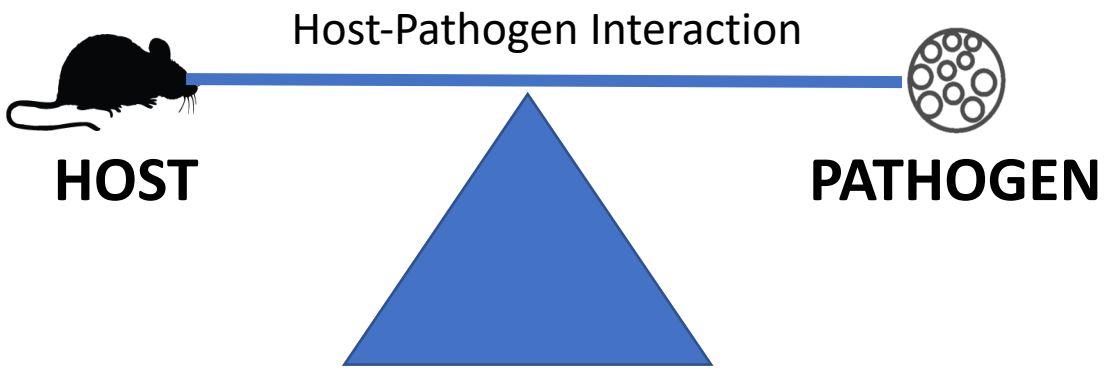


Host-*Coccidioides* Battle

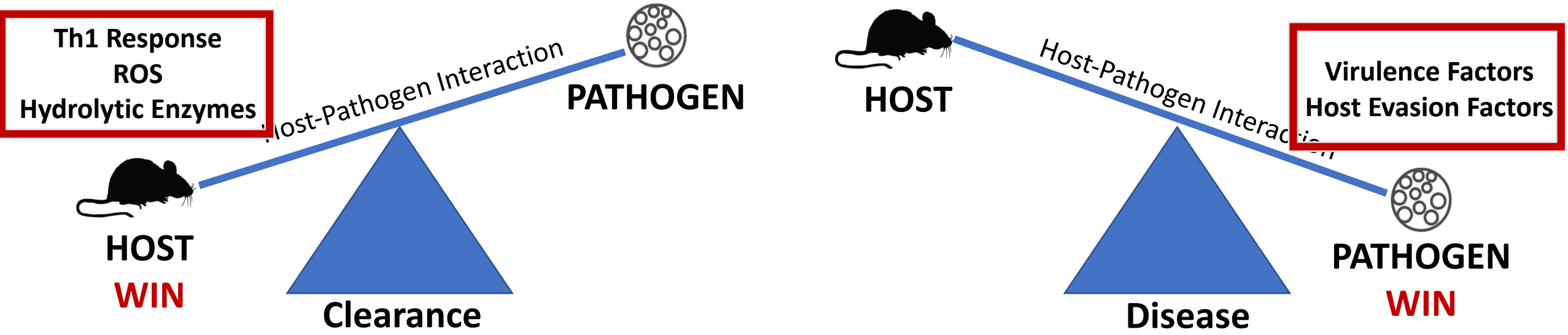


Adapted from Leibniz Institute for Natural Product Research and Infection Biology

Host-Pathogen Interactions



Host-Pathogen Interactions



Rationale:



How do we currently study host-pathogen interaction in Cocci?

In mouse models

Are mice comparable to mice?

Yes, within inbred strains.

Are mouse models comparable to humans?

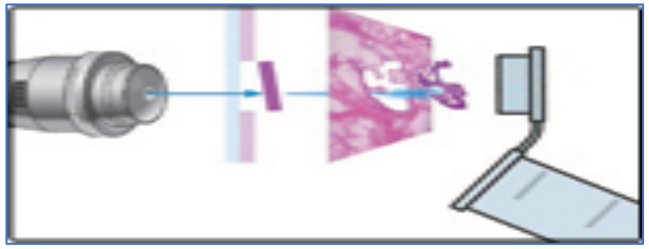
Not really (different immune systems, flora, genetics…)

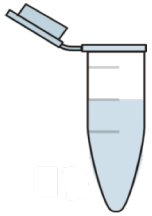
Are humans comparable to humans?

Somewhat. Hopefully not inbred. Multiple co-morbidities may exist though.

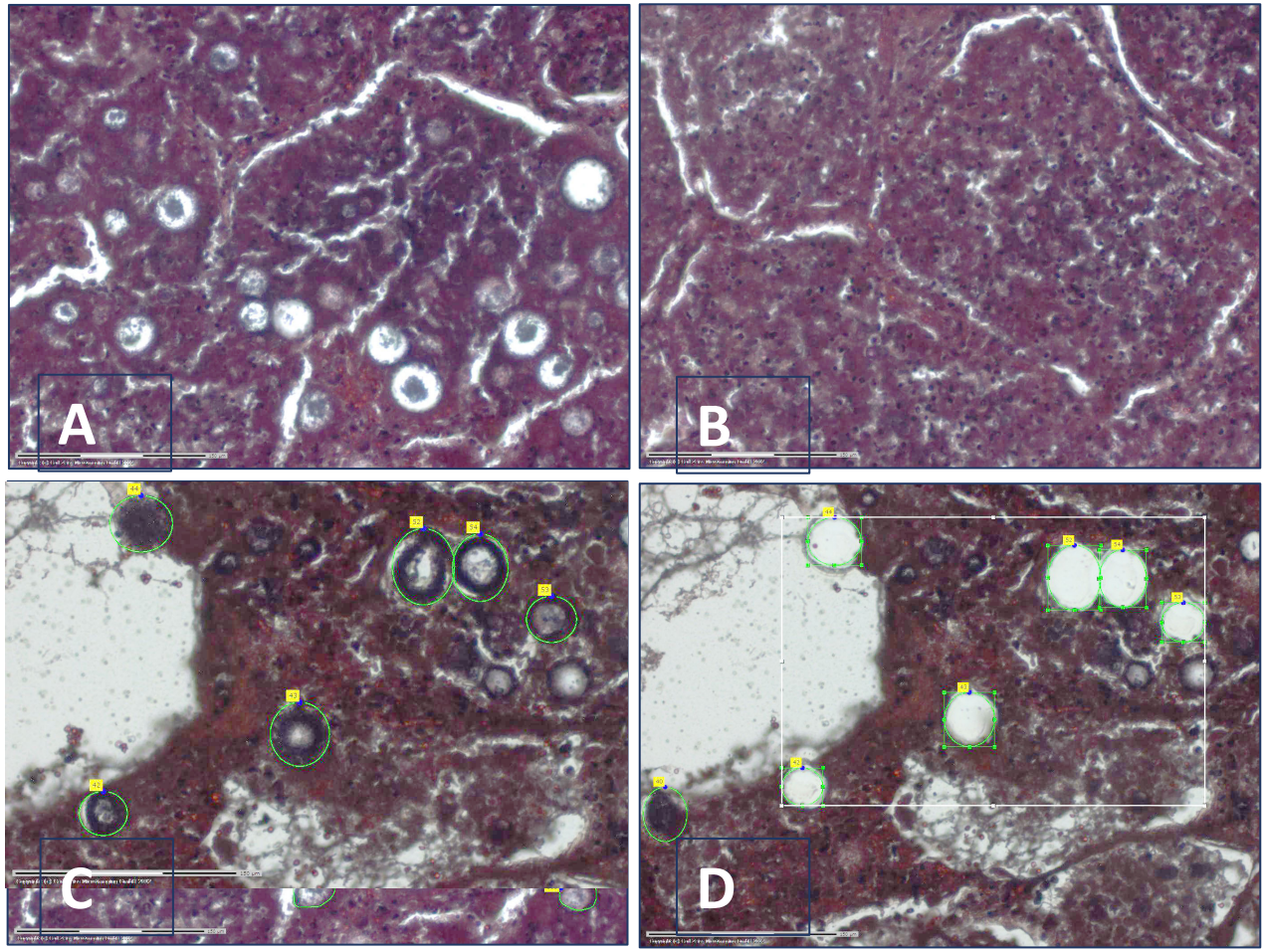
Laser Capture Microdissection (LCM)

-Spherule capture





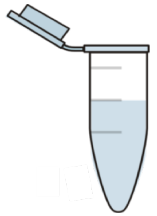
Protein Digestion



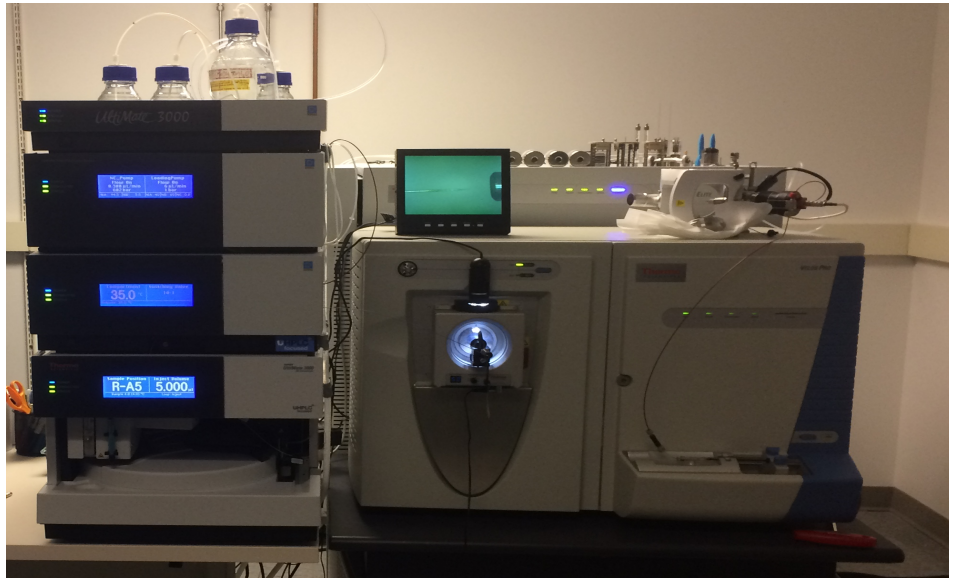
20x Human Lung Tissue
10um thick, H&E stained

Laser Capture Microdissection (LCM)

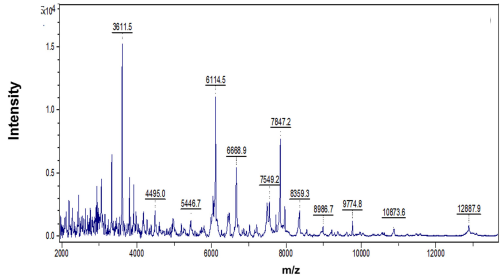
-Proteomics



Digested Protein



Nano LC-MS/MS



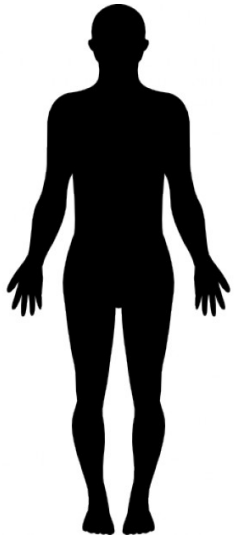
Acquisition of spectra and protein Identification

$$dNSAF_i = \frac{dSAF_i}{\sum_{i=1}^{N} dSAF_i}$$

Normalization of spectral abundance

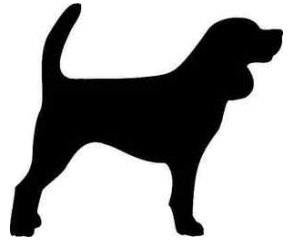
Laser Capture Microdissection (LCM)

-Experimental Samples



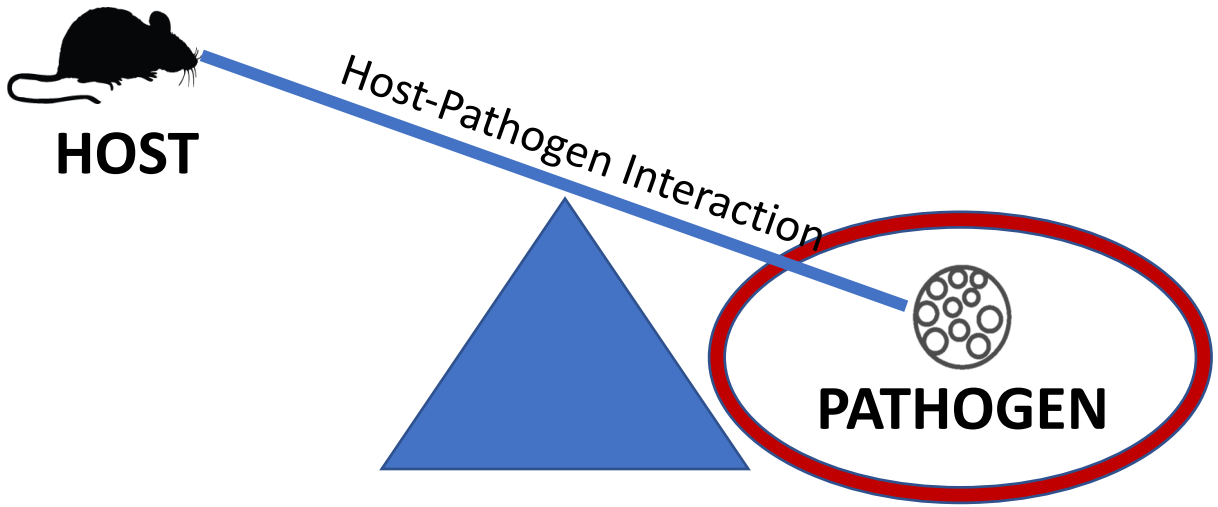
4 Diseased, 3 Control
2 HIV+ patients
1 Adalimumab
1 Immunocompetent

3 Diseased, 3 Control
All Balb/c mice

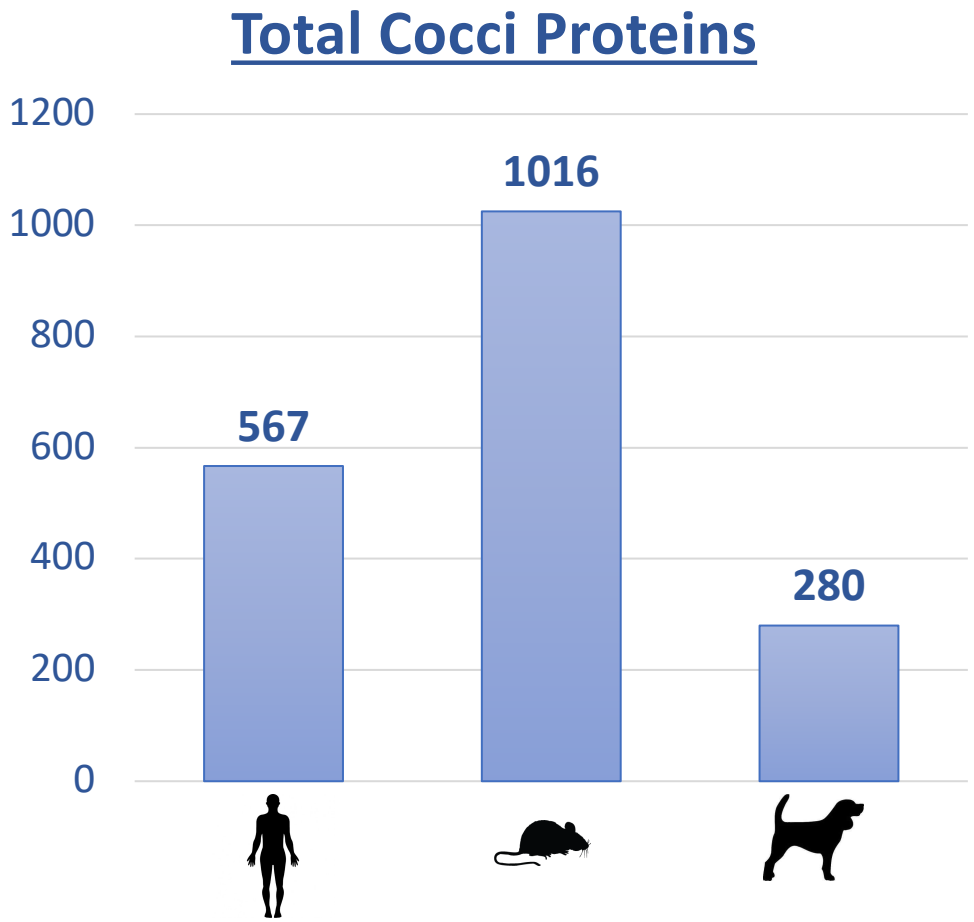


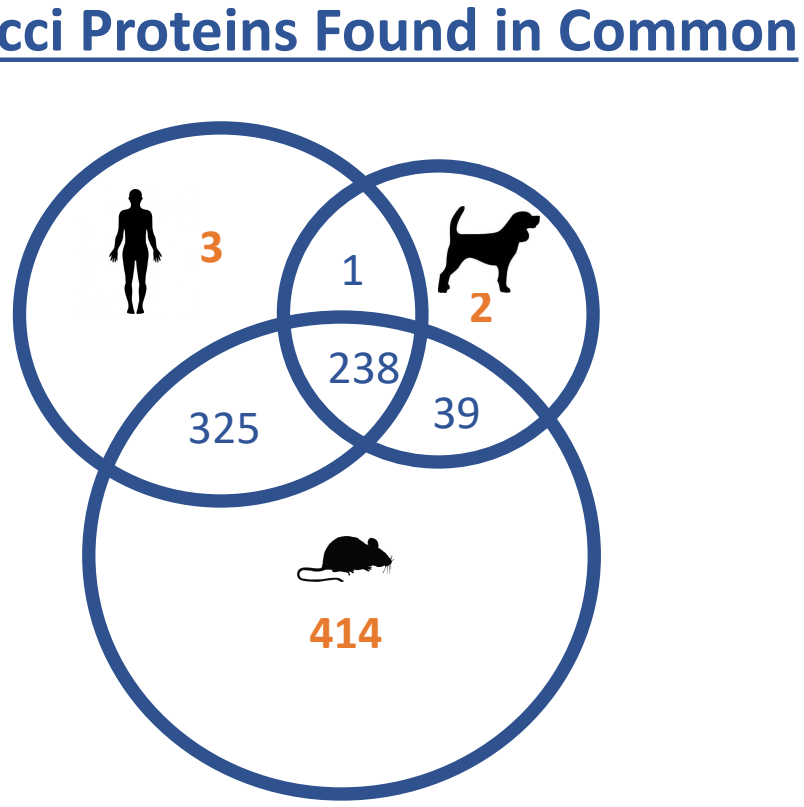
1 Diseased, 1 Control
Diseased unknown breed
Control was a Beagle

Coccidioidal Proteins

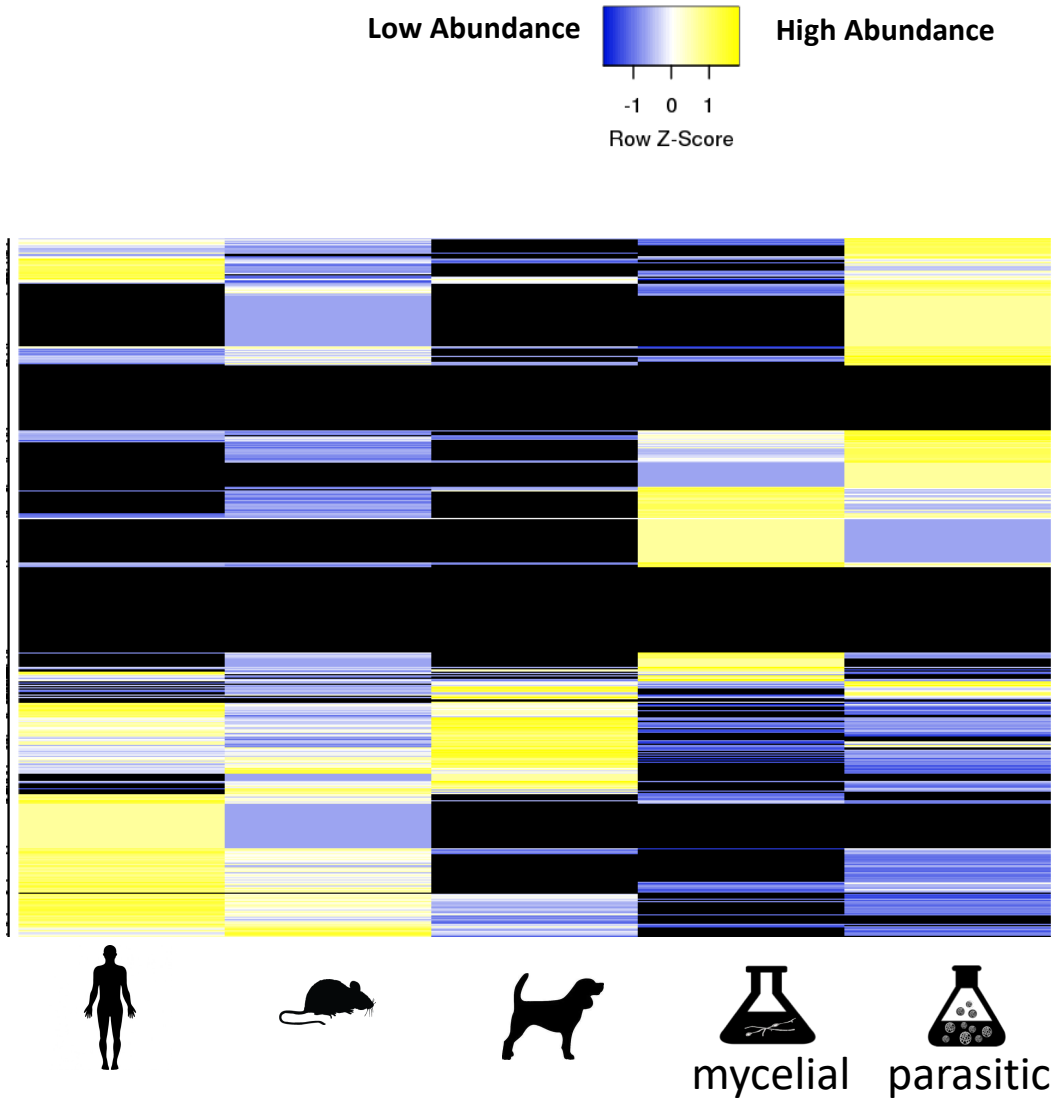


Coccidioidal Proteins





Lung Grown ≠ Lab Grown



www.heatmapper.ca

Lung Grown ≠ Lab Grown

Gene Ontology (Biological Process)

Protein Production

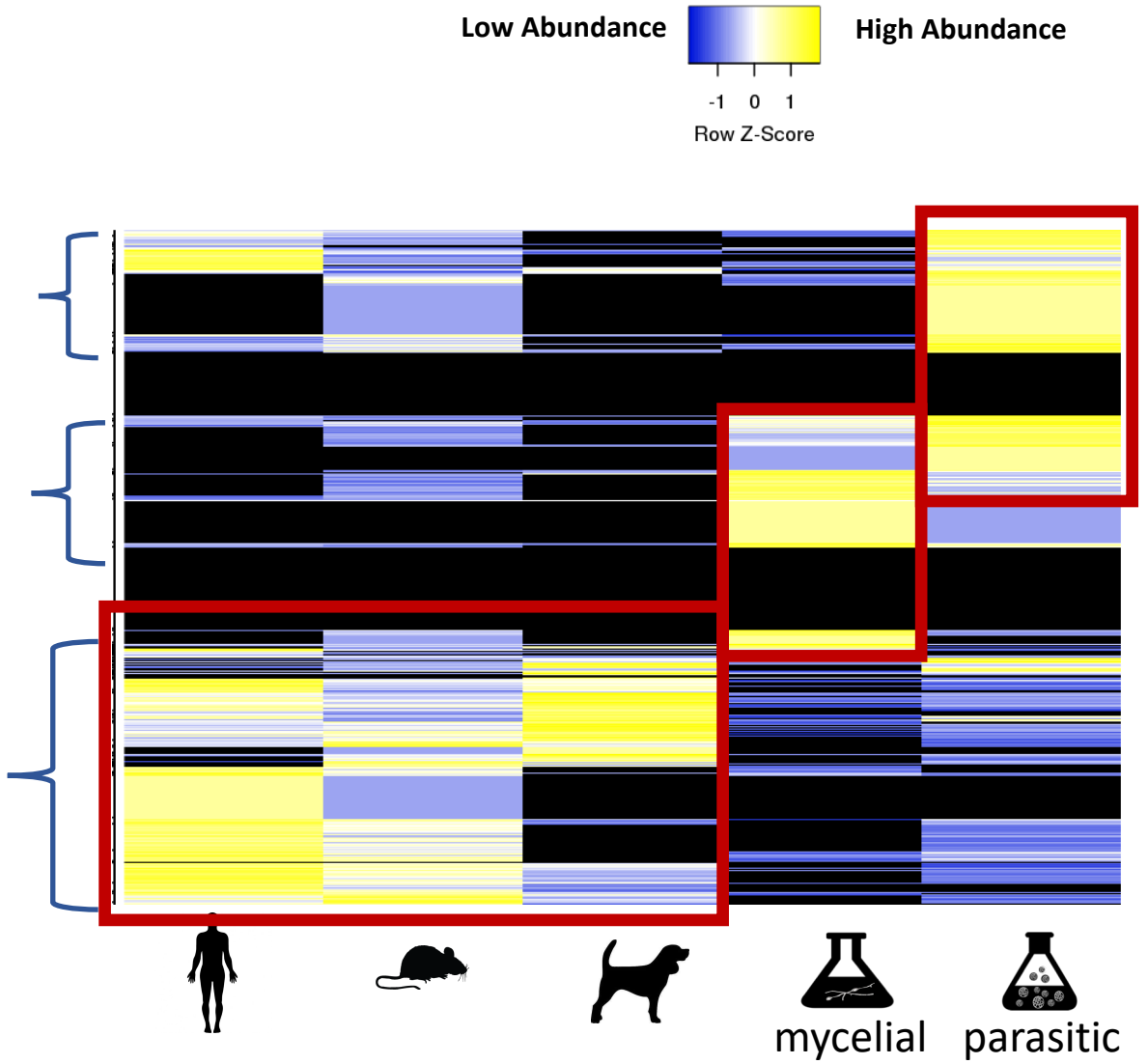
- Translation [GO:0006412]
- Cellular Amino Acid Biosynthetic Process [GO:0008652]
- mRNA Splicing, via Spliceosome [GO:0000398]

Carbohydrate Metabolism

- Metabolic Process [GO:0008152]
- Carbohydrate Metabolic Process [GO:0005975]
- Polysaccharide Catabolic Process [GO:0000272]

General Metabolic Processes

- Translation [GO:0006412]
- ATP synthesis Coupled Proton Transport [GO:0015986]
- Carbohydrate Derivative Metabolic Process [GO:1901135]
- Cell Redox Homeostasis [GO:0045454]
- Protein Folding [GO:0006457]
- TCA cycle [GO:0006099]



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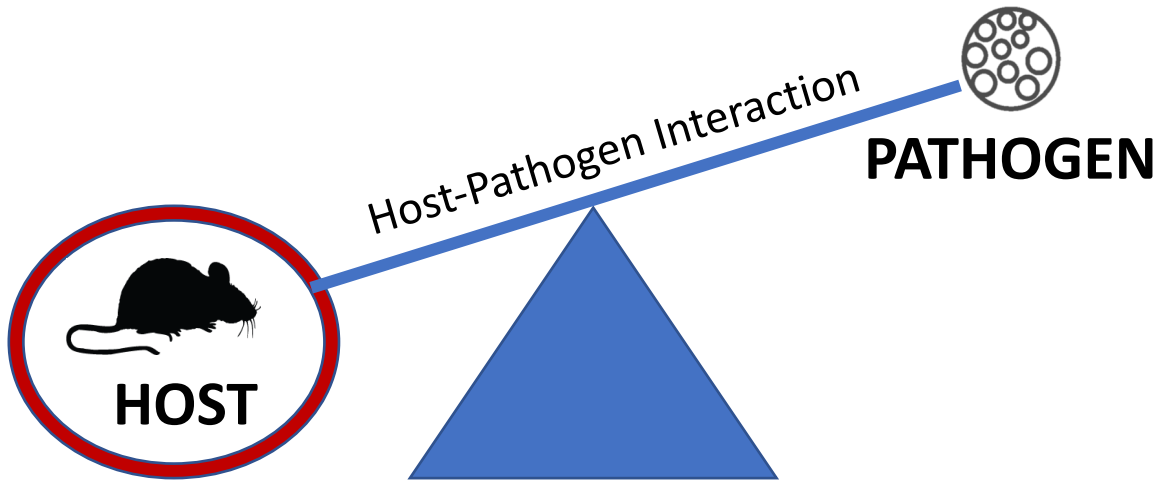
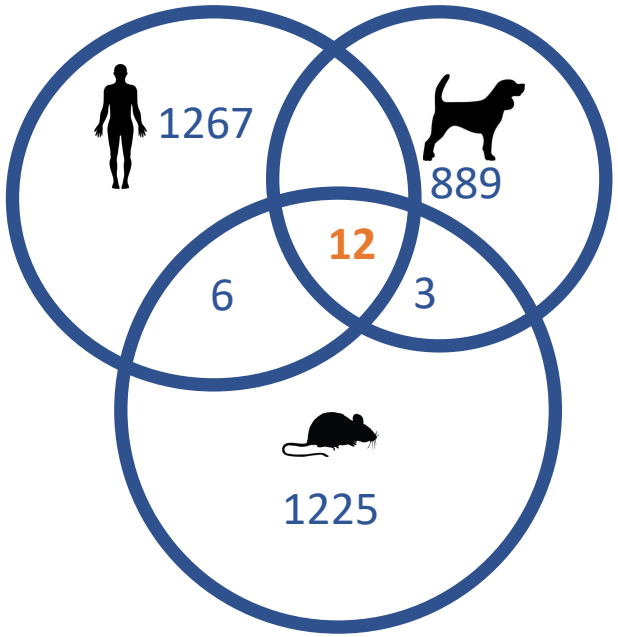
Host Proteins

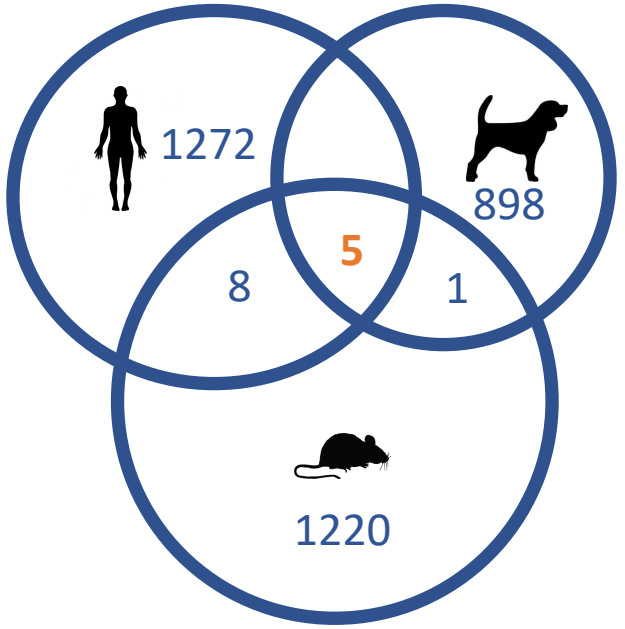


Host Proteins

Significantly Higher Abundance

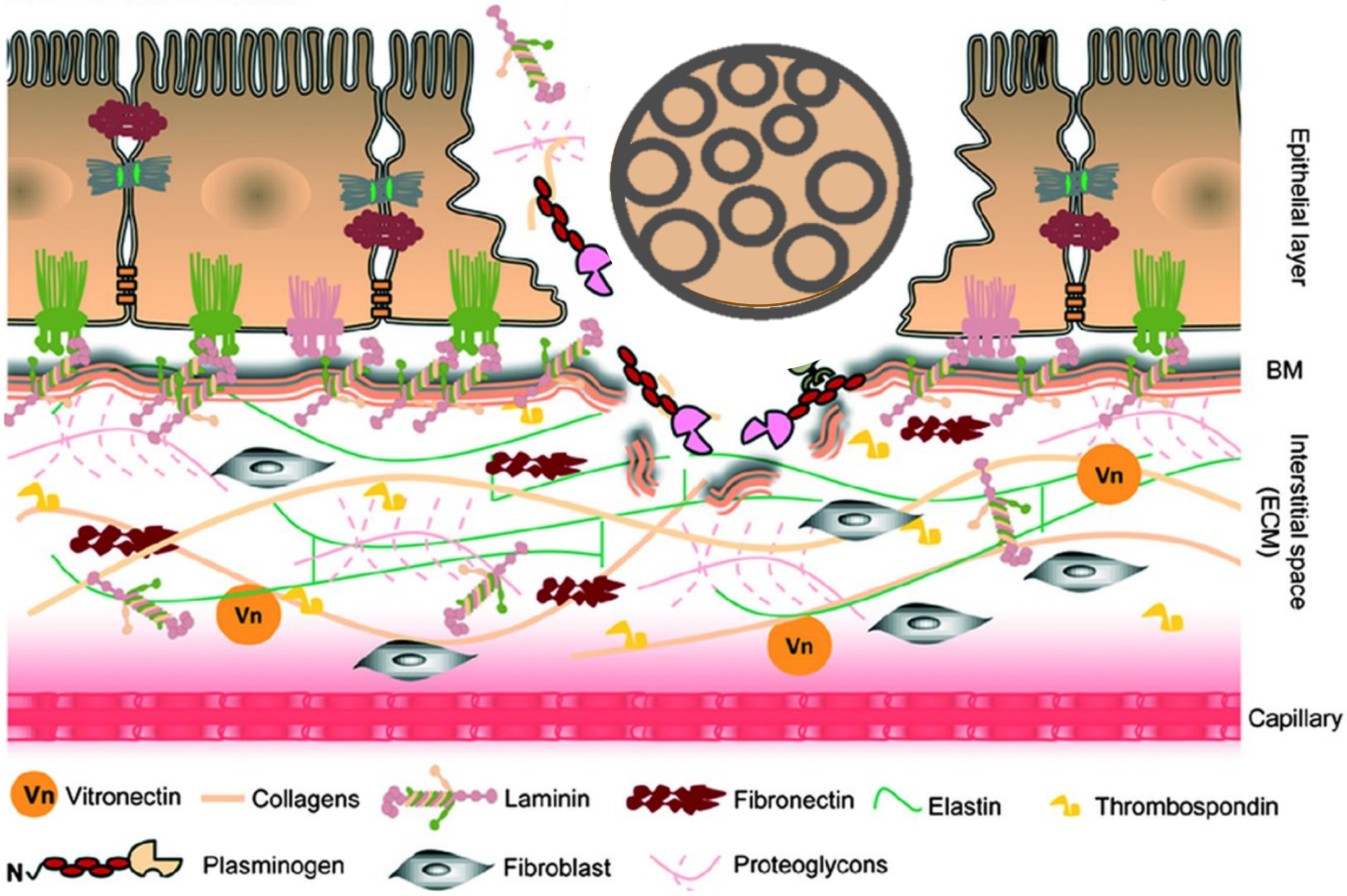


Significantly Lower Abundance



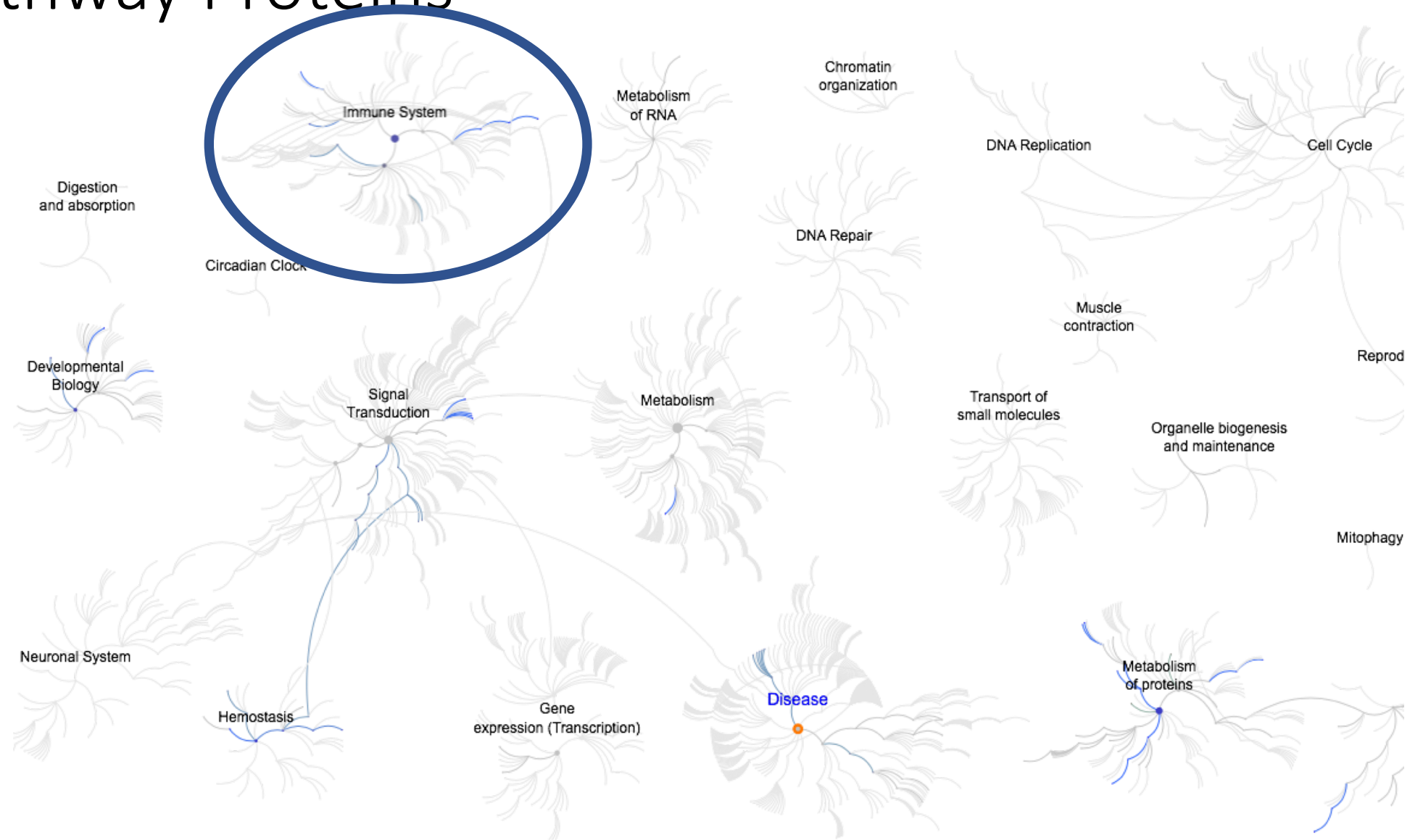
Significant Reduction in ECM Proteins

- Laminin
- Fibrillin
- Collagen



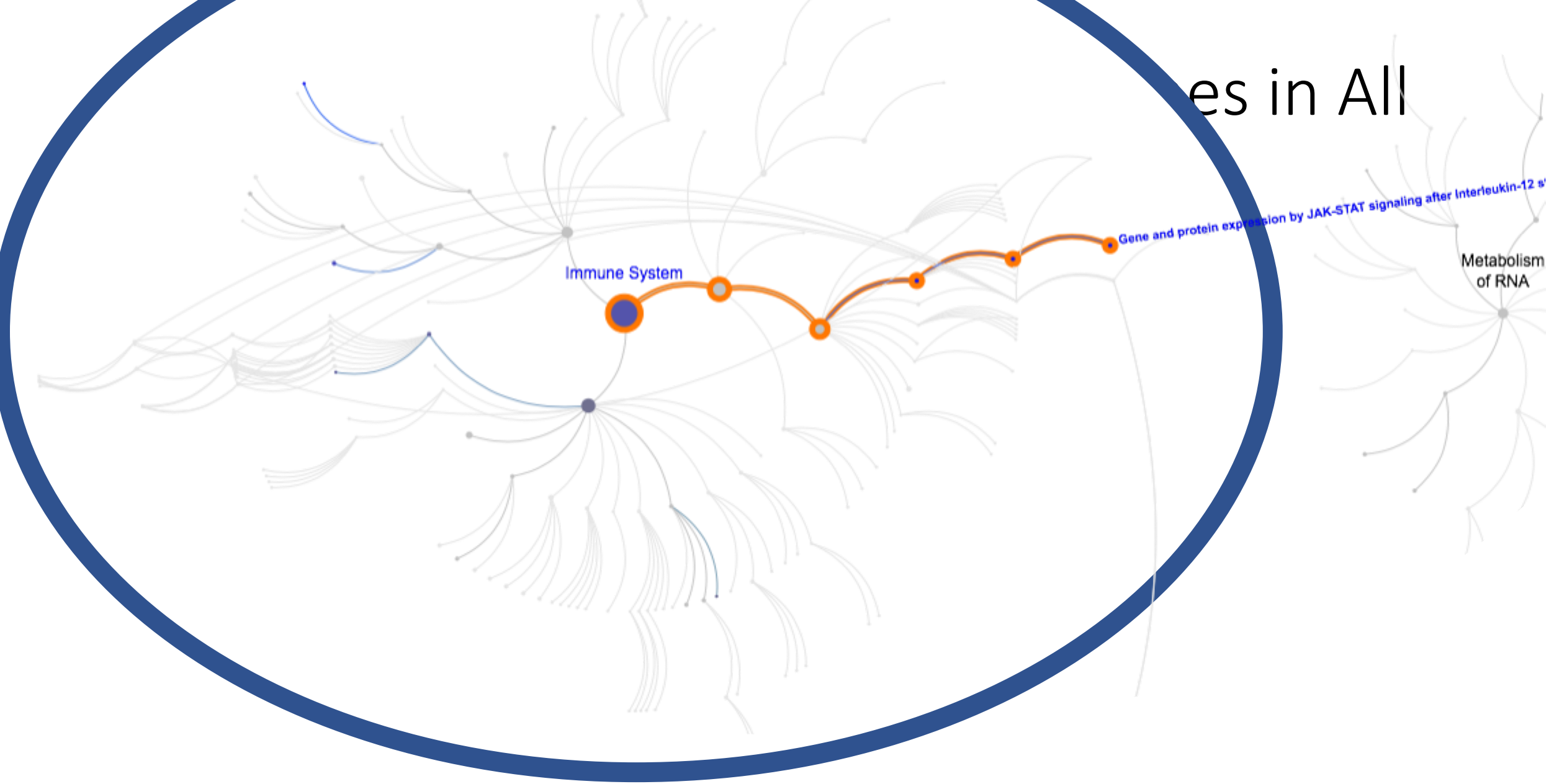
Adapted from Singh, *et al*; Human pathogens utilize host extracellular matrix proteins laminin and collagen for adhesion and invasion of the host, *FEMS Microbiology Reviews*, Volume 36, Issue 6, 1 November 2012, Pages 1122–1180

Significant Increase in Immune System and Disease Pathway Proteins

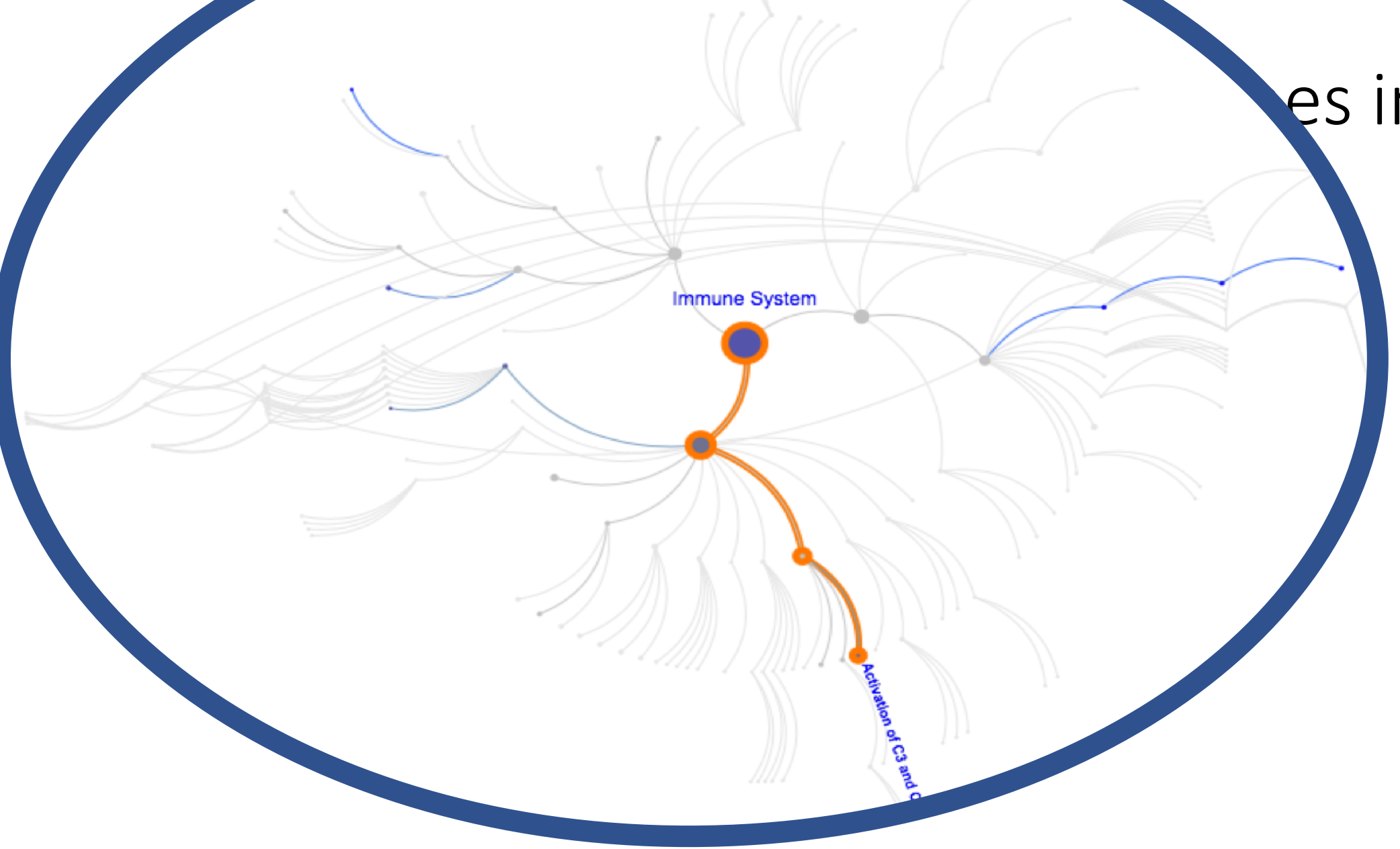


www.reactome.org

es in All


Immune System
Gene and protein expression by JAK-STAT signaling after Interleukin-12 s
Metabolism
of RNA

es in All



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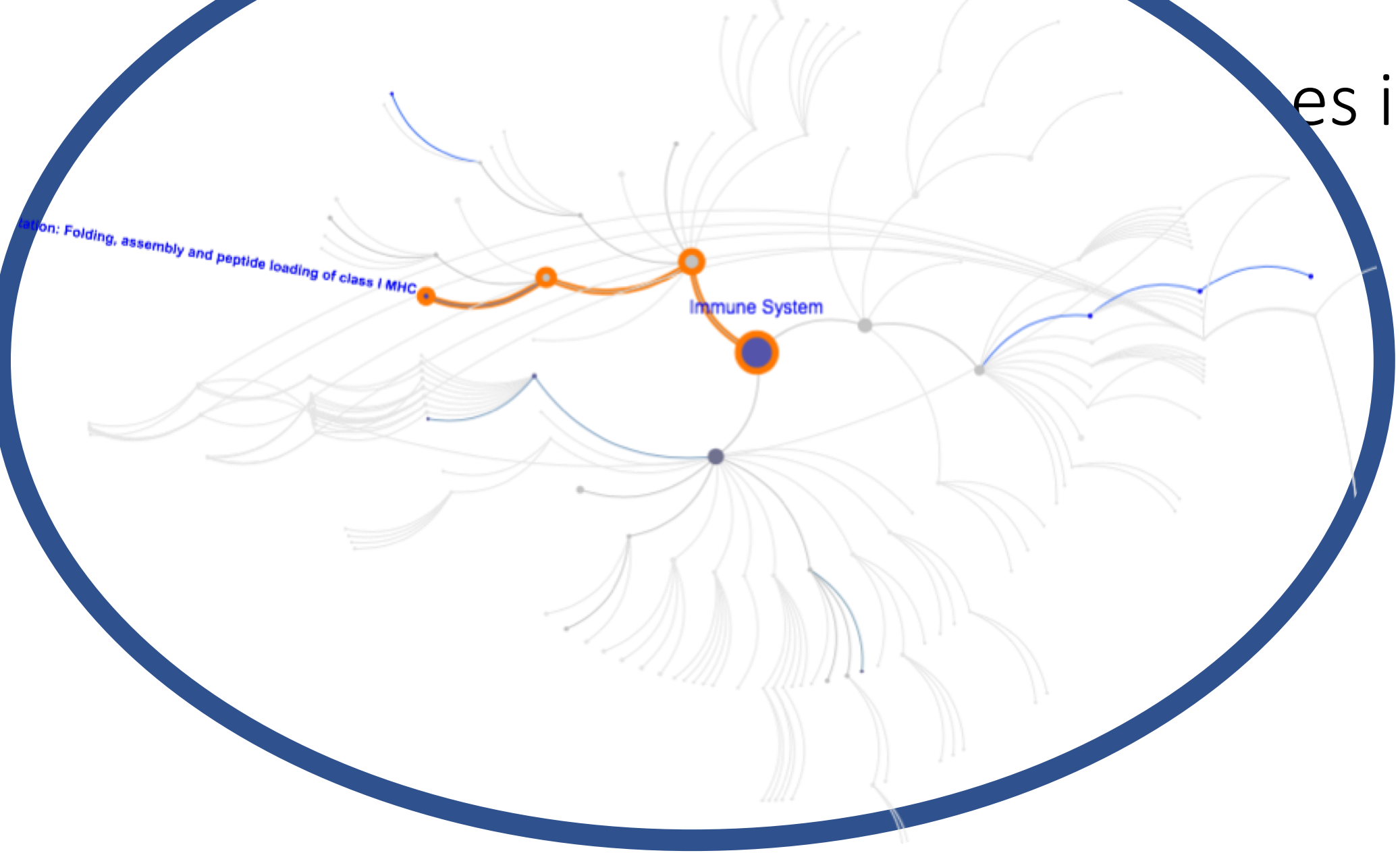
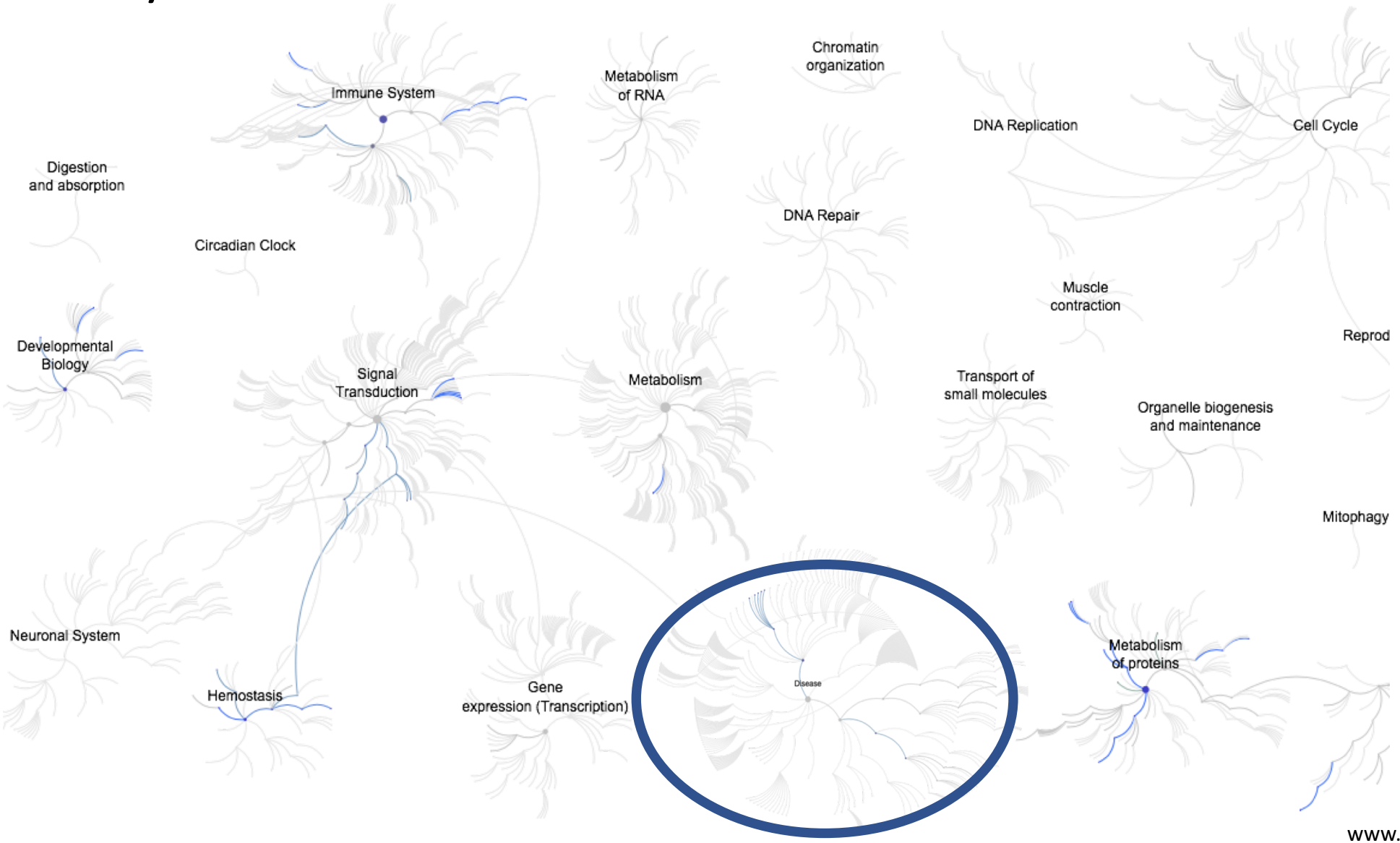

ation: Folding, assembly and peptide loading of class I MHC
Immune System

Significant Increase in Immune System and Disease Pathway Proteins



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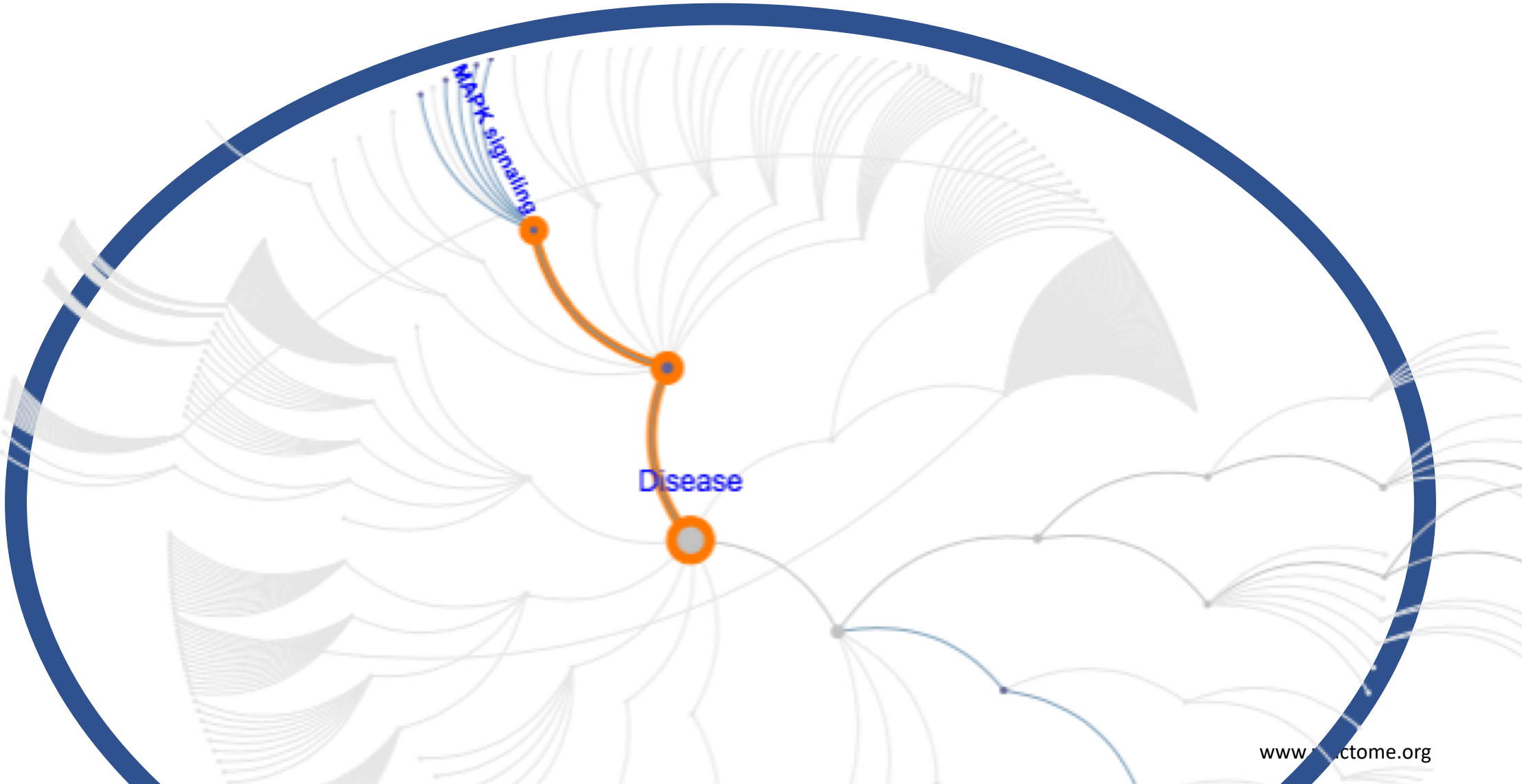
Significant Increase in Immune System and Disease Pathway Proteins



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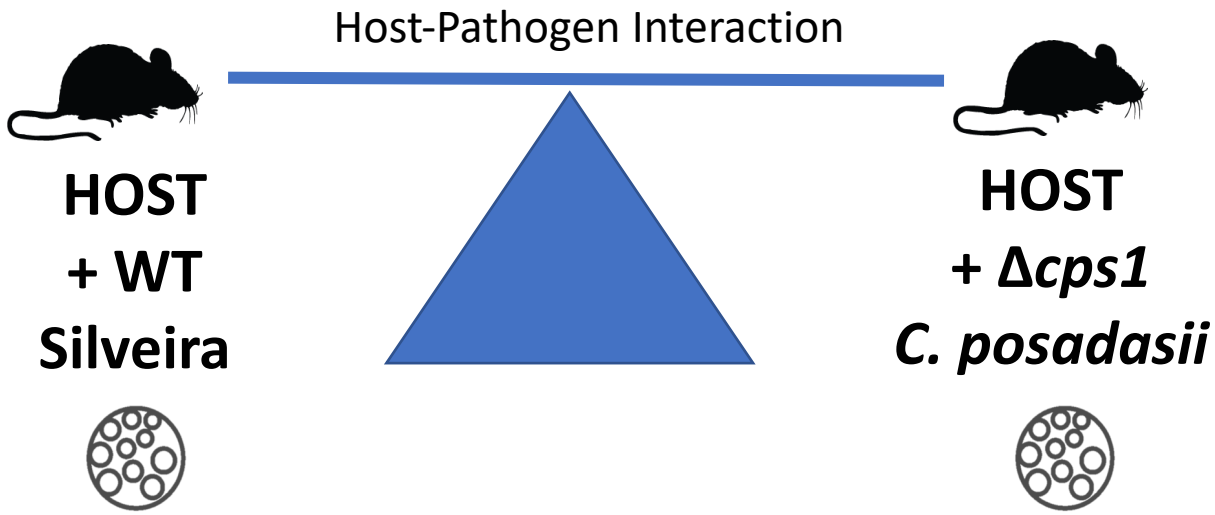
Same Host

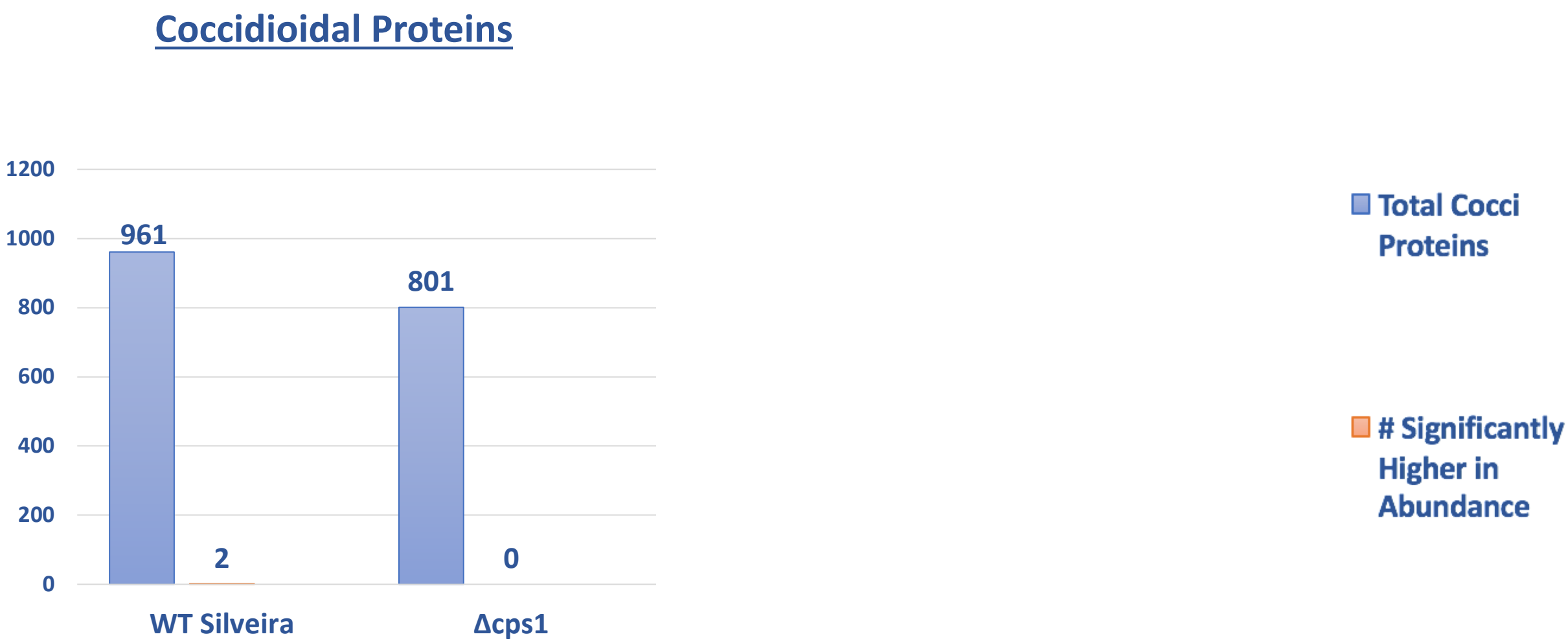
-2 different Cocci strains

?



Δ*cps1* mutant vs. WT

– Coccidioidal Proteins Similar ; Host Proteins are Different



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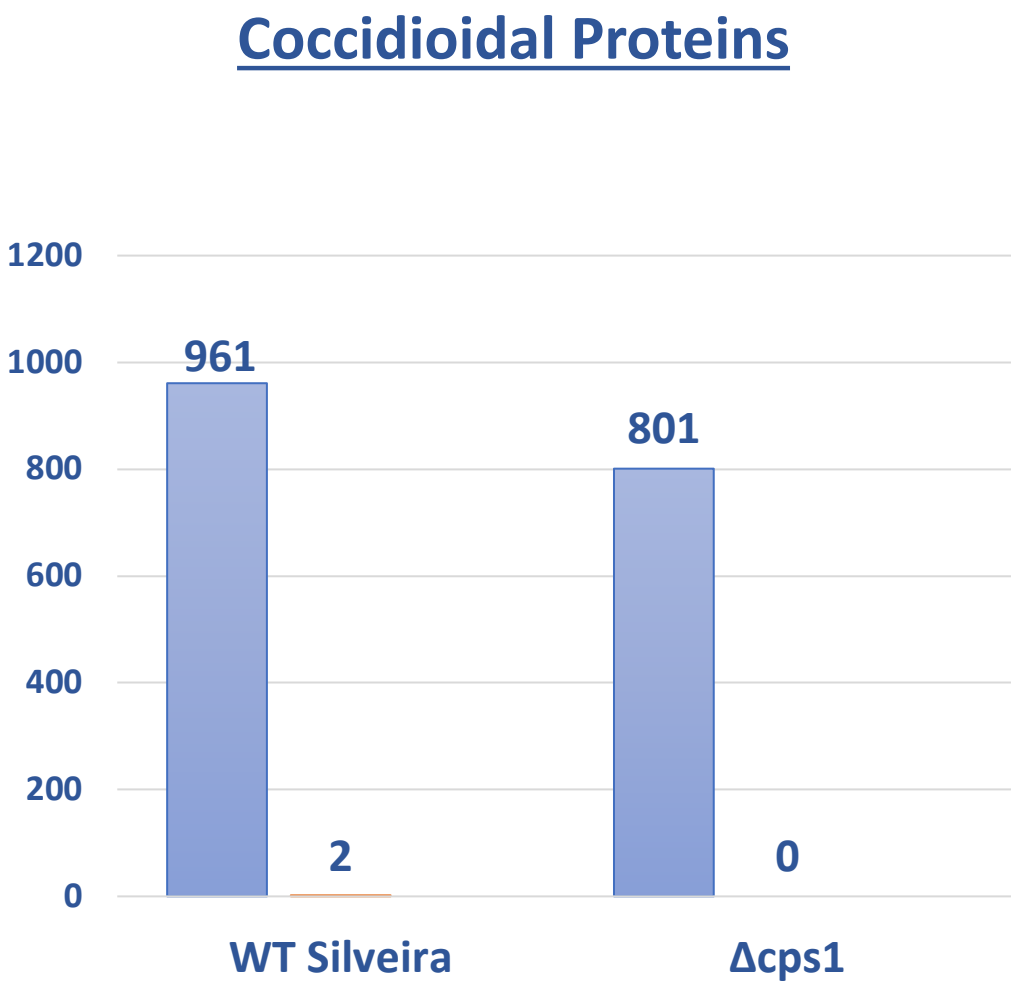
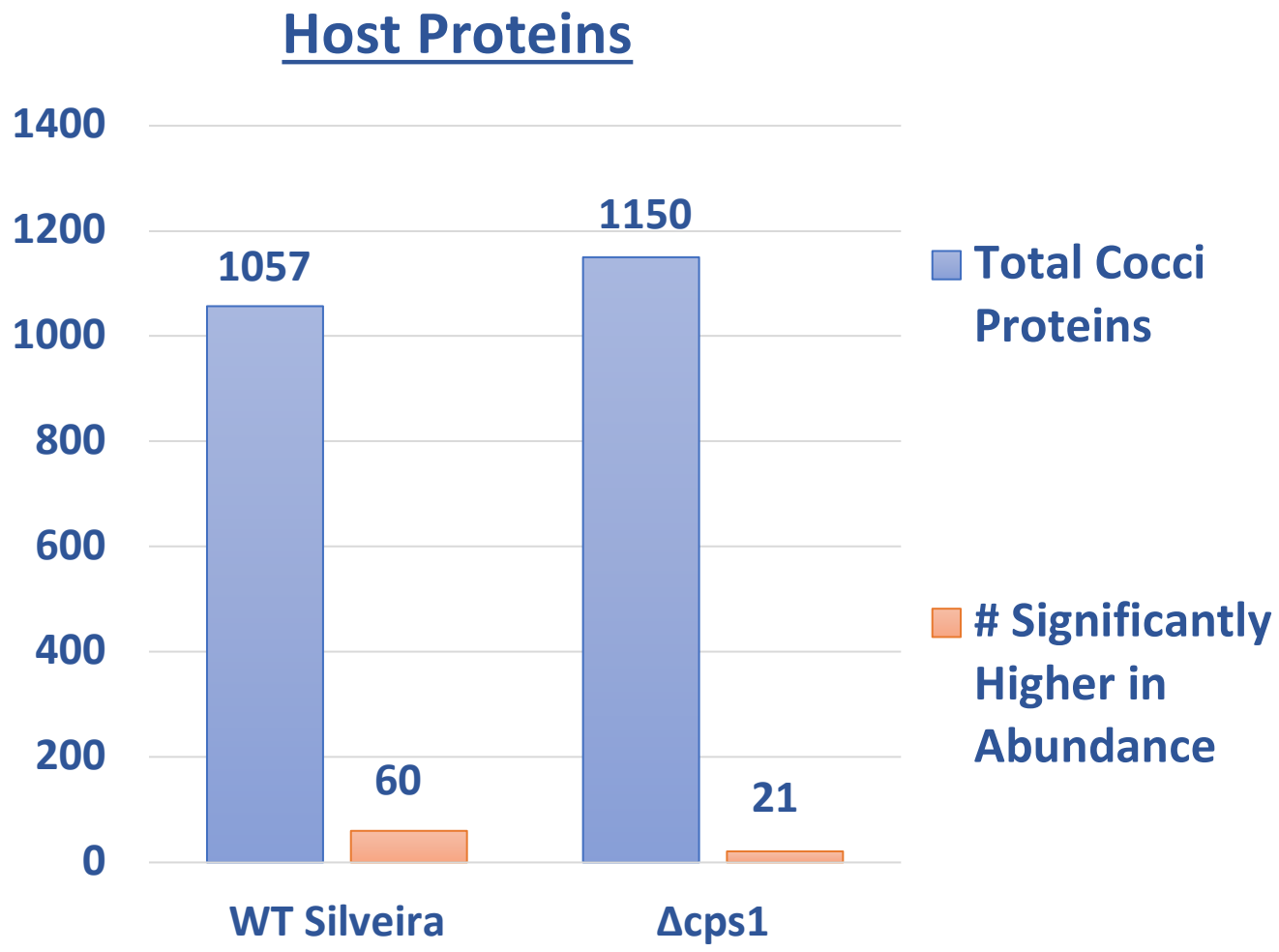




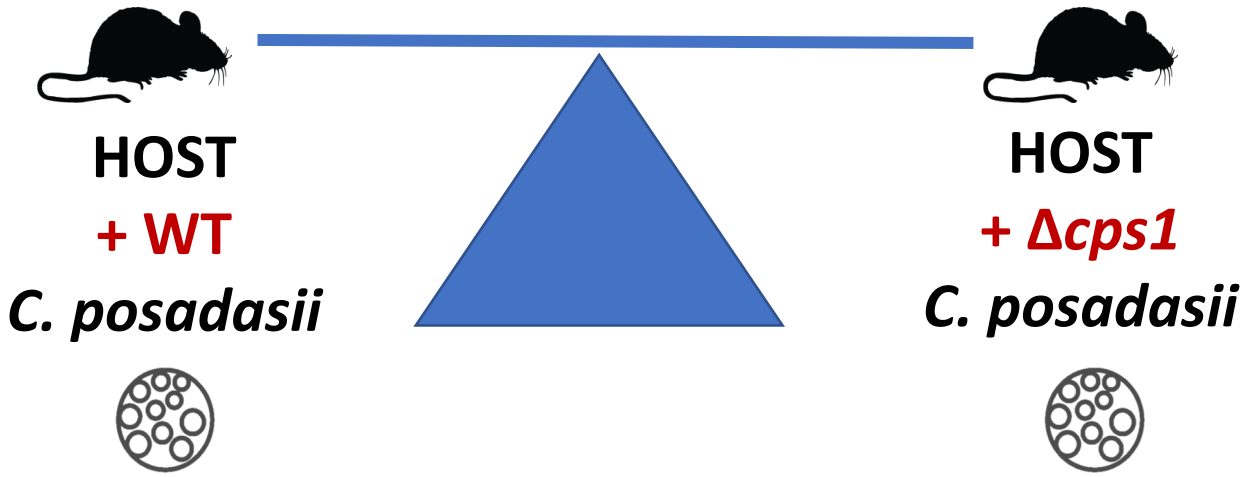
Δ*cps1* mutant vs. WT
– Significant Host Proteins are Different

Th2 Associated Proteins

Antibodies
Eosinophil Cationic Proteins

Th1 Associated Proteins

MHC Class I Protein
Interferon Gamma Inducible Factors



Conclusions

- In the limited samples tested, *in vivo* Coccidioidal proteins and abundances were similar in human, mouse and dog lungs.
- Cocci grown in human, mouse and dog lungs were more similar than in vitro grown Cocci.
- Significant differences in host proteins was observed, suggesting caution should be taken when extrapolating findings about immune responses from mouse models.
- Also provided is data suggesting that the *Δcps1* strain may alter the host proteins towards a protective Th1 response rather than altering expression of Coccidioidal proteins in mice.

Acknowledgements

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Lisa Schubitz (UofA)

Hein Trinh (UofA)

Cheryl Meyers (Mayo Clinic Phoenix)

Douglas Lake Lab:

Yvette Ruiz

Alexa Roeder

Amber Fifield

Calvin Koelbel

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Christine Charlesworth

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