

PPIs in host-microbe interactions: An interplay between pathogens and our cells

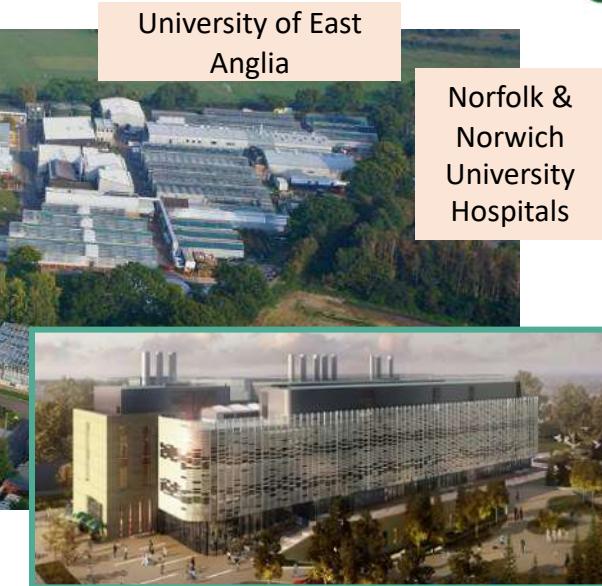
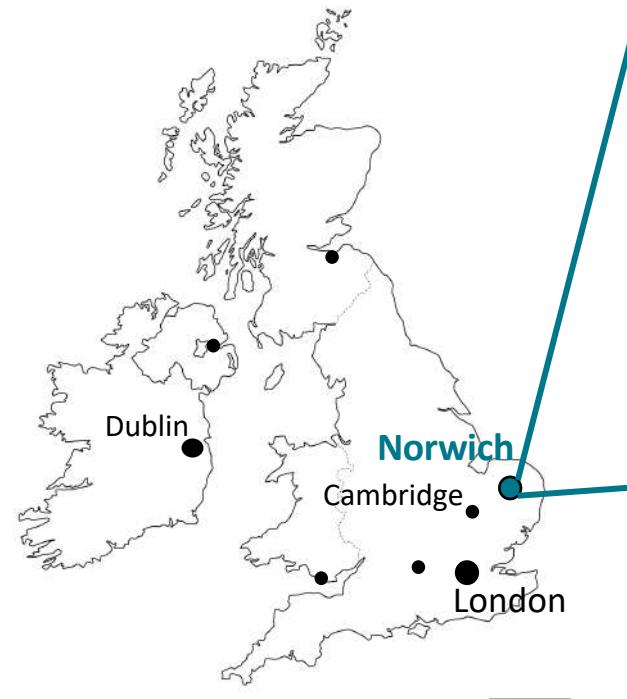
TAMAS KORCSMAROS
Group leader

EMBO Practical course
Bangalore, India



Quadrant
Institute

Earlham Institute & Quadram Institute

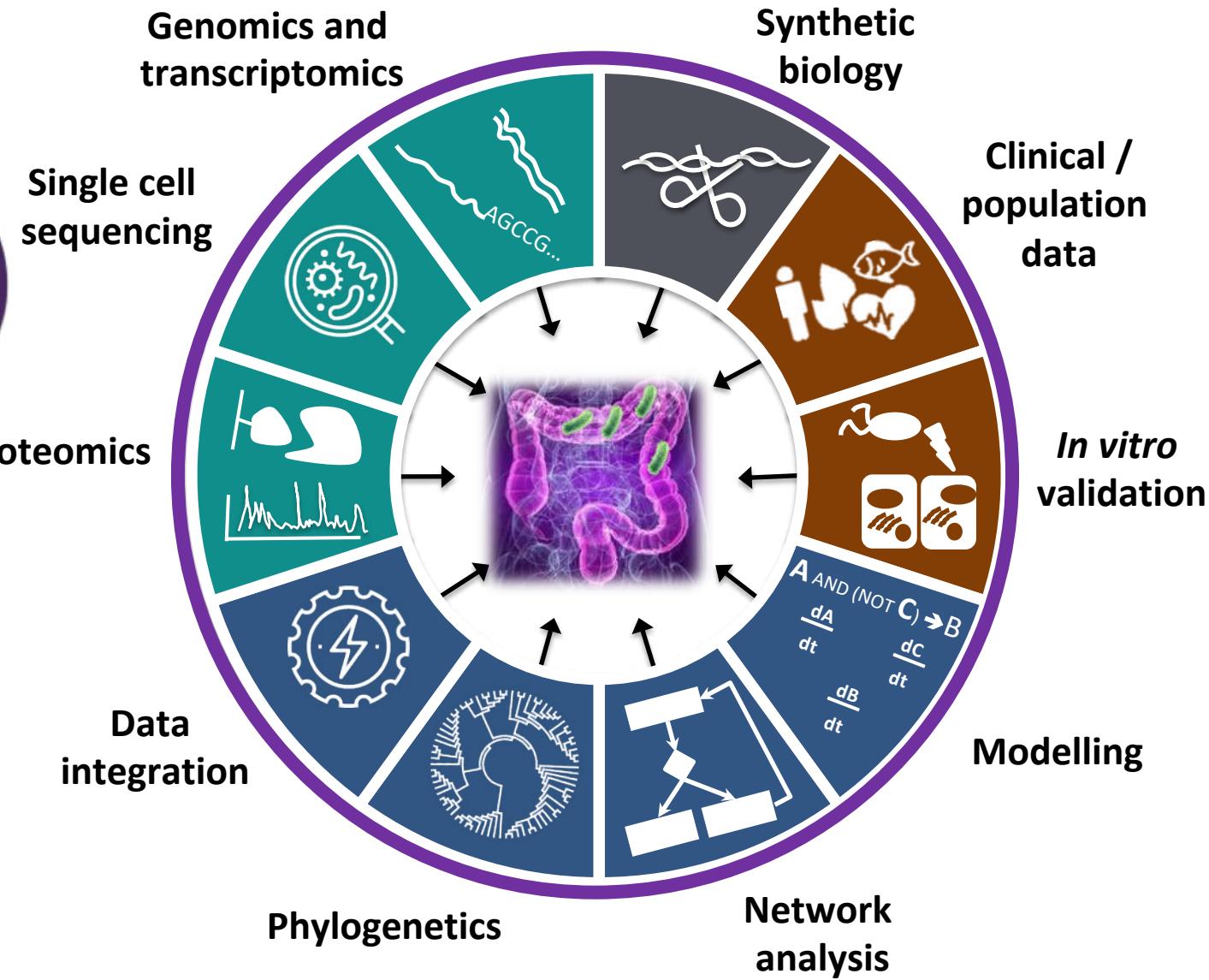
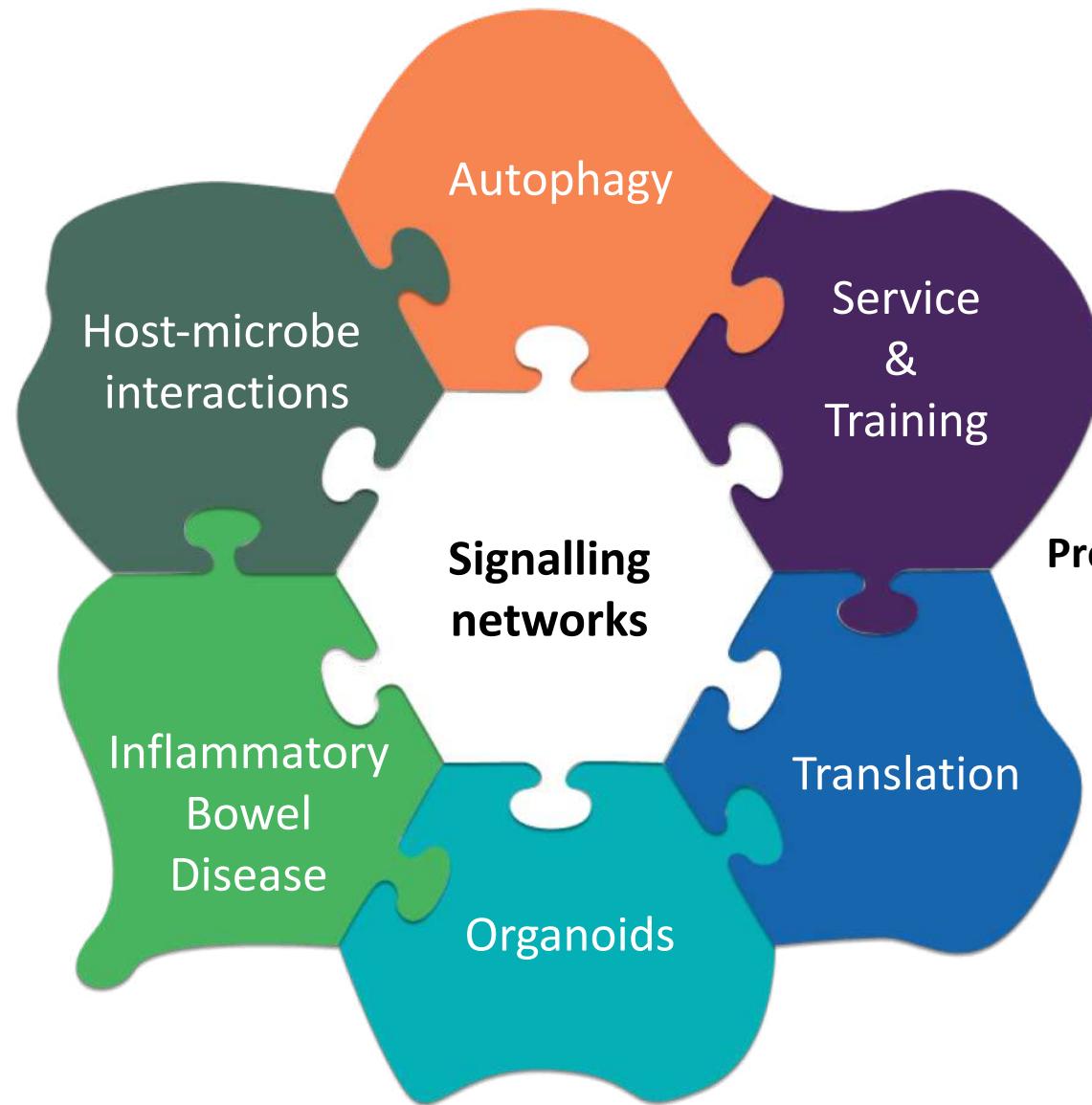


- BRINGS TOGETHER EXPERTISE IN BIOINFORMATICS, SYSTEMS BIOLOGY, SEQUENCING AND HIGH PERFORMANCE COMPUTING
- TO UNDERSTAND COMPLEX BIOLOGICAL SYSTEMS IN HUMANS AND THEIR INTERACTIONS WITH MICROBES

Quadram Institute

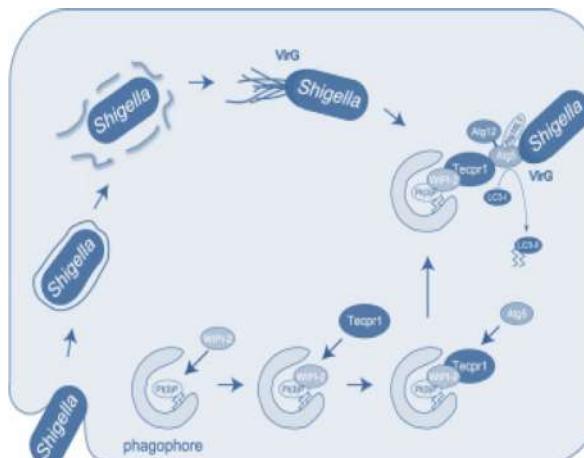
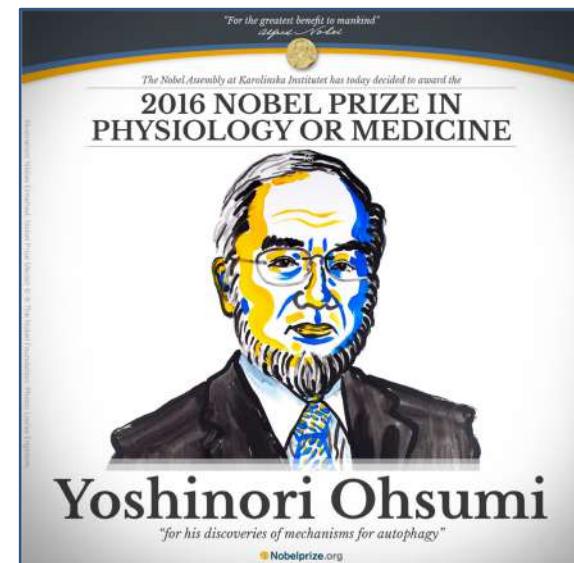
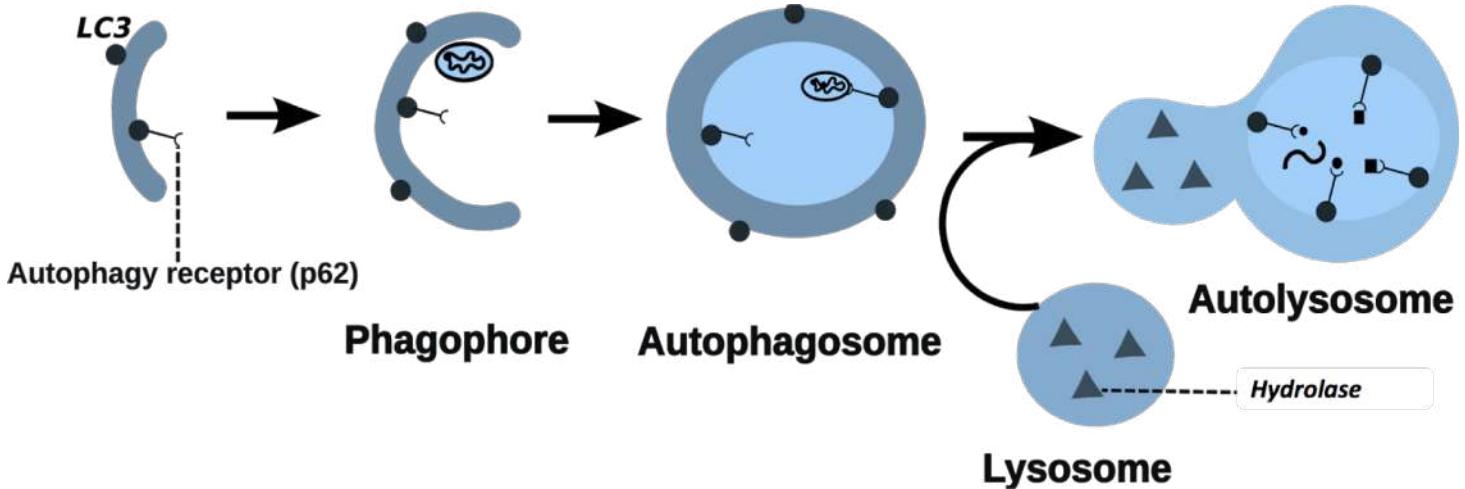
- CREATING A STEP CHANGE FOR GUT HEALTH RESEARCH
- LARGE ENDOSCOPY UNIT BENEATH THE LABORATORIES
- FOCUS ON COMMENSAL AND PATHOGENIC MICROBES AND ON THE MICROBIOME

The Korcsmaros group



Autophagy

- Stress response mechanism
 - starvation
 - hypoxia
 - protein misfolding
 - damaged mitochondria
- Target microbes to lysosomal degradation (p62, NDP52, LC3)
- Also involved in intestinal homeostasis, normal development, inflammation, immune recognition, regeneration, and ageing
- Neurodegenerative diseases, diabetes, cancer, IBD (Crohn's disease)
- Several intracellular bacteria can stimulate and/or inhibit autophagy
 - *Helicobacter pylori*
 - *Salmonella enterica*
 - *Listeria monocytogenes*
 - *Shigella flexneri*

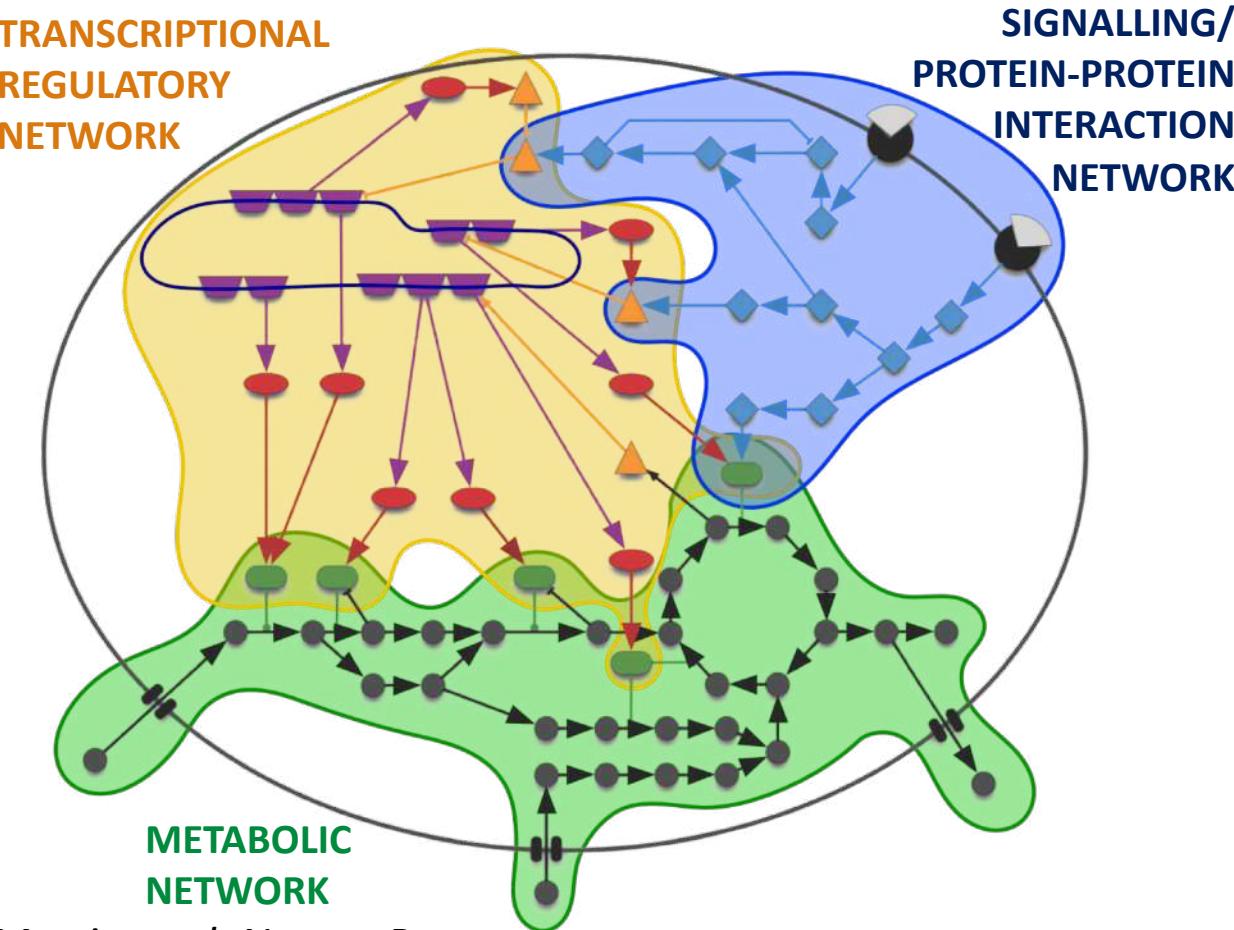


Co-authored publication:
Klionsky, (...), Korcsmaros, Ohsumi, (...), *Autophagy*, 2016

How autophagy is regulated upon infection?

The War of the Motifs

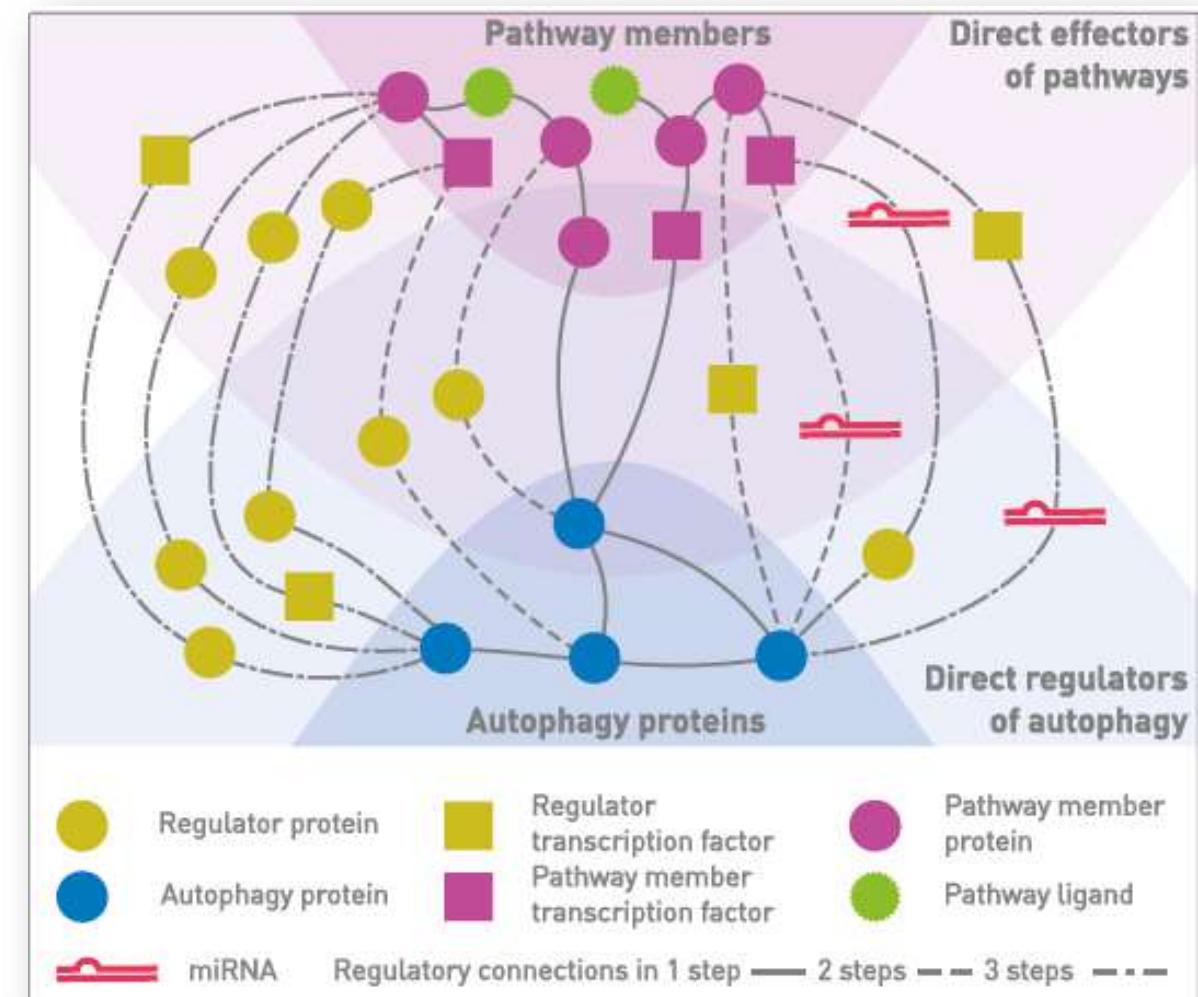
SalmoNet: an integrated *Salmonella* network



Metris et al. *Nature Partner Journals Systems Biology and Applications* (2017)
<http://SalmoNet.org>

Networks for >10 strains
allow comparative and
strain-specific studies

Autophagy Regulatory Network: Multi-layered mechanisms regulating autophagy

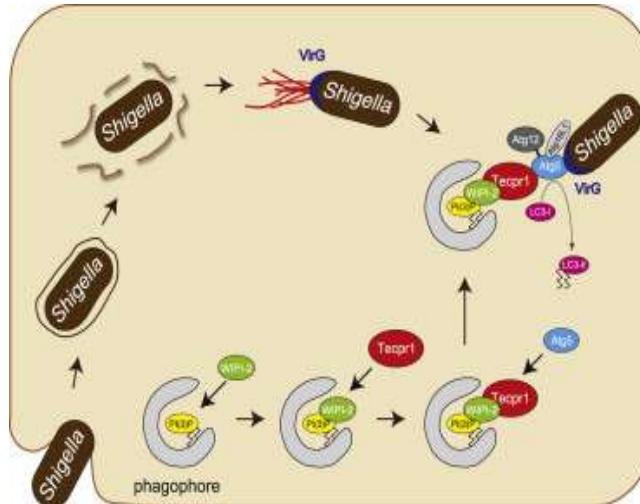


Turei et al, *Autophagy*, 2015

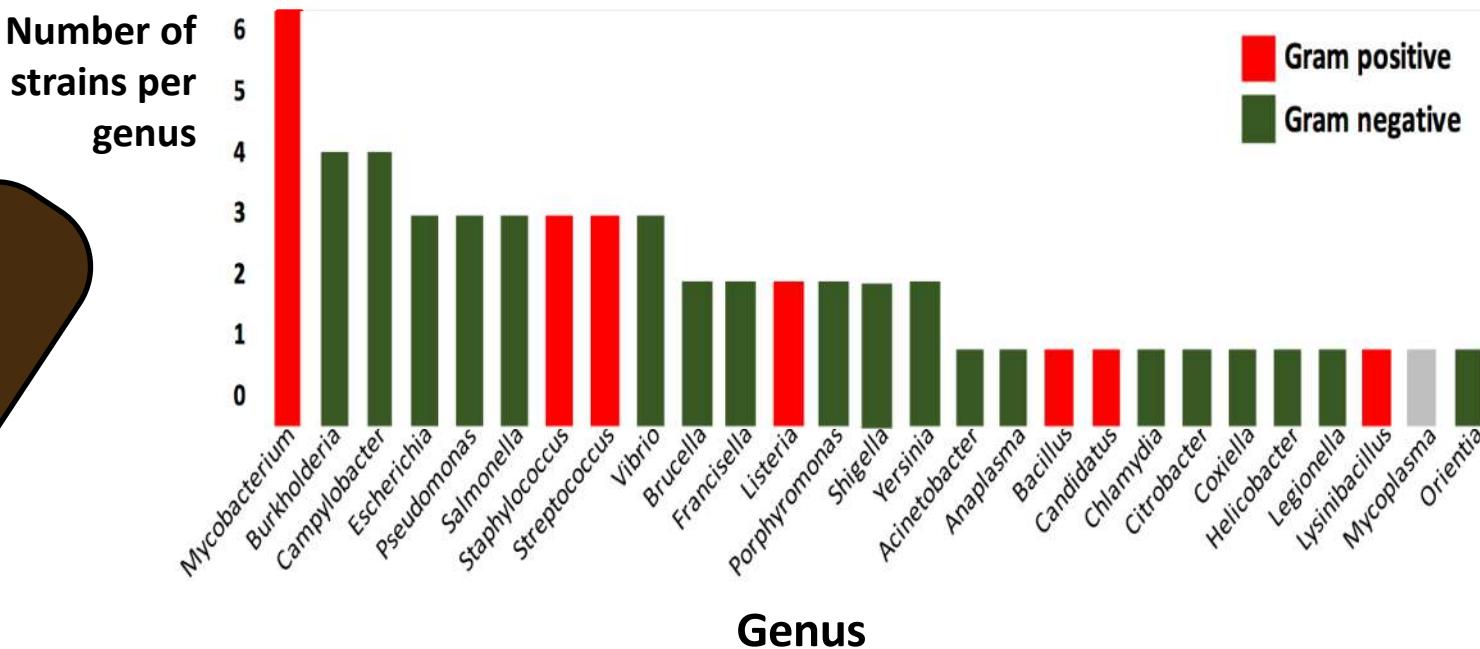
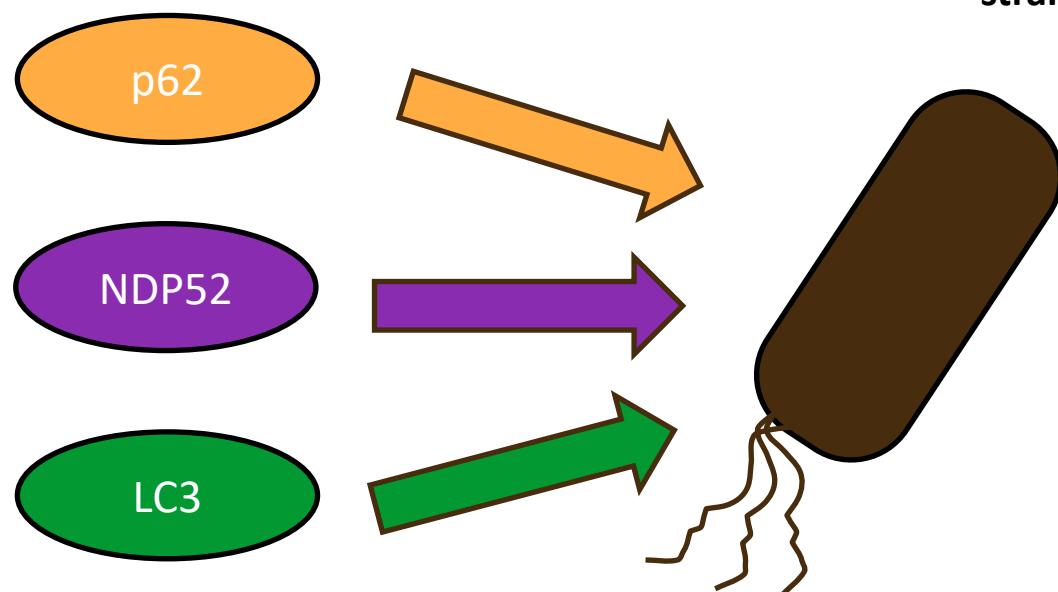
<http://AutophagyRegulation.org>

Modelling interactions between gut pathogens and the host

Autophagy is an essential process of intestinal homeostasis and clearance of pathogens

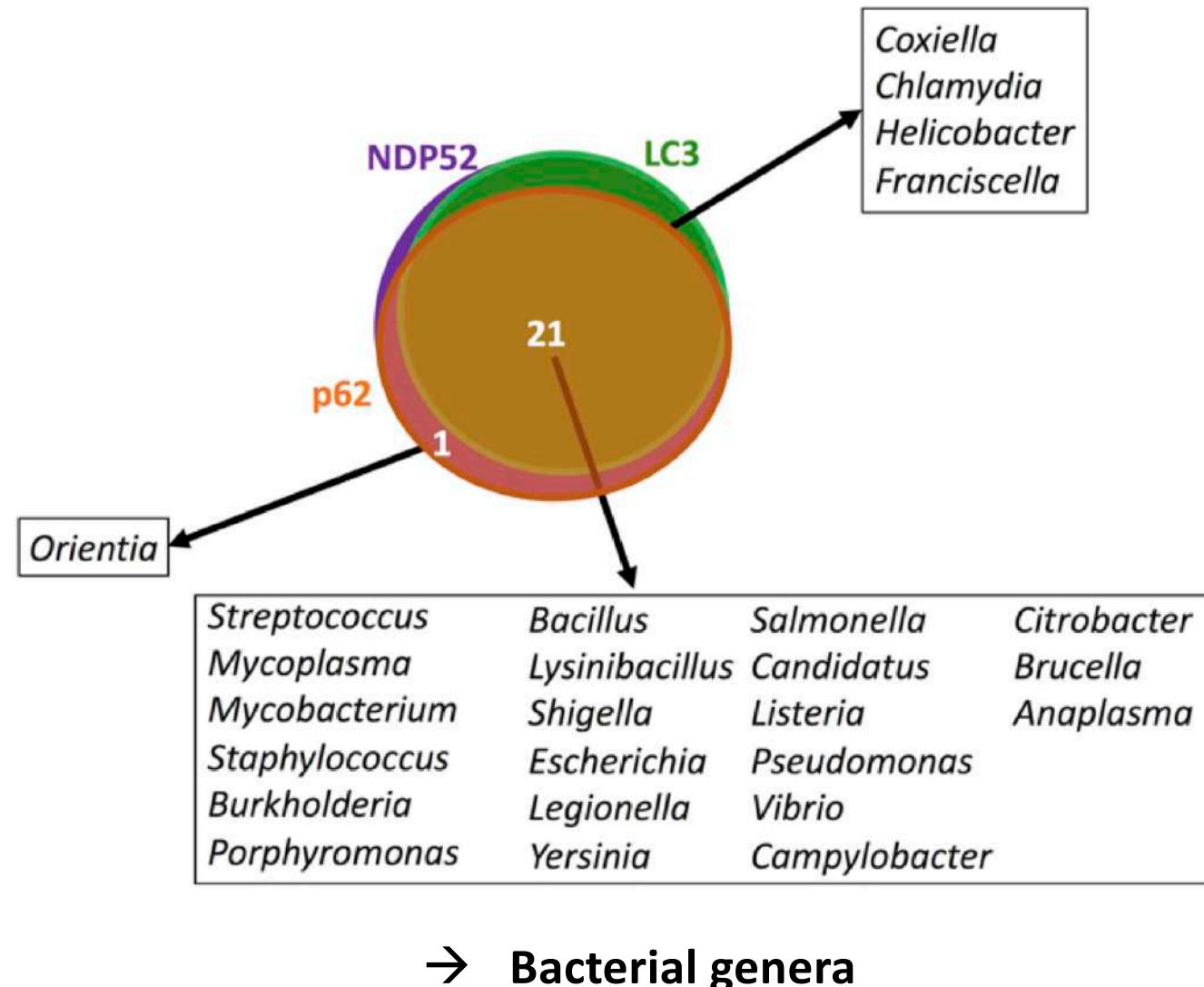
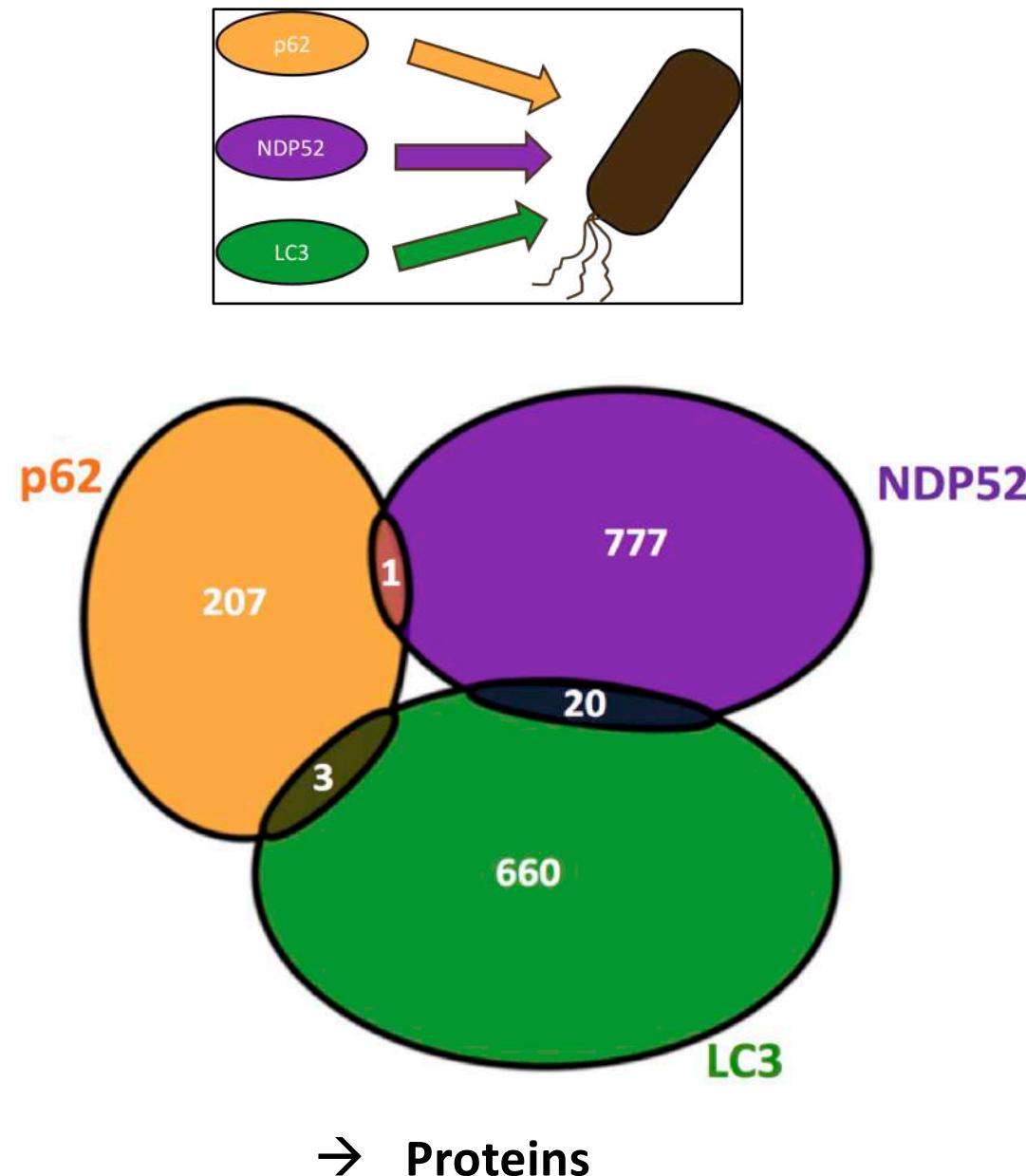


- How do bacterial pathogens modulate host autophagy?
- Is there a conservation of pathogen-autophagy interplay?

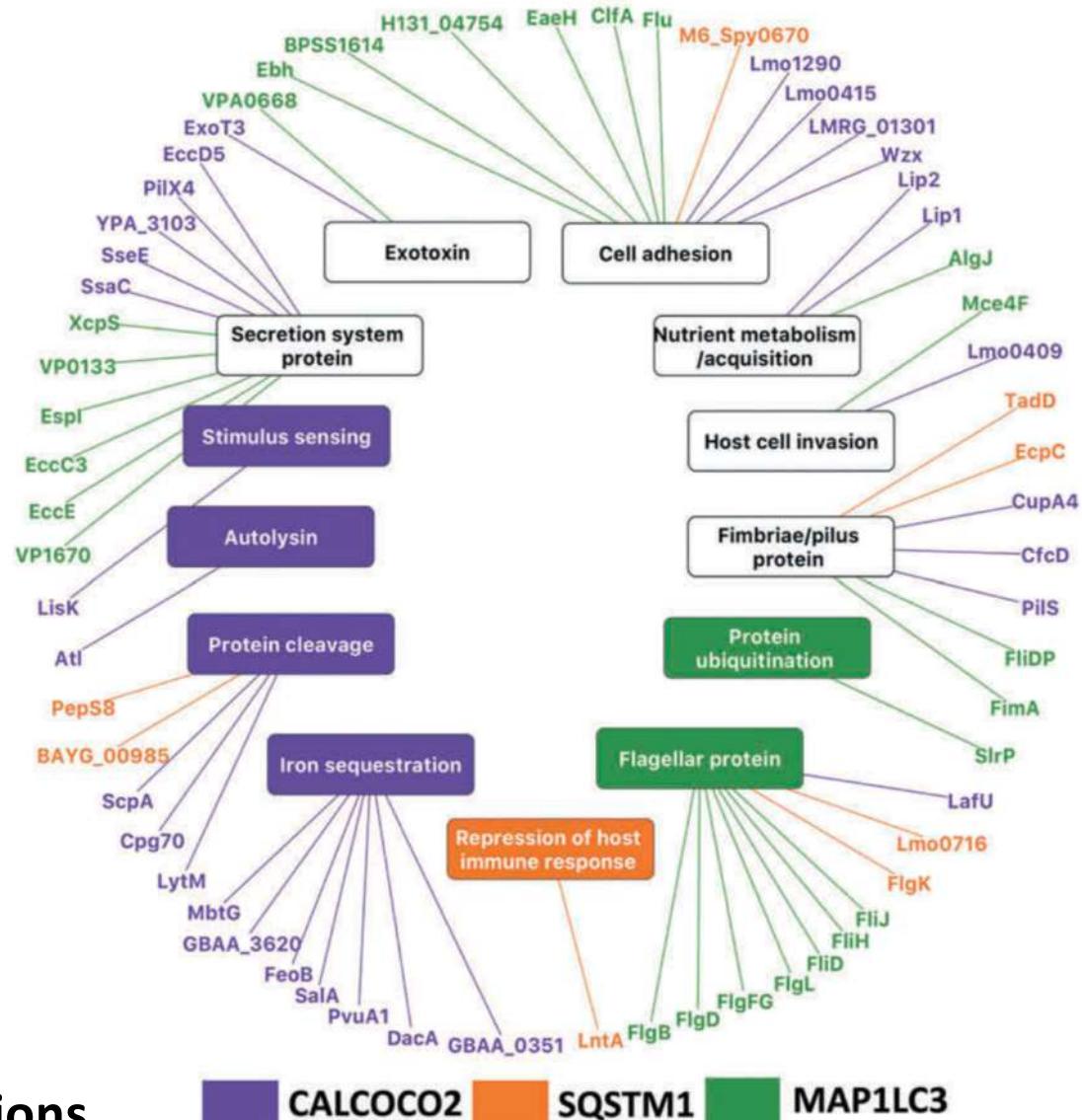
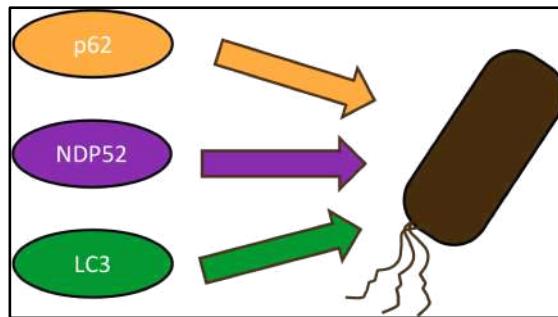


→ We analysed 56 strains from 26 genera

Pathogens and their proteins are recognised by autophagy receptors and adaptors



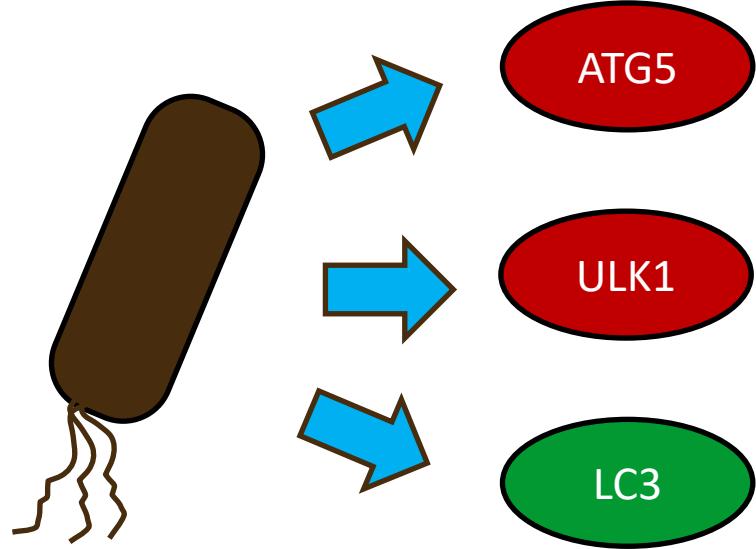
Pathogenic virulence factors recognised by autophagy receptors and adaptors



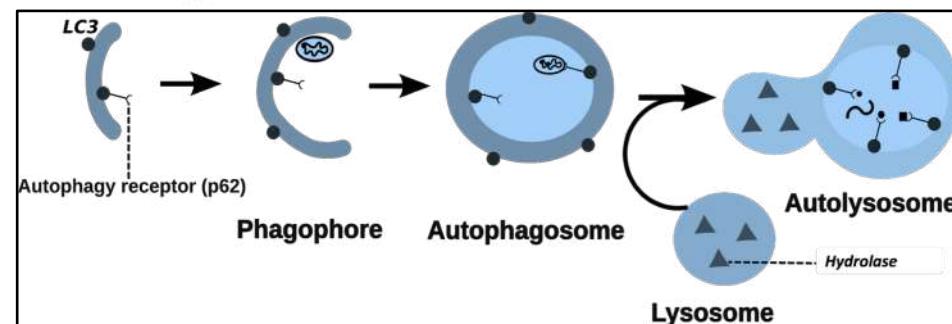
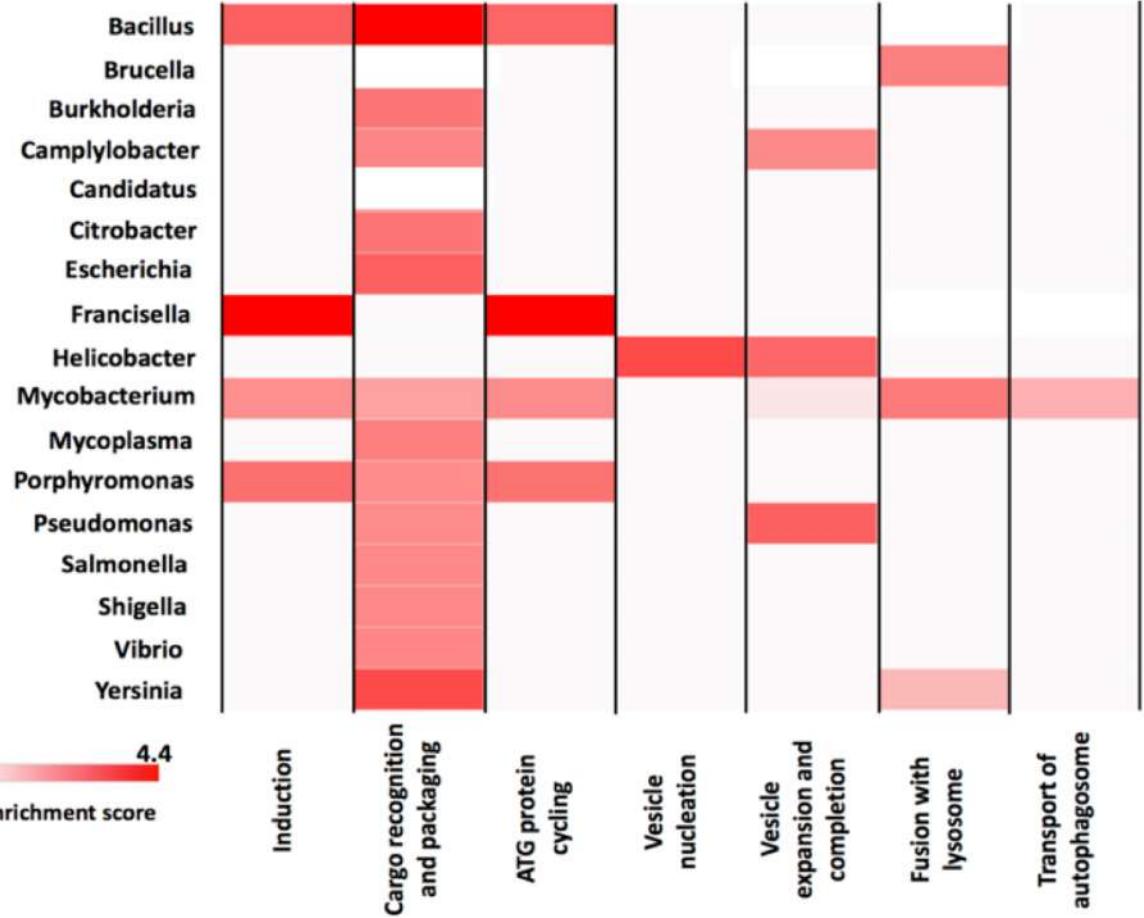
→ Virulence factor's functions

 CALCOOCO2 SQSTM1 MAP1LC3

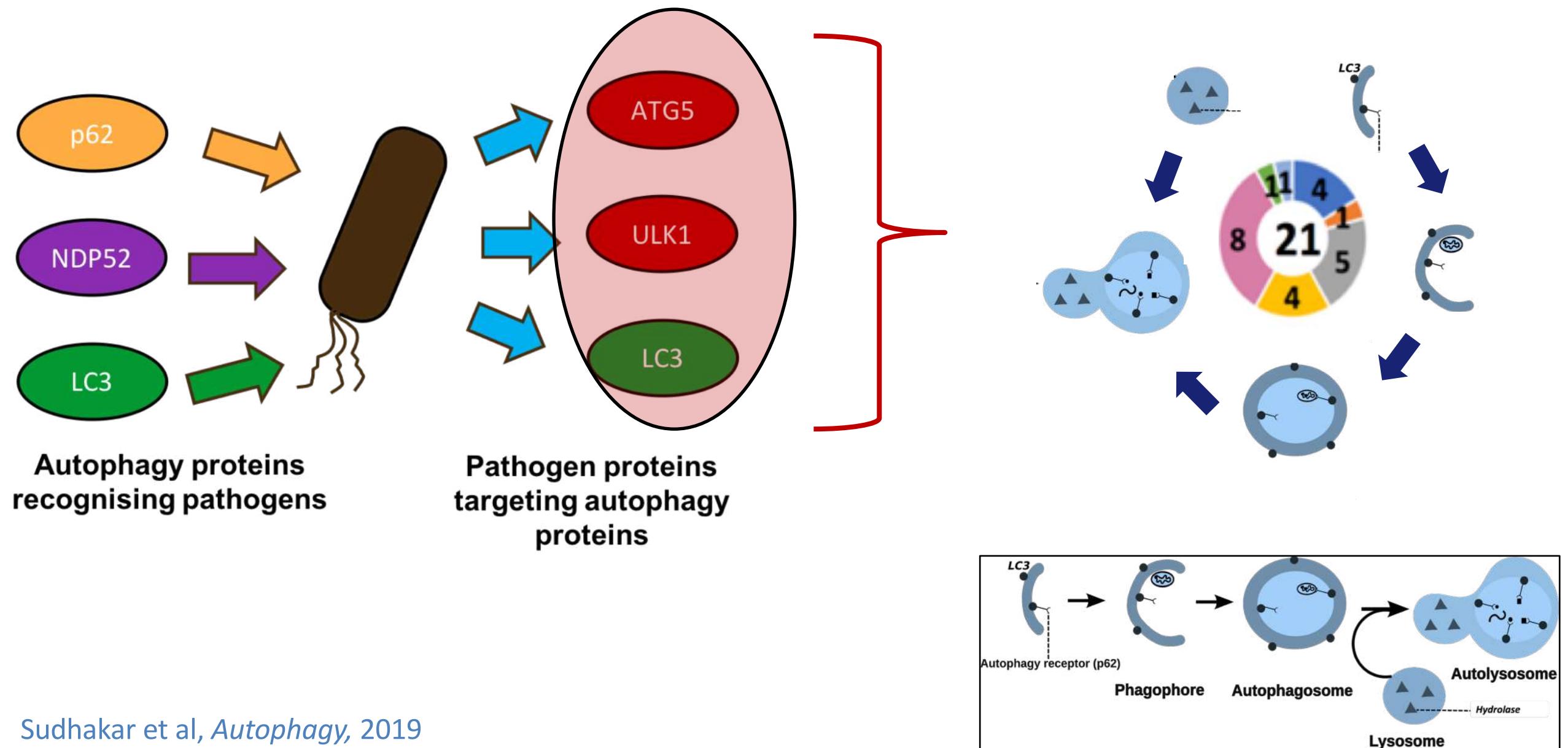
Pathogens and their proteins could modulate autophagy



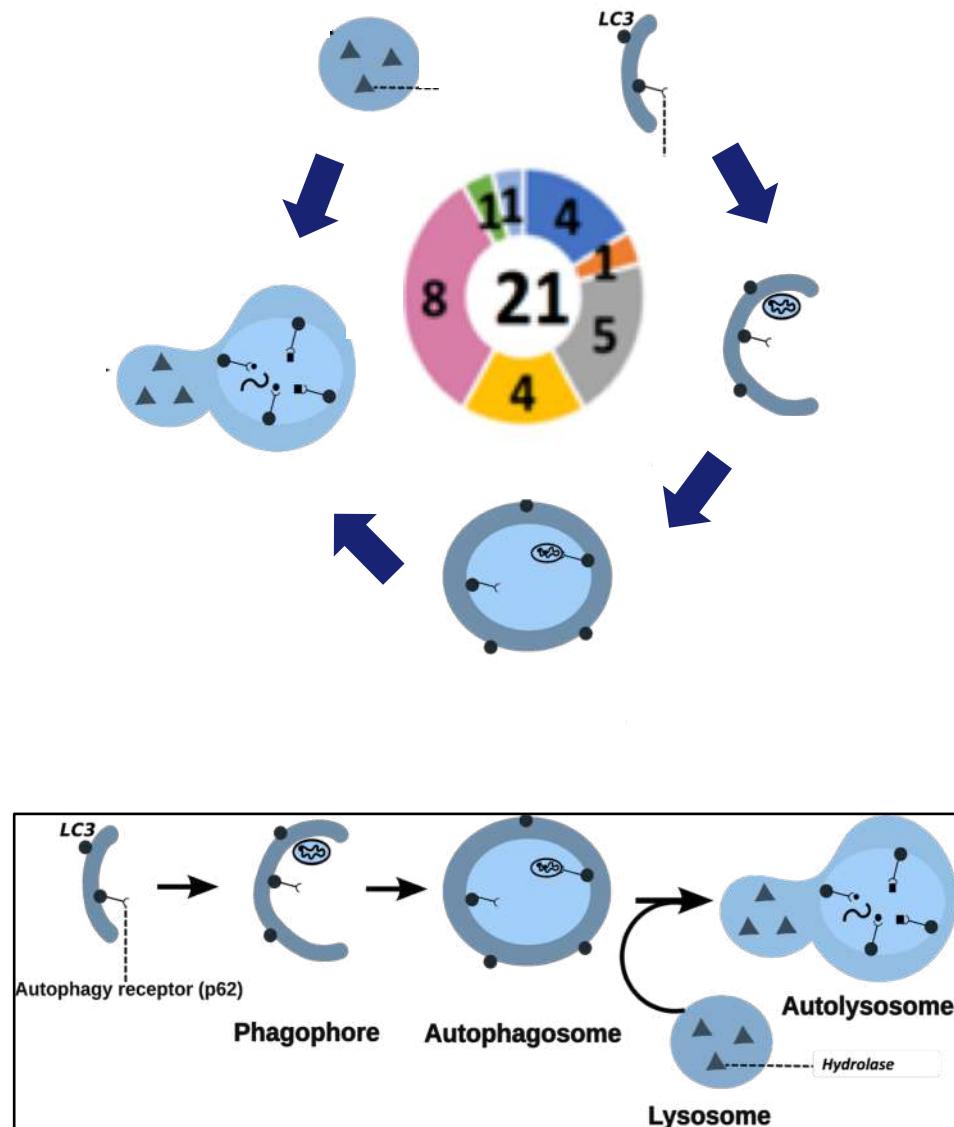
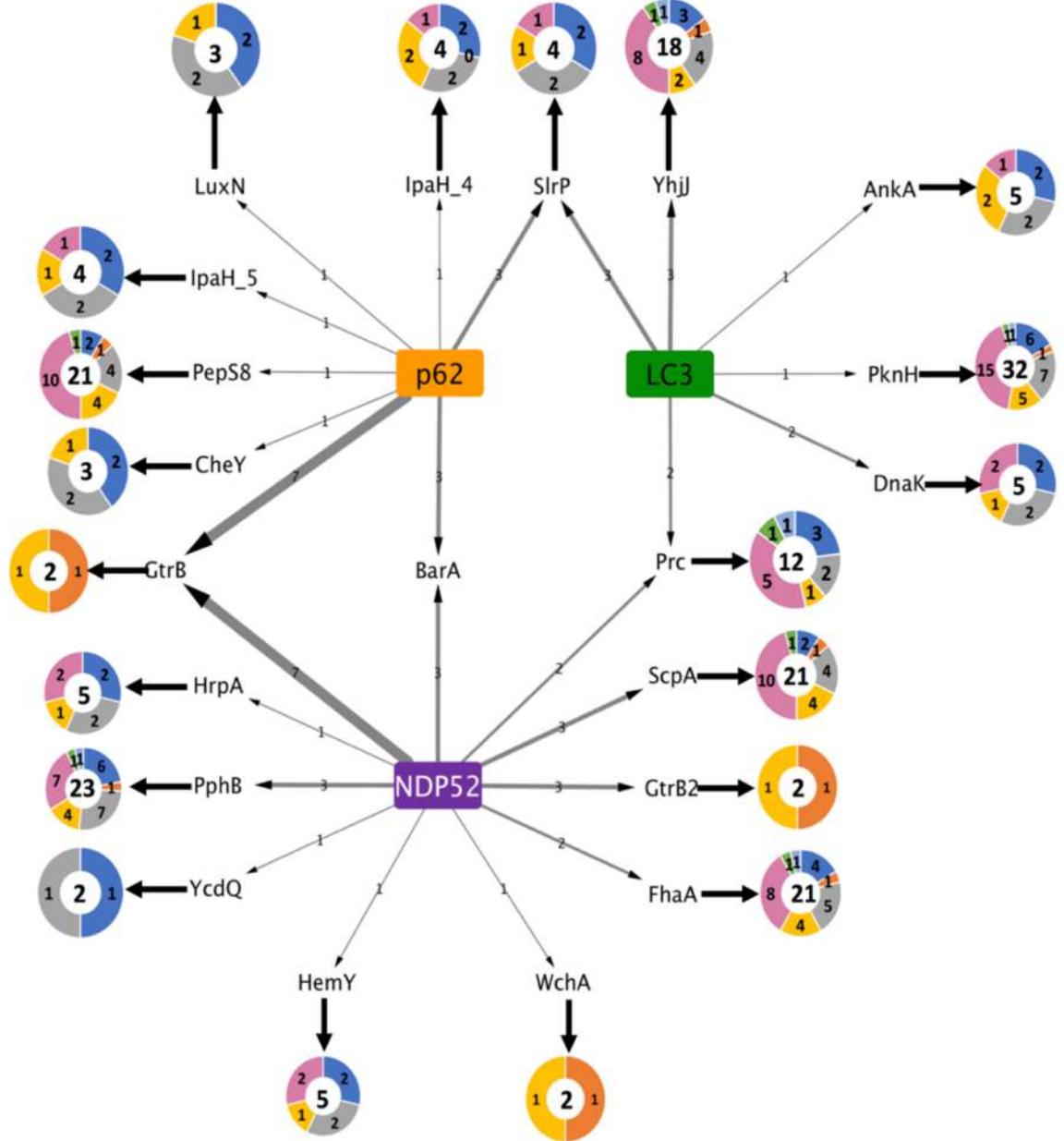
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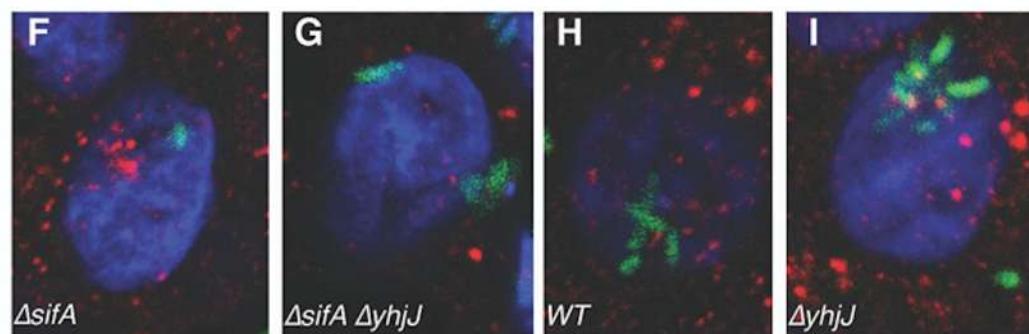
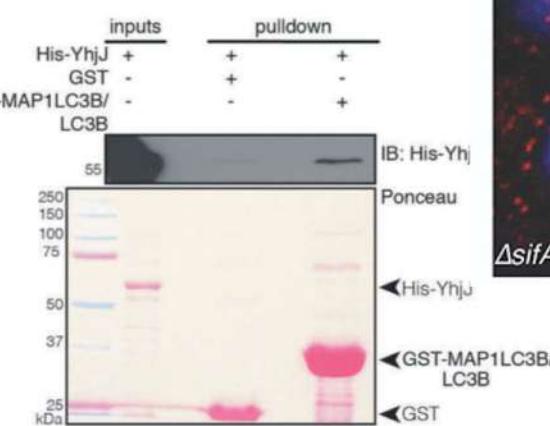
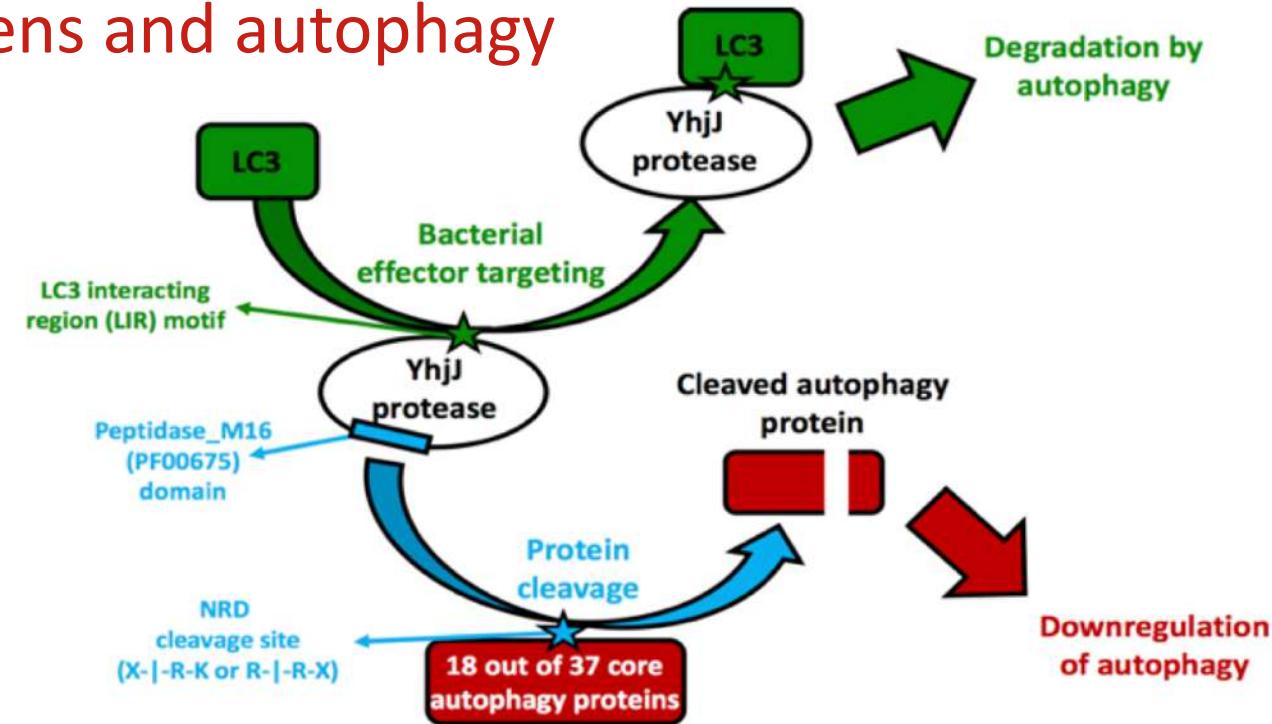
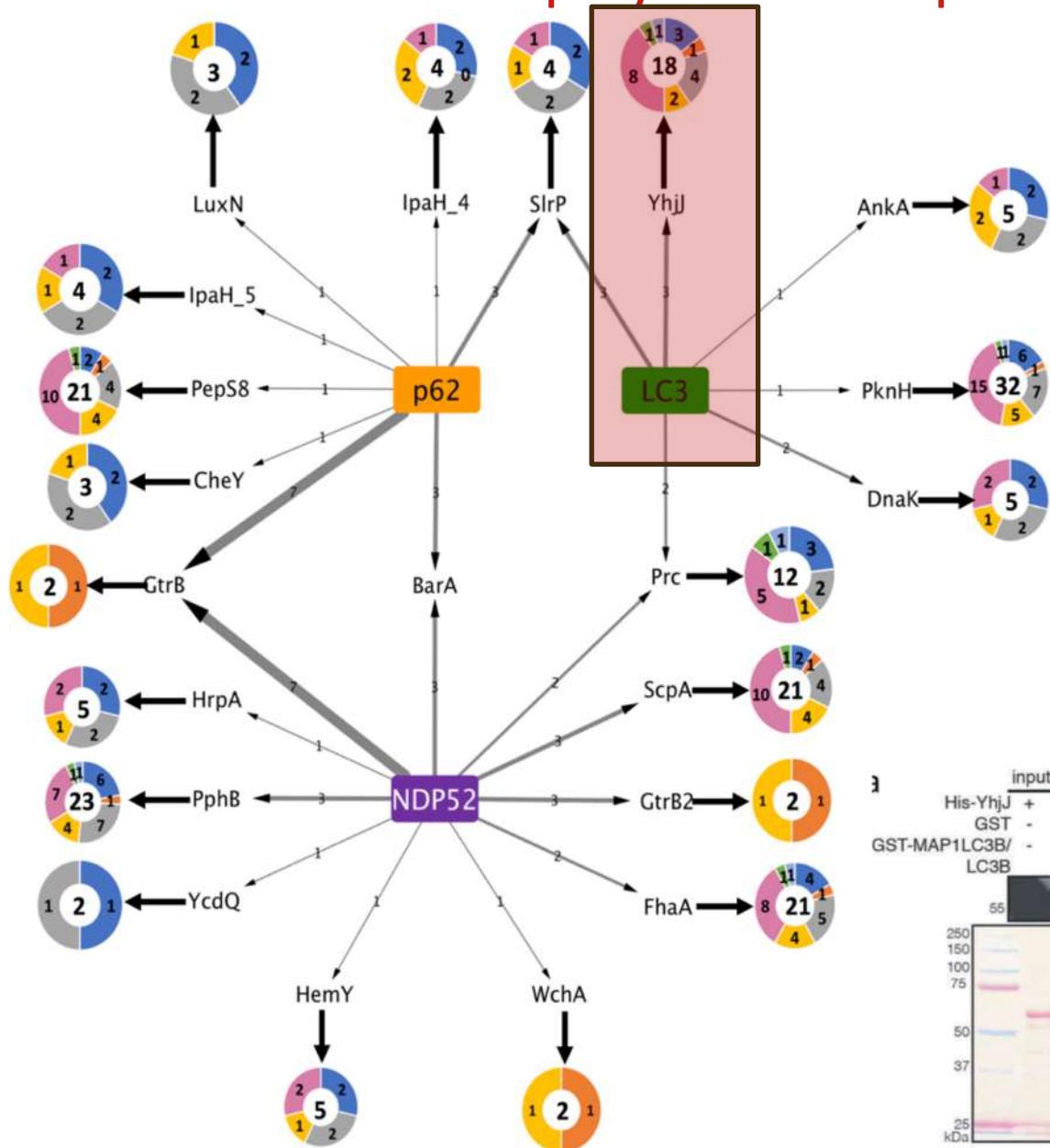
There is an interplay between pathogens and autophagy



There is an interplay between pathogens and autophagy

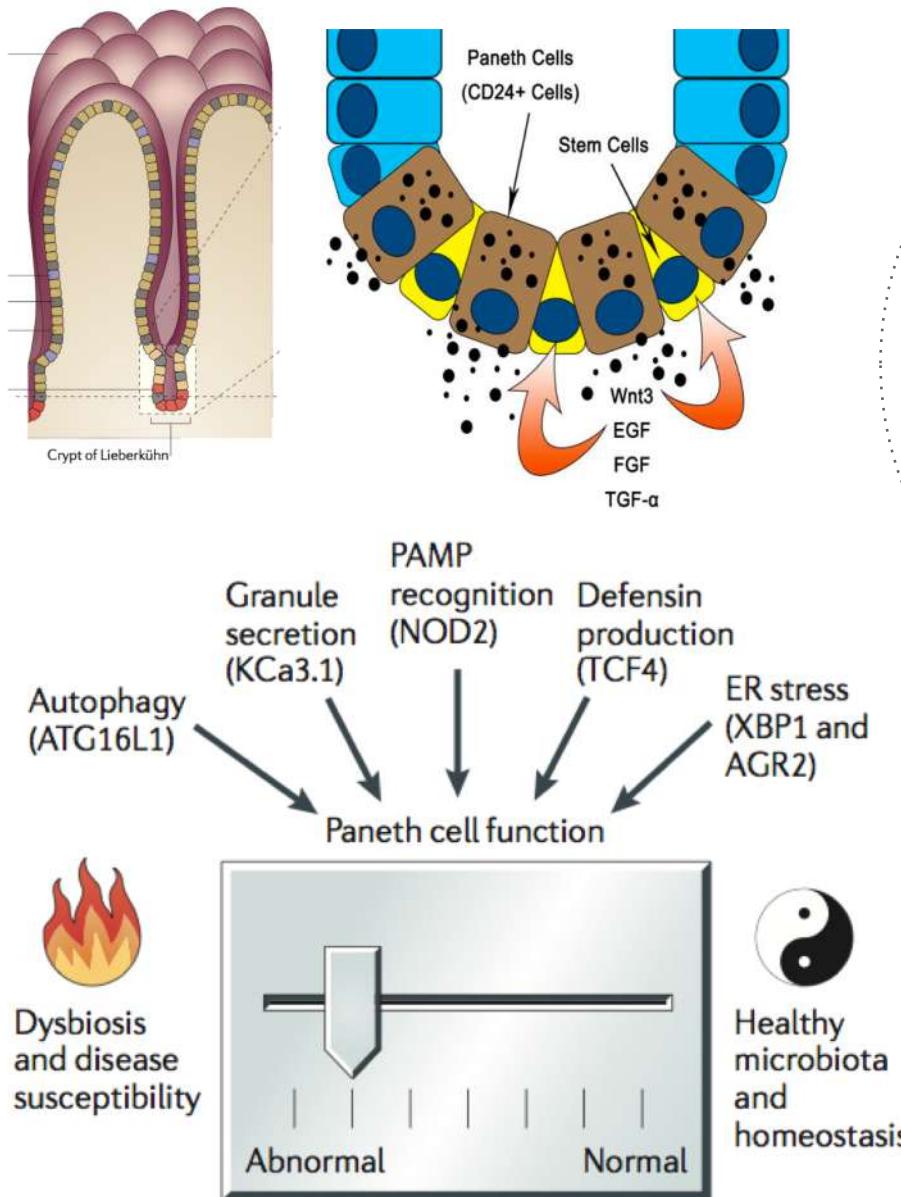


There is an interplay between pathogens and autophagy

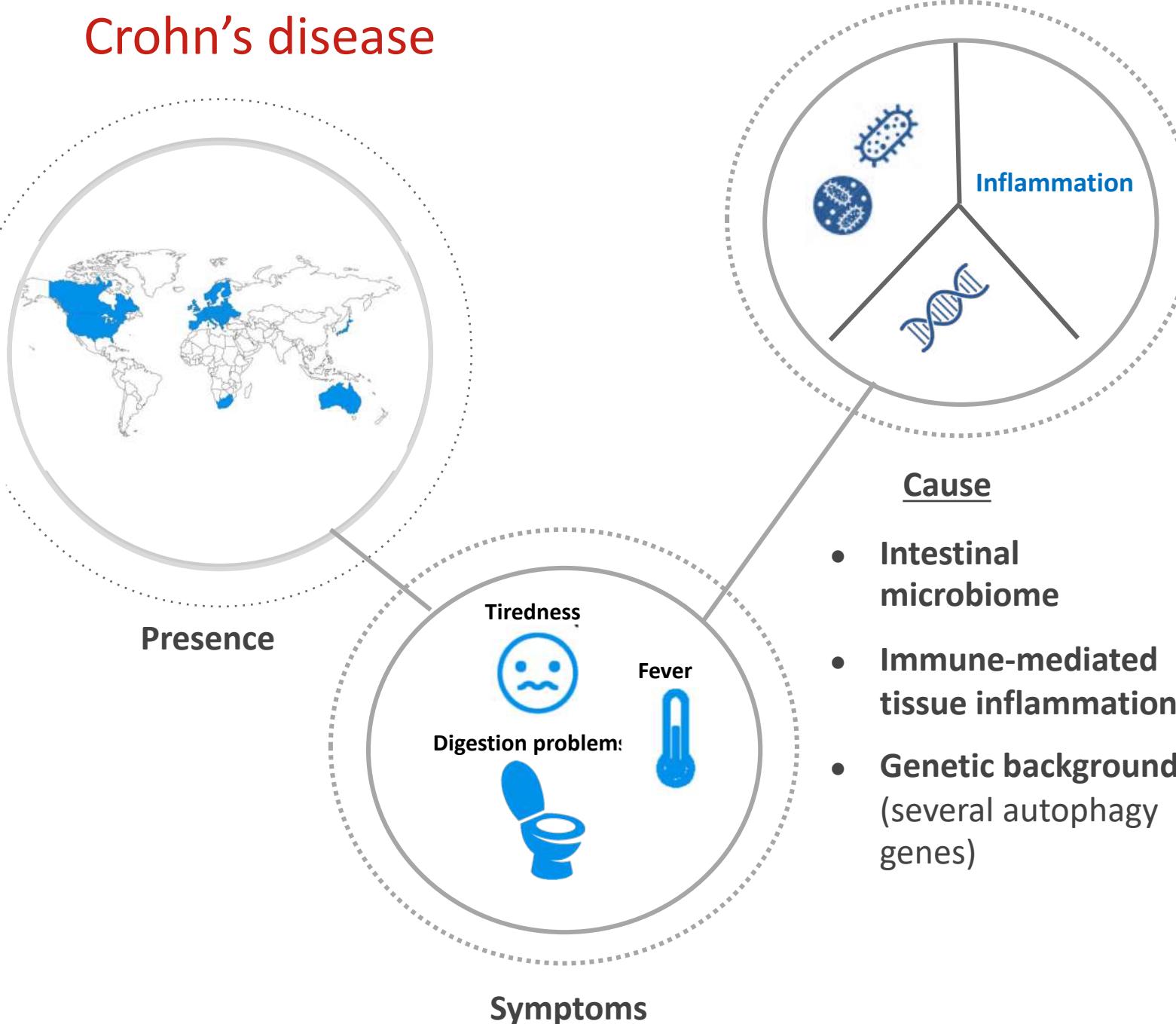


What are the autophagy
dependent cellular processes
in the gut?

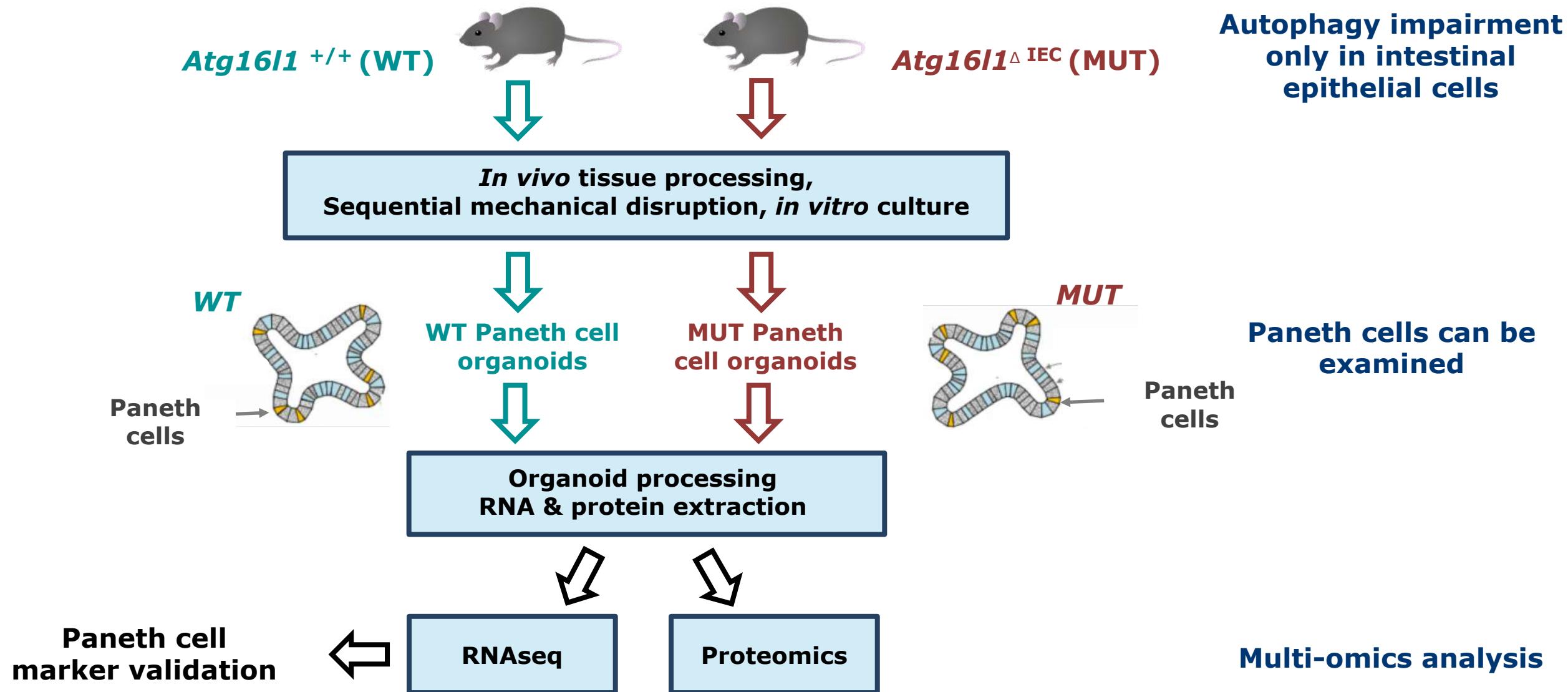
Paneth cells

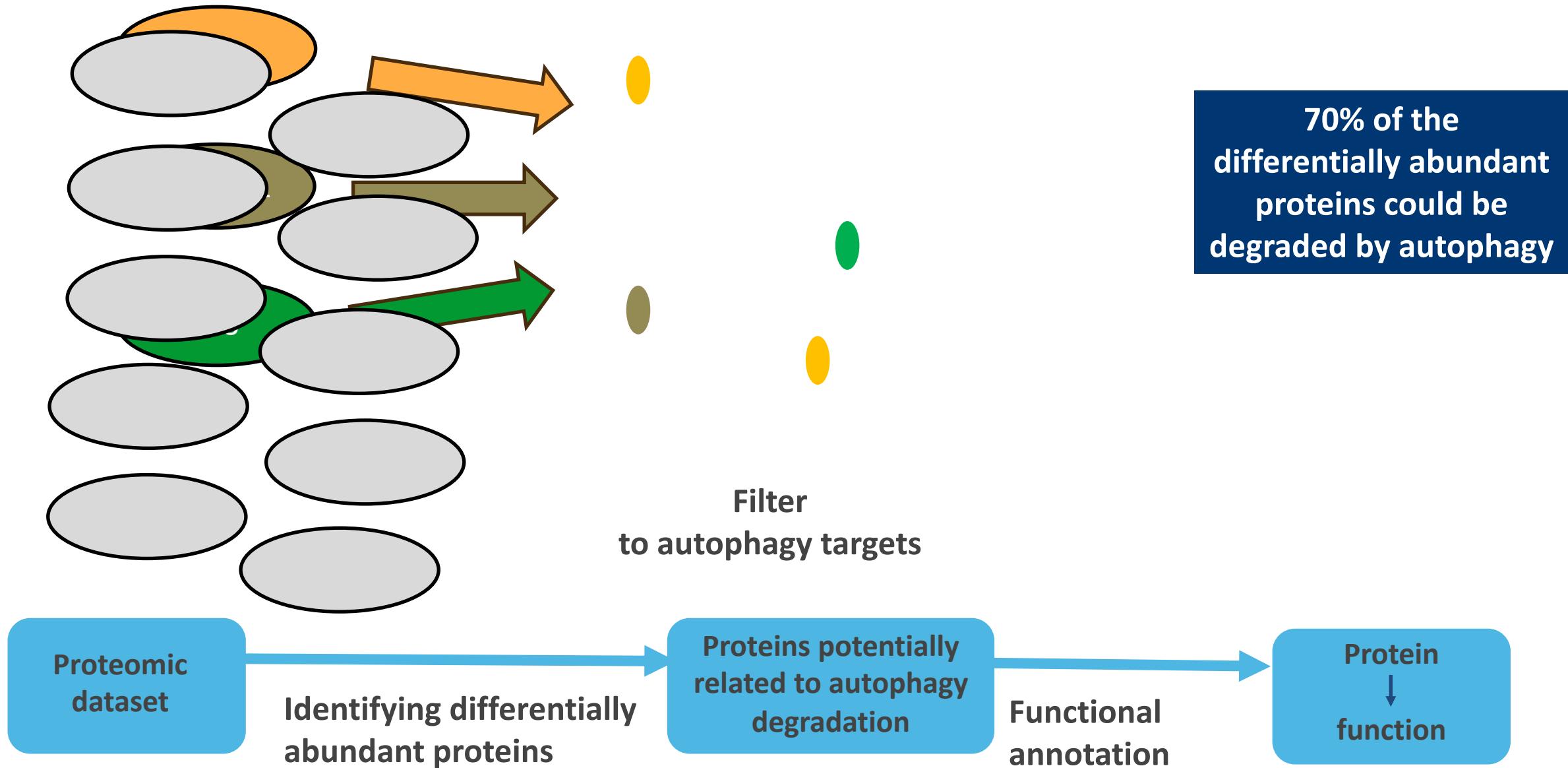


Crohn's disease



Workflow





Protein level change

UP



Prediction of the function change

Activation

UP



Inhibition

DOWN



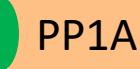
Inhibition

DOWN



Activation

Manual curation



PP1A



Immune response



CDK1



Exocytosis



SCG2



Apoptosis



CDC5L



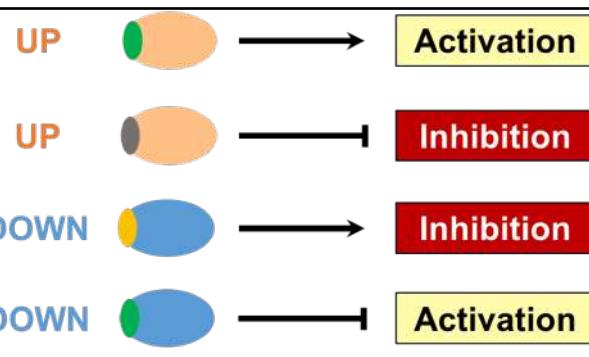
DNA Damage

Proteins potentially related to autophagy degradation

Functional annotation

Protein ↓ function

Functions affected by autophagy impairment



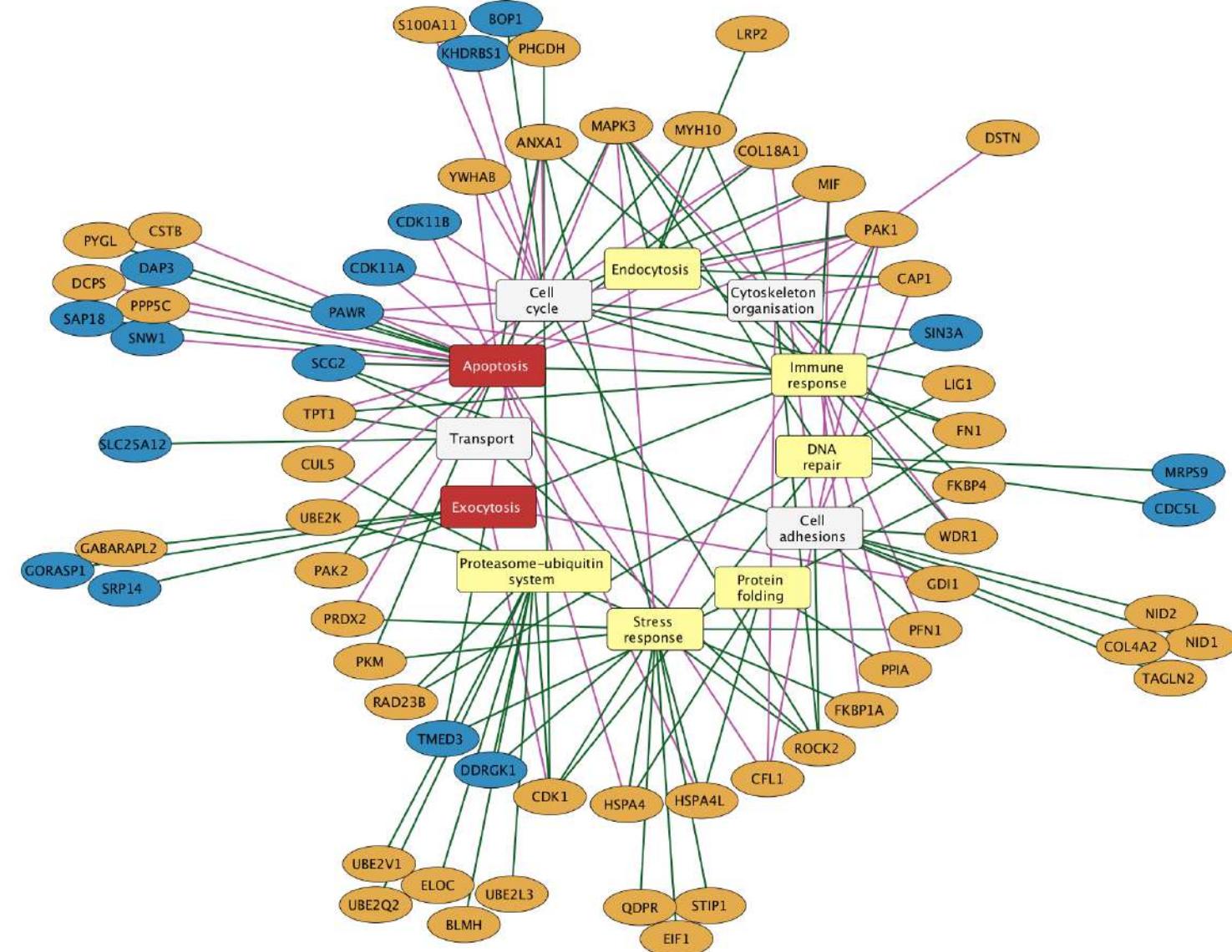
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✓

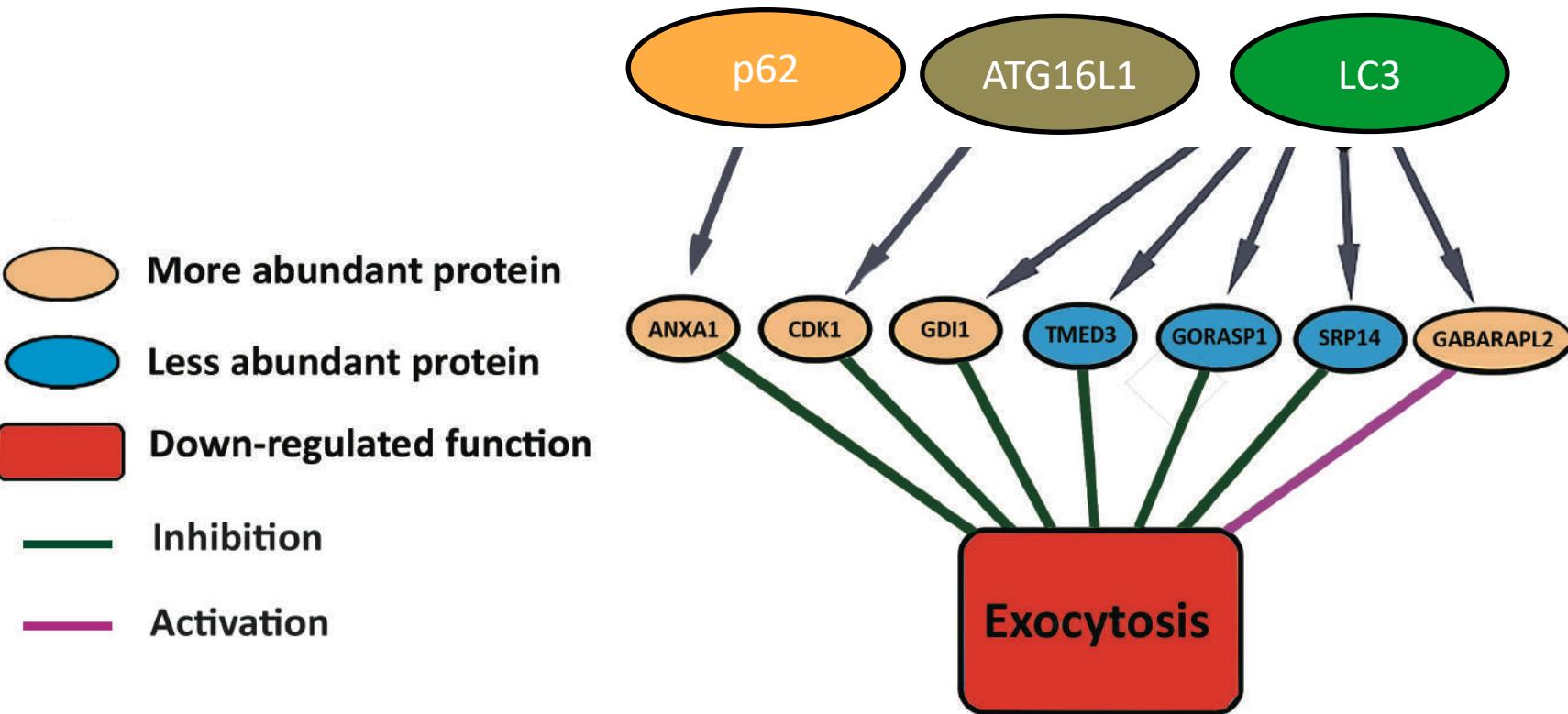
✓

✓

- Up-regulated protein
- Down-regulated protein
- Activation
- Inhibition
- Up-regulated function
- Down-regulated function
- Up- & down-regulated function

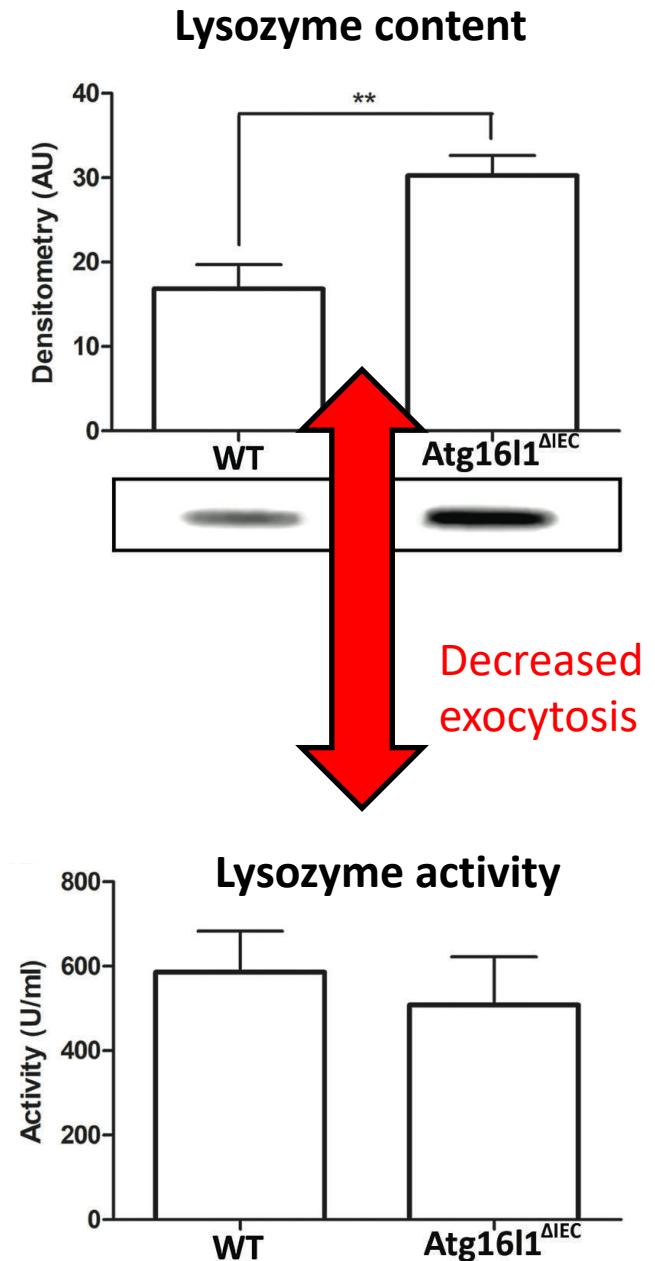


RNAseq data: the changes of putative autophagy target proteins are due to protein and not gene level changes



Jones *et al*, Disease Models & Mechanisms, 2019

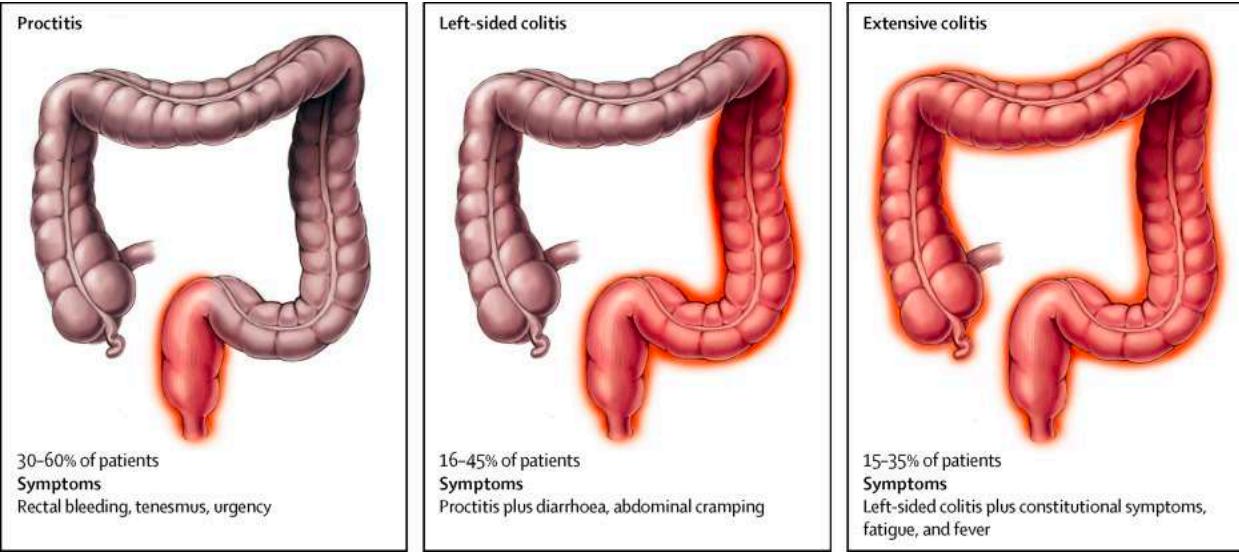
Treveil *et al*, Molecular Omics, 2019



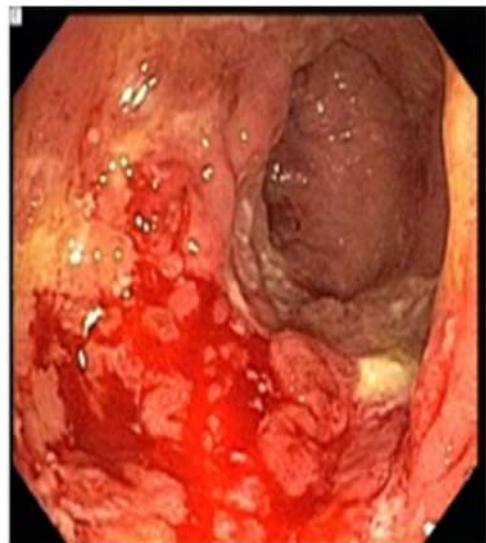
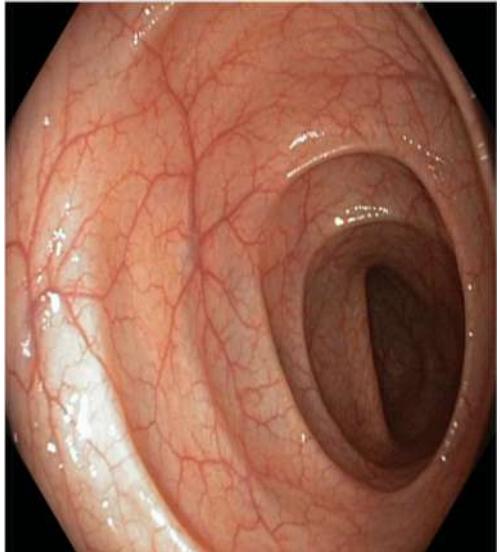
How can we combine genomics
information and PPI networks
on a patient-specific level?

Ulcerative colitis

- A type of Inflammatory Bowel Disease (IBD)
- 1:400 people in the UK;
- Costs the NHS >£700 million per annum
- Pathogenesis: mostly unknown
- Treatments: ineffective
- Patient stratification is based on therapeutic response and progression - no real markers



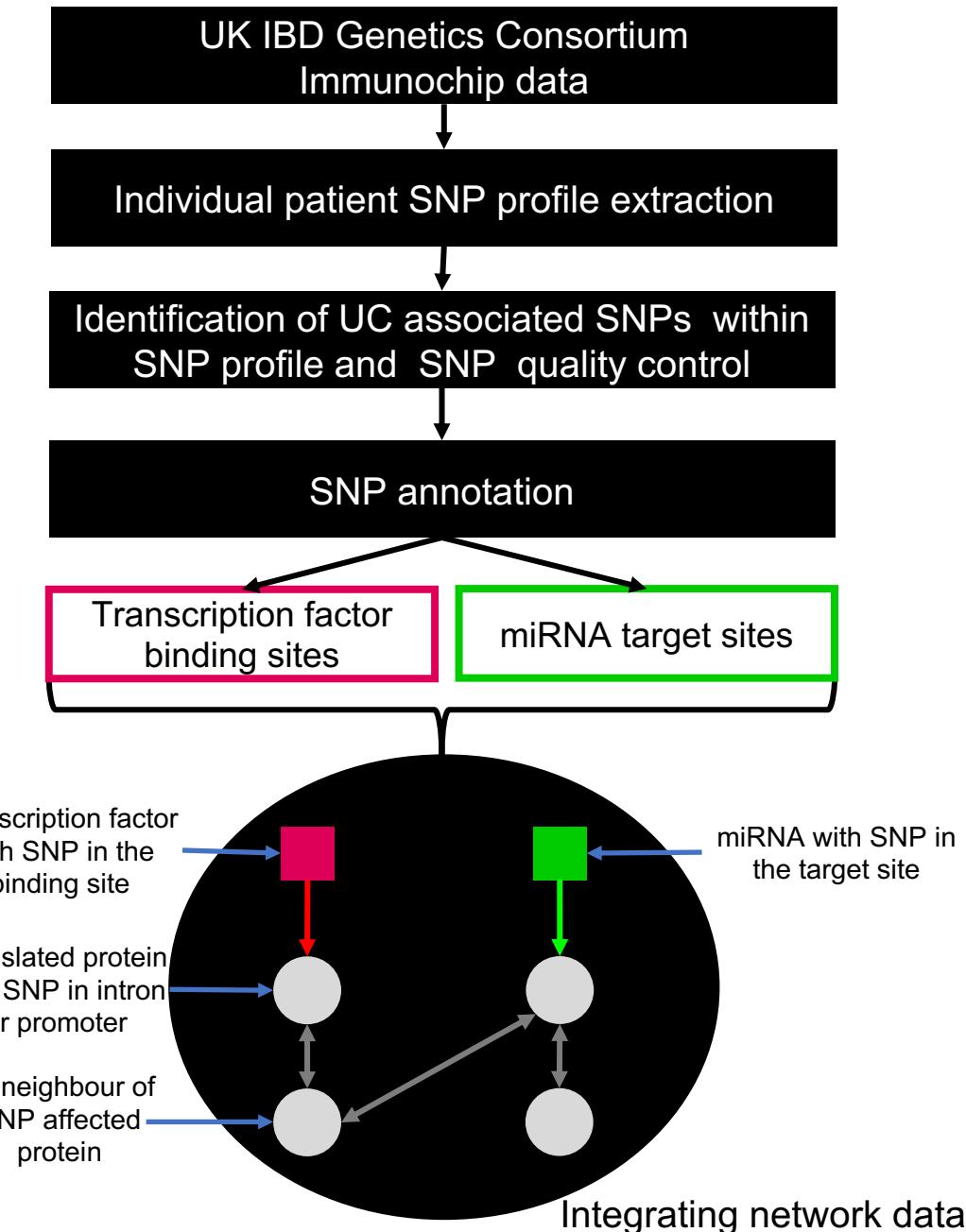
Ungaro *et al*, Lancet, 2017

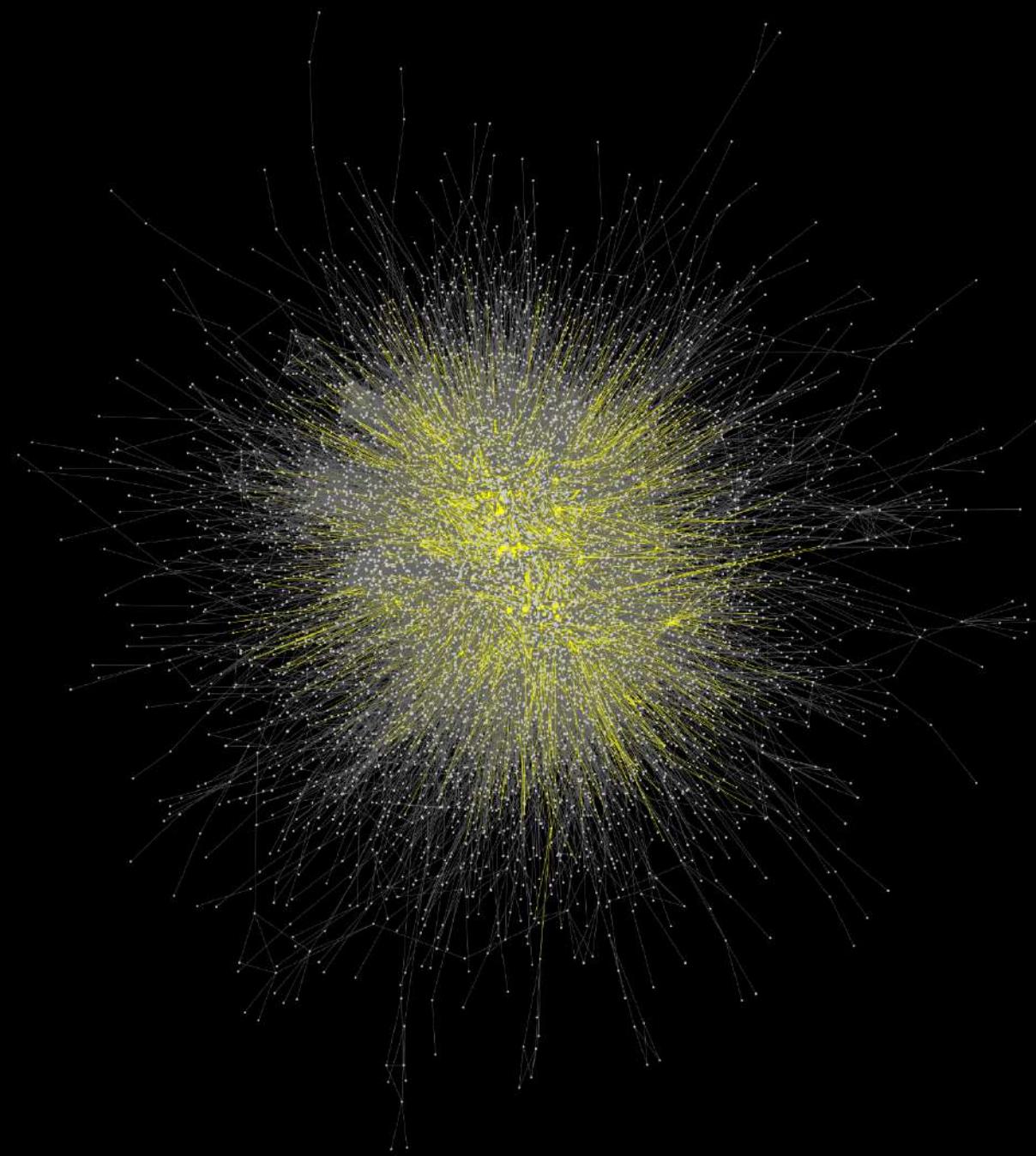


Can we understand better the pathogenesis and treatment options of ulcerative colitis with PPI networks?

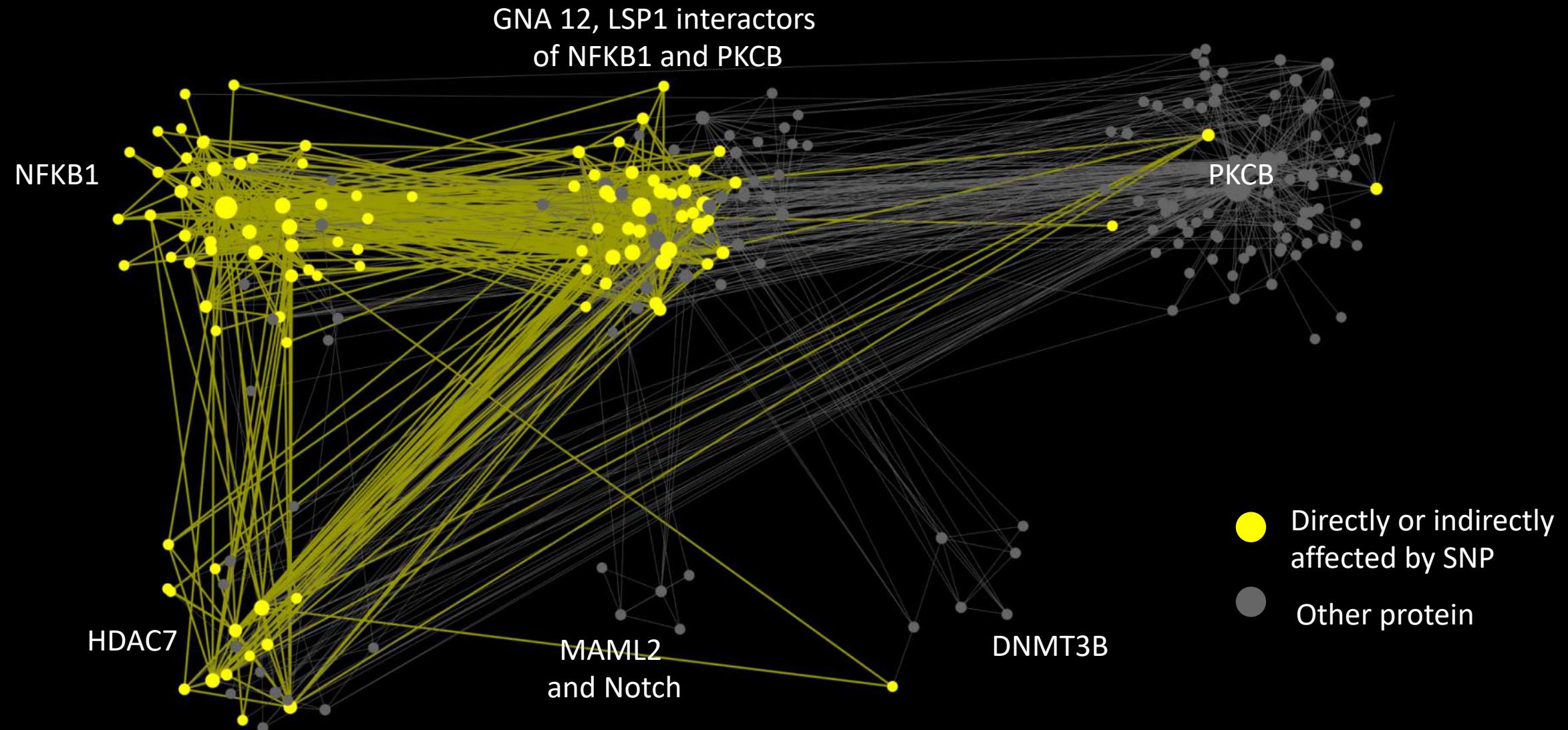
Patient-specific networks for ulcerative colitis patients

- GWAS – no major clinical impact
- 90% of SNPs are non-coding





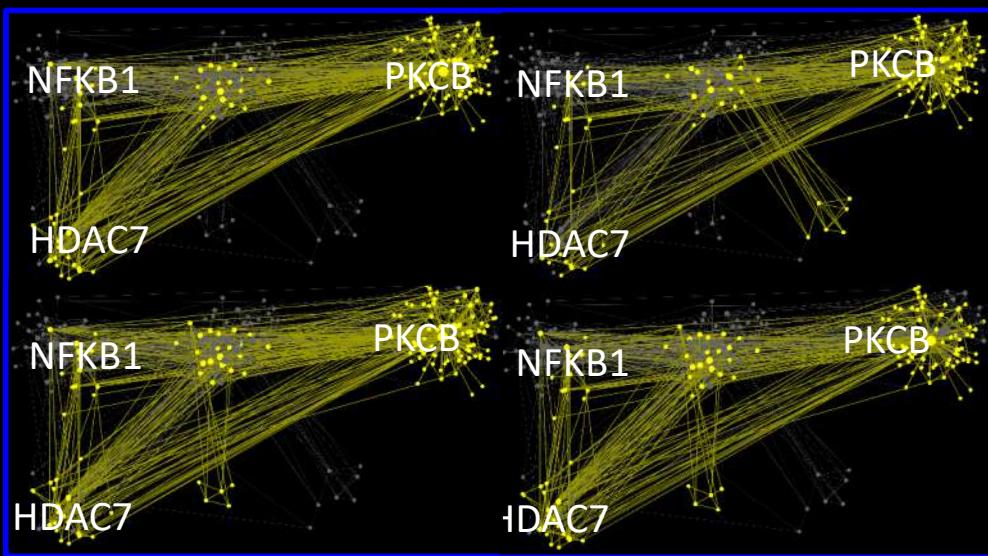
Patient-specific network footprint



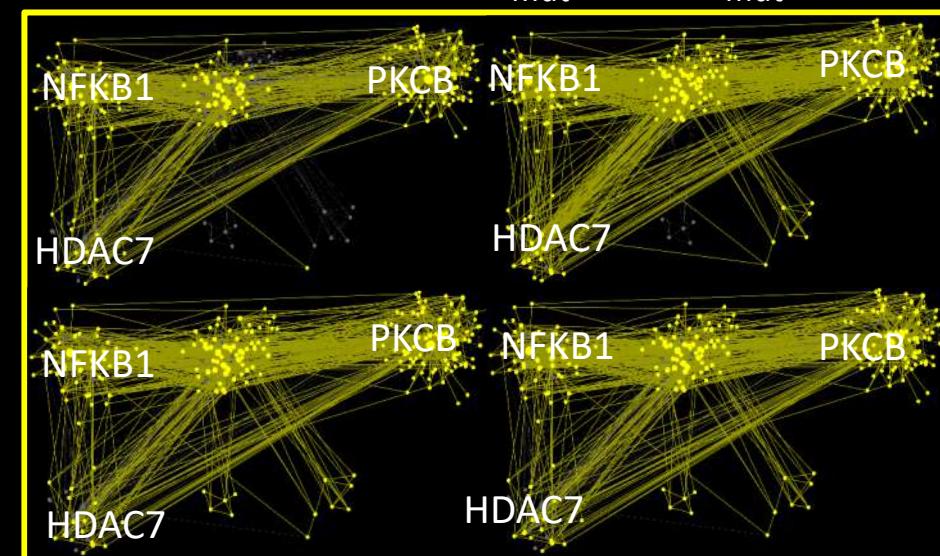
Clustering of patient-specific network footprints

Brooks et al, under revision

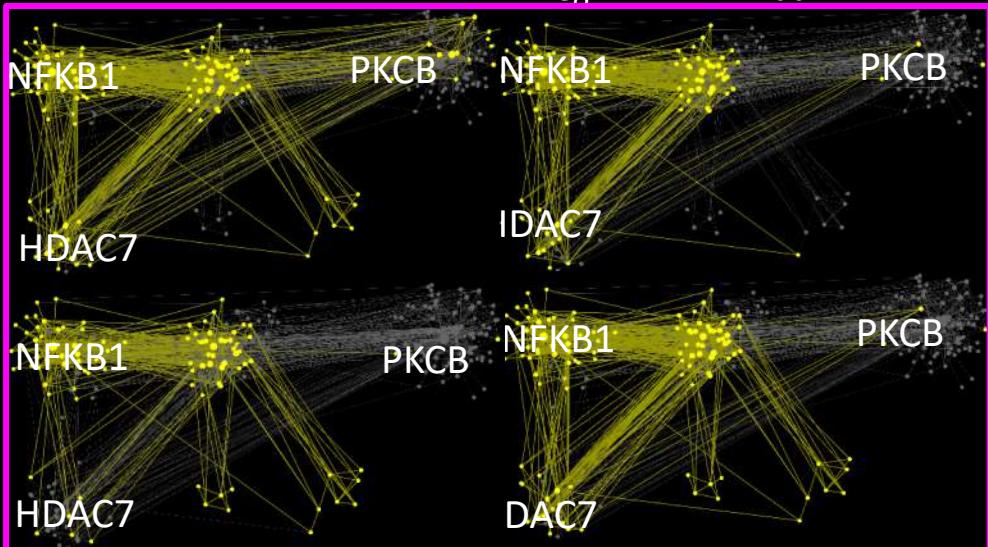
Cluster 1: PKCB_{mut} $\text{NFKB1}_{\text{neg}}$



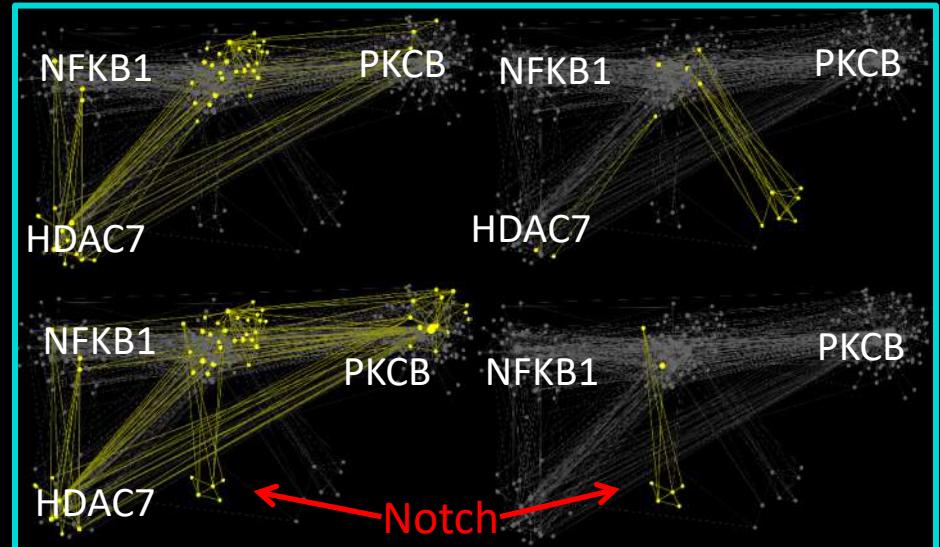
Cluster 3: PKCB_{mut} $\text{NFKB1}_{\text{mut}}$



Cluster 2: PKCB_{neg} $\text{NFKB1}_{\text{mut}}$



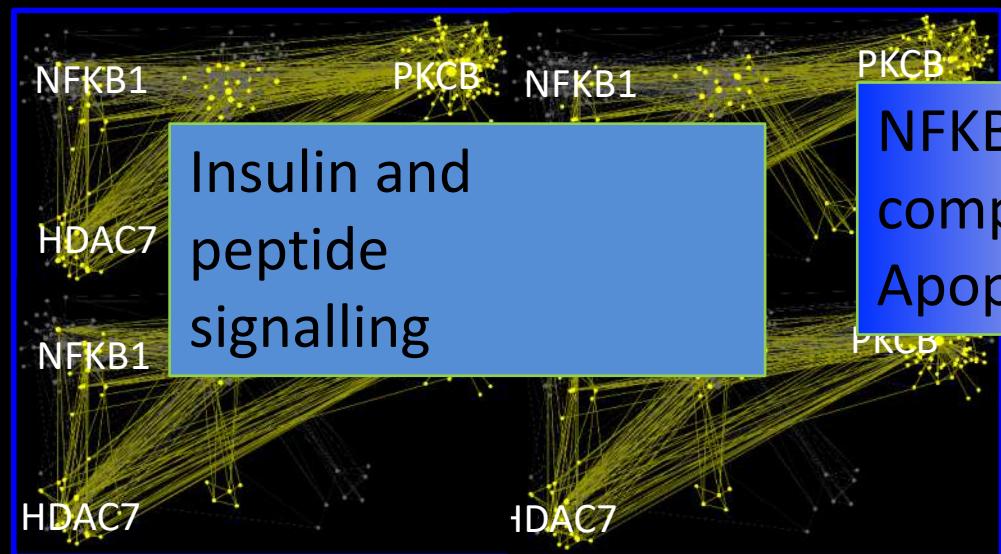
Cluster 4: PKCB_{neg} $\text{NFKB1}_{\text{neg}}$



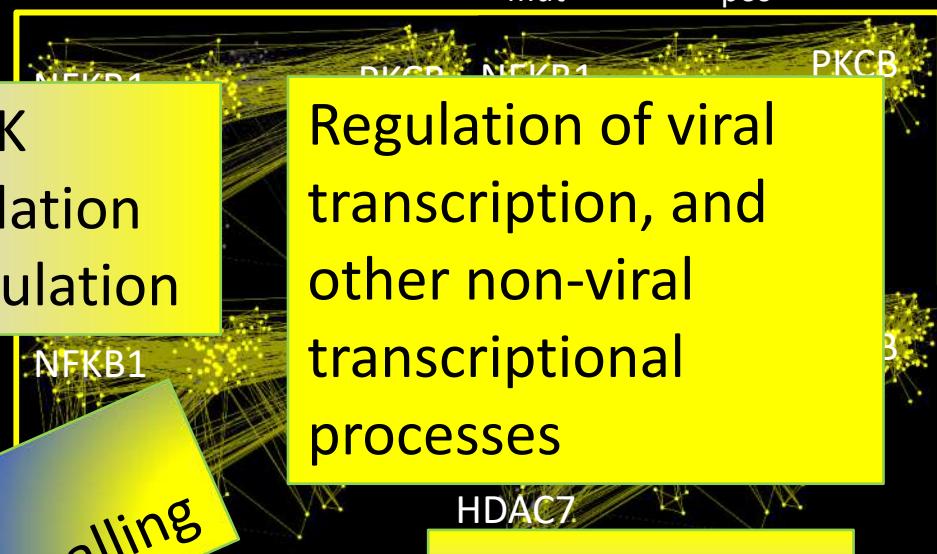
Clustering of patient-specific network footprints

Brooks et al, under review

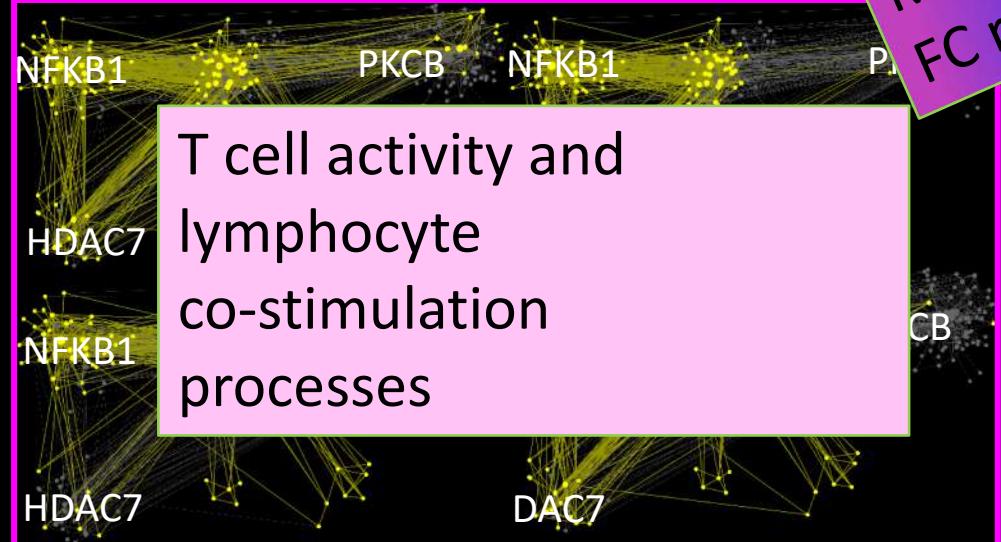
Cluster 1: PKCB_{mut} $\text{NFKB1}_{\text{neg}}$



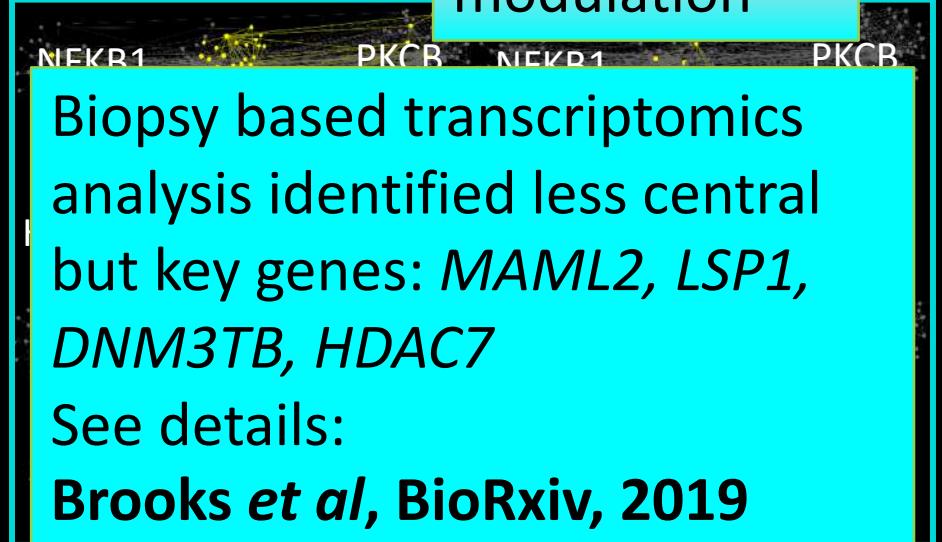
Cluster 3: PKCB_{mut} $\text{NFKB1}_{\text{pos}}$



Cluster 2: PKCB_{neg} $\text{NFKB1}_{\text{mut}}$



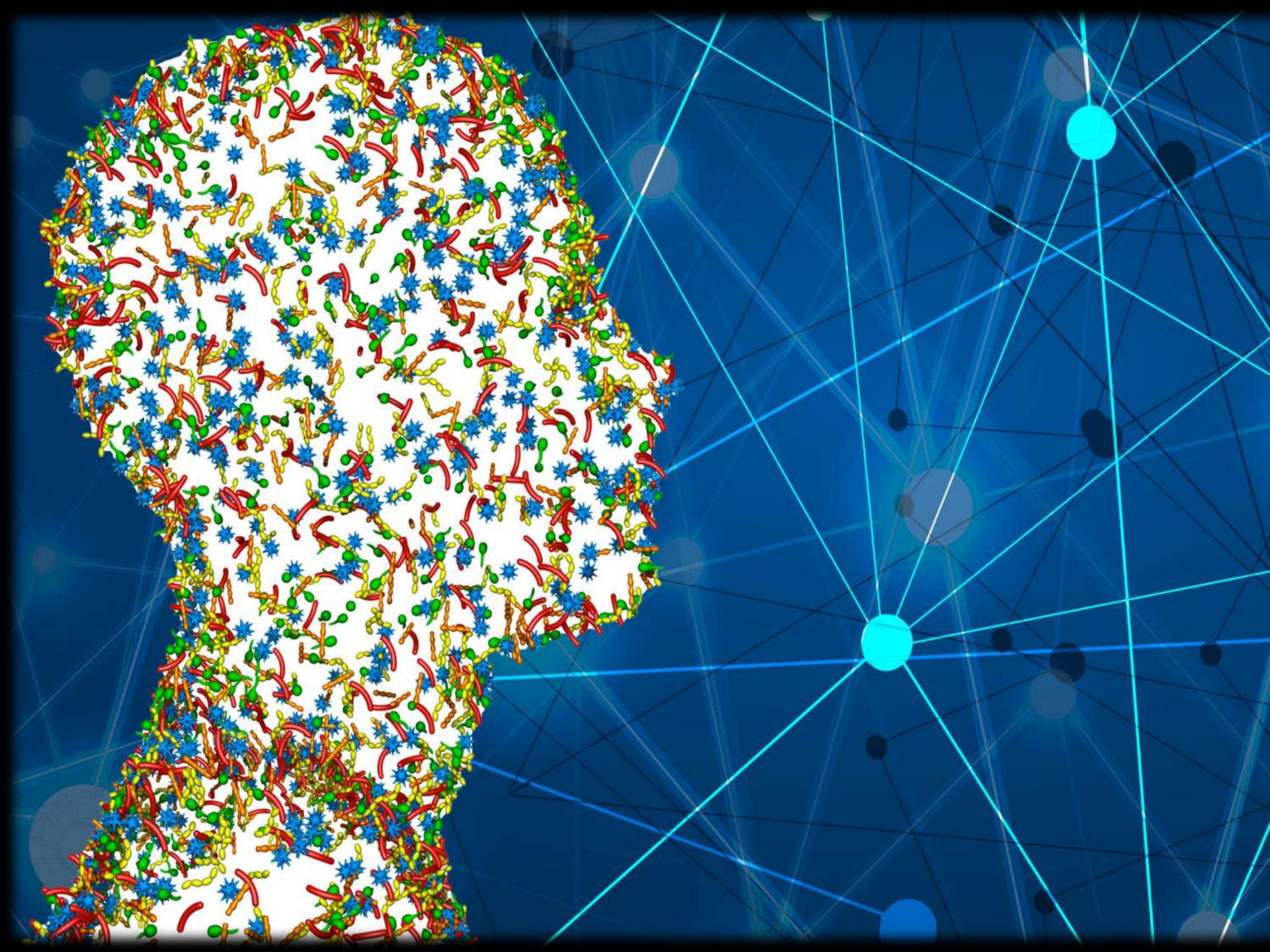
Cluster 4: PKCB_{neg} $\text{NFKB1}_{\text{pos}}$



Current and future plans



Host-microbe interactions in IBD

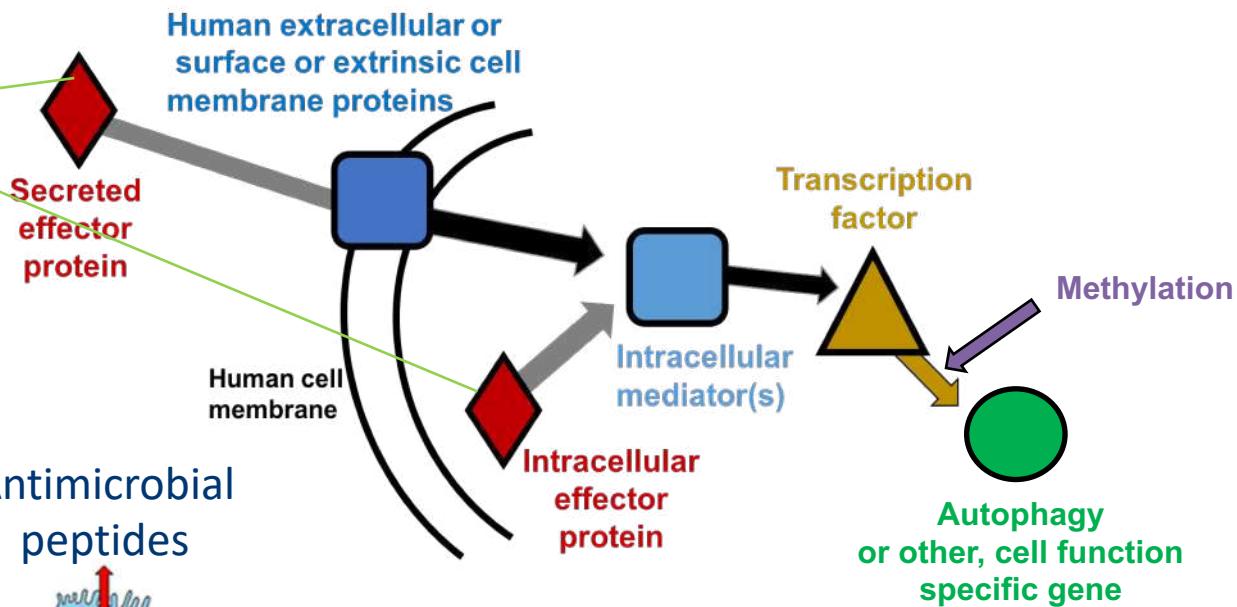
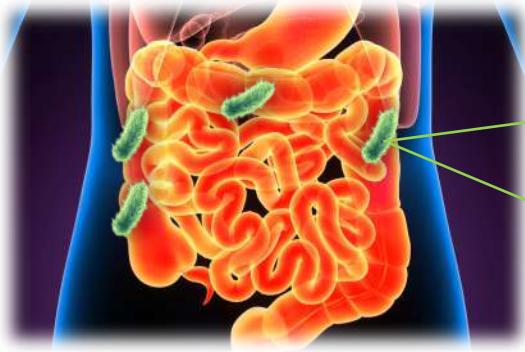


**How do signals
from microbes
modulate host
processes?**

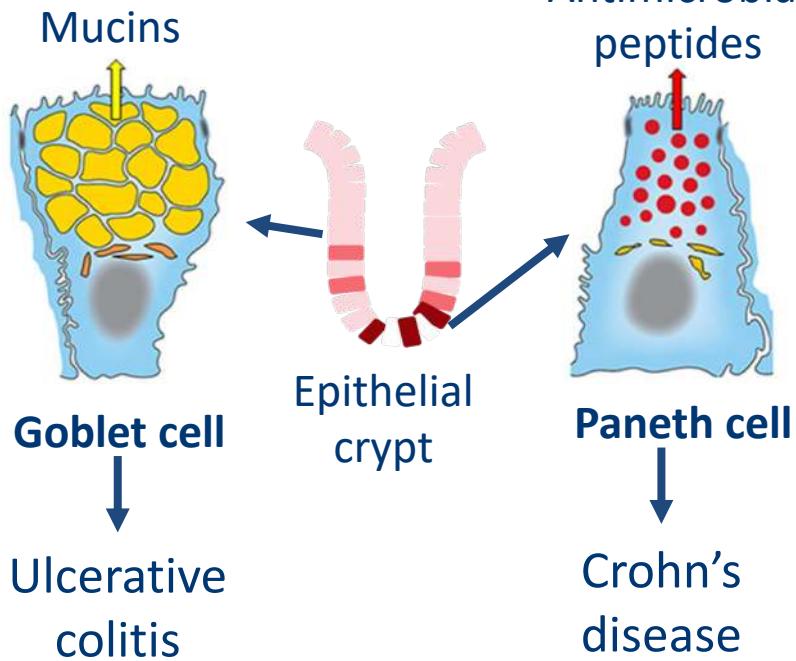
**How do our cells
rely on microbial
signals for their
normal functions?**

**How do host
mutations affect
microbial signal
regulated processes?**

Combining host-microbe interactions and systems genomics



Key cell types of interest



Approaches

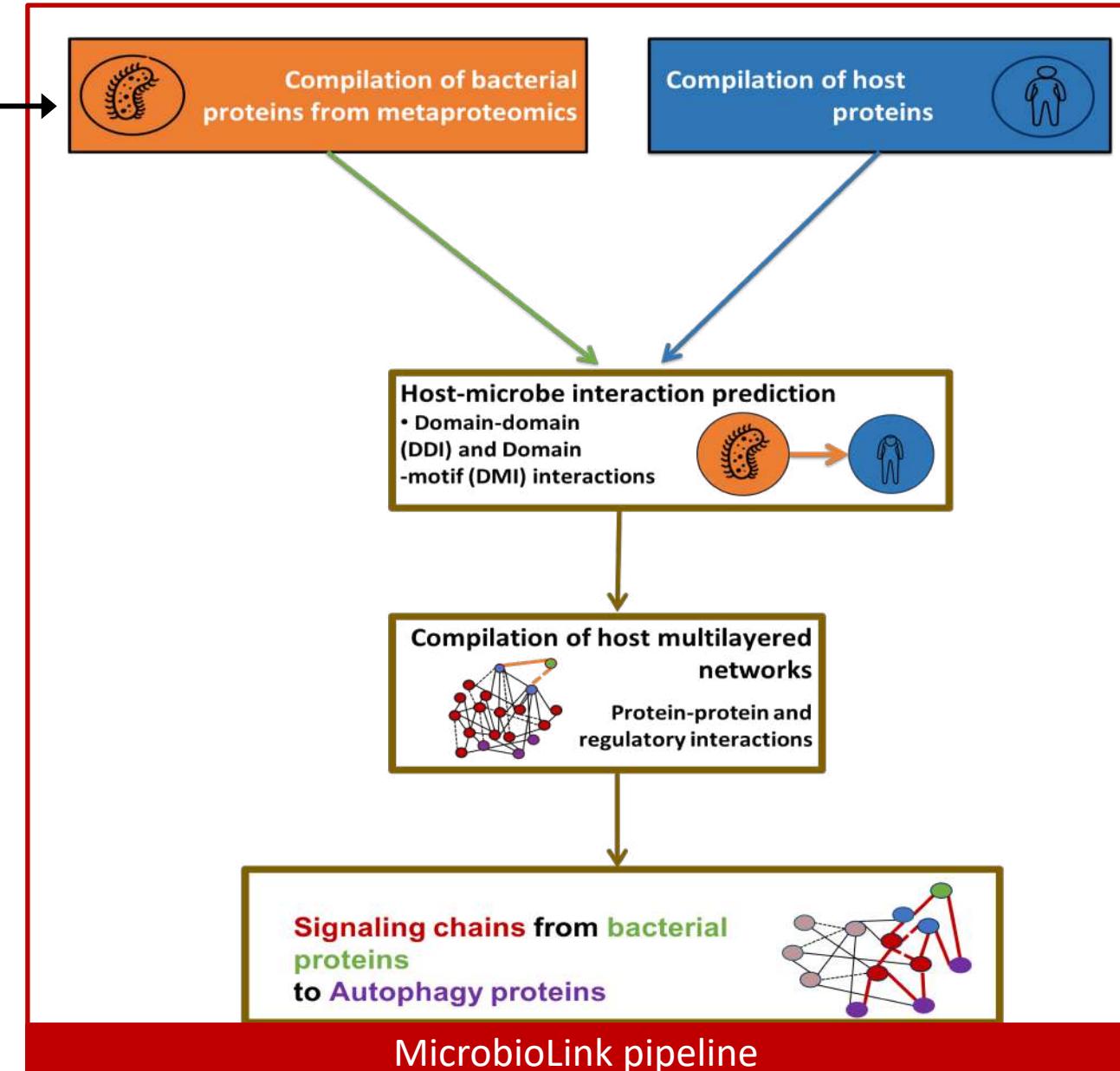
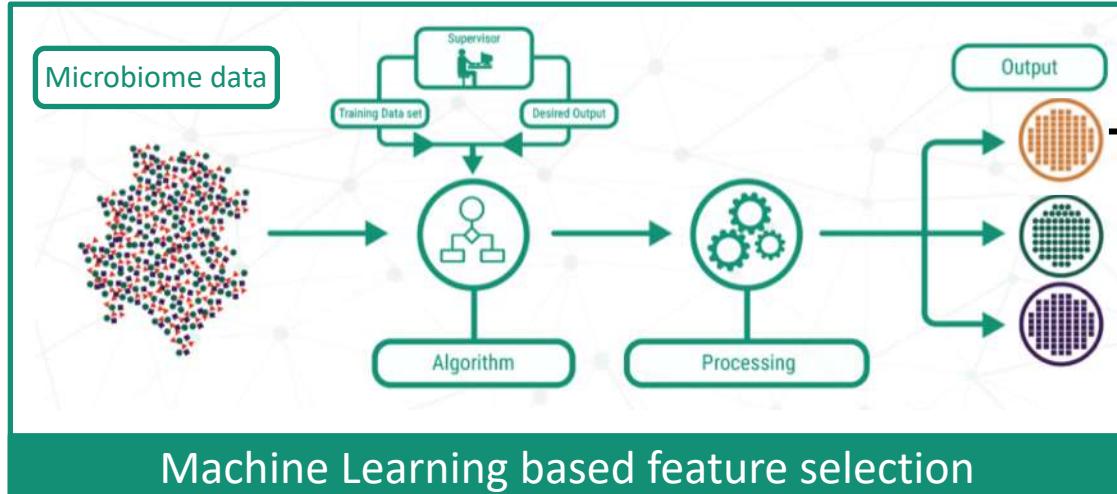
1. Microbiome analysis with machine learning and systems biology
2. Organoid monolayers and organoid array to test host-microbe interactions
3. Imaging and single cell/low-input parallel genomics and transcriptomics (G&T) profiling

How do signals from microbes modulate host processes?

How do our cells rely on microbial signals for their normal functions?

How do host mutations affect microbial signal regulated processes?

1. Uncovering host-microbe interactions and predicting their effect on the host



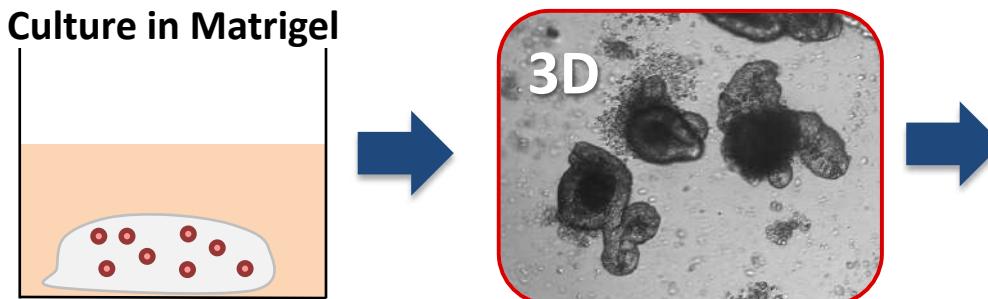
- Integrate host signaling and regulation with host-microbe molecular mechanisms
- Validation and modeling of networks with 'omics read-outs from the host
- Scalable, agnostic to microbial species

2. Validating host-microbe interaction with organoids

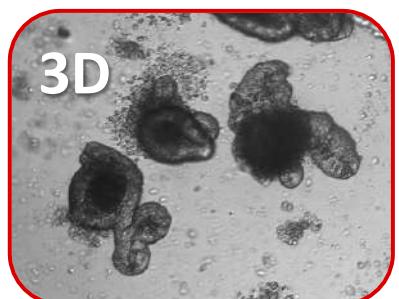
In vivo crypts



Culture in Matrigel

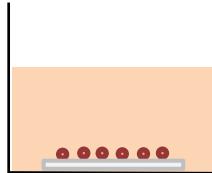


In vitro 3D organoid

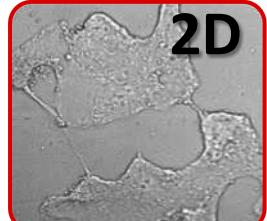


Study response to
Basolateral challenges

Culture on thin
Matrigel layer



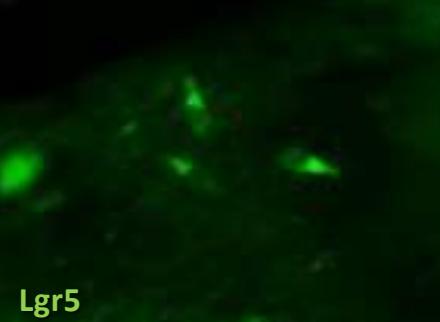
In vitro organoid
monolayer



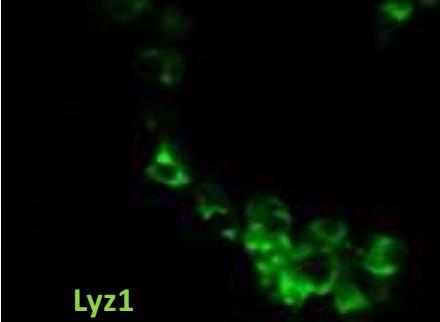
Study response to
Luminal challenges

Specific intestinal cell types represented

Stem Cells



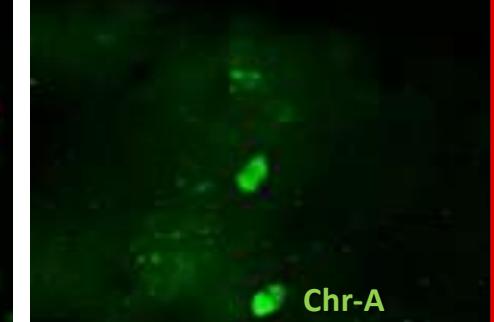
Paneth cells



Goblet cells

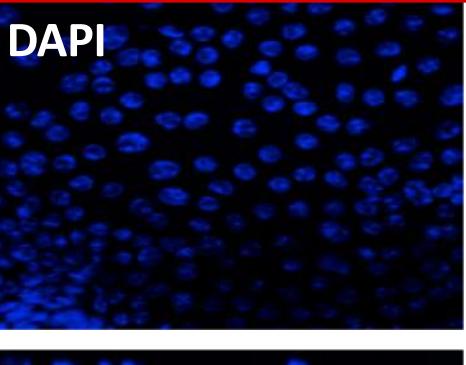


Enteroendocrine cells:

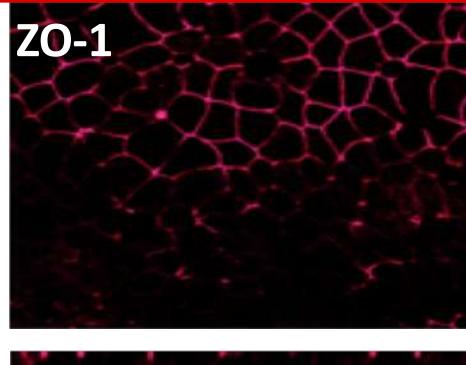


Polarised 2D Intestinal Organoid models

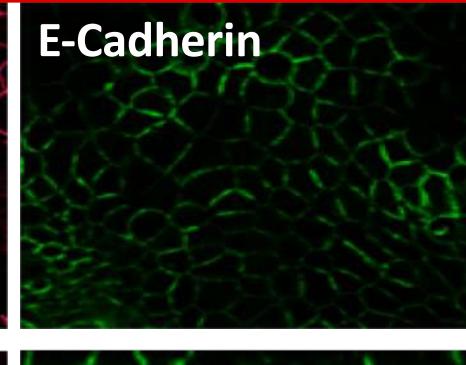
DAPI



ZO-1



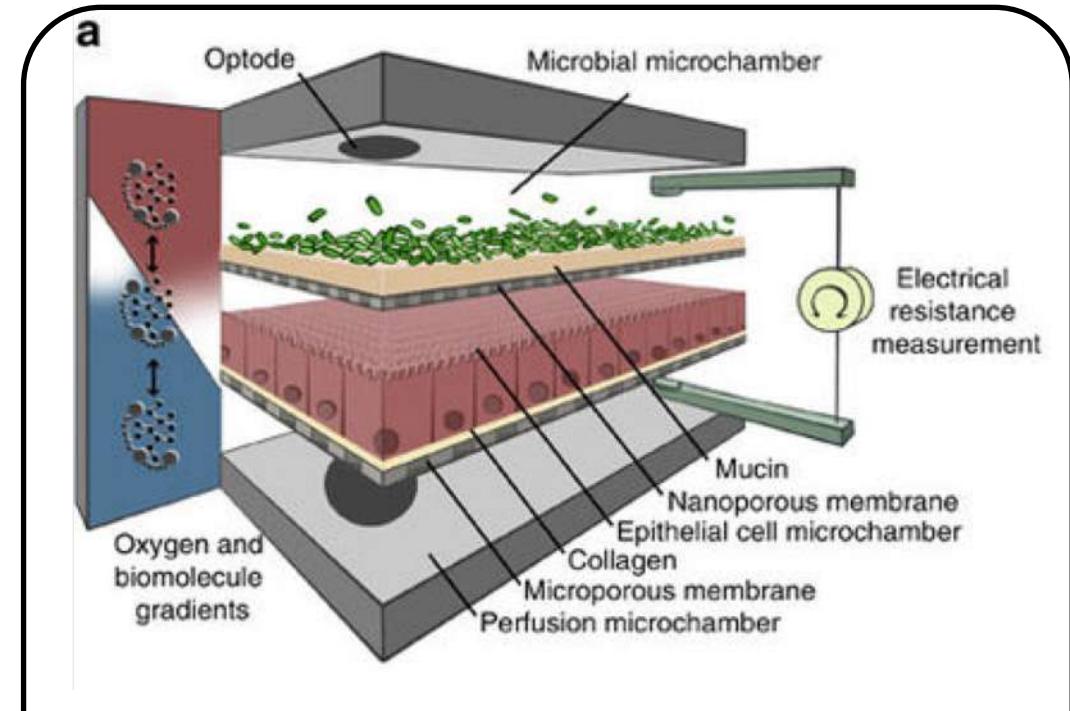
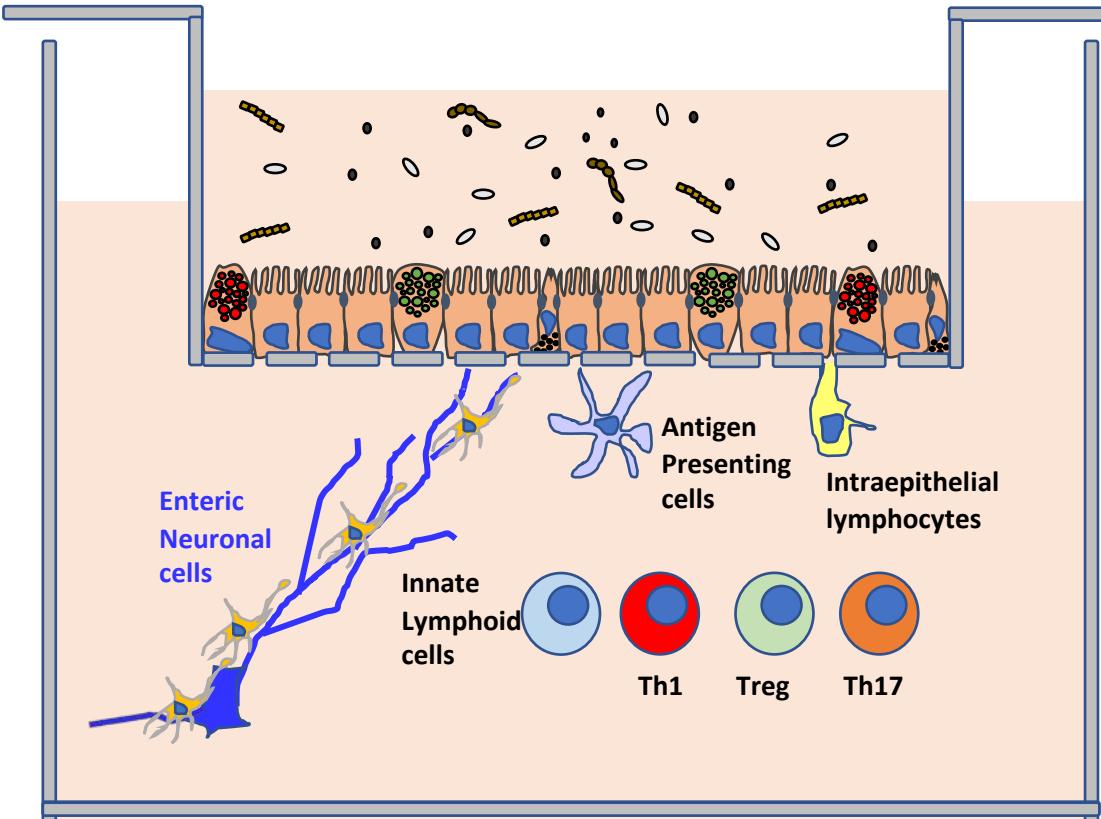
E-Cadherin



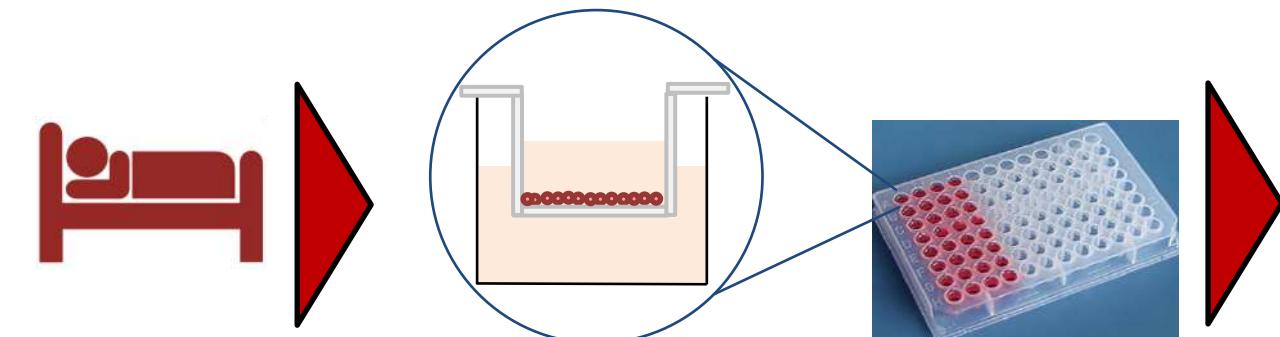
B-Catenin



3. Analysing the effect of microbes, microbial products and host cells on the intestinal epithelium

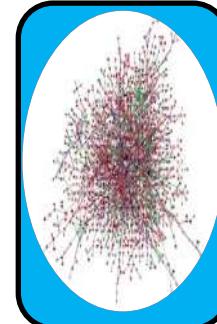


HuMiX: Shah *et al* (Paul Wilmes, Luxemburg)
Nature Communications 7, 11535 (2016)



Multi-omics analysis

- ❖ High-Content Screening based imaging (KCL)
- ❖ Transcriptomics
 - ❖ mRNAs, miRNAs & lncRNAs
 - ❖ Batch or on a single-cell level
- ❖ Genomics (methylation)



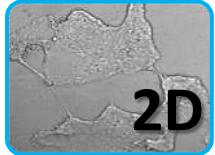
A systems biology pipeline for personalised microbial studies



Patients

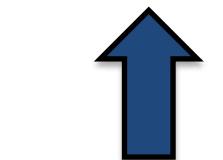


In vitro organoids

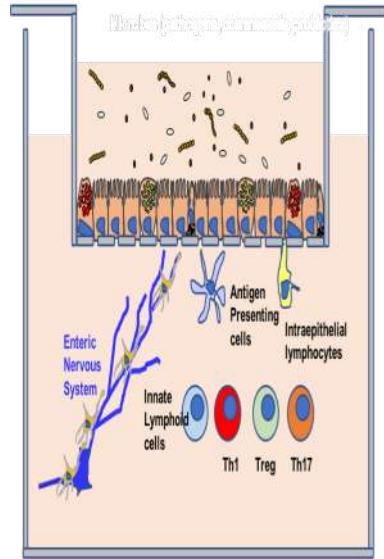


2D

- Cell type specific
- Flat organoid



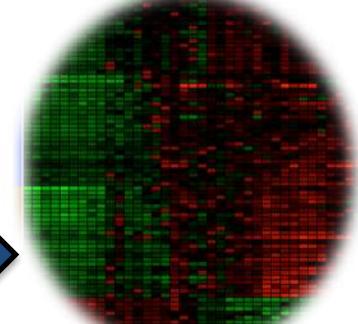
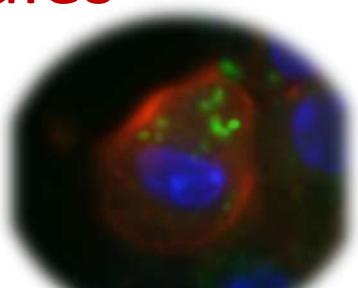
Murine disease
models



- Treatment with microbes or microbial products
- Co-culture with other cell types

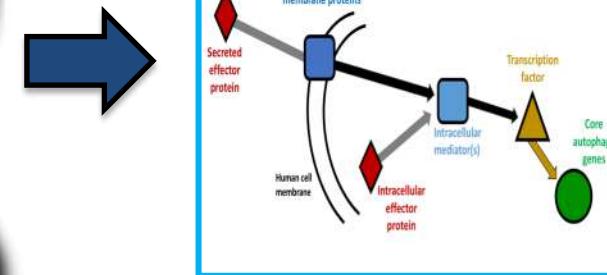
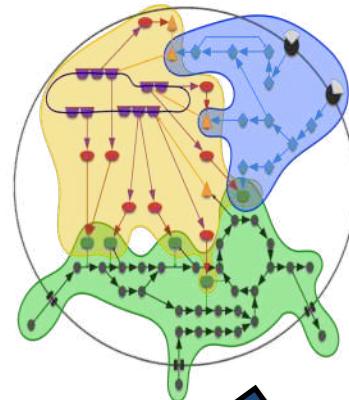


- High-throughput



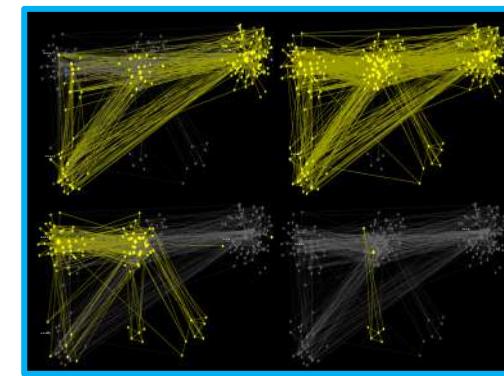
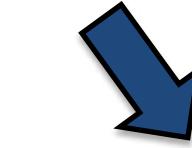
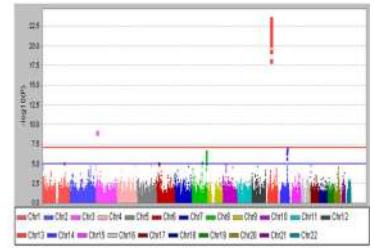
- Imaging (incl. HCS)
- FACS
- Transcriptomics
- Single-cell
- Proteomics

Integrated networks



- Cell and condition specific host-microbe interaction analysis
- Identification of master regulators and affected host processes

Mutation data



Patient-specific networks



Precision selection of beneficial and disadvantageous microbes and therapies

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Thank you!

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