Targeting Human Host and Microbe Interactions in Drug Discovery

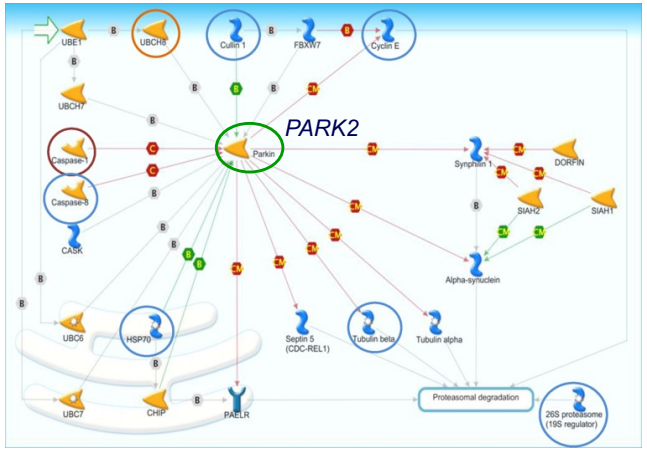
James R. Brown, M.Sc., Ph.D.

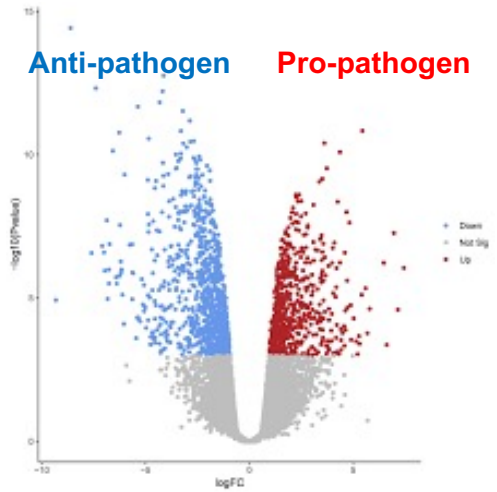
Visiting Scholar, Drexel University

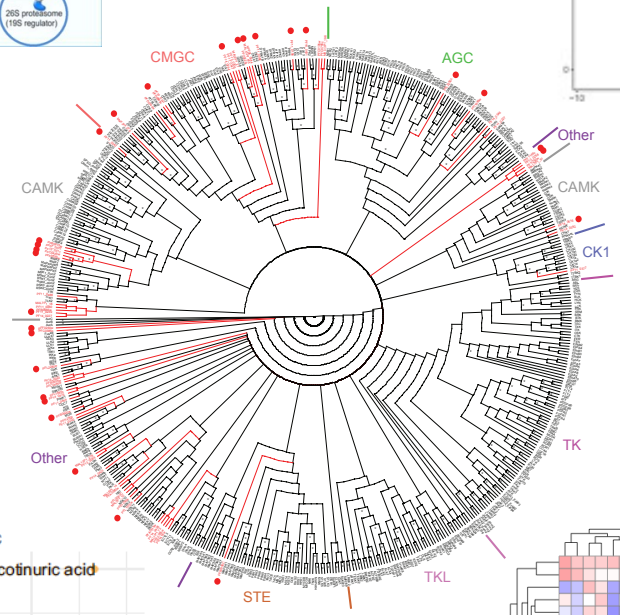
Courtesy Professor, U. Florida Gainesville

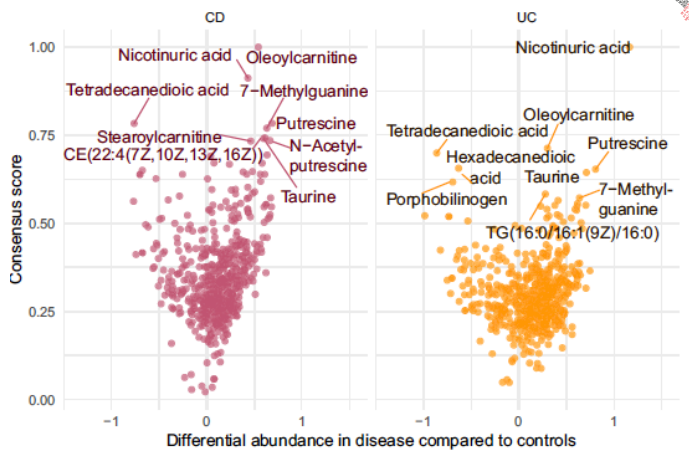
JRBrown Bio Consulting LLC

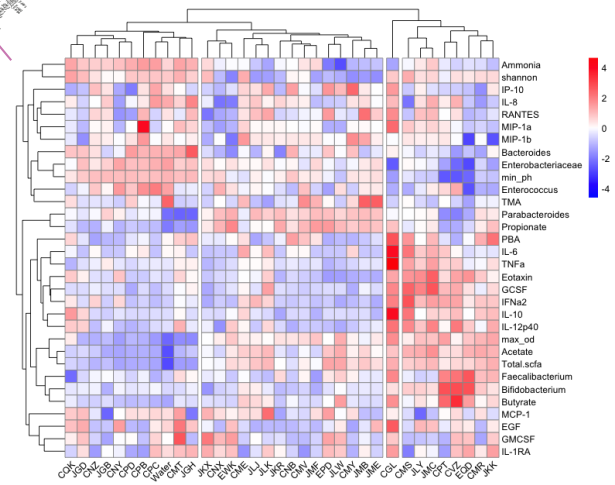
Former affiliations: GSK , Kaleido Biosciences, Novasenta, Dalhousie U., Simon Fraser U., McGill U.











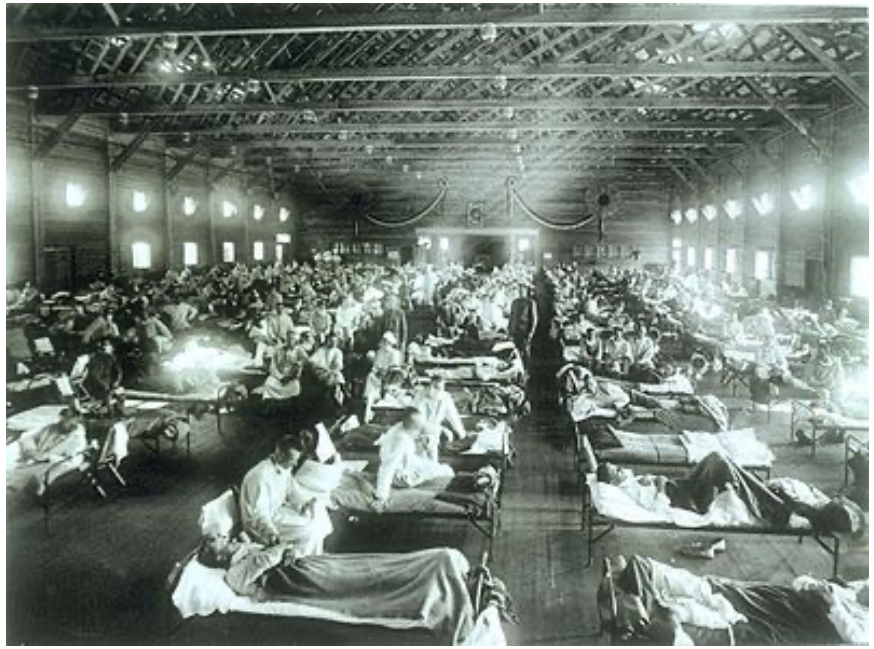
Outline

1. Targeting human host factors for infectious diseases

2. Multi-omics data analysis of human protein-metabolite interactions

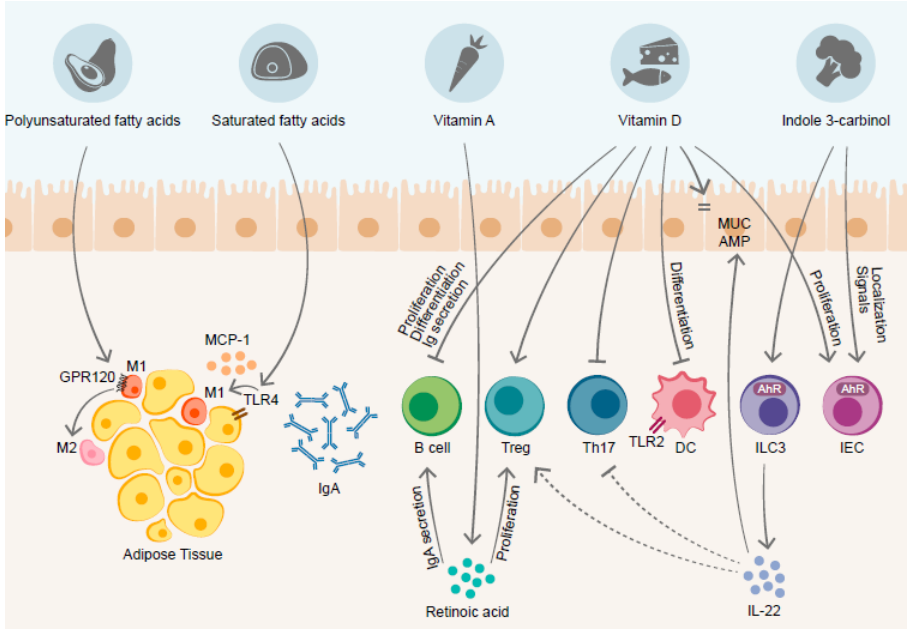
Host-microbe Interactions: The Immune System Balancing Act

- Microbiome and pathogens interact with the host in different ways.
- Complex immune pathways have evolved to orchestrate an effective defense against a wide range of ***pathogens*** while still promoting colonization of ***beneficial microbes*** for dietary energy and immune homeostasis.



1918 Flu Pandemic

Immune response

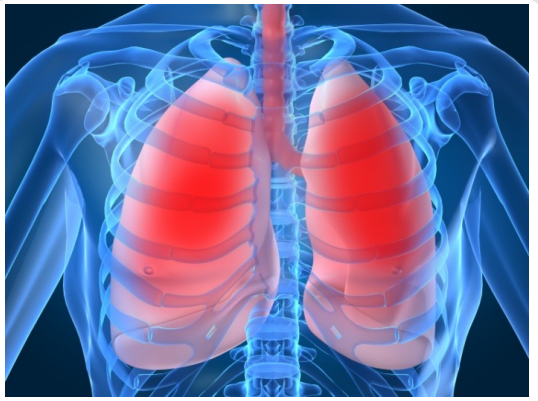


Microbial conversion of food stuffs & GI tract immune homeostasis

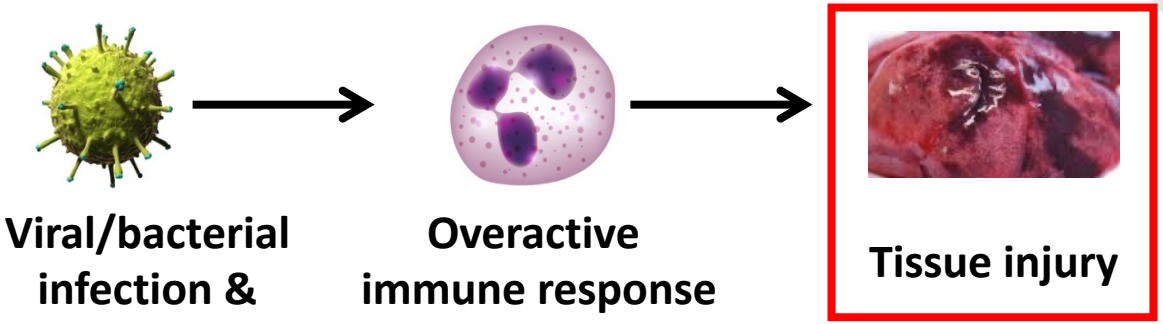
The Rationale for Host Defense Targets

- Pathogens can readily mutate into multi-drug resistant strains while interactions with human targets are less susceptible to selection pressures.
- Potential to mitigate collateral tissue damage caused by overactive immune response to infection.
- Potential for broad applicability across multiple, genetically diverse pathogens.
- Broader range of human drug targets and chemical matter in pharma inventories.
- Compared to drugs targeting the pathogen, entities that modulate human targets have lower effective dosing levels (potentially de-risking drug toxicity).
- Minimize collateral damage to the microbiome.

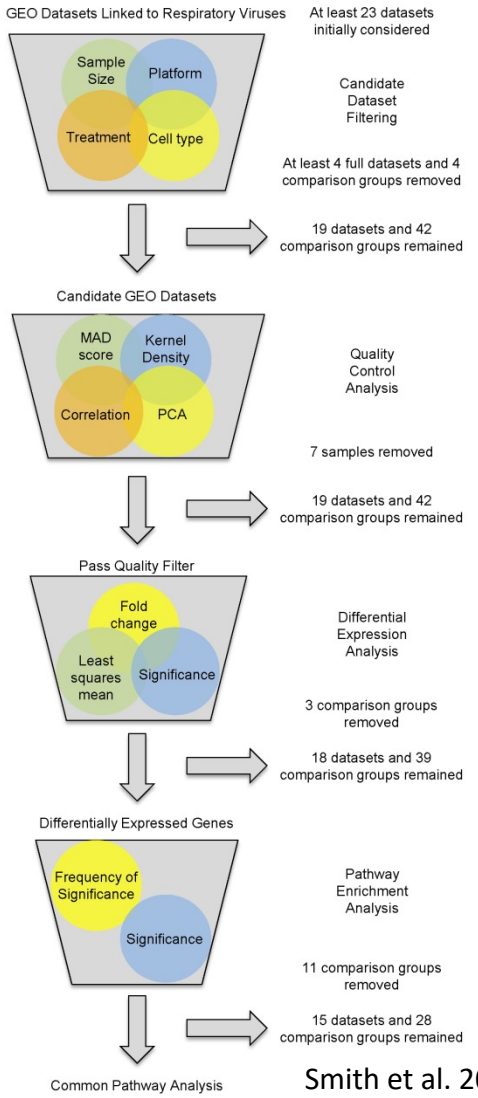
Severe respiratory infections
(viral & bacterial)



Opportunities for intervention



Host Response to Respiratory Viral Infections



OPEN ACCESS Freely available online PLoS one

Identification of Common Biological Pathways and Drug Targets Across Multiple Respiratory Viruses Based on Human Host Gene Expression Analysis

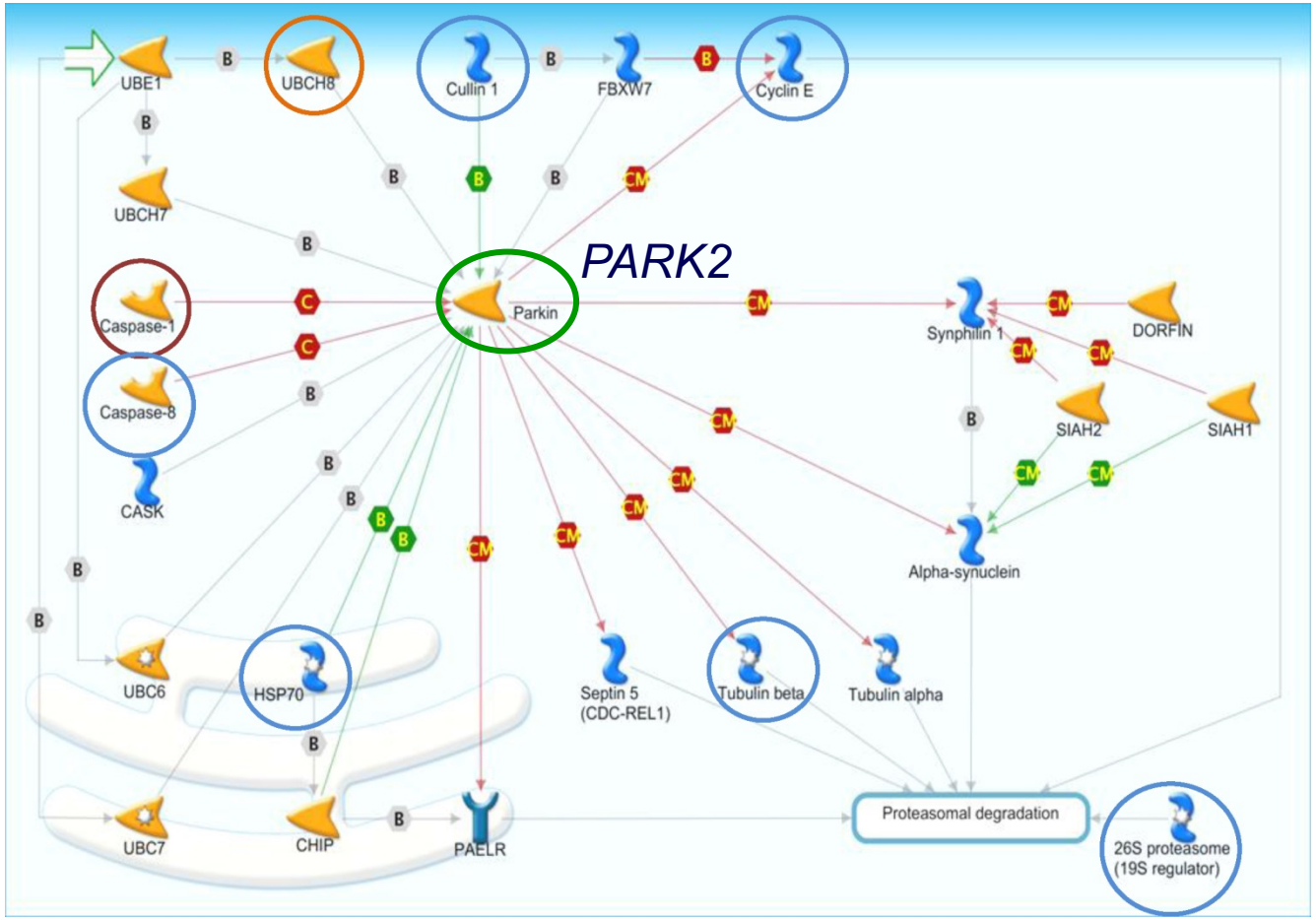
Steven B. Smith[1,2], William Dampier[3], Aydin Tozeren[3], James R. Brown[4*], Michal Magid-Slav[2] 2012

Steve Smith (M.Sc. Student; Data Scientist, Labcorp)
Will Dampier
Aydin Tozeren

- Analysis of human gene expression studies across seven common respiratory tract viruses
  - Respiratory synovial virus (RSV); Metapneumonia virus; Influenza A virus; Coronavirus (SARS); Rhinovirus; Coxsackievirus; Cytomegalovirus
  - Public RNA-array datasets with matched infected and un-infected human cell-types
  - Extensive QC criteria
  - Performed pathway enrichment and druggable target analyses
- 67 pathways in common among all seven viruses
- Multiple novel anti-viral and tissue damage targets (from Drug Bank and literature)
  - IL1B – Antagonists such as Canakinumab
  - TNF – Antagonists such as Pranlukast
  - CASP1 – Antagonists to reduce inflammatory damage
  - MMP9 – Antagonists to modulate NLRP3 inflammasome

Smith et al. 2012 ***PLoS One.*** e33174

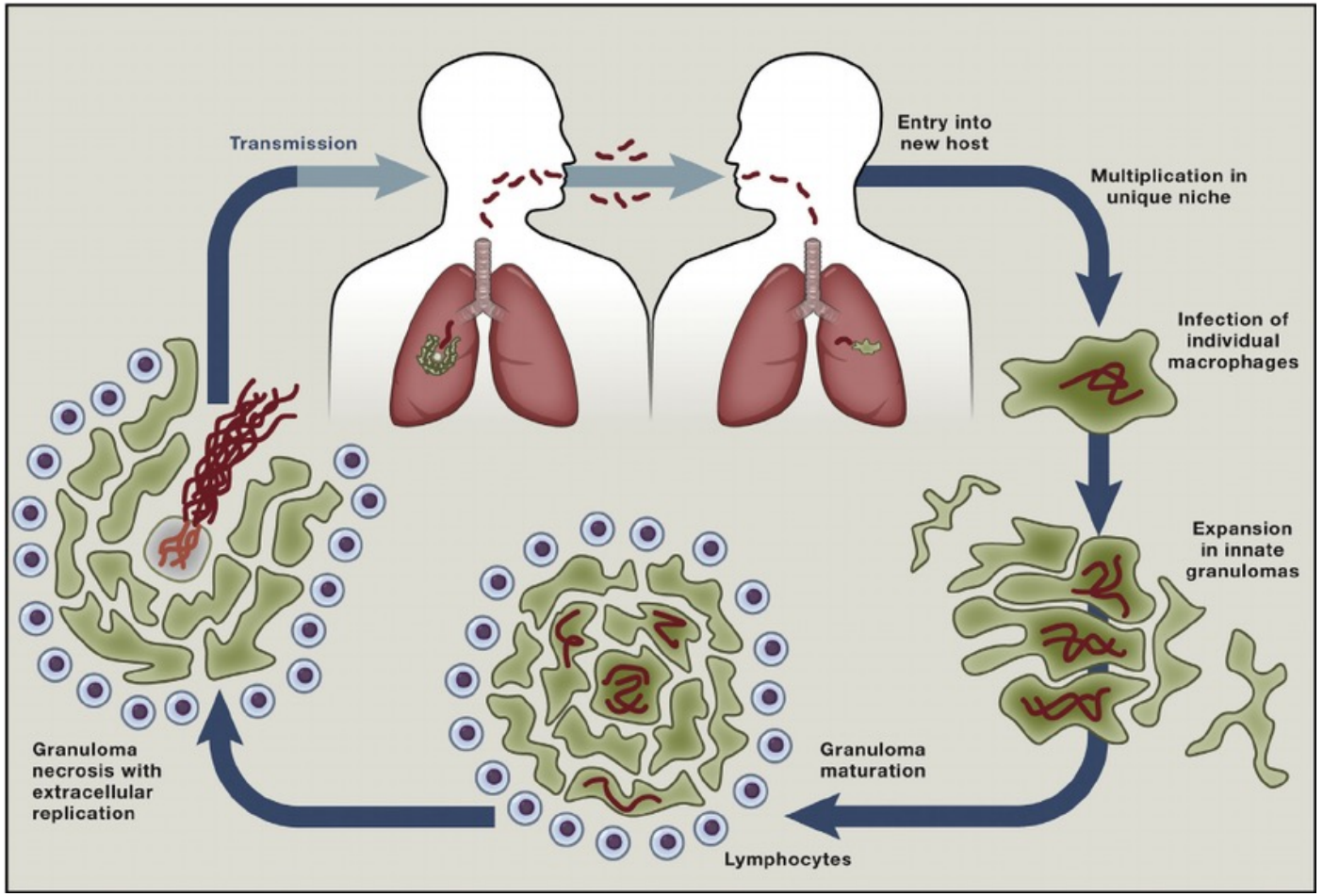
Novel Pathways for Infectious Diseases



Smith et al. 2012 ***PLoS One.*** e33174

- *PARK2* (now called *PRKN*) encodes parkin RBR E3 ubiquitin protein ligase, a component of the Parkin-Ubiquitin Proteasomal System (Parkin-UPS) pathway.
- Pathway enriched across 5 of 7 viruses based on human mRNA microarray analysis.
- Mutations in *PARK2* are known to cause Parkinson disease and autosomal recessive juvenile Parkinson disease.
- In humans, *PARK2* gene variants are also associated with susceptibility to leprosy, typhoid and paratyphoid fever (Ali et al 2006 Clin. Exp. Immunol. 144:425).

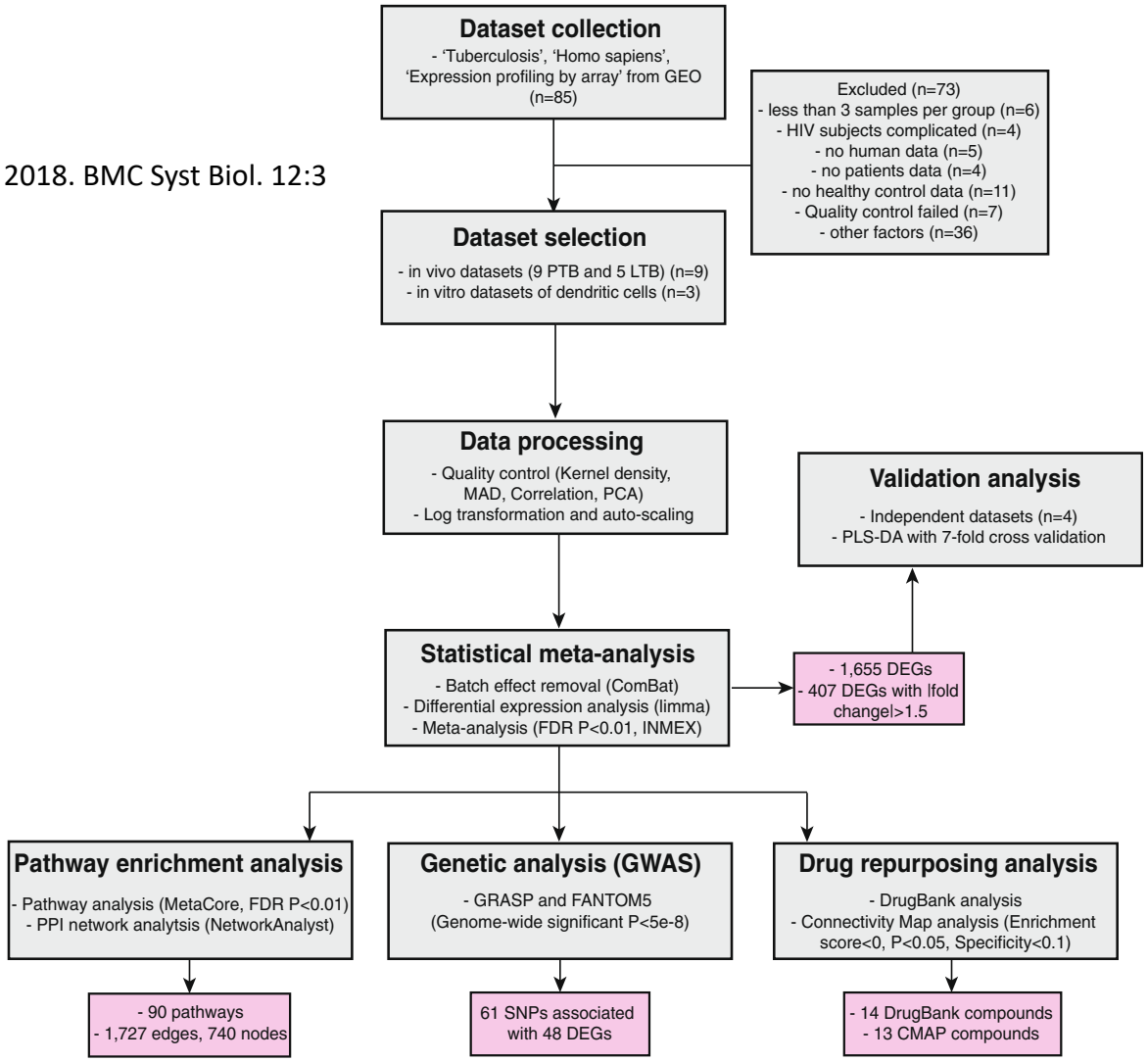
Tuberculosis (TB) Infection Interactions with Host Immunity



- Globally TB is one of the most prevalent infectious diseases (WHO).
  - 1.8 billion people infected
  - In 2022, 10.6 million fell ill and 1.6 million died
  - High unmet medical need
- The bacterium *Mycobacterium tuberculosis* (MTB) is the causative agent of TB.
- Intra-cellular pathogen of lung macrophages.
- Latent MTB can be a long term infection requiring several months of treatment with multiple antibiotics:
  - Increase in multidrug-resistant (MDR) TB strains
  - Urgent need for new therapies
- Similar to viruses, MTB proliferation depends upon:
  - Evasion and/or subversion of host immune responses
  - Manipulation of the macrophage microenvironment

Meta-analysis of Human Gene Response to *M. tuberculosis*

Wang et al. 2018. BMC Syst Biol. 12:3

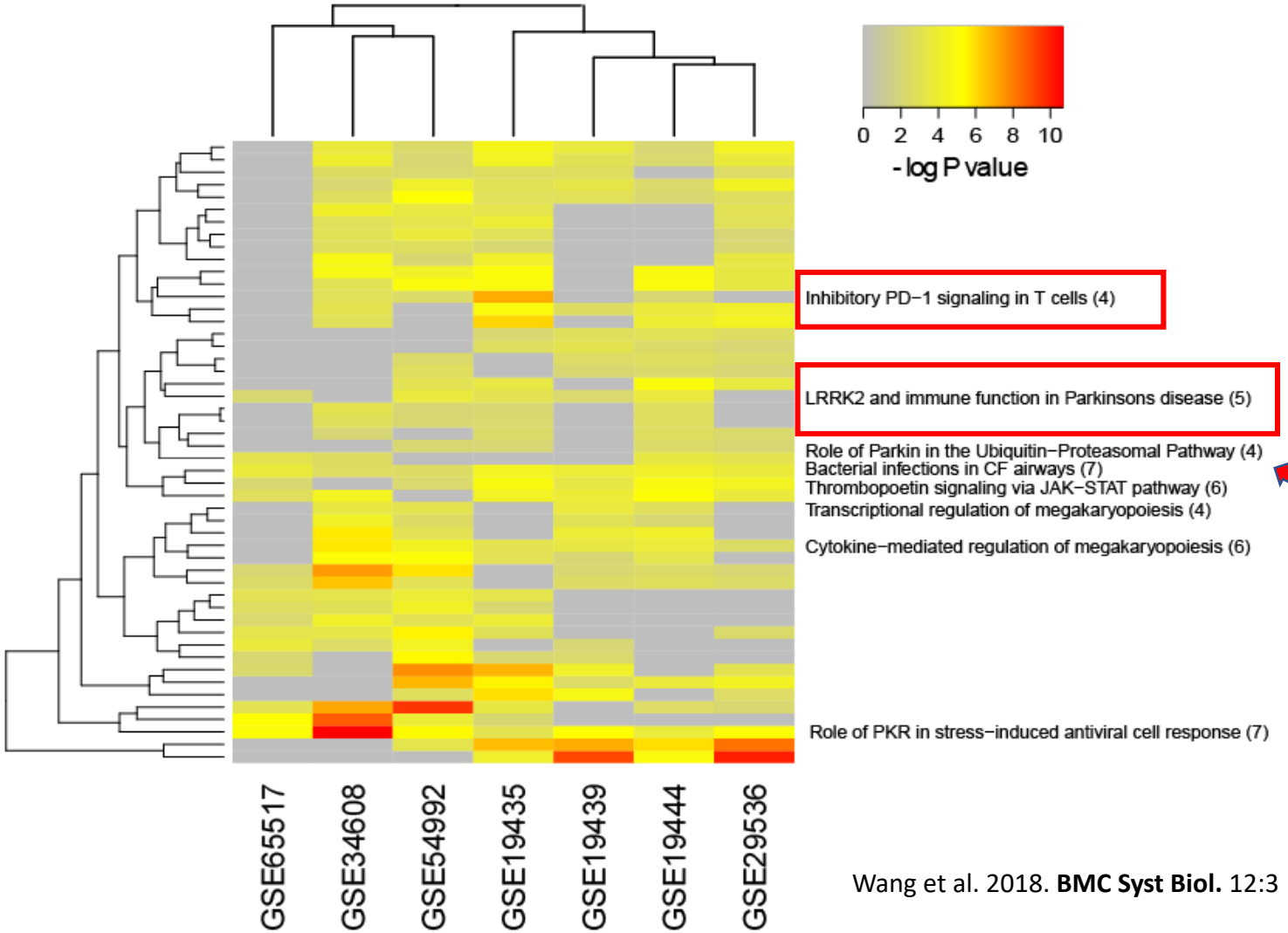


Dr. Seda Arat
Co-op Graduate Student;
Comp. Toxicologist, Pfizer

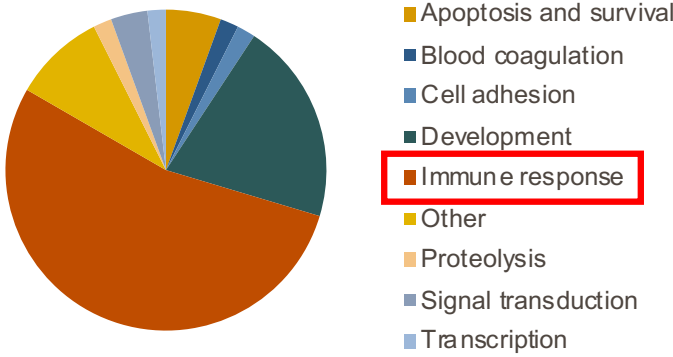
Dr. Zhang Wang,
Early Talent PDF;
CB Scientist GSK;
Professor Southern China Normal U.

- Human transcriptome meta-analysis of 7 published human transcriptome during active pulmonary TB infection (PTB) datasets
  - Health control groups
  - No co-occurring infections
  - Pass QC and sample size criteria
- Complete re-analysis of RNA-seq datasets for differentially expressed genes (DEGs)
- Meta-analysis of individual studies then looking at overlapping gene sets
- Pathway enrichment
- Targets reviewed for genetic (GWAS) associations
- Drug repurposing analysis

Enriched Human Pathways in PTB Infections



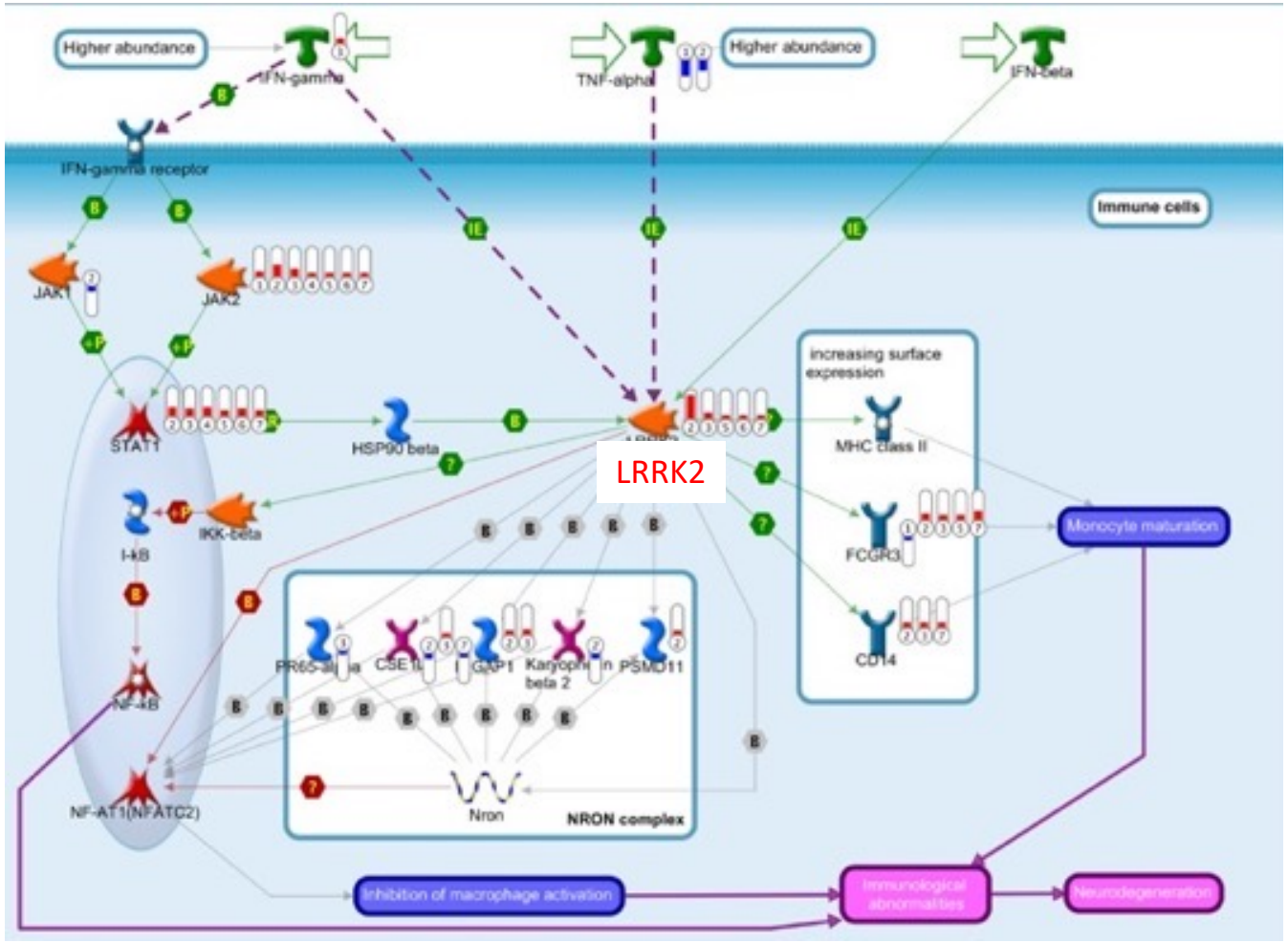
- 54 pathways found enriched for 4 or more out of 7 datasets



- Parkin-Ubiquitin Proteasomal System, involved in the progression of Parkinson disease

Wang et al. 2018. **BMC Syst Biol.** 12:3

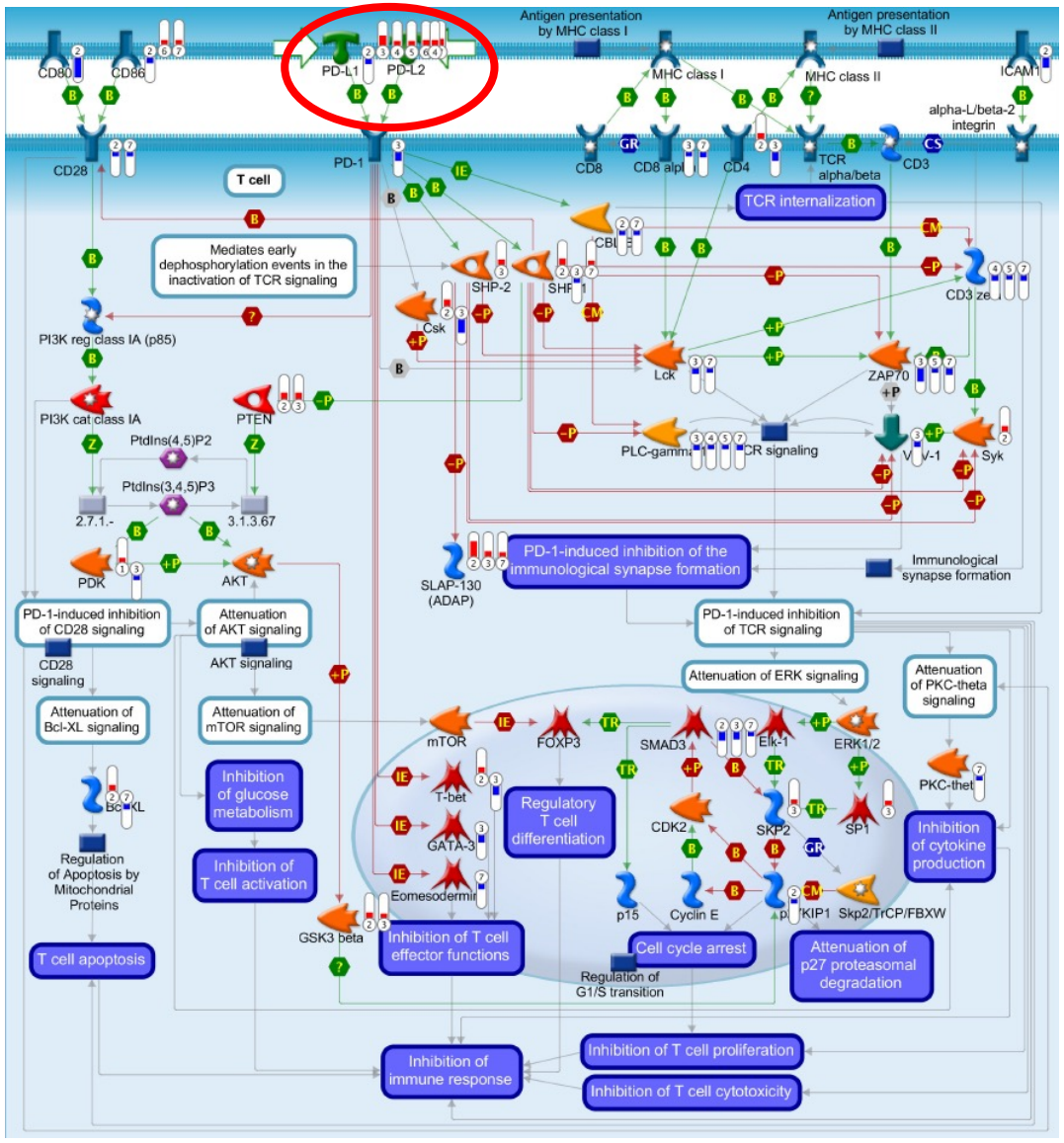
LRRK2 in Parkinson's Disease (PD) and Tuberculosis



Wang et al. 2018. BMC Syst Biol. 12:3

- Several Parkinson's Disease core pathways are modulated in TB.
  - 1.38-fold risk of Parkinson's Disease in TB patients independent of other clinical factors (Shen et al. 2016. Medicine [Baltimore] 95:e2883).
- **58 genetic variants associated with PD** proximal to 407 Differential Expressed Genes (DEGs) in TB.
- **LRRK2** (leucine rich repeat kinase 2) mutations associated with PD; considered a potential target.
  - LRRK2 has wide immune regulatory functions and associates with the mitochondria
  - LRRK2 highly expressed in the lung and linked to gut-brain immunity (Peter & Strober. 2023. J. Parkinsons Dis.)
- GSK/Crick collaboration support LRRK2 as a potential TB target
  - LRRK2 deficiency in mice resulted in a significant decrease in *M. tuberculosis* burdens early during the infection (Hartlova et al. 2018. EMBO J. 37).

Inhibitory PD-1 Signaling in T-cells



- Pathway significantly enriched in 4 PTB datasets. ***PD-L1*** gene significantly up-regulated in 5 PTB datasets.
- The PD-1/PD-L1 pathway has been shown to inhibit T cell effector function during PTB infections (Yin et al. 2014. **Tuberculosis** 94:131)
  - Suggests *Mtb* exploits PD-1/PD-L1 pathway to evade host immune response.
- Overcoming T-cell exhaustion is the basis of cancer immuno-therapy and might be a strategy for TB.
  - Potential to test PD-1 check point inhibitors clinically used for immuno-oncology (i.e., Pembrolizumab [Keytruda]) for activity against active PTB.

Wang et al. 2018. BMC Syst Biol. 12:3

Potential Drug Repurposing For TB Therapy

Targets and compounds proposed in this study		
Compounds	Targets/ Pathways	Evidence
LRRK2 inhibitor	*LRRK2* pathway	*LRRK2* pathway significantly upregulated in TB. *LRRK2* genetically associated with susceptibility of *M. leprae* infection. Cormobidities between TB and Parkinson's disease.
PD-L1 inhibitor (Atezolizumab)	*PD-1/PD-L1* pathway	*PD-1/PD-L1* significantly upregulated in TB, and inhibit TB-specific T-cell and macrophage functions.
Carfizomib	*PSMB8*, *PSMB9*, *PSMB10*, *PSMB2*	*PSMB8*, *PSMB9* significantly upregulated in TB, with strong genetic association with TB infection.
Intraveneous Immunoglobulin (IVIg)	*FCGR2A*, *FCGR3A*, *C5*	*FCGR2A*, *FCGR3A*, *C5* significantly upregulated in TB. Efficacy of IVIg in reducing bacterial load in TB infection.
Disopyramide	*SCN5A*, *ORM1*	Top compound in CMAP analysis. *SCN5A* regulates spatial and temporal calcium signaling during *Mtb* phagocytosis.
Flunarizine	*HRH1*, *CACNA1G*, *CACNA1H*, *CACNA1I*, *CALM1*	Top compound in CMAP analysis. Potential efficacy in restricting *Mtb* growth.

- Drug repurposing hypotheses – two methods.
- 407 DEGs searched for associations with known drugs listed in the Drug Bank database https://go.drugbank.com/ .
  - 19 drug-target links identified involving 14 drugs and 16 differentially expressed genes (DEGs).
- Connectivity MAP (L1000 CMAP https://clue.io/) analysis utilizes the anti-correlation relationships between gene expression (RNA-seq) signatures in diseases and drug perturbations.
  - 13 drugs with significantly anti-correlated signatures to the PTB signature

Wang et al. 2018. BMC Syst Biol. 12:3

Outline

1. Targeting human host factors for infectious diseases

2. Multi-omics data analysis of human protein-metabolite interactions

Metabolites: The Currency of Microbial Crosstalk with Host Signaling Functions

GSK

Commensal bacteria make GPCR ligands that mimic human signalling molecules

Louis J. Cohen[1,2], Daria Esterhazy[3], Seong-Hwan Kim[1], Christophe Lemetre[1], Rhiannon R. Aguilar[1], Emma A. Gordon[1], Amanda J. Pickard[4], Justin R. Cross[4], Ana B. Emiliano[5], Sun M. Han[1], John Chu[1], Xavier Vila-Farres[1], Jeremy Kaplitt[1], Aneta Rogoz[3], Paula Y. Calle[1], Craig Hunter[6], J. Kipchirchir Bitok[1] & Sean F. Brady[1]

48 | NATURE | VOL 549 | 7 SEPTEMBER 2017

A Forward Chemical Genetic Screen Reveals Gut Microbiota Metabolites That Modulate Host Physiology

Haiwei Chen,[1] Phu-Khat Nwe,[2] Yi Yang,[1] Connor E. Rosen,[1] Agata A. Bielecka,[1] Manik Kuchroo,[3] Gary W. Cline,[4] Andrew C. Kruse,[5] Aaron M. Ring,[1] Jason M. Crawford,[2,6] and Noah W. Palm[1,7,*]

Cell *177*, 1217–1231, May 16, 2019

OPEN ACCESS Freely available online

PLOS | BIOLOGY

Production of α-Galactosylceramide by a Prominent Member of the Human Gut Microbiota

Laura C. Wieland Brown[1,2☯], Cristina Penaranda[3☯], Purna C. Kashyap[4], Brianna B. Williams[1], Jon Clardy[2], Mitchell Kronenberg[5], Justin L. Sonnenburg[4], Laurie E. Comstock[6], Jeffrey A. Bluestone[3*], Michael A. Fischbach[1*]

July 2013 | Volume 11 | Issue 7 | e1001610

(Natural Killer Cell Agonists)

PLOS | ONE

RESEARCH ARTICLE

Human gut bacteria as potent class I histone deacetylase inhibitors *in vitro* through production of butyric acid and valeric acid

Samantha Yuille, Nicole Reichardt, Suchita Panda, Hayley Dunbar, Imke E. Mulder*

4DPharma Research Ltd., Aberdeen, United Kingdom

PLOS ONE | https://doi.org/10.1371/journal.pone.0201073 July 27, 2018

MucosalImmunology www.nature.com/mi

ARTICLE

A screen of Crohn's disease-associated microbial metabolites identifies ascorbate as a novel metabolic inhibitor of activated human T cells

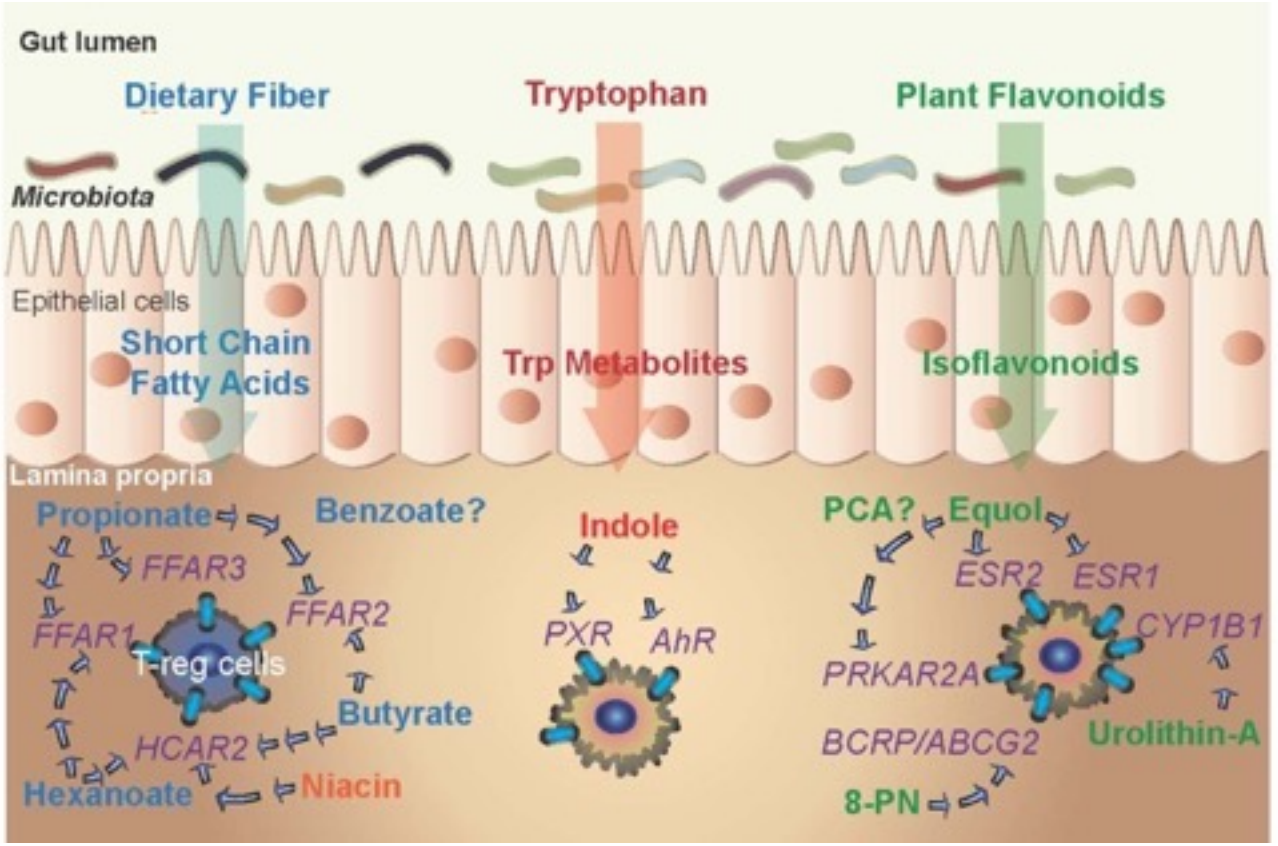
Yu-Ling Chang[1,2], Maura Rossetti[2], Hera Vlamakis[3], David Casero[2], Gemalene Sunga[2], Nicholas Harre[2], Shelley Miller[2], Romney Humphries[2], Thaddeus Stappenbeck[4], Kenneth W. Simpson[5], R. Balfour Sartor[6], Gary Wu[7], James Lewis[8], Frederic Bushman[9], Dermot P. B. McGovern[10], Nita Salzman[11], James Borneman[12], Ramnik Xavier[3], Curtis Huttenhower[3] and Jonathan Braun[2]

Mucosal Immunol. 2018 Apr 25. doi: 10.1038/s41385-018-0022-7.

14

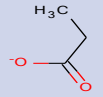
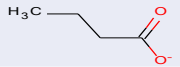
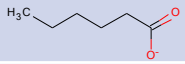
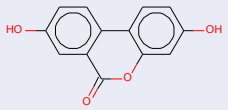
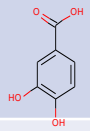
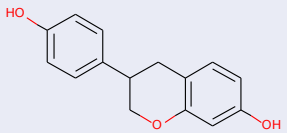
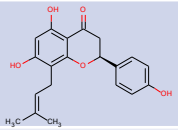
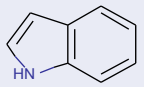
The Apothecary Within: Targeting Human-Microbial Crosstalk

- Microbiome metabolism of dietary fibers generates many diverse metabolites with positive immuno-modulatory effects.
- Metabolites are advantageous starting points for drug discovery:
  - Known modulators of host immunity (i.e., Cohen et al. 2017. **Nature** 549:48).
  - Well-tolerated as endogenous molecules.
  - Evolutionary optimized metabolite-receptor pairing for selectivity and specificity.
  - Many successfully launched drugs have “metabolite-like” properties (Dobson et al. 2009 **Drug Discovery Today** 14:31).
- Challenge: Low-throughput of current experimental approaches to identify potential metabolite ligand-receptor linkages.
- *Can we accelerate the discovery of useful metabolite-protein ligand pairings via in silico hypothesis generation?*



Saha et al. 2016. **Drug Discovery Today** 21:692

Metabolites Reported As Immuno-modulators

Microbial Metabolite	Structure	Known Target (if any)
Propionate		G-protein coupled receptors
Butyrate		G-protein coupled receptors
Hexanoate		G-protein coupled receptors
Benzoate		Unknown
Niacin		G-protein coupled receptors
Urolithin -A		Cytochrome P450s family 1B1
Protocatechuic Acid (PCA)		Unknown
Equol		cAMP-protein kinase A Estrogen receptors
8-Prenylnaringenin (8-PN)		Unknown
Indole		Voltage Gated K+ channels

CCC(=O)[O-]
SMILE String 2D Conversion

> 0.8 Tanimoto score

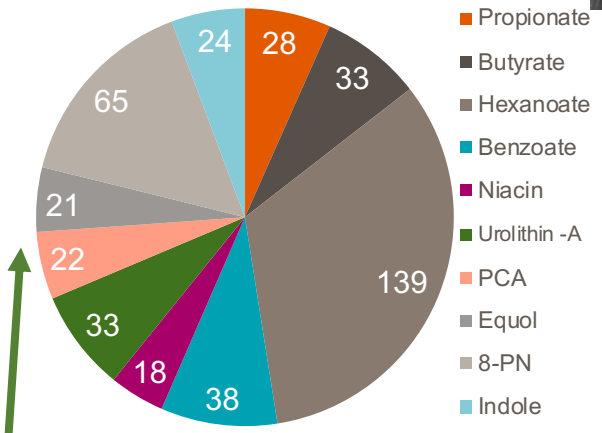
Search GSK 4.5 M compound collection

Retrieve metabolite-mimics

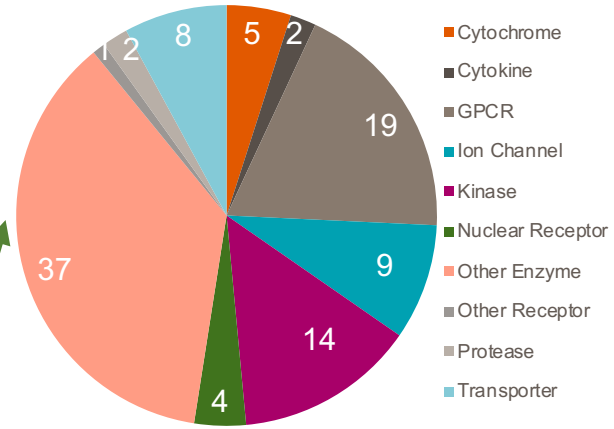
Retrieve associated human protein assay data

Output:
1. Cpds highly similar to microbial metabolites.
2. Their 2D chemical structures.
3. Putative protein ligands.

Dr. Somdutta Saha, Early Talent PDF
Bix Scientist, SpringWorks



421 “metabolite-mimics”



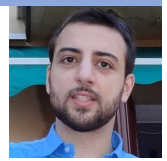
101 putative metabolite-receptor ligand relationships

Saha et al. 2016. Drug Discovery Today. 21:692

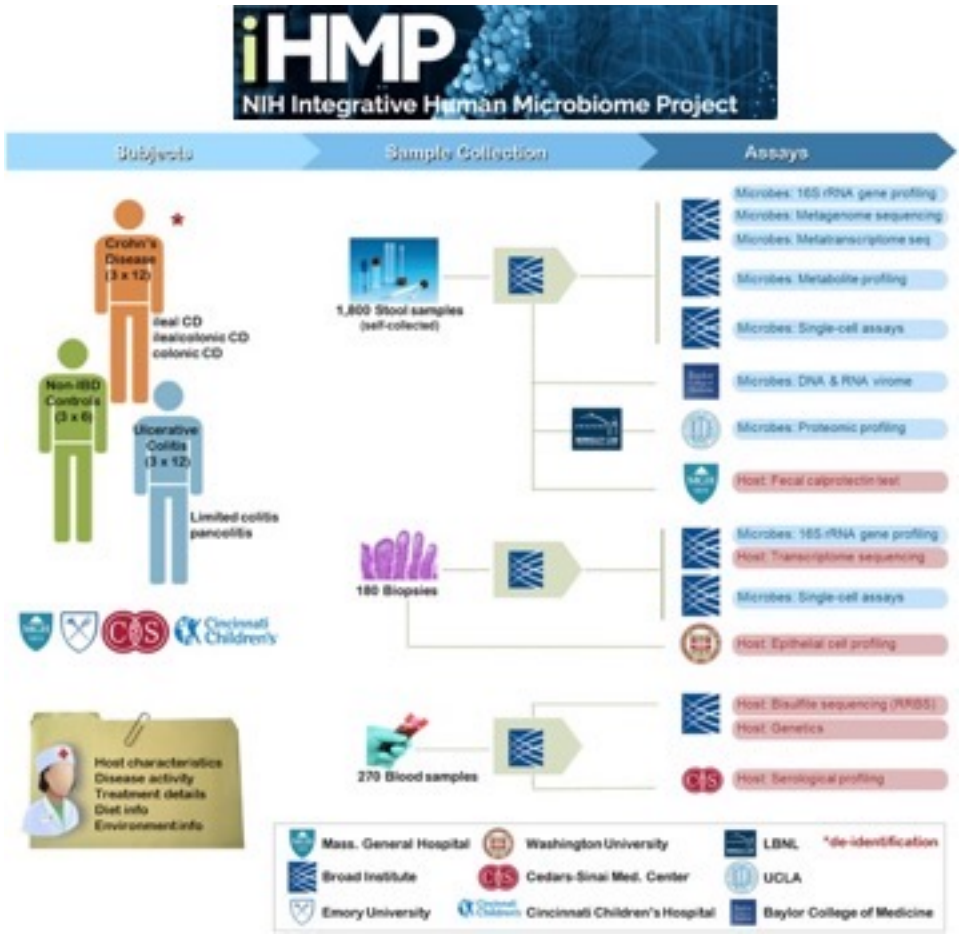
The Human Microbiome Project 2 (HMP2)

- Inflammatory bowel disease (IBD) patients:
  - CD: Crohn's disease
  - UC: Ulcerative colitis
- Multi-omics longitudinal assays:
  - Human host genetics (though underpowered for GWAS)
  - RNASeq from human biopsies
  - Metagenome, metatranscriptome, metaproteome & stool metabolome

Dr. Andrea Nuzzo,
Early Talent PDF;
Assoc. Dir., GSK



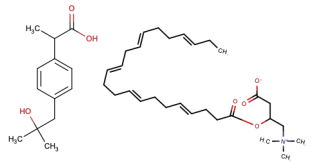
	Controls (nonIBD)	Crohn's disease (CD)	Ulcerative colitis (UC)	*Tot*
Participants	26	49	30	*105*
Metagenomic samples	429	750	459	*1638*
Metabolomic samples	135	265	146	*546*
RNAseq samples	51	127	74	*252*



Cell Host & Microbe, 2014, 16:3, 276-289, https://doi.org/10.1016/j.chom.2014.08.014

Computational and In vitro Validation Workflow

Metabolomics
(548 metabolites verified in HMDB, Human Metabolome Database)



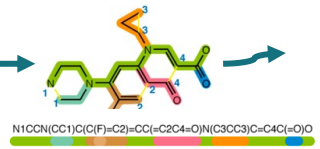
Machine learning
(XGBoost & consensus scoring)

Relevant metabolites

Genome-wide association studies

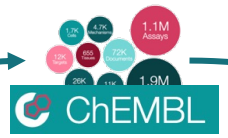
GWAS Catalog

biobank uk
Improving the health of future generations



Chemoinformatics
metabolite analogs
Tanimoto similarity ≥ 0.85
Tversky$_{\alpha=0.05}$ similarity ≥ 0.95

Analog cpds

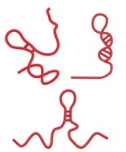


Functional assays databases

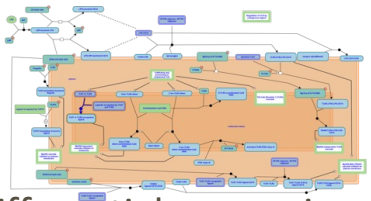
gsk

Cpd-target pair w/MOA

Metabolite-target pair evaluation

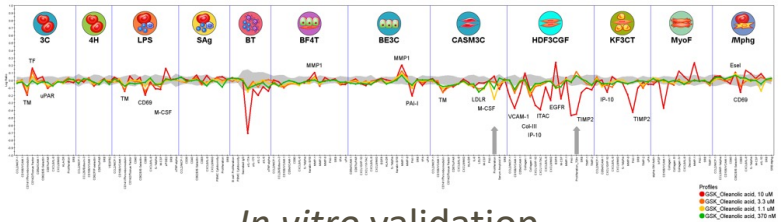


Human biopsy transcriptomics
(43870 transcripts)



Differential expression and pathway enrichment analysis

Relevant targets



In vitro validation

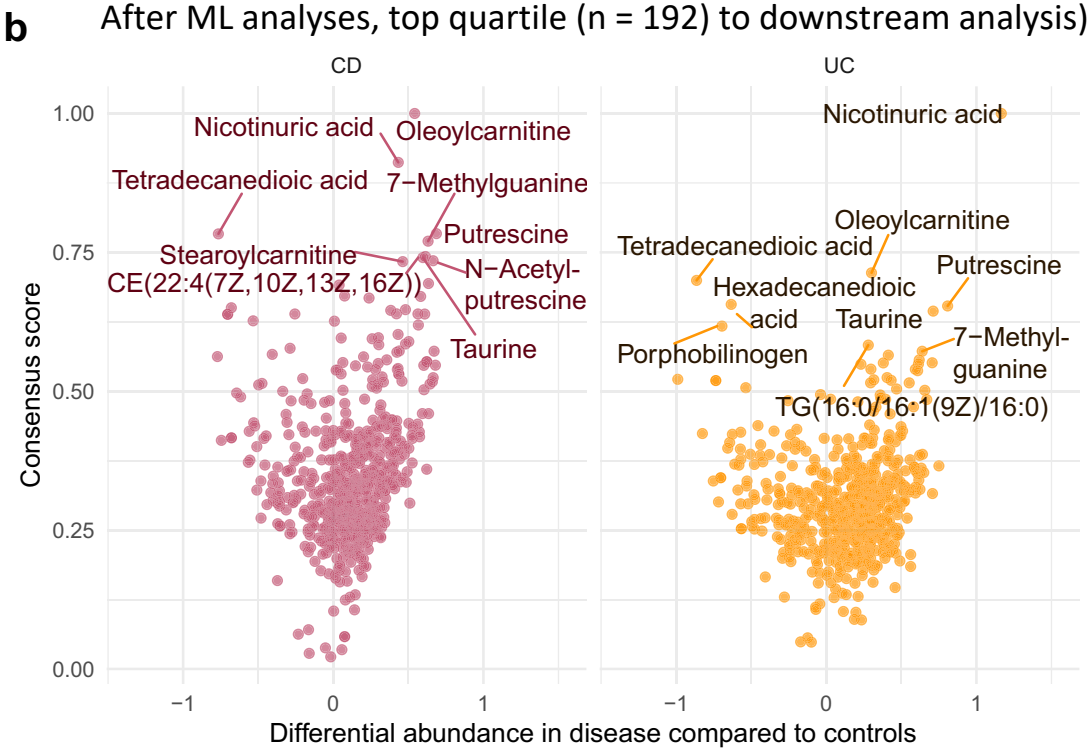
* identified in the Human Metabolome Database [HMDB]

Nuzzo…Brown. 2021. Commun. Biol. (Nature). 4:288

Metabolomics and Transcriptomics in IBD Samples

Metabolites

After ML analyses, top quartile (n = 192) to downstream analysis)



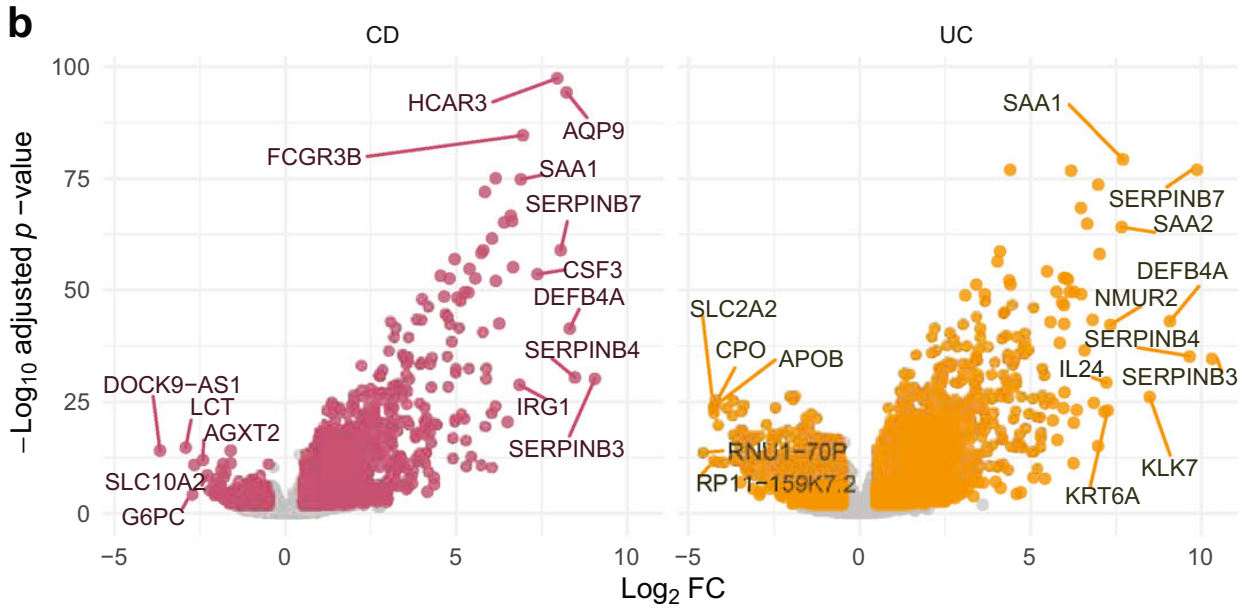
diagnosis

CD

UC

Gene Expression

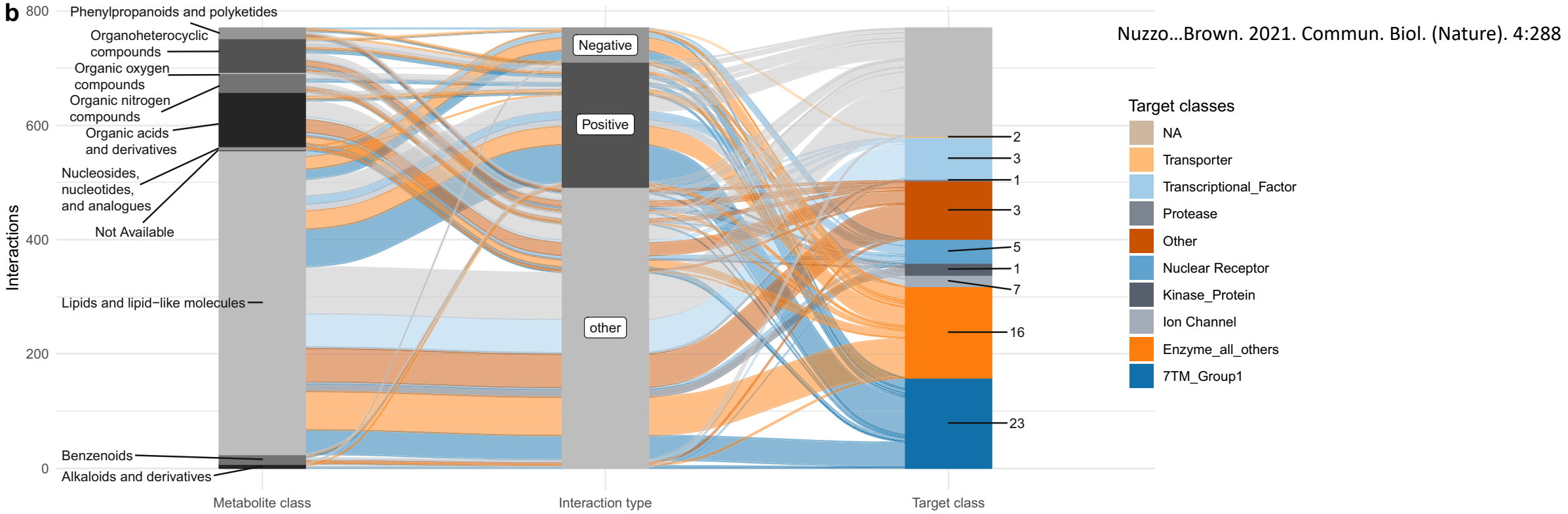
Total DEGs n = 2107 of which 820 DEGs shared between CD & UC



- Differential abundance of metabolites and gene RNA-seq in CD and UC patients compared to non-IBD subjects
- Prioritized known metabolites reported in the Human Metabolome Database.
- Gene transcripts were aligned to Genome Reference Consortium Human Build 37 (GRCh37).

Nuzzo…Brown. 2021. Commun. Biol. (Nature). 4:288

Connecting Metabolites and Drug Targets

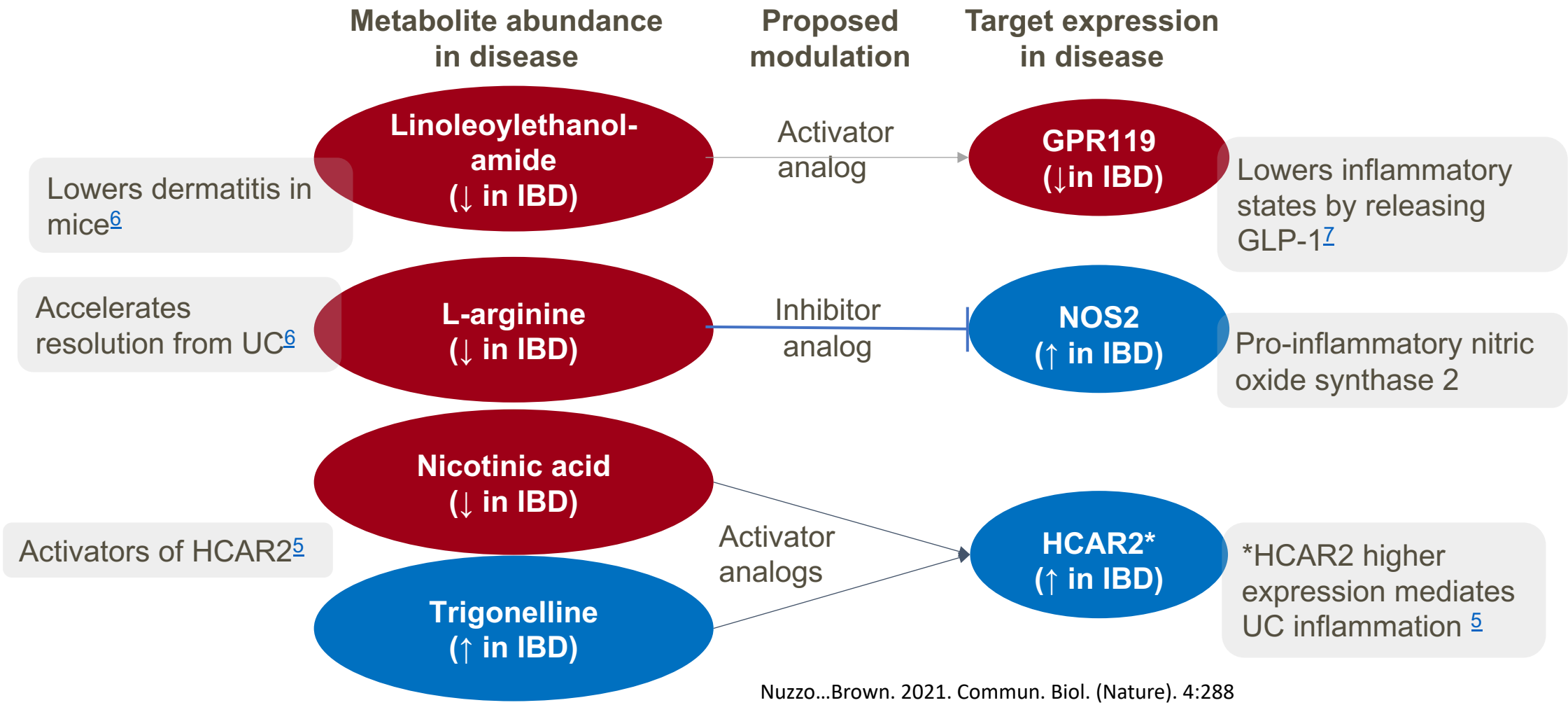


Nuzzo…Brown. 2021. Commun. Biol. (Nature). 4:288

- After filtering, 135 metabolites provisionally connected to 80 perspective proteins.
- Distribution of connections between metabolite classes, modulation type and drug target classes (numbers represent unique targets per drug target class [*n* = 61]). Some genes and metabolites have multiple interactions)
  - Filtered for metabolite-protein pairs with high binding affinity (i.e., either pIC50 or pEC50 values ≥5.5)
  - Highly pleiotropic metabolites and targets (≥ 20 predicted interactions) were removed.

Metabolite Co-directionality with Target Gene Expression

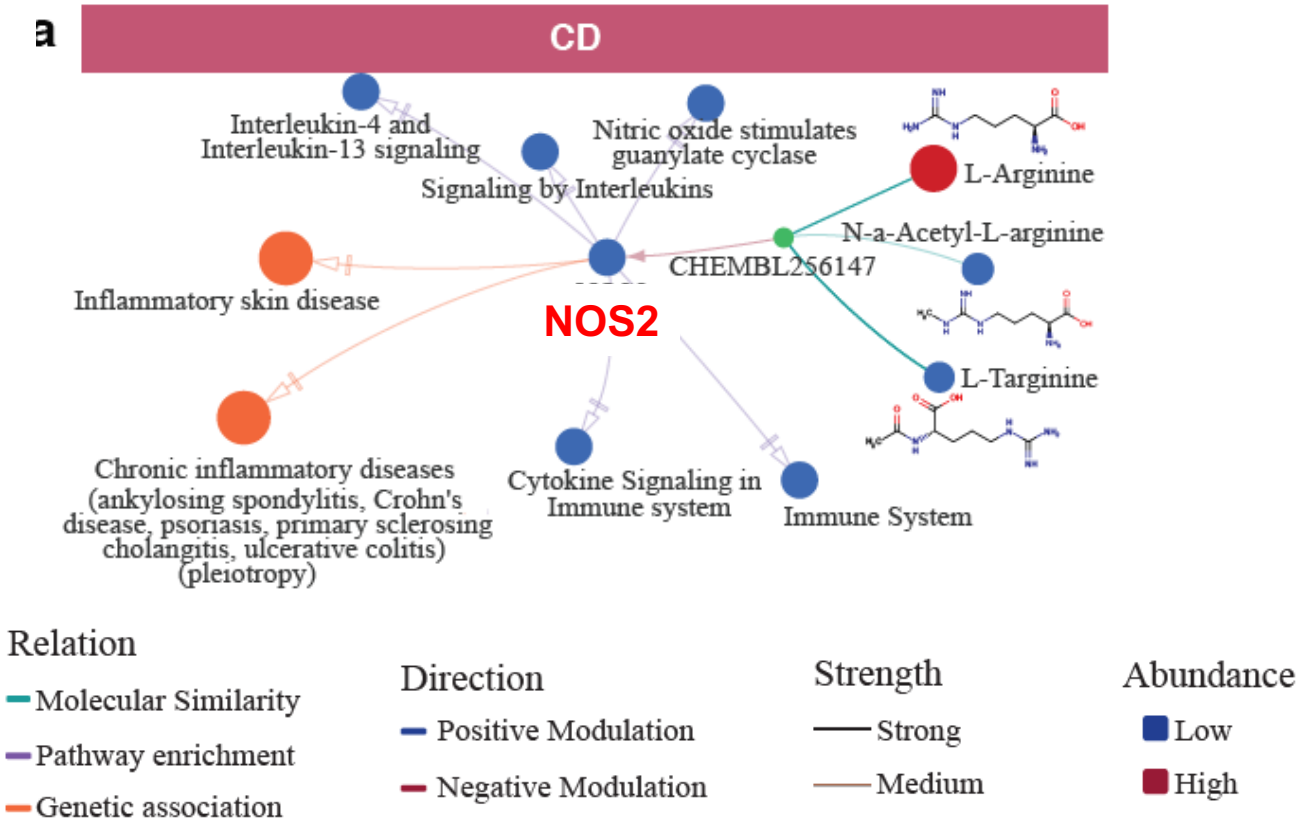
- Reversing transcriptomic disease signature using candidate modulators

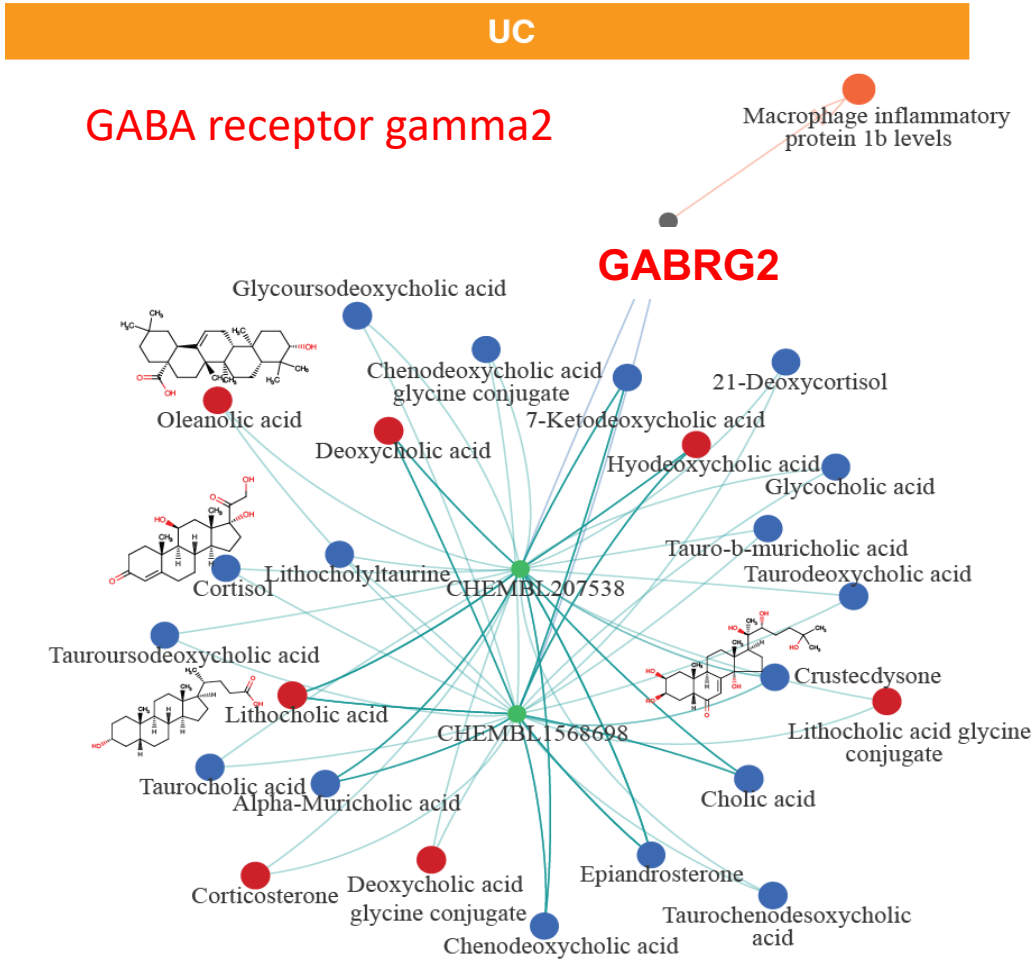


Nuzzo…Brown. 2021. Commun. Biol. (Nature). 4:288

Linkages to Disease Genetics

- Metabolites passing thresholds and tractable targets with genetic evidence
- Retrieved 808 genes with genetic associations to IBD
- Identified 464 potential pairings between genetic targets with metabolite modulators, 13 with known modulation mechanisms



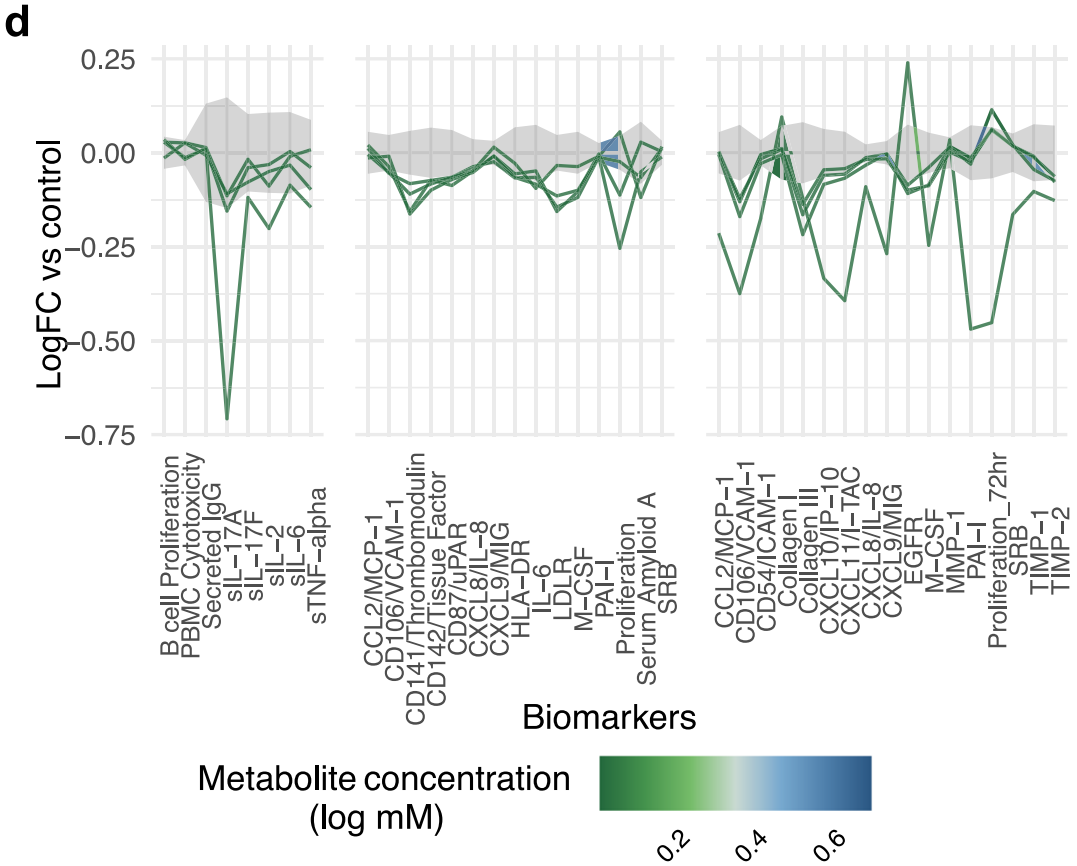


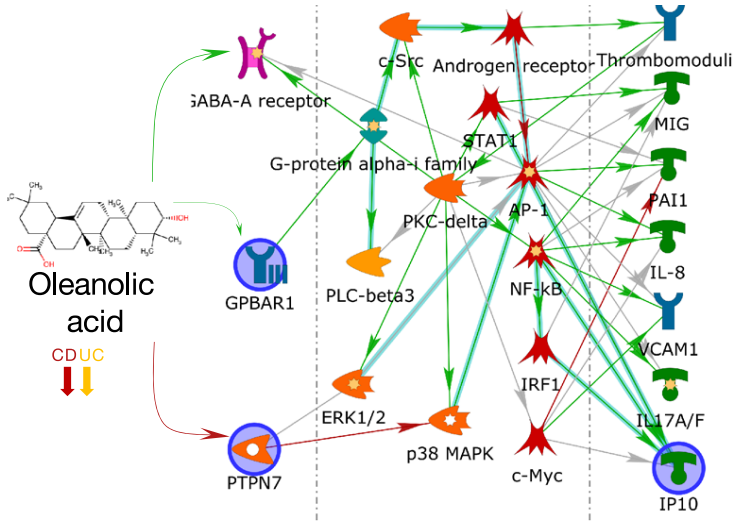
Nuzzo…Brown. 2021. Commun. Biol. (Nature). 4:288

in vitro Validation Assays for Selected Metabolites

- Selected 11 metabolites for profiling in human primary cell-based phenotypic assays (BioMAP® Diversity PLUS panel)
- 8 metabolites showed significant modulation of immune biomarkers in one or more cellular systems.
- Summary
  - 135 metabolites provisionally connected to 80 different targets in IBD
  - 983 potential metabolite-target interactions identified
  - Immuno-modulating metabolites and targets are potential starting points for drug discovery

Nuzzo…Brown. 2021. Commun. Biol. (Nature). 4:288





- Oleanolic acid (OA) showed activity in T-cell dependent B-cell activation (BT), coronary artery smooth muscle (CASM3C), fibroblasts (HDF3CGF) assays
- OA is a connected ligand of GABRG2, PTPN7 and GPBAR1

Summary and Future Directions

- Multi-omics analyses of human-microbe interactions can assist in drug discovery:
  - Novel targets.
  - Mechanism of action.
  - Biomarkers.
  - Drug repositioning.
  - Precision medicine – Identify potential disease subtypes in patient populations.
  - Find common targets and pathways across diverse disease etiologies.

- Future areas
  - New frontier for AI enabled target discovery:
    - Large language models (LLMs) trained on diverse chemical, biological and clinical datasets.
    - Understanding feature selection and the underlying drivers of AI model predictions could be insightful.

- For any computational hypothesis, it is essential to have experimental and clinical validation.

Acknowledgments

- GSK Students / Post-docs / Scientists
  - **Andy Nuzzo**
  - **Zhang Wang**
  - **Seda Arat**
  - **Steve Smith**
  - **Somdutta Saha**
  - **Carol Costa Sa**
- GSK Computational Biology, Infectious Disease and Global Health colleagues
- Contact info: jb4633@drexel.edu