
U.S. AIR FORCE
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Accelerating synthetic biotic development with gut-on-a-chip technology
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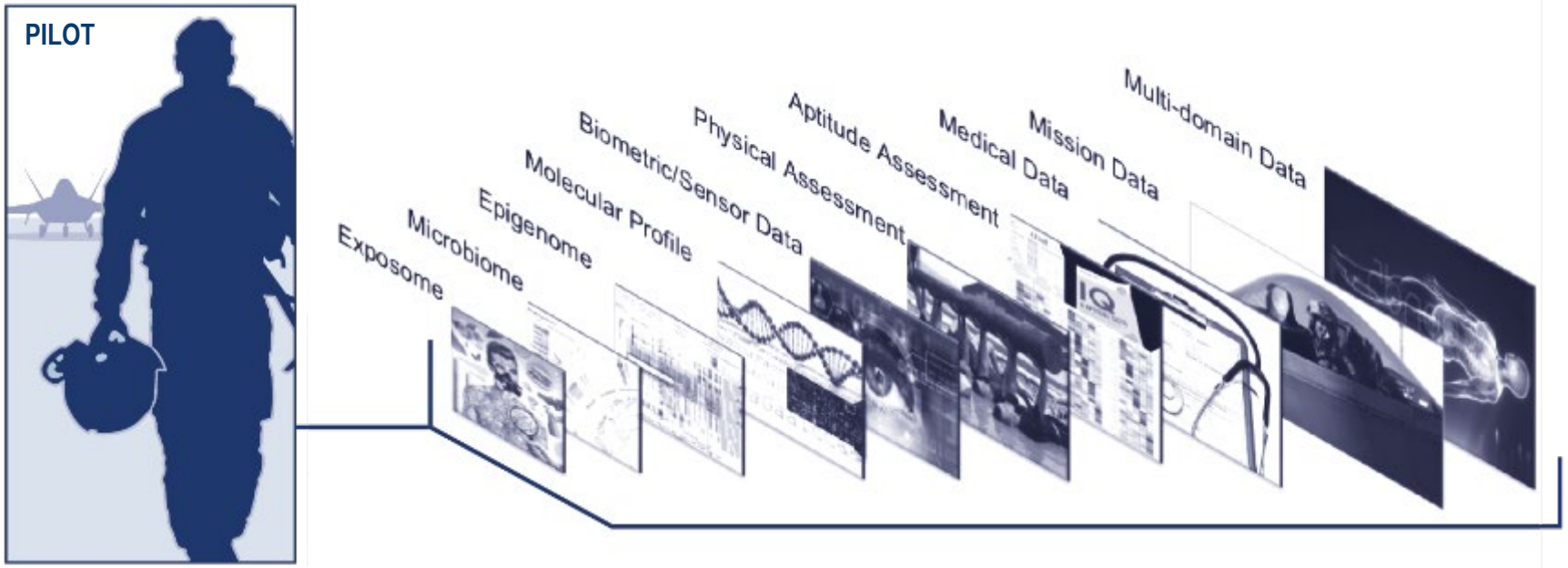
711th Human Performance Wing, WPAFB, OH

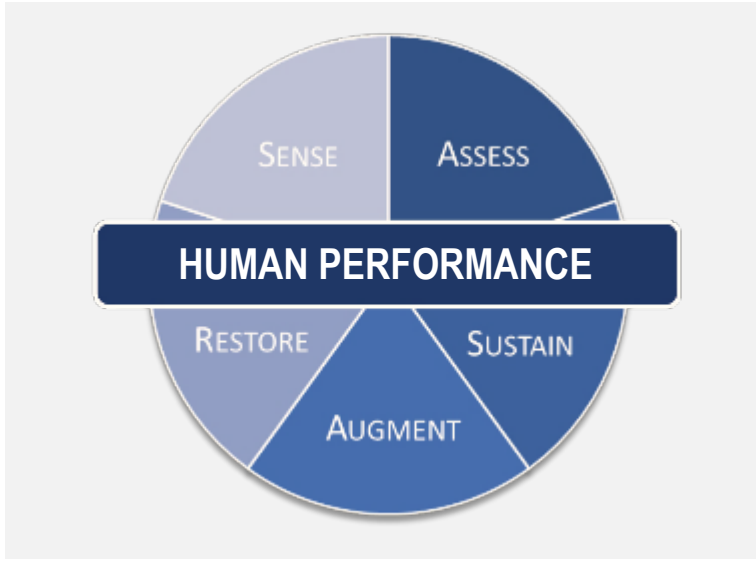
The views presented are those of the speaker and do not necessarily represent the views of the DoD or its components

Outline

- Characterization of a clinical synbiotic for the treatment of PKU in a human gut-on-a-chip
- Analysis of a Novel Sense & Respond Synbiotic in a Gut-on-a-chip system
- Future Directions
- Summary
- Acknowledgements

Airmen / Guardians as a “System of Systems”





The Medical and Operational Biosciences CTC utilizes multivariant, **SYSTEMS BIOLOGY** *approaches to provide advanced science and technology solutions to understand the warfighter’s biologic state and the underlying mechanism of responses*

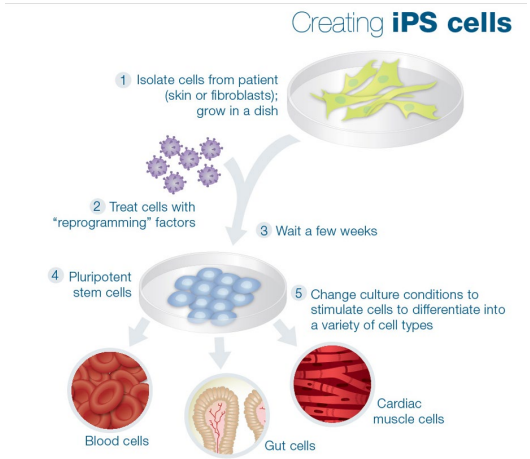
Delivering personalized predictions of response to stressors & optimized interventions

Understanding the individual by putting the pieces together

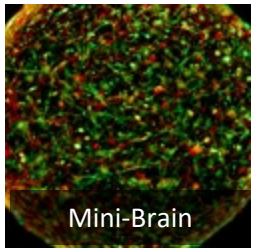
Warfighter Benefits

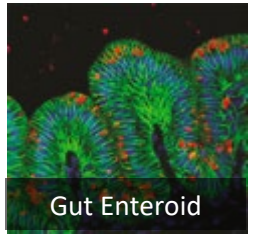
Model Development and Mechanistic Analysis Work-Flow

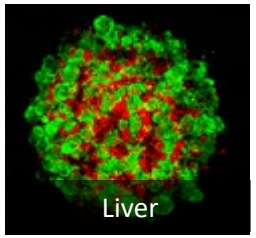
Organoid/iPSC Development



JHU (CRADA)



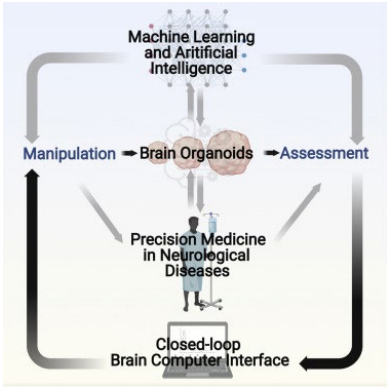


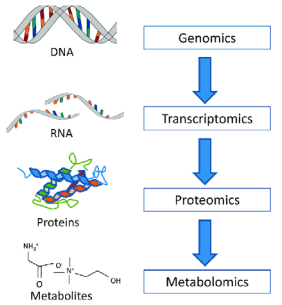


Organ-Specific Model Development

Data Acquisition

Knowledge Transition



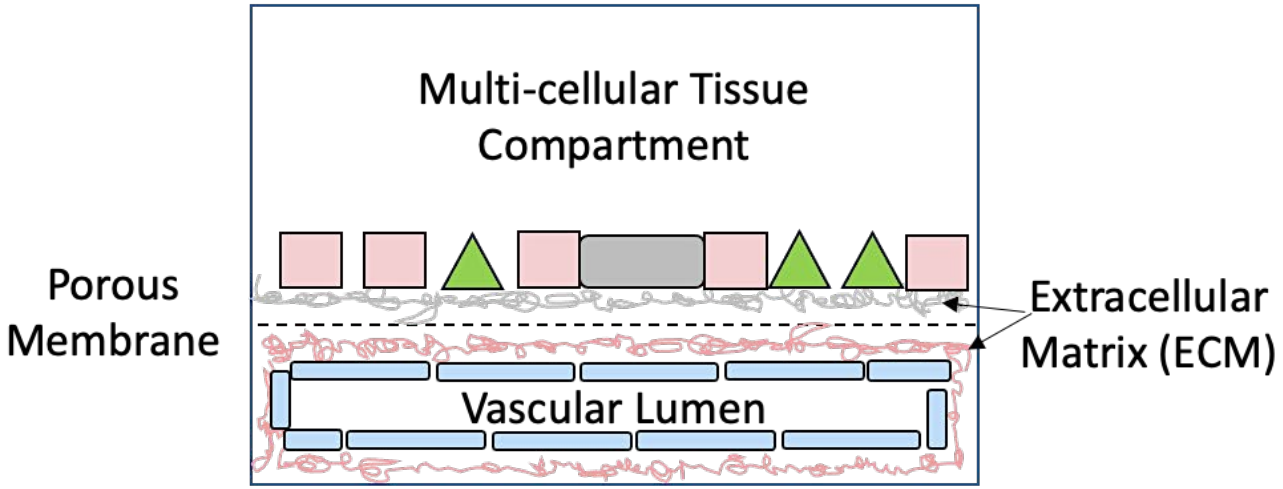


Analysis of a Clinical Synbiotic in a Human Gut-on-a-Chip

Gut-on-a-Chip Development

Microfluidic Device

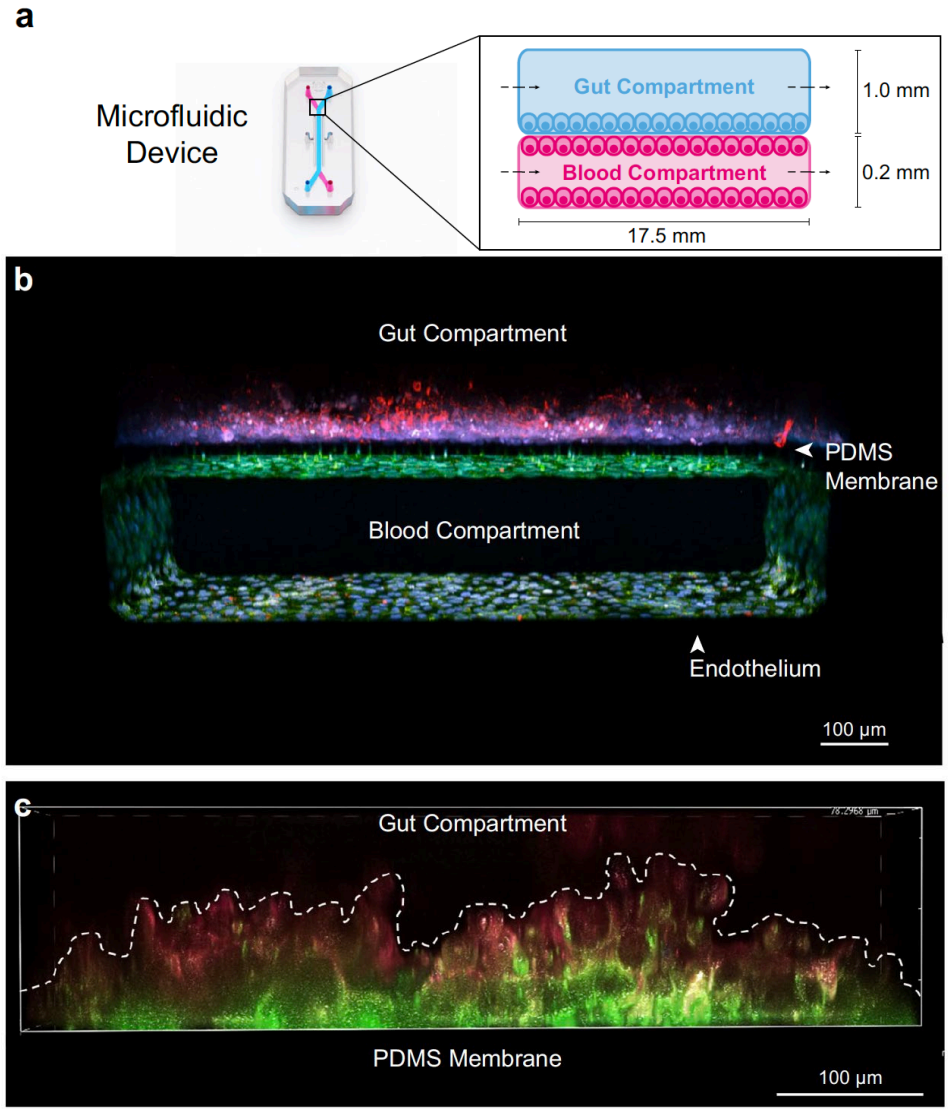
Organ-on-a-Chip Schematic (Side View)

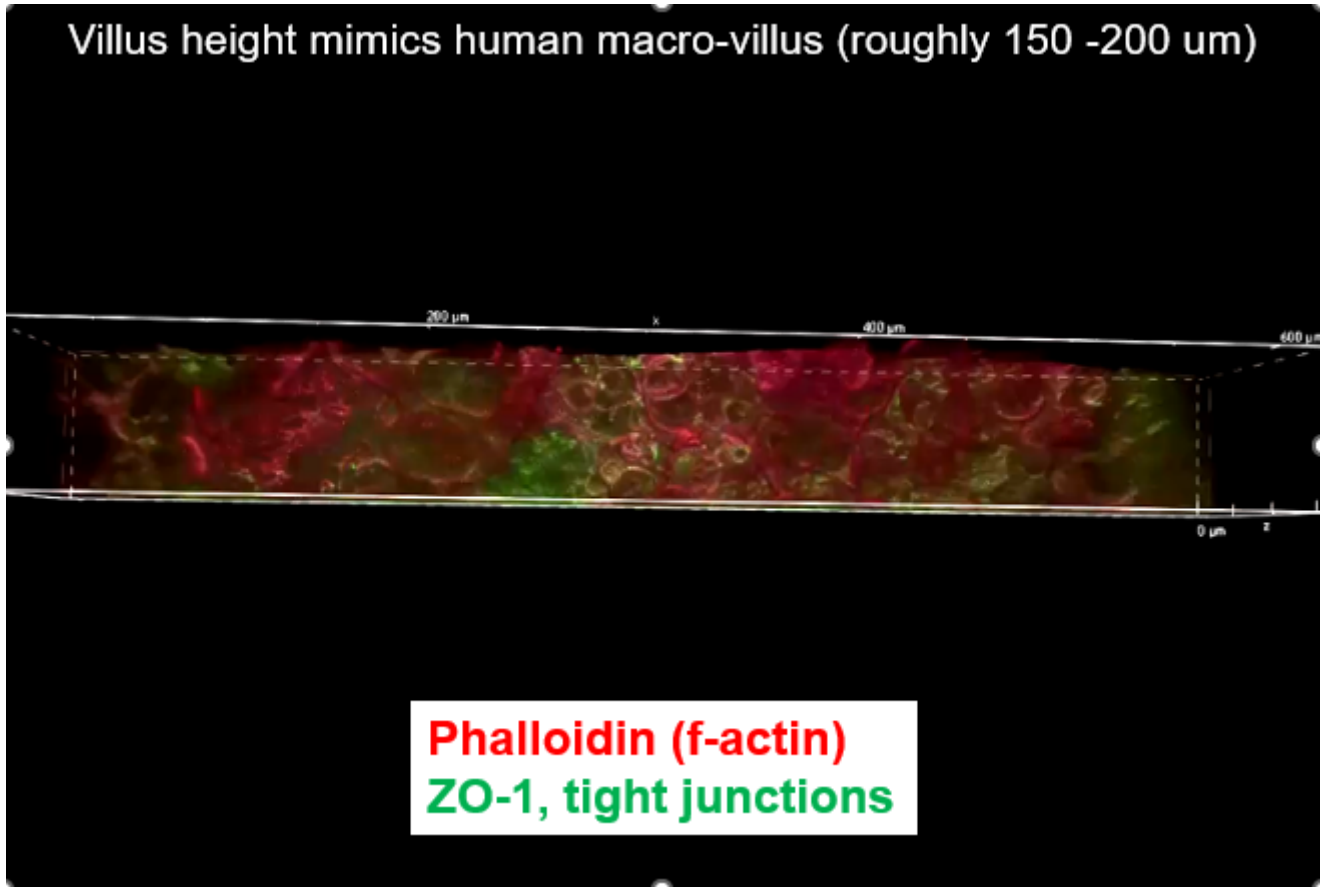


OOC Pumping System

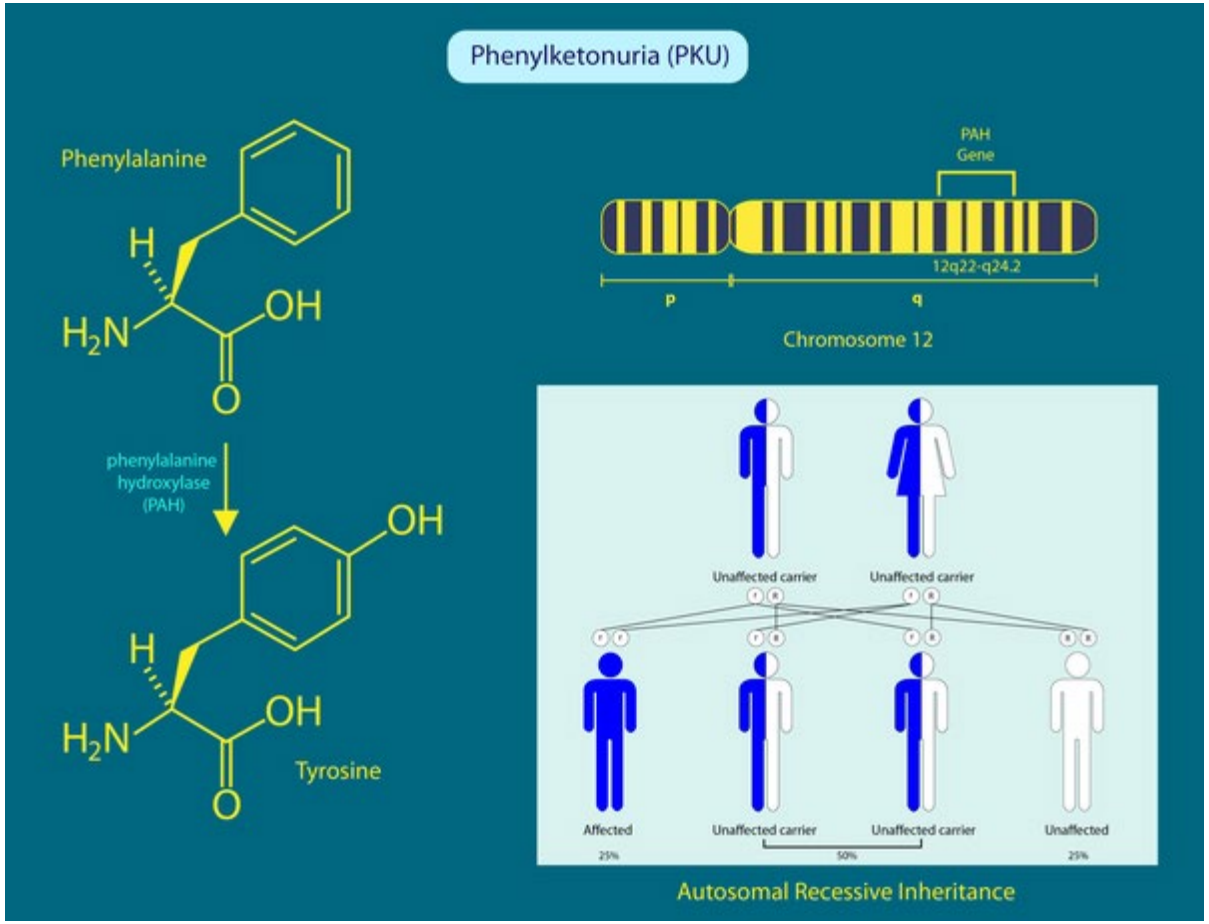
- Microfluidic devices (PDMS) are purchased from Emulate Bio
- The surfaces are activated with amine rich solution and UV light
- ECM is a blend of growth factor reduced Matrigel and type I Collagen (100 µg/mL)
- Caco2-C2BBE enterocyte-like and HT-29 MTX goblet-like gut cell lines mixed at a 4:1 ratio composes the gut-channel
- Human microvascular intestinal endothelial cells are grown in the lower compartment on all surfaces forming a lumen
- The organ-on-a-chip pumping system (Human Emulation System, Emulate Bio) uses pneumatics to precisely control flow
- Parallel air-filled channels running next to the cell culture compartments enable application of stretch forces

Gut-Chip Characterization





Phenylketonuria (PKU) and Synlogic Inc. Synbiotic Treatment

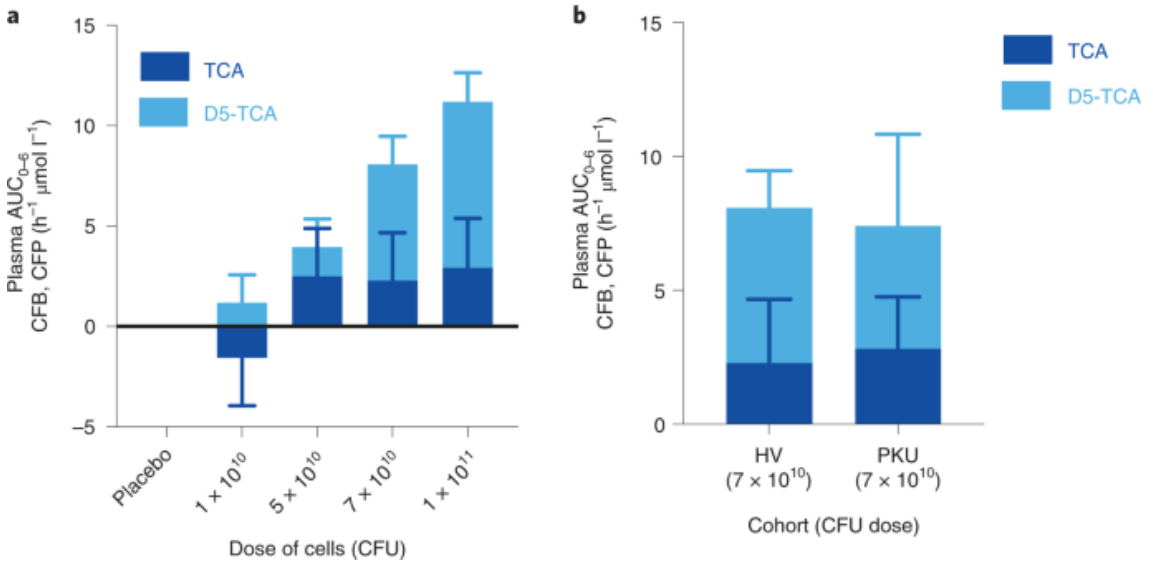


- 1 in 80 individuals are a carrier for PKU, current treatments lack robustness to reduce serum levels
- Phenylalanine (Phe) build-up causes cognitive disfunction and death

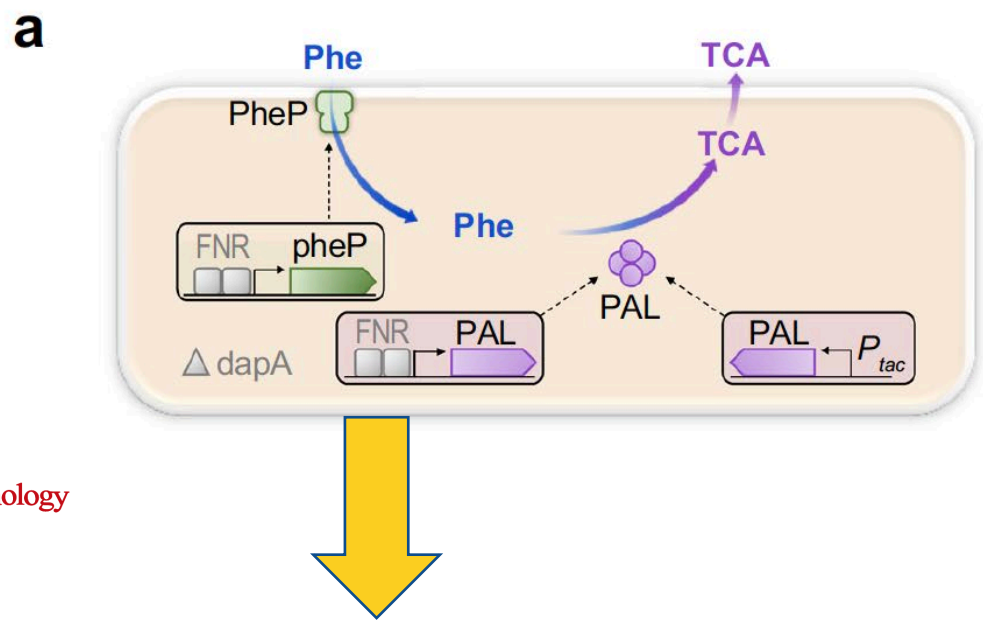
nature metabolism
ARTICLES
https://doi.org/10.1038/s42255-021-00430-7
Check for updates

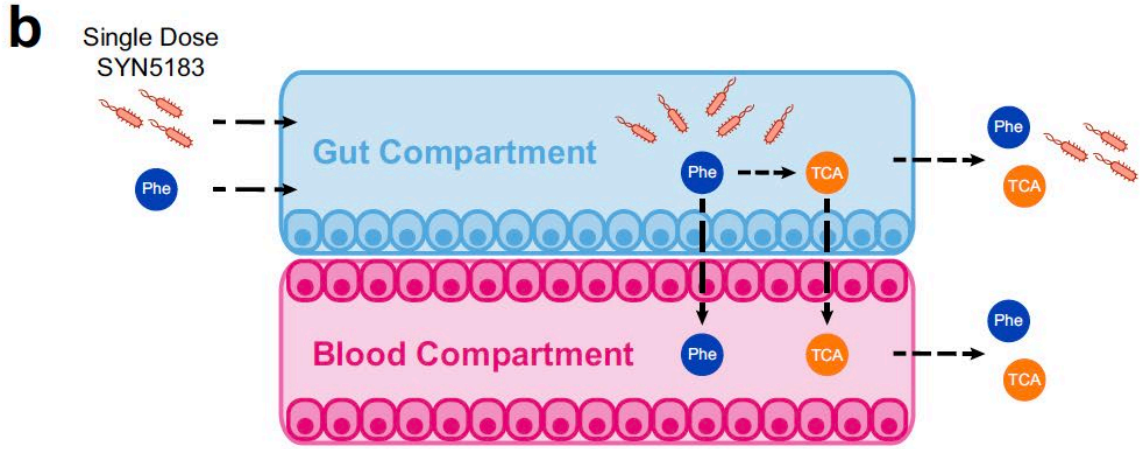
Safety and pharmacodynamics of an engineered *E. coli* Nissle for the treatment of phenylketonuria: a first-in-human phase 1/2a study

Marja K. Puurunen[1]✉, Jerry Vockley[2,3], Shawn L. Searle[4], Stephanie J. Sacharow[5,6], John A. Phillips III[7], William S. Denney[8], Benjamin D. Goodlett[5,6], David A. Wagner[9], Larry Blankstein[1], Mary J. Castillo[1], Mark R. Charbonneau[1], Vincent M. Isabella[1], Vasu V. Sethuraman[1], Richard J. Riese[1], Caroline B. Kurtz[1] and Aoife M. Brennan[1]



Evaluating SYNB5183 in a human gut-chip





nature biotechnology

Development of a synthetic live bacterial therapeutic for the human metabolic disease phenylketonuria

Vincent M Isabella[1], Binh N Ha[1], Mary Joan Castillo[1], David J Lubkowicz[1], Sarah E Rowe[1,2], Yves A Millet[1], Cami L Anderson[1], Ning Li[1], Adam B Fisher[1], Kip A West[1], Philippa J Reeder[1], Munira M Momin[1], Christopher G Bergeron[1], Sarah E Guilmain[1], Paul F Miller[1], Caroline B Kurtz[1] & Dean Falb[1]

- Engineered *E. coli* Nissle 1917 with encoded phenylalanine ammonia lyase (PAL) enzyme
- Pre-clinical efficacy results from non-human primate studies

- SYNB5183 at high ($1.25x10^9$), moderate ($6.5x10^8$), or low ($1.25x10^8$) colony forming units (CFU/mL) was inoculated in the gut-chip under static conditions without Phe
- Phe at a physiological concentration of 5 µM was administered continuously via the fluidic reservoirs
- Simulated intestinal fluid (SIF) was utilized in the gut compartment (pH = 6.2, containing digestive and intestinal enzymes)
- Physiological flow rate (60 µl/hour, 0.0003 dynes/cm^2)

nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-021-23072-5 OPEN

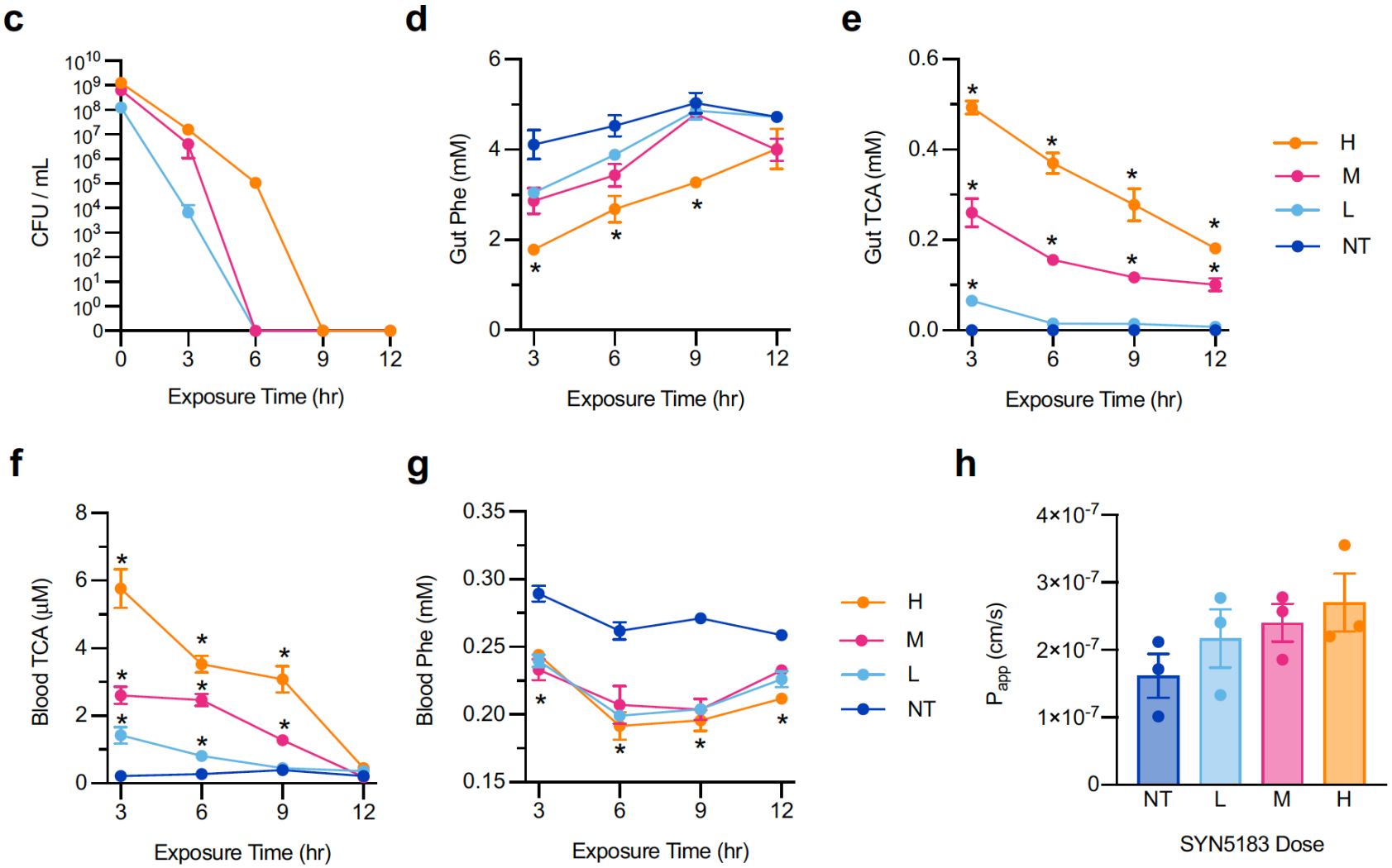
Check for updates

Characterization of an engineered live bacterial therapeutic for the treatment of phenylketonuria in a human gut-on-a-chip

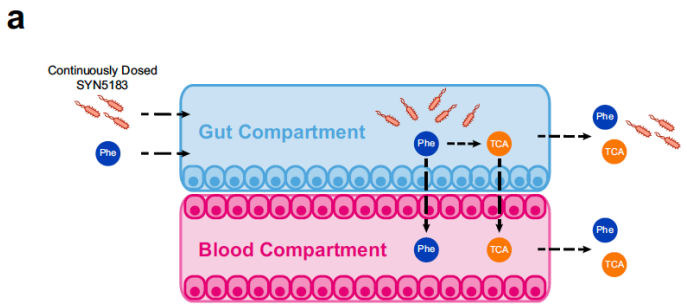
M. Tyler Nelson[1,6✉], Mark R. Charbonneau[2,6], Heidi G. Coia[1,3], Mary J. Castillo[2], Corey Holt[1], Eric S. Greenwood[1,4], Peter J. Robinson[1,5], Elaine A. Merrill[1], David Lubkowicz[2] & Camilla A. Mauzy[1]

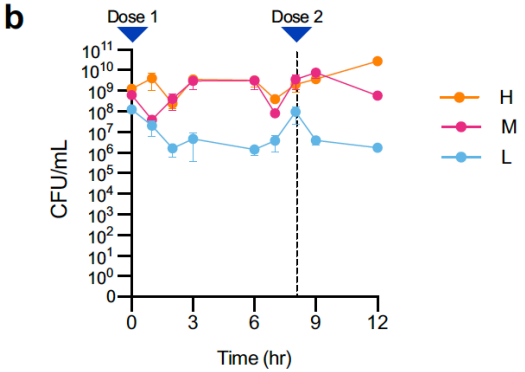
SYNB5183 Pharmacokinetic Assessment in a human gut-chip

- **A bolus dose of SYNB5183** was added to the gut chip and exposed directly to gut-compartment Phe
- **SYNB5183 fully cleared the gut-chip** between 6-9 hours (clinically a single dose was found to clear within 8 hours)
- **Gut Phe lowering** and corresponding biomarker production was observed in a dose/time-dependent manner
- Most important **blood Phe levels were reduced**

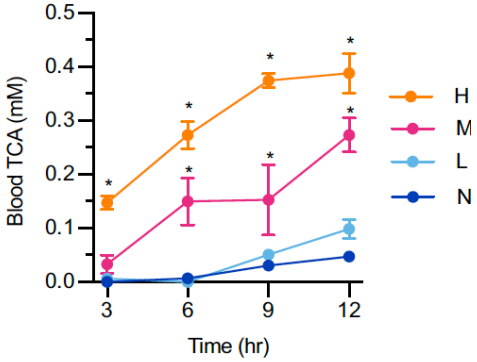


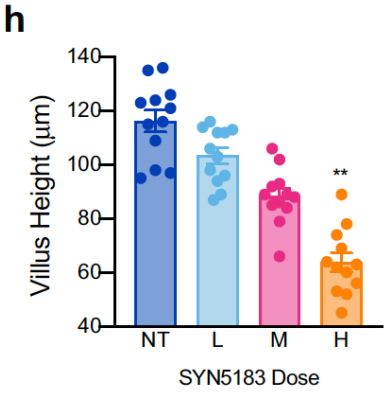
Supra-Physiological Dosing and Characterization of SYNB5183

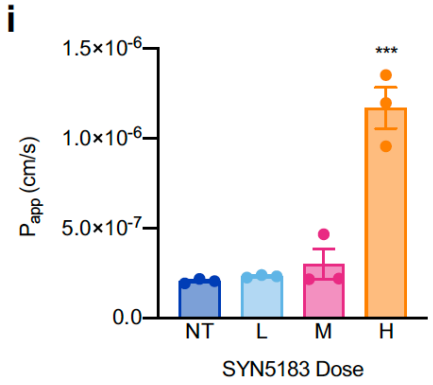


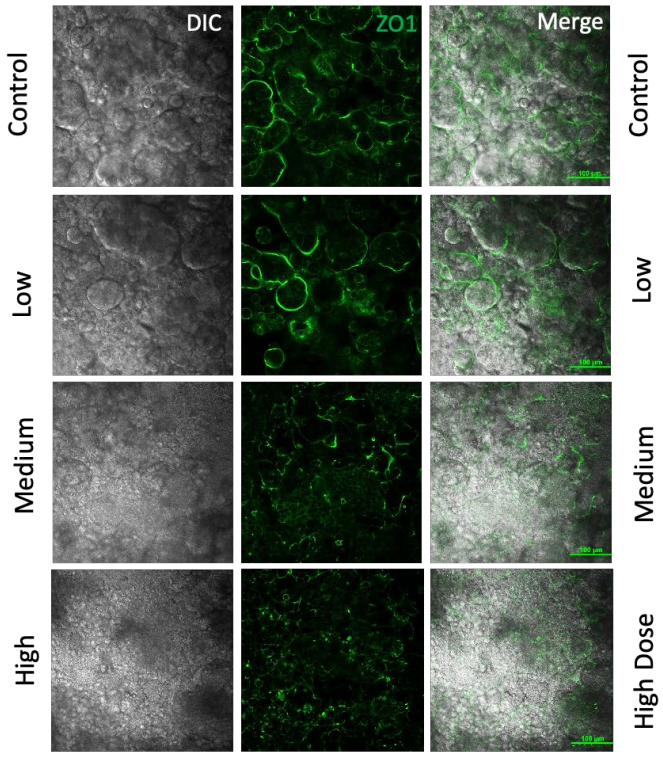


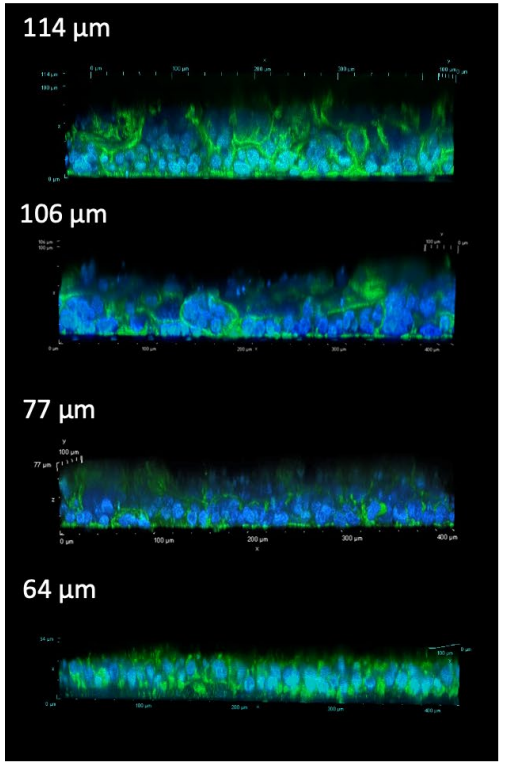
- Continuous dosing of SYNB5183 prevented strain clearance simulating extreme limits of dosing otherwise not possible
- Far greater biomarker production and transport was observed
- Due to the build-up of bacteria and the sustainment of the host-microbial interaction a dose-dependent decrease in macrovillus height and an increase in apparent permeability was observed





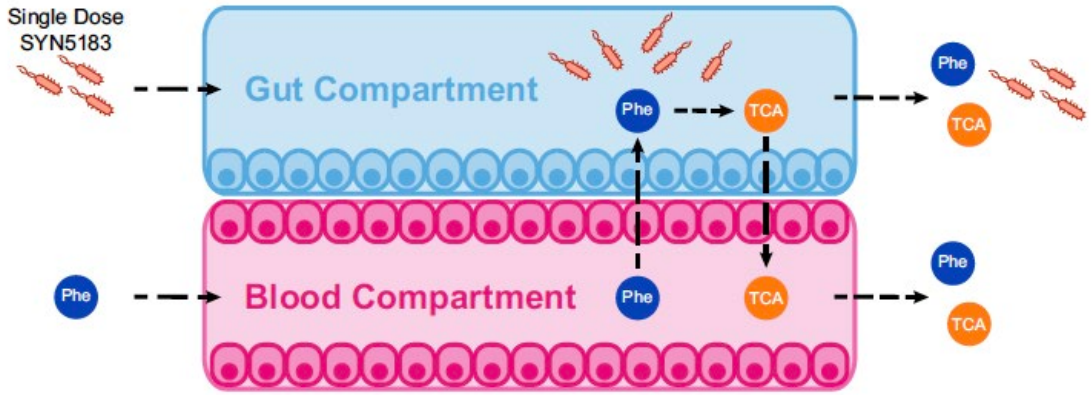


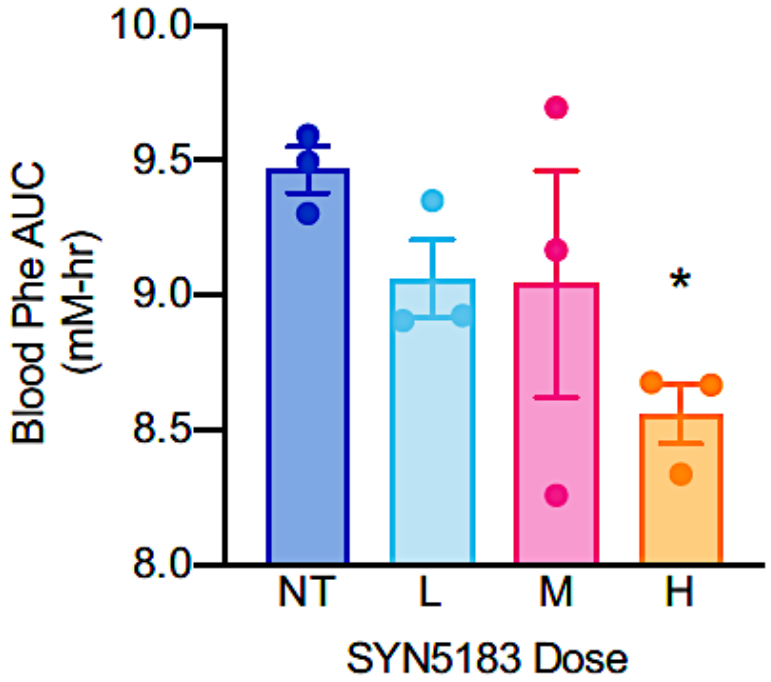




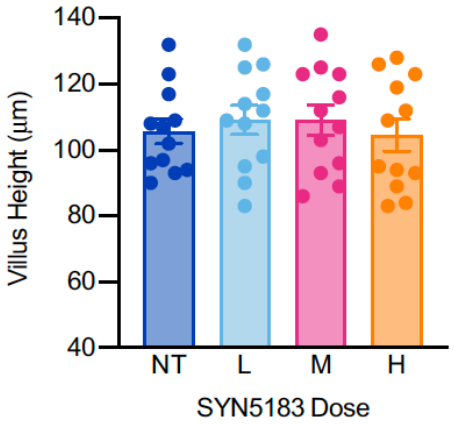
- Macrovillus morphology alters with increasing dose
- Tight-junction, zona occludin-1 (ZO-1) has diminishing expression and diffuse organization with increasing dose
- Macrovillus height decreased by 56%

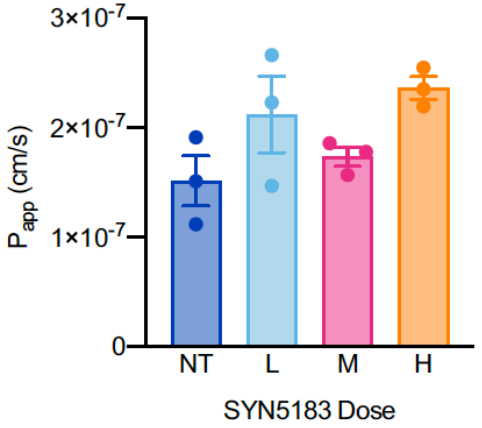
SYNB5183 Lowers Circulating Phe Levels in a Gut-chip





- Pharmacologics are unable to impact circulating levels of Phe
- Simulating enterorecirculation, Phe was dosed in the vascular compartment

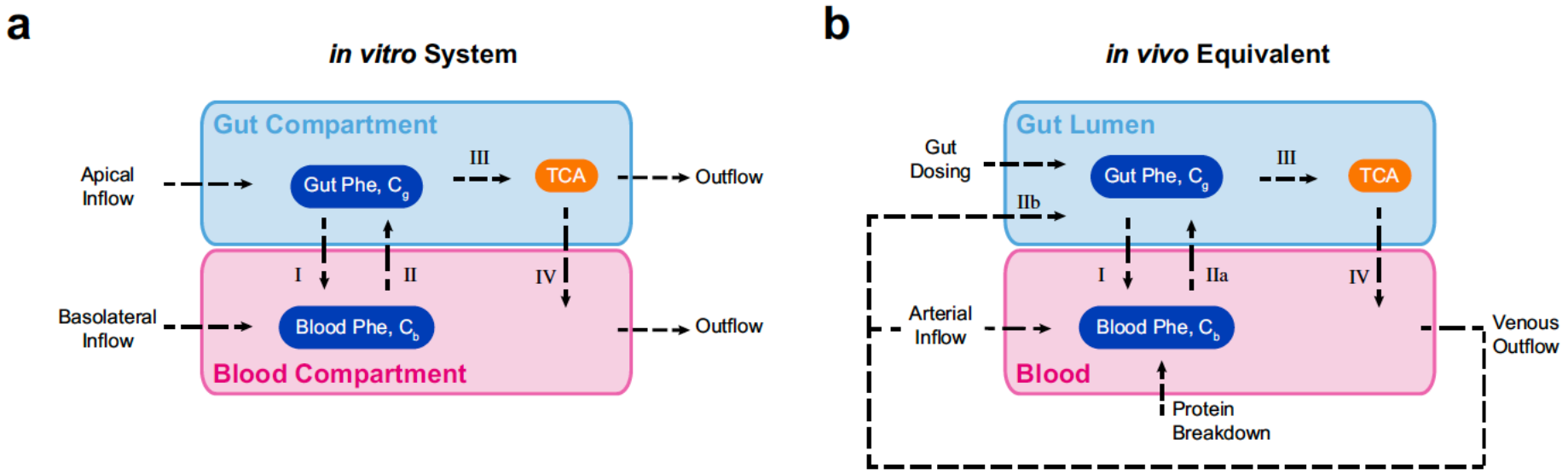




- No impact of villus height or morphology was observed
- Gut-chip barrier integrity was maintained at all doses

In Vitro to *In Vivo* Extrapolation of a human gut-chip

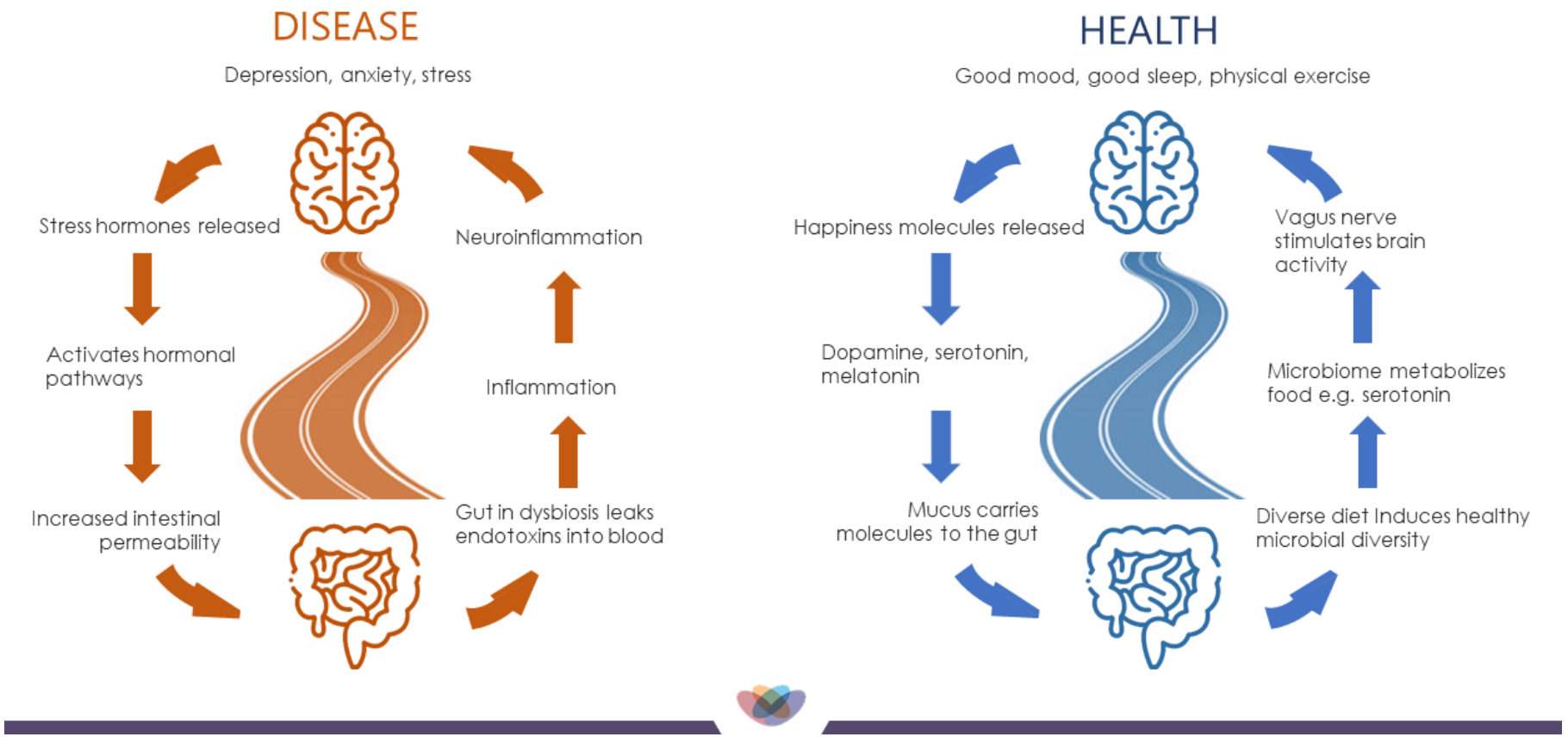
- Data was previously collected in a pre-clinical non-human primate assessment
- Using computational methods simulations of the gut-chip and non-human primate model were constructed following a PBPK approach



Major outcome = Gut-chip extrapolation resulted in an impressive Pearson's Correlation of 0.72

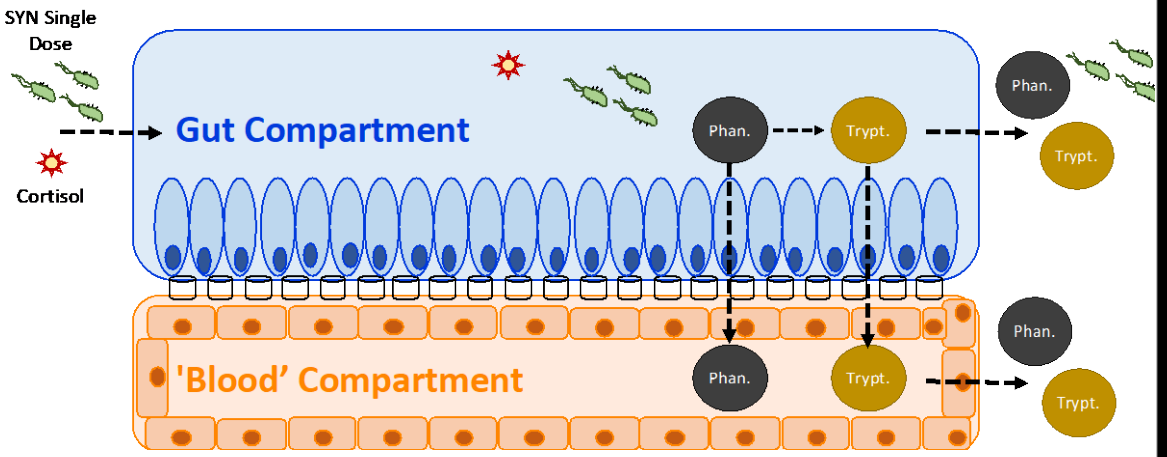
Analysis of a Novel Sense & Respond Synbiotic in a Gut-Brain Axis organ-on-a-chip system

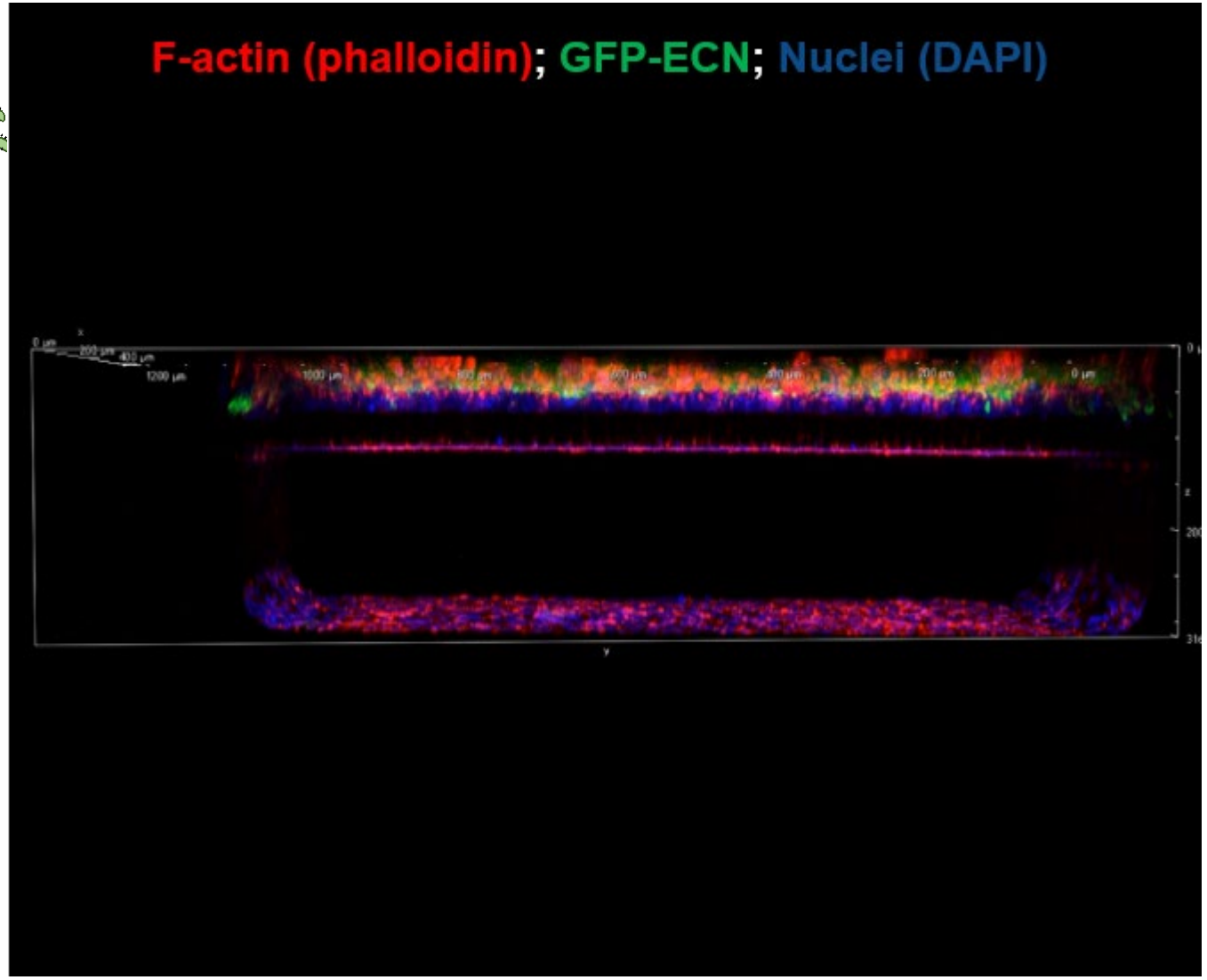
Characterization of a Cortisol Sensing Tryptamine Producing Synbiotic for Cognitive Augmentation



Li Y, Hao Y, Fan F, Zhang B. The Role of Microbiome in Insomnia, Circadian Disturbance and Depression. Front Psychiatry. 2018;9:669. Published 2018 Dec 5. doi:10.3389/fpsyt.2018.00669

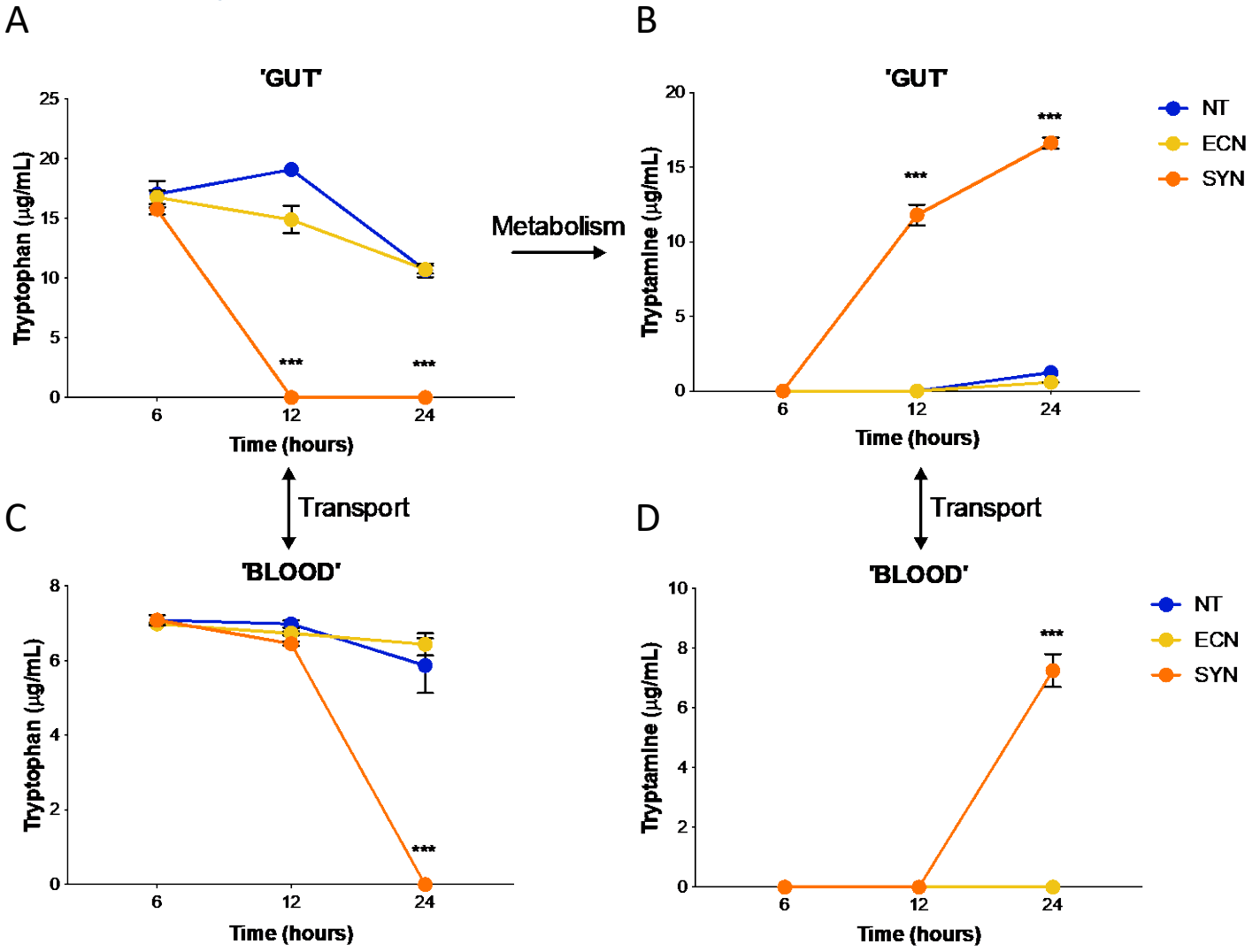
Physiologically Relevant Efficacy Screening of a Synbiotic in a Human Gut-on-a-chip



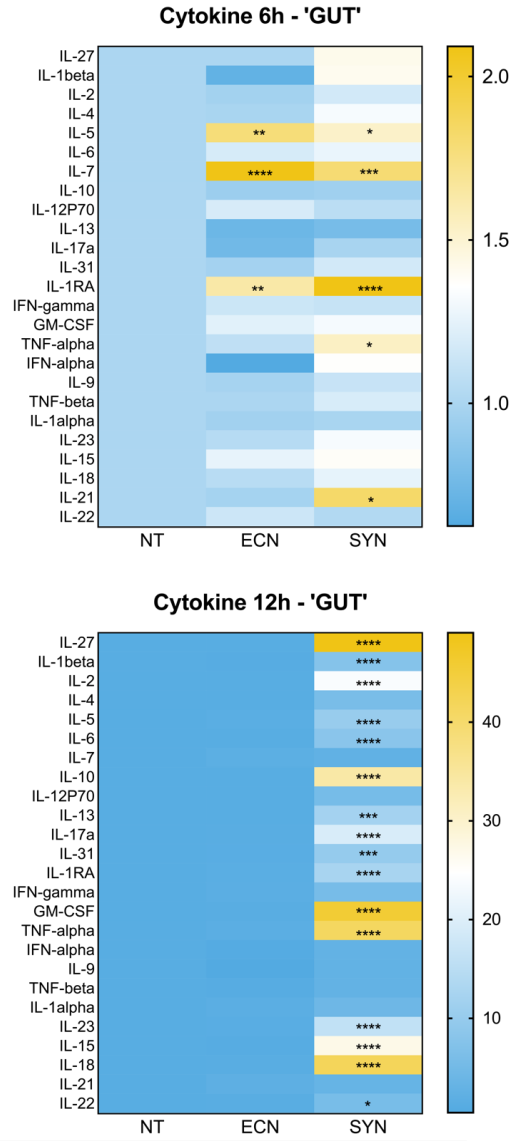


- SYN (E. Coli Nissle 1917 – engineered to sense cortisol and upon activation produce tryptophan decarboxylase metabolizing tryptophan to tryptamine
- 5 µM cortisol was added to the gut medium
- Physiological L-tryptophan is present in the medium

Tryptamine production and transport assessment in a human gut-chip

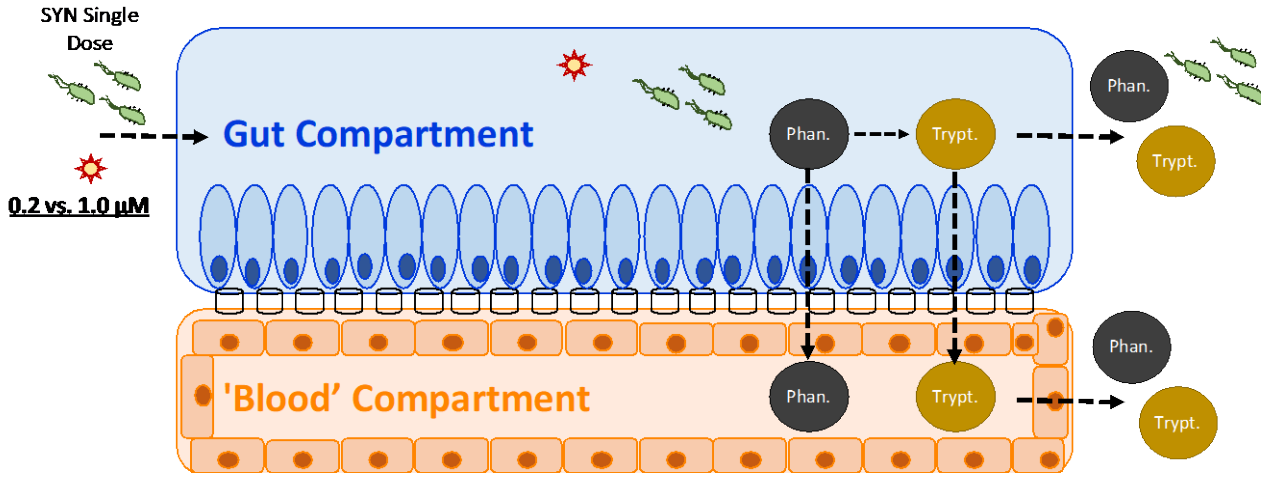


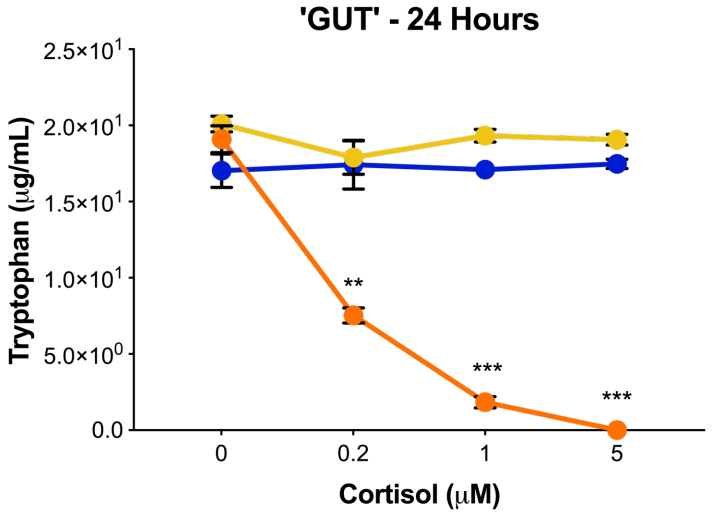
- Tryptophan is rapidly metabolized by SYN and converted to tryptamine
- Transport lags production, but nearly 50% of what was produced was detected in the blood compartment
- L-tryptophan is an essential amino acid for human cells, without it stress responses dominate
- Cytokines increase when L-tryptophan is depleted (12 hours)

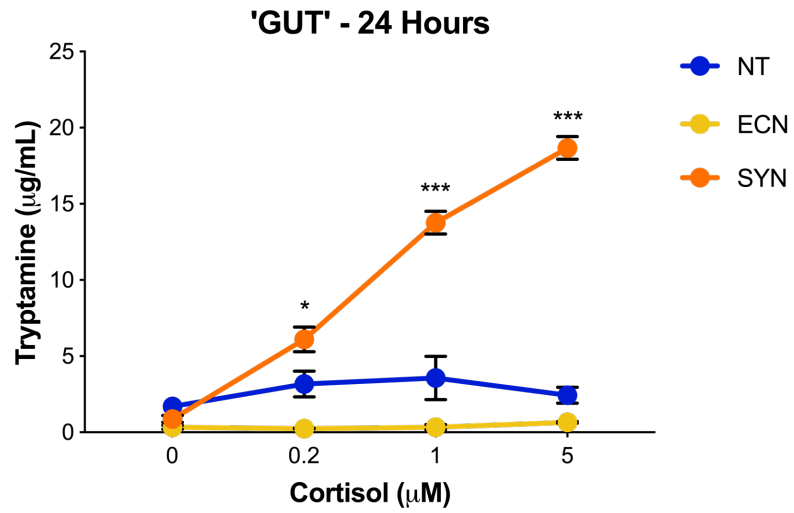


Cortisol Concentration Impact on Tryptamine Production

- Determine the sensitivity level of the synbio sense and respond elements
- Reduced Cortisol [ ]
- Physiological L-tryptophan is present in the medium
- 5 µM cortisol completely activates and depletes the bioavailable tryptophan within 24 hours
- While, only 80 and 55% of the bioavailable tryptophan was depleted for the 1 and 0.2 µM doses
- The a similar linear relationship in tryptamine production was observed

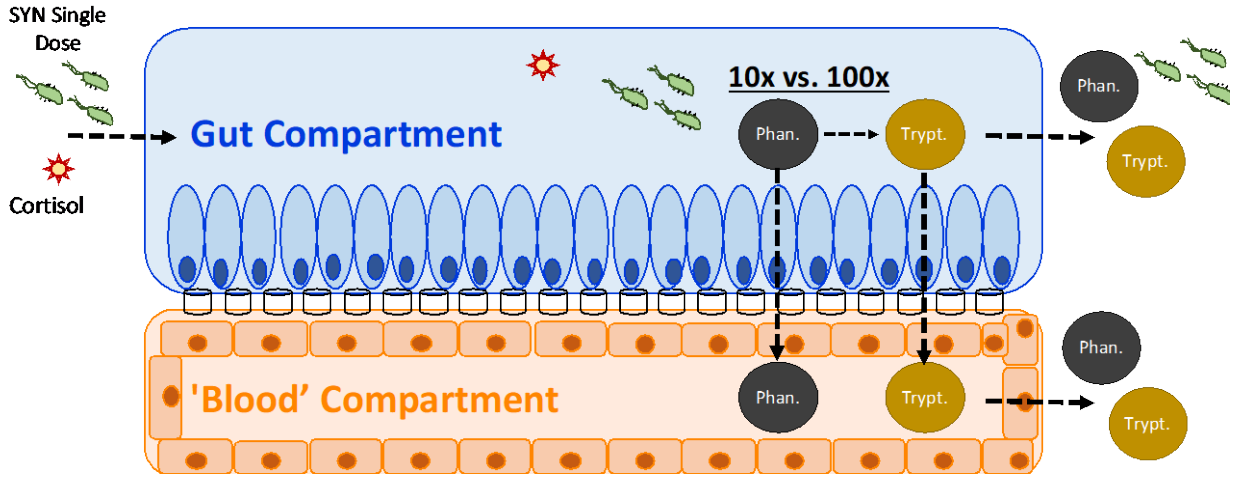


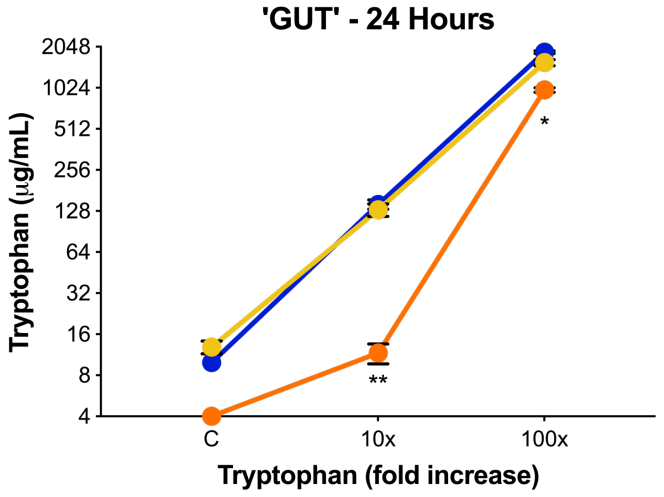


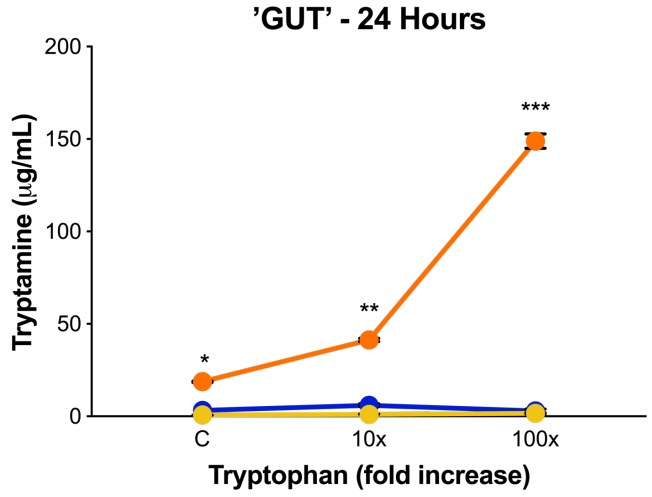


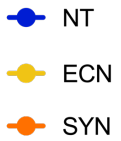
Tryptophan Concentration Drives Tryptamine Production

- Tryptophan is concentration limiting for the production of tryptamine
- Increases above the physiological L-tryptophan present in the medium
- 5 µM cortisol activation of the synbio circuit
- Tryptamine production increased substantially
- Excess Tryptophan improved tryptamine production and homeostasis of gut-chip





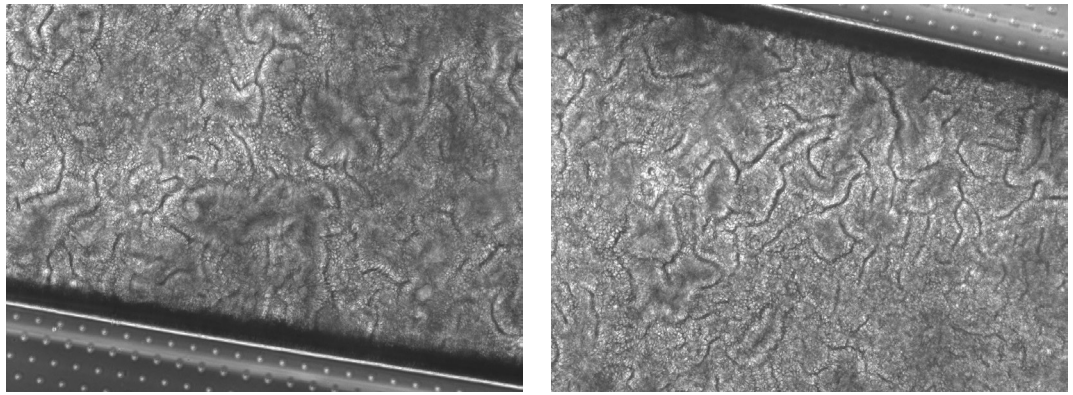




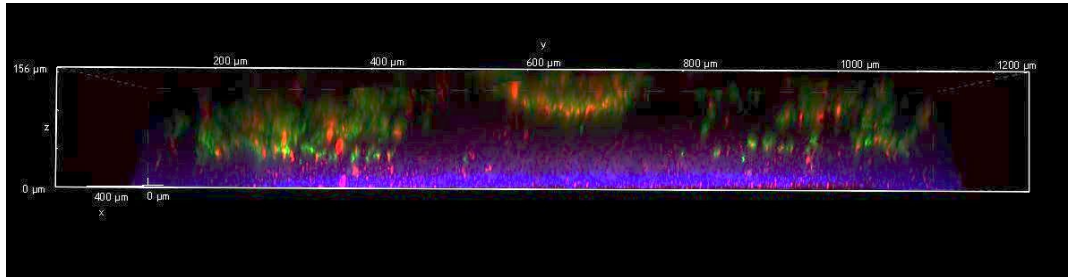
Future Direction(s)

When to Utilize More Complex Organoid-Derived Intestine-Chips?

- Advantages/Disadvantages of simplified Caco2 gut-chips as compared to intestine-chips
  - ADVANTAGES: Caco2 gut-chips are in general terms easier, faster, and cheaper
  - DISADVANTAGES: Caco2 gut-chips lack full intestinal cellularity, mainly mucus secreting goblet cells, and Caco2 gut-chips lack small/large intestine identity in terms of morphology/function (transporters/gene expression…)
- Does the problem require increased cellularity/complexity?

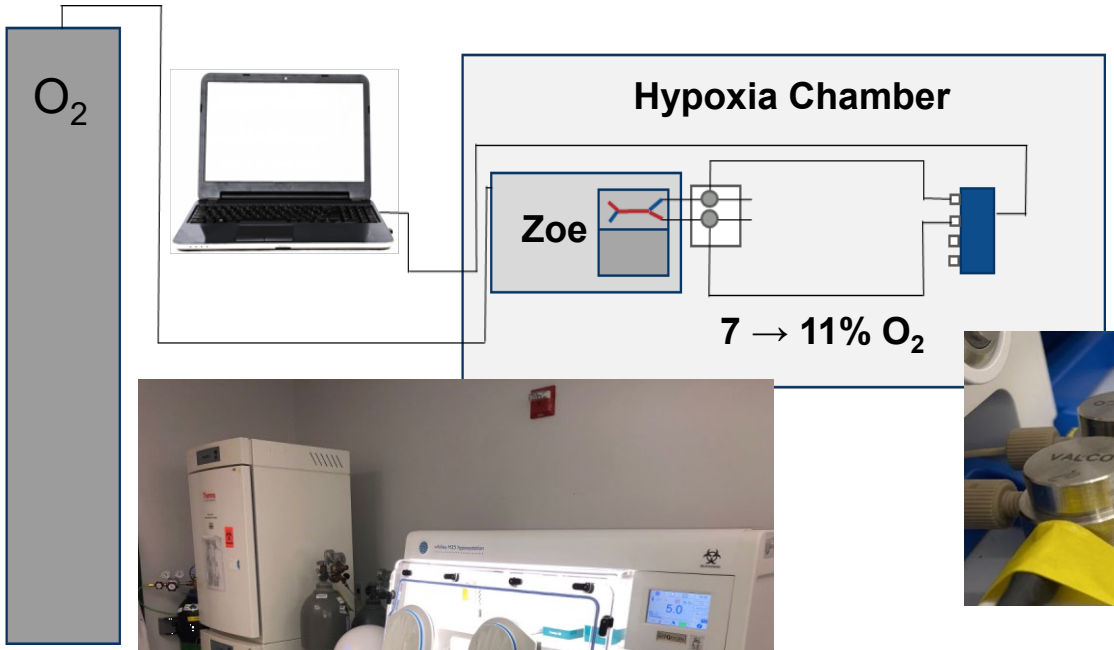


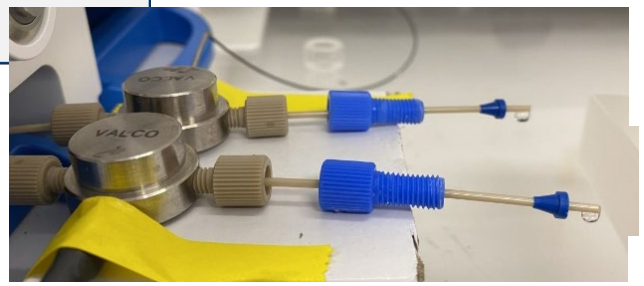
TOP LEFT: jejunum-on-a-chip (Day 8); RIGHT: colon-on-a-chip, both derived from fragmented human intestinal organoids.

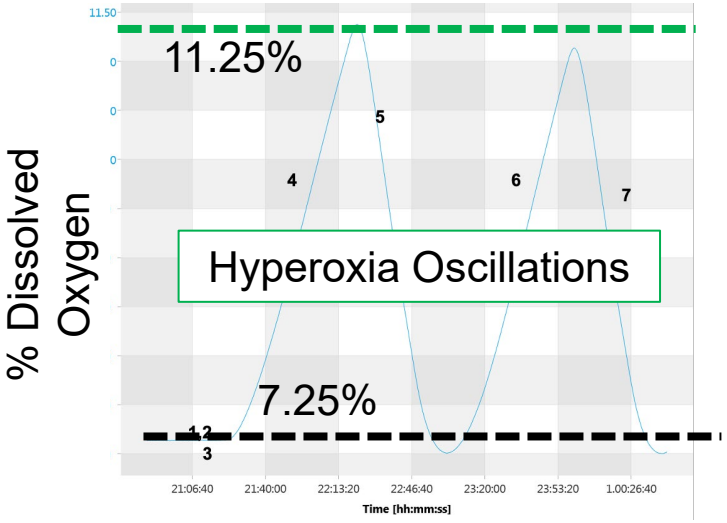


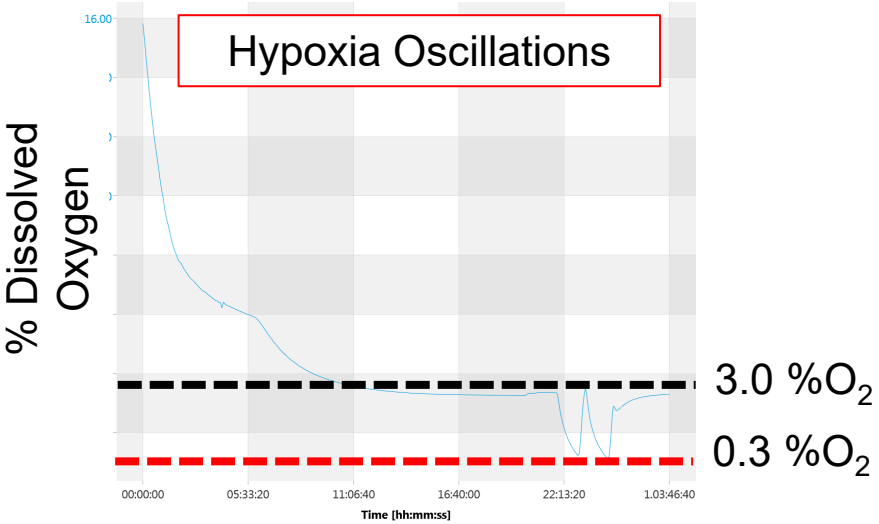
ABOVE: Cross-section 3D reconstructed confocal image of colon-chip Immunostained: e-cadherin (magenta), villin (green), and muc2 (red/orange)

Intestine-on-a-Chip In-Line Oxygen Sensing and Oxygen Control

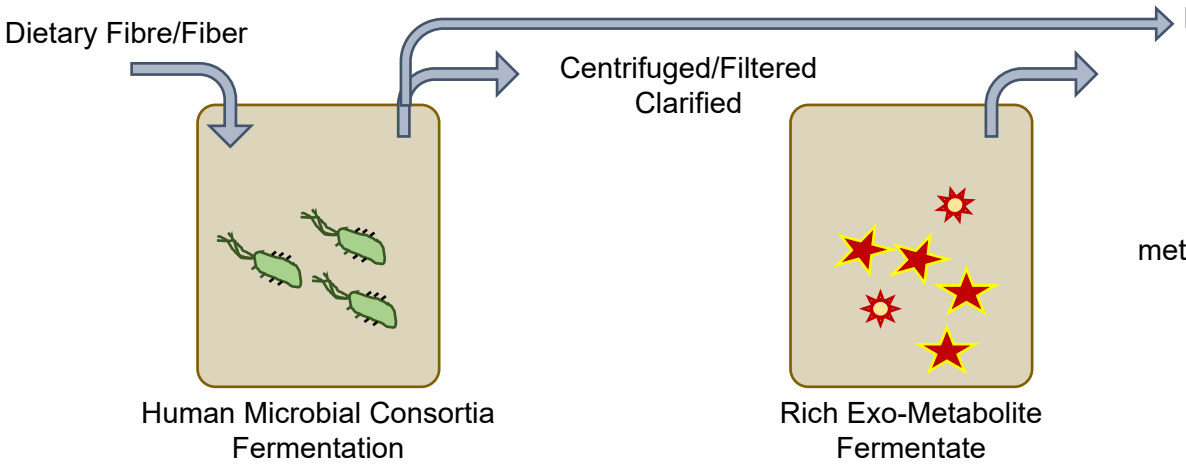


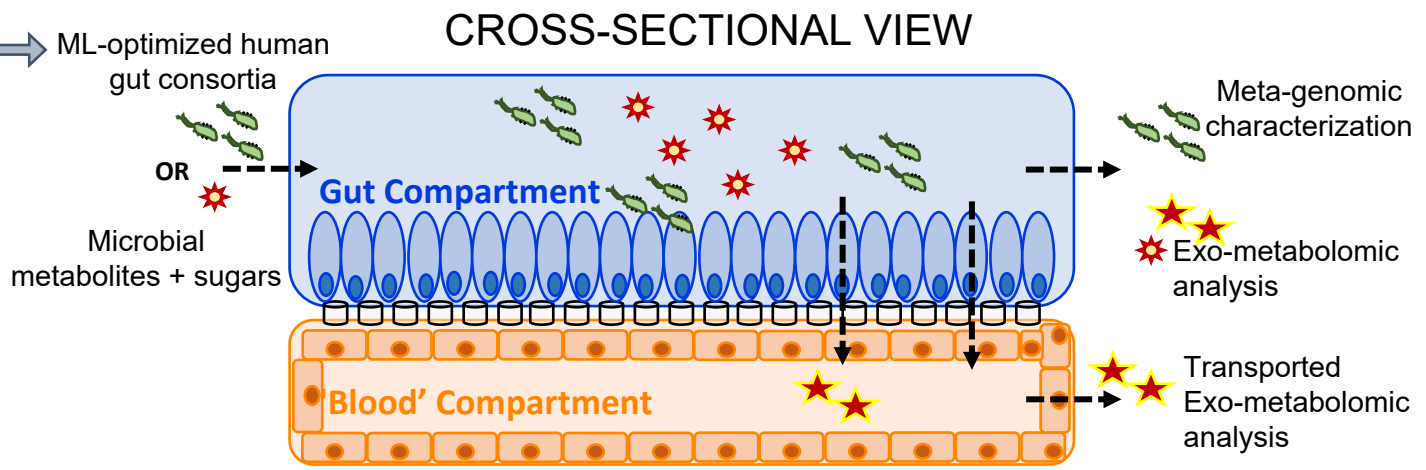






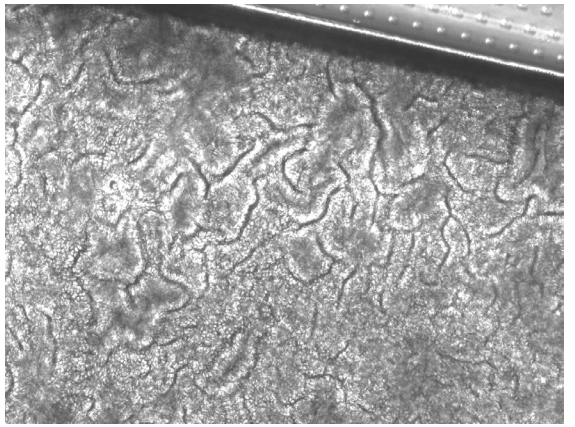
Intestine and Microbiome-on-a-Chip

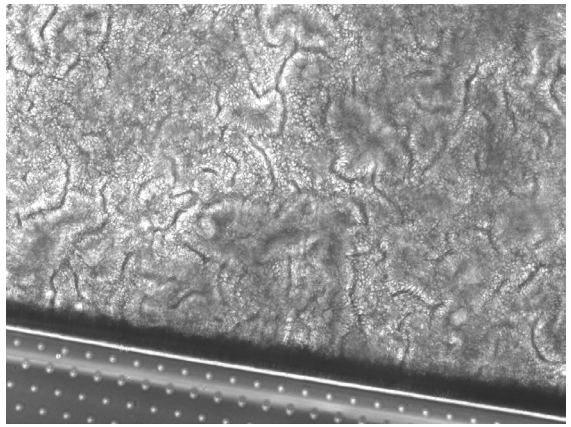




- Integrating batch fermentation and organ-on-a-chip
- Using patient crypt-based enteroids
- Full fecel microbiome cultivation on-a-chip
- Personalized intestine+microbiome-on-a-chip

Small Intestine-on-a-Chip





Summary

- Dynamic organ-on-a-chip gut models provide a robust platform to screen and test potential synbiotics, reducing animal needs
- Gut-chip models displayed a high degree of translatability when paired with computational in silico analyses
- Integration of additional organ systems-on-a-chip (Brain, muscle, and lung for example) could serve as useful test bed to characterize not only primary synbiotic function but target organ impacts

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