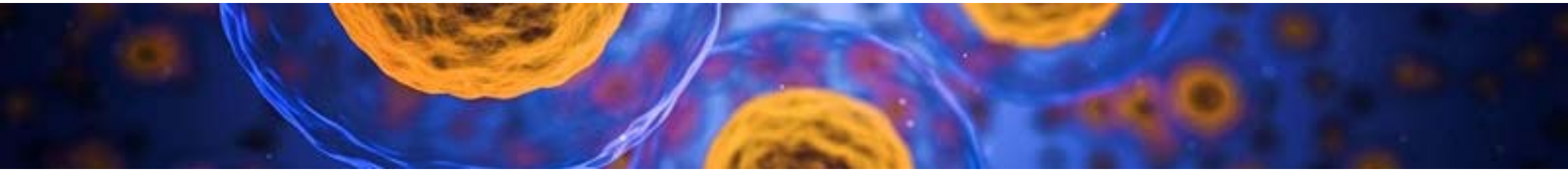


3D Cell Culture Models for Drug PK, Safety, and Efficacy Assessment

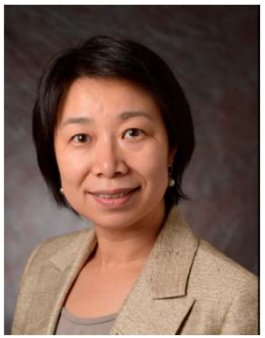
Sessions Three and Four

FDA and M-CERSI Collaborative Workshop

CERSI
University of Maryland
Center of Excellence in Regulatory Science and Innovation

Session Three:

Multi-organ 3D Models: Intestine, Liver and Beyond



Session Chair: Dr. Grace Guo

Associate Professor
Department of Pharmacology and Toxicology
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3D Hepatic and Enteric Experimental Systems for Drug Metabolism, Drug Drug Interactions, and Drug Toxicity

Albert P. Li, Ph. D.

In Vitro ADMET Laboratories

Acknowledgment

Hong Wei and her mentor
Gregg L Semenza, Johns Hopkins University
2019 Nobel Prize in Physiology or Medicine

- Hong (Ivy) Wei
- Qian Yang
- David Ming Chih Ho
- Walter Mitchell

IVAL Mission

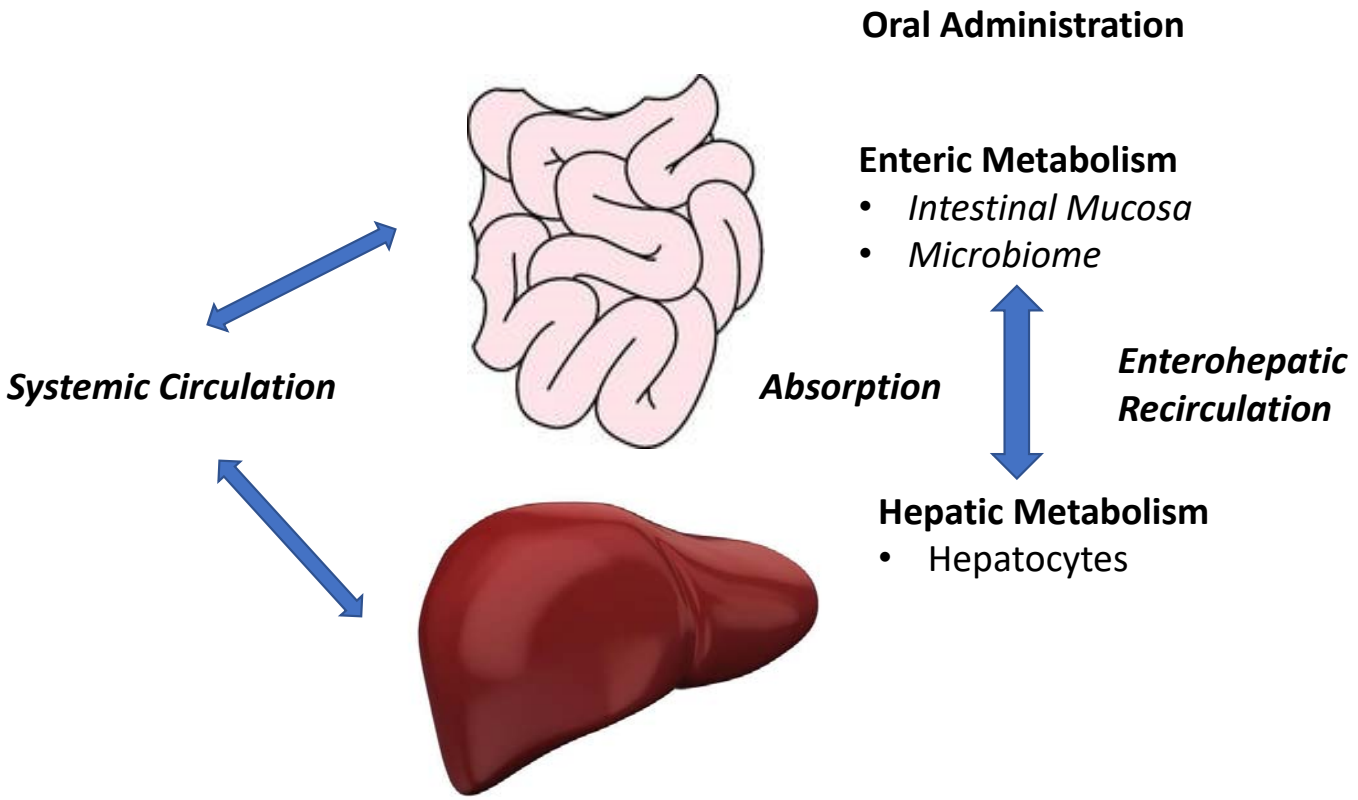
Provision of Products and Contract Research Service for Accurate Assessment of Human Drug Properties

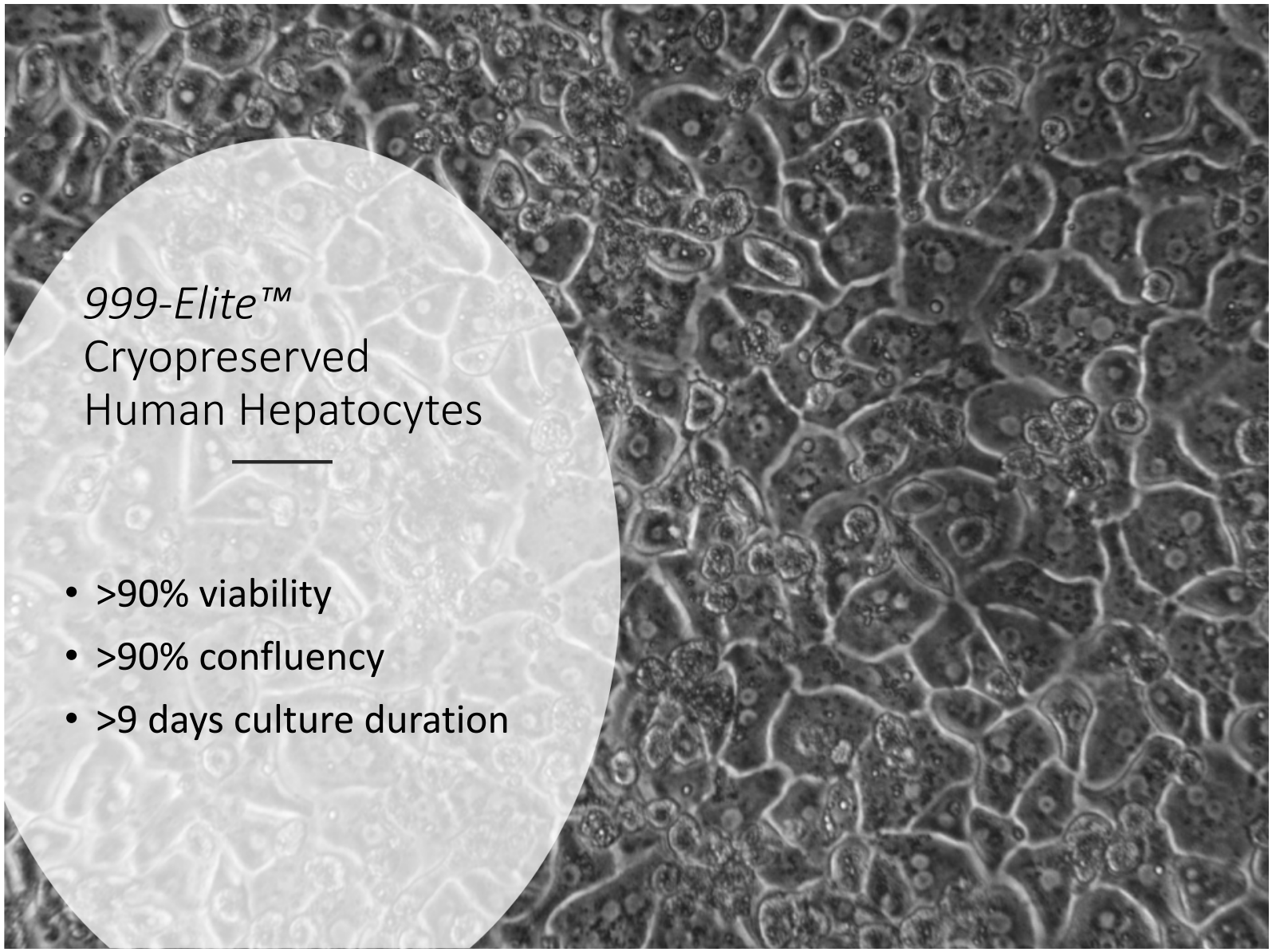
Key Properties of In Vitro Experimental Systems

Organ-specific properties

Species-specific properties

IVAL Focus: Liver and Intestine

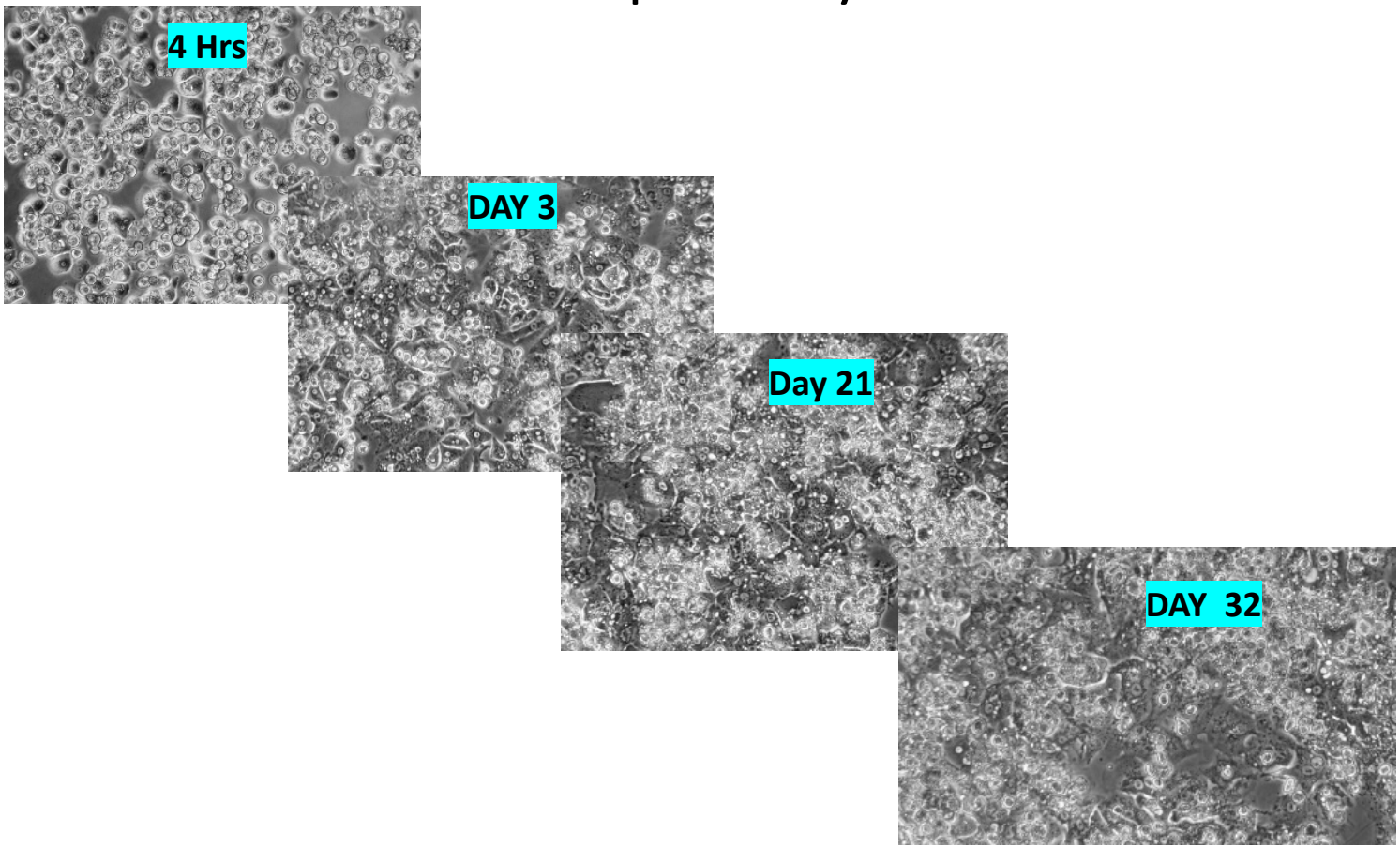




999-Elite™
Cryopreserved
Human Hepatocytes

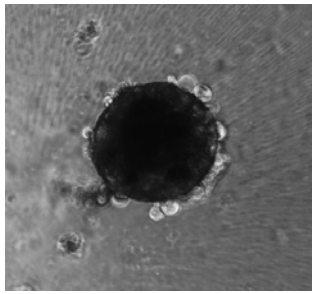
- >90% viability
- >90% confluency
- >9 days culture duration

999Elite™ Human Hepatocytes: HH1144

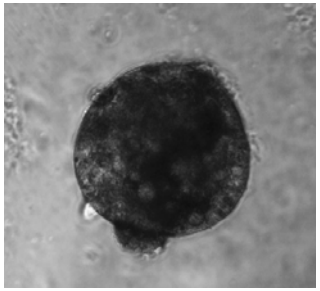


999Elite™ Human Hepatocyte Spheroids

HH1051



HH1085



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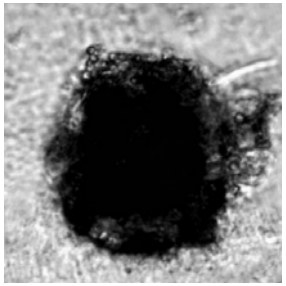
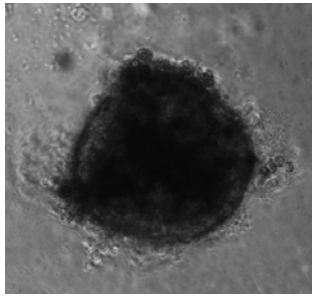
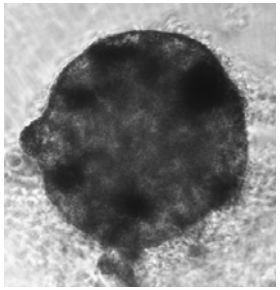
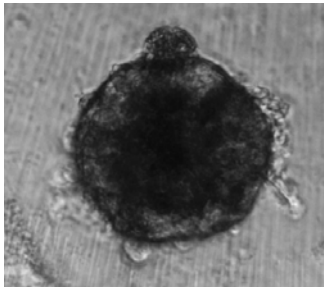




HH1136

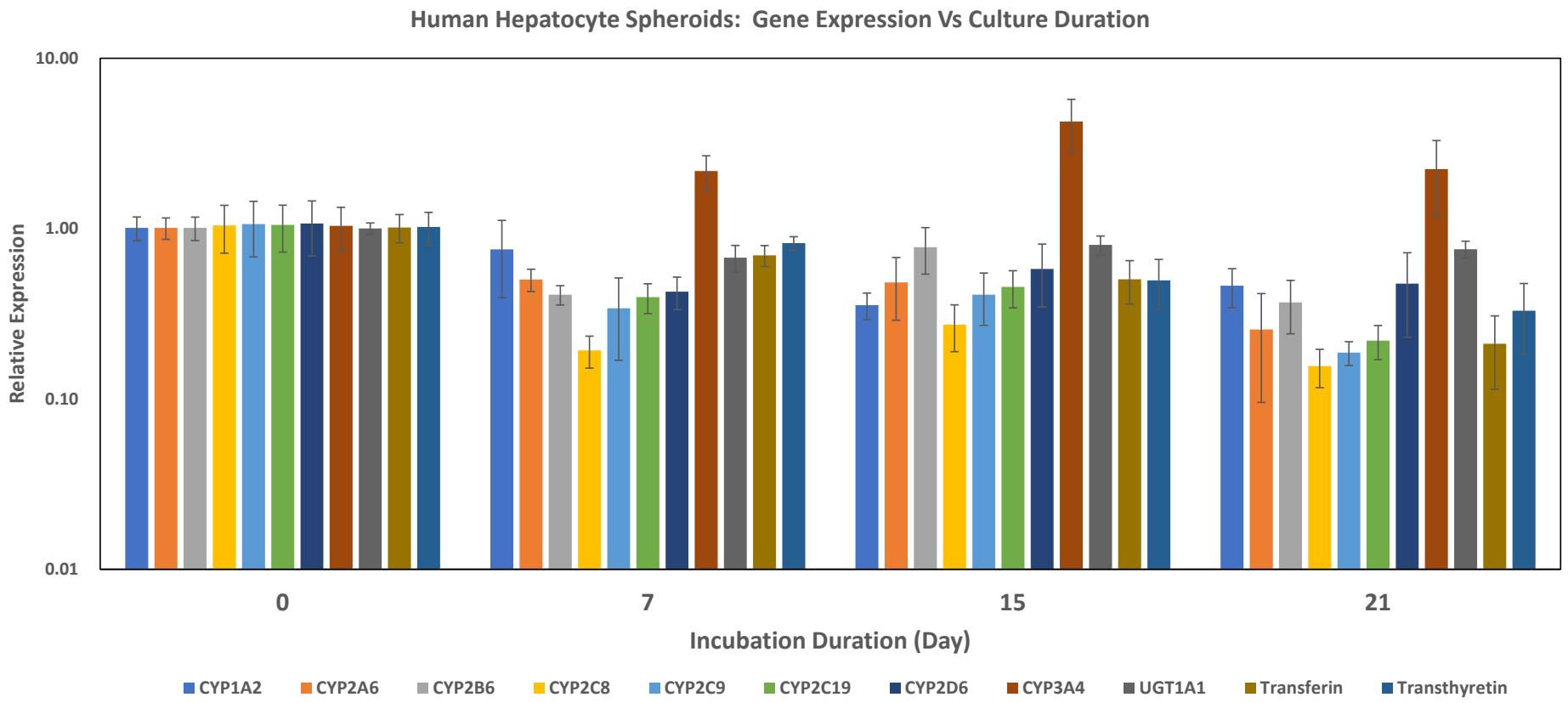


HH1142

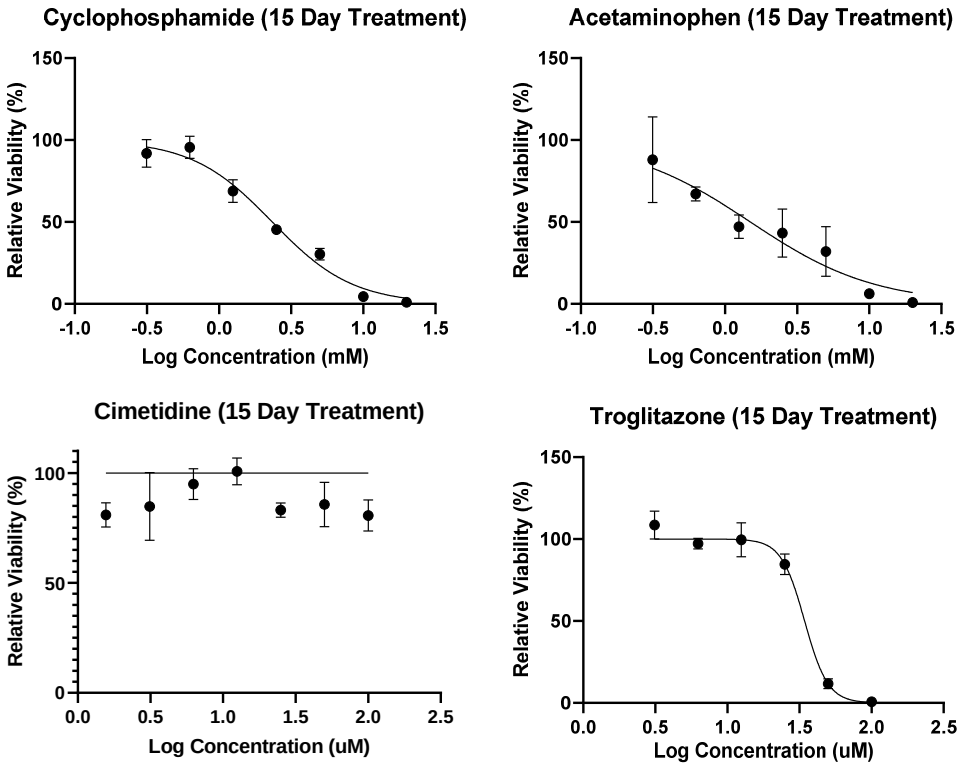


HH1176

Effects of Culture Duration on Hepatic Gene Expression



Spheroid 15-Day Hepatotoxicity Assay



Conclusion Hepatocyte Spheroids

- 999Elite™ human hepatocytes can be cultured as spheroids
  - Spheroids can be cultured for >21 days
- Gene expression results show stabilized gene expression for drug metabolizing enzymes and key hepatic biomarkers (>21 days)
- Human Hepatocyte Spheroid Hepatotoxicity have been established
  - Acceptable spheroid to spheroid variations
  - Dose-dependent hepatotoxicity observed with known hepatotoxicants
  - Non-hepatoxicity for nonhepatotoxicants
  - Applicable towards prolonged treatment durations

3D-Enteric System

Cryopreserved Human Intestinal Mucosa (CHIM)

Critical Challenge for In Vitro Enteric Systems

- All In Vitro Crypt, IPS, Fetal, and Cell line-based Enteric Systems Are Deficient in Drug Metabolizing Enzyme Activities

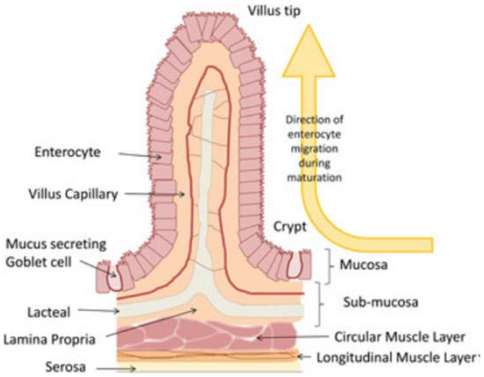
Solution:

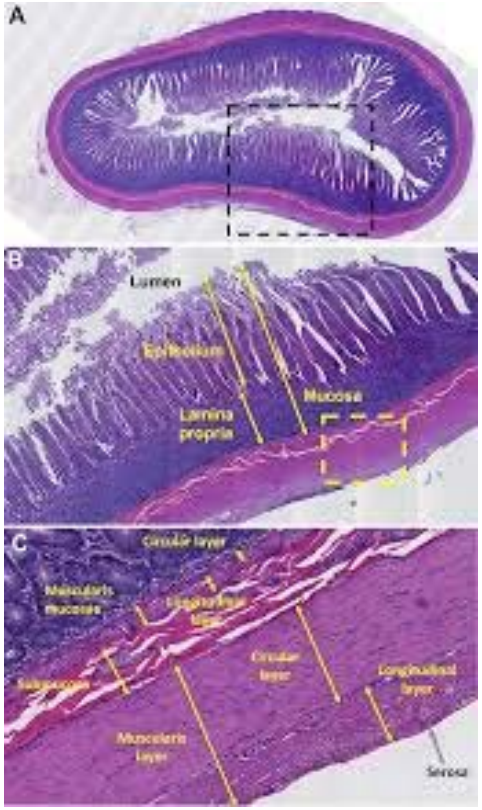
Isolation and cryopreservation of enterocytes from human and animal intestines for use immediately after thawing

IVAL In Vitro Enteric Systems

- **Cryopreserved Human Enterocytes**
  - Ho, M.C.D., Ring, N., Amaral, K., Doshi, U. and Li, A.P., 2017. Human Enterocytes as an In Vitro Model for the Evaluation of Intestinal Drug Metabolism: Characterization of Drug-Metabolizing Enzyme Activities of Cryopreserved Human Enterocytes from Twenty-Four Donors. *Drug Metabolism and Disposition*, *45*(6), pp.686-691.
- **MetMax™ Cryopreserved Human Enterocytes (Patent Pending)**
  - Li, A. P., Amaral, K., & Ho, M. C. D. (2018). A Novel In vitro Experimental System for the Evaluation of Enteric Drug Metabolism: Cofactor-Supplemented Permeabilized Cryopreserved Human Enterocytes (MetMax™ Cryopreserved Human Enterocytes). Drug metabolism letters, 12(2), 132-137.
- **3D System: Cryopreserved Human Intestinal Mucosa (CHIM™; Patent Pending)**
  - Li, A. P., Alam, N., Amaral, K., Ho, M. C. D., Loretz, C., Mitchell, W., & Yang, Q. (2018). Cryopreserved Human Intestinal Mucosal Epithelium: A Novel In Vitro Experimental System for the Evaluation of Enteric Drug Metabolism, Cytochrome P450 Induction, and Enterotoxicity. Drug Metabolism and Disposition, 46(11), 1562-1571.

Intestinal Mucosa



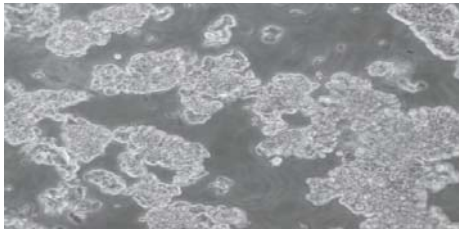


Preparation of CHIM from a Human Intestine

Collagenase Digestion

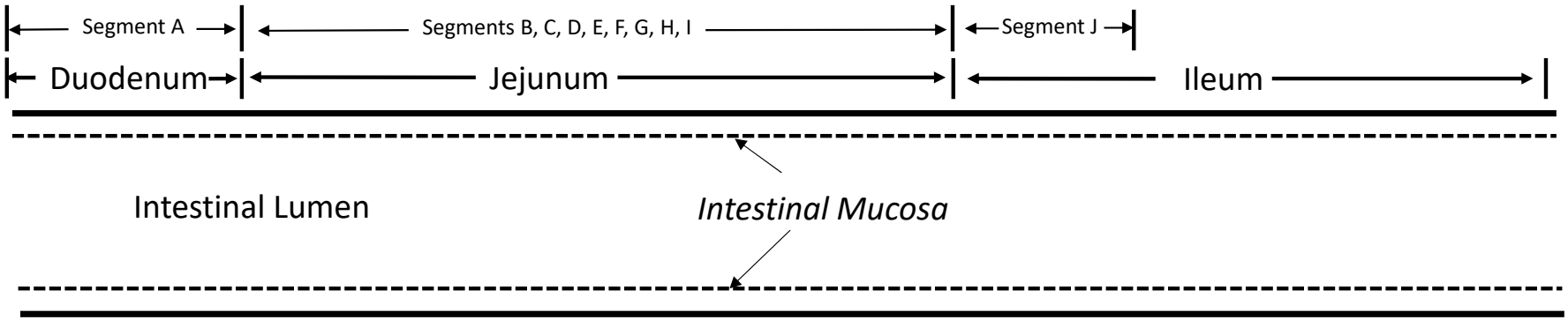
Intestinal Villi

Gentle Homogenization

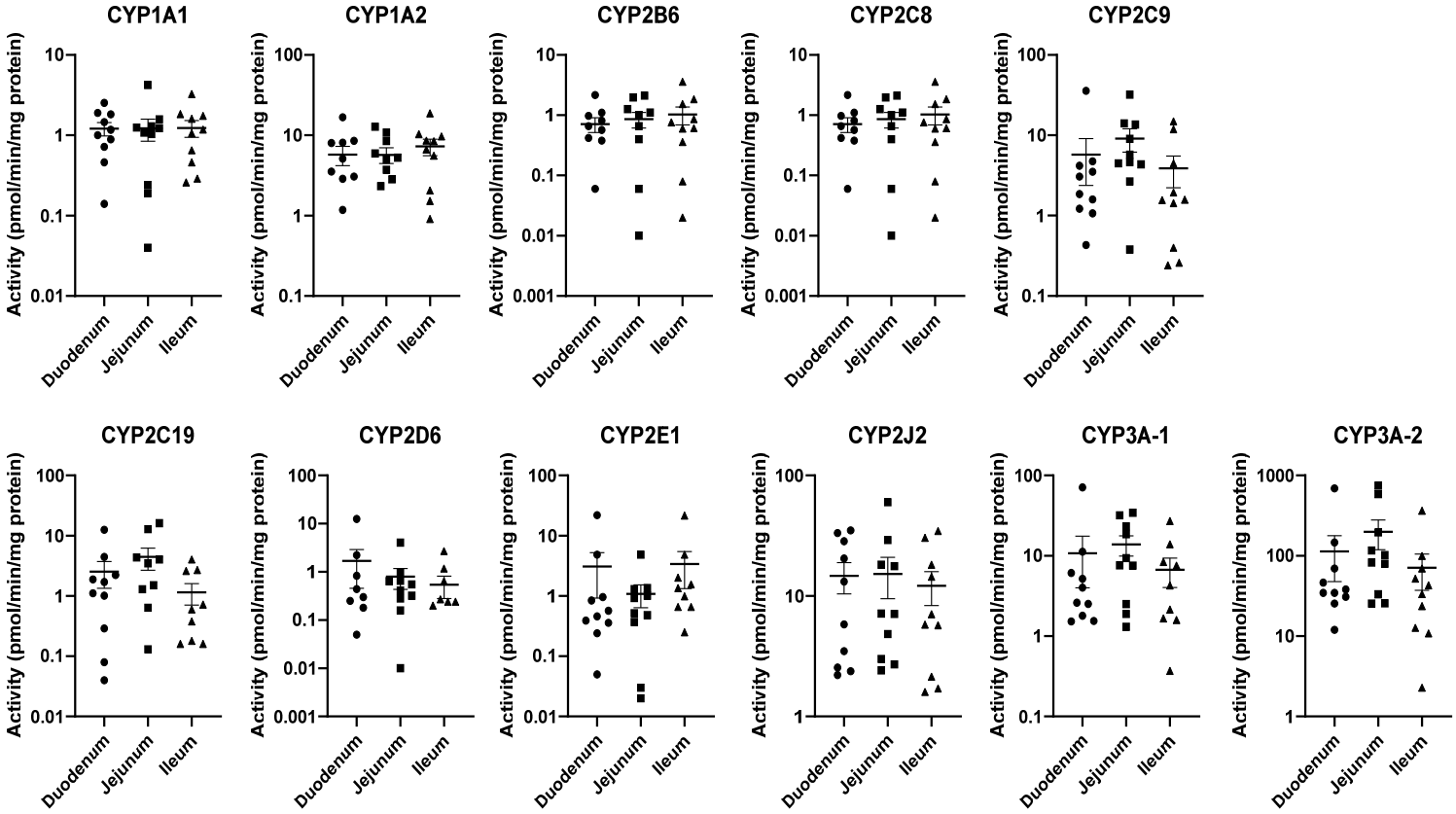


Cryopreservation

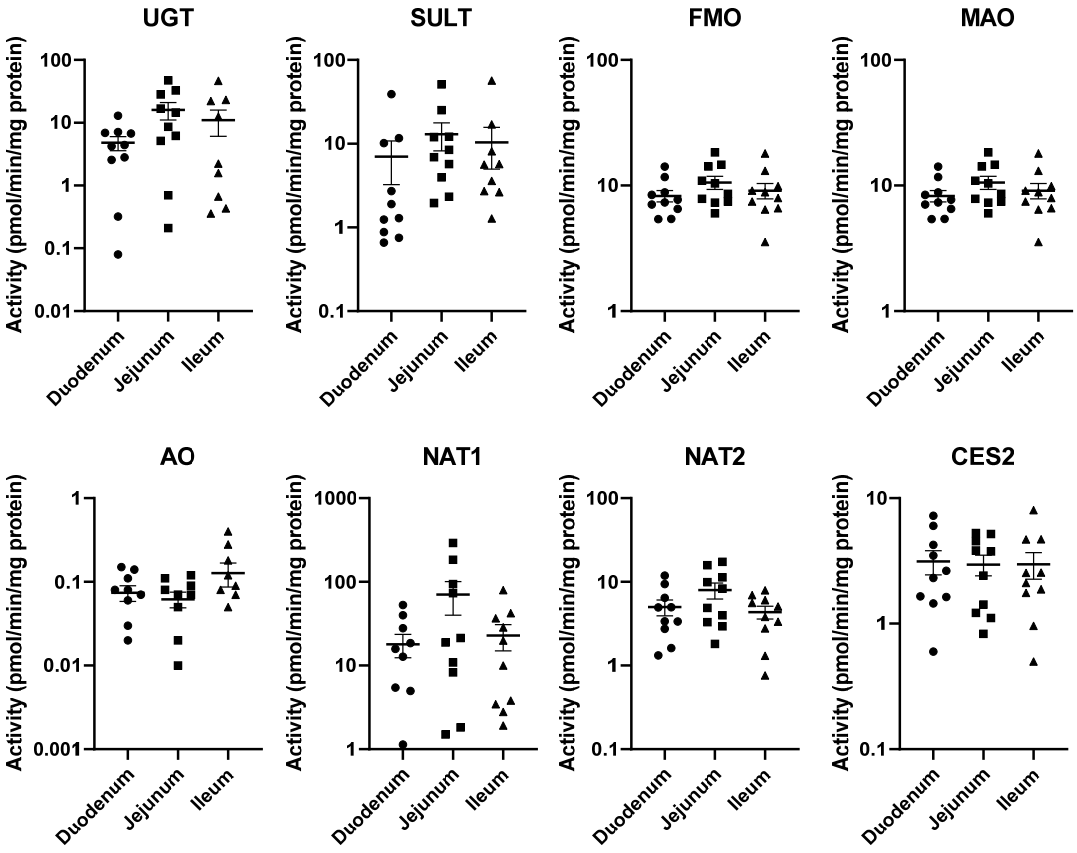
Inter-individual and Inter-regional Variations in Enteric Drug Metabolism (Li et al., PR&P (2020))



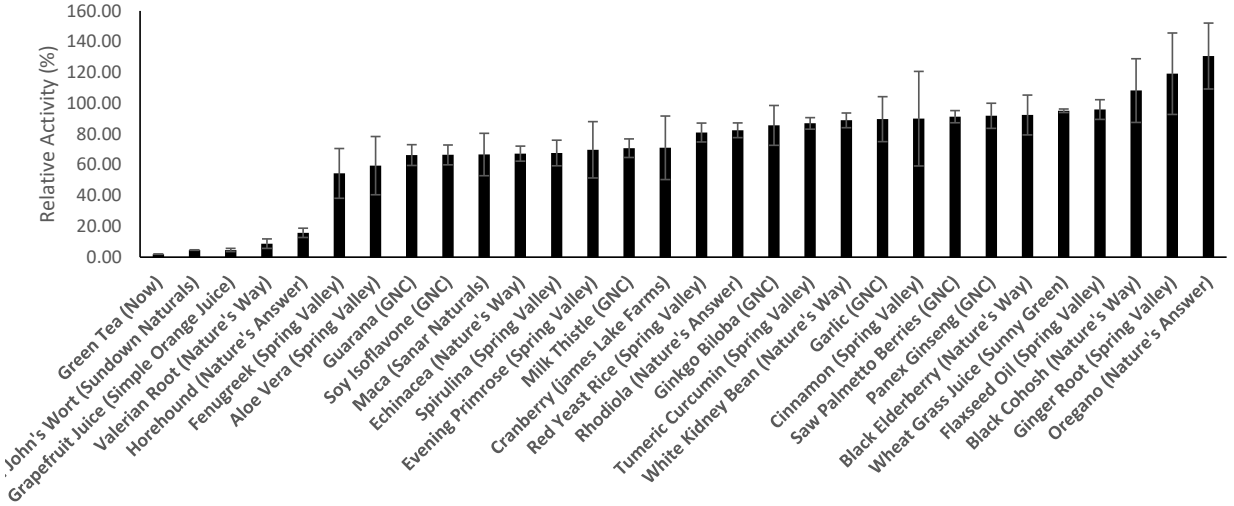
P450 Isoforms (Li et al., PR&P (2020))



Non-P450 DME (Li et al., PR&P (2020))



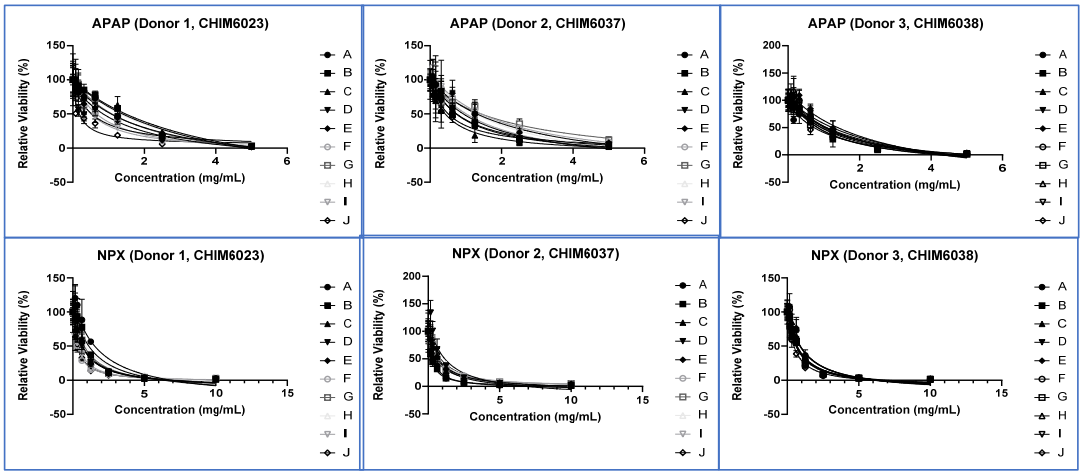
Herb Drug Interactions: Herbal Supplement Inhibition of Enteric CYP3A Activity (Midazolam 1'-Hydroxylation)

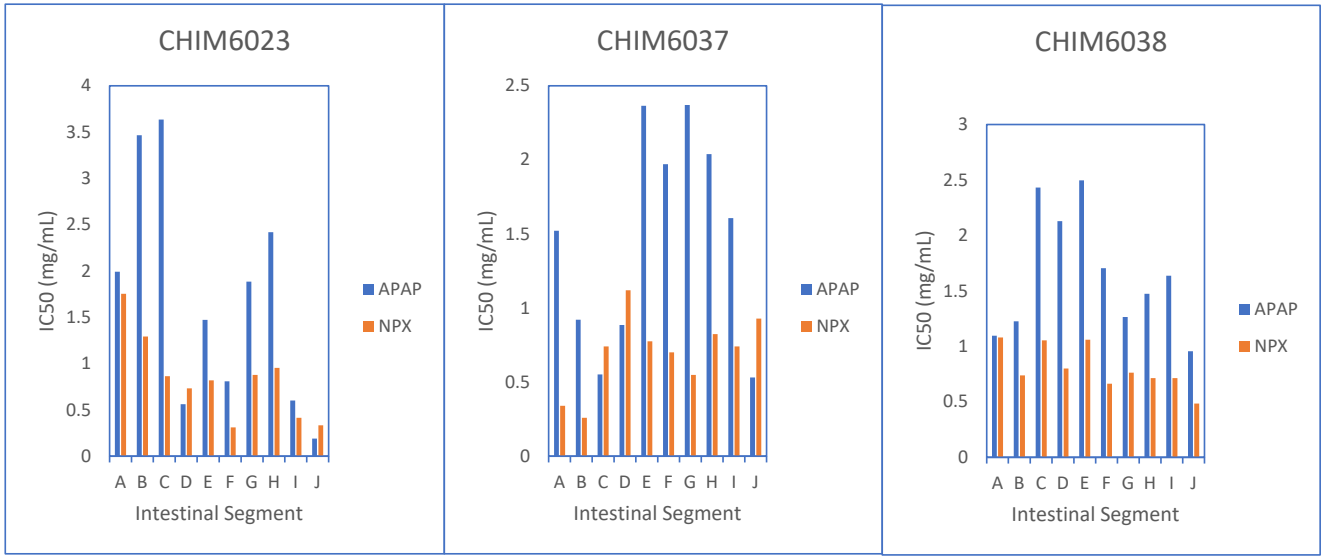


- Loretz C, Ho DD, Alam N, Mitchell W, Li AP. Application of cryopreserved human intestinal mucosa and cryopreserved human enterocytes in the evaluation of herb-drug interactions: Evaluation of CYP3A inhibitory potential of grapefruit juice and commercial formulations of twenty nine herbal supplements. Drug Metabolism and Disposition. 2020 (in press)

Enteric Drug Toxicity

NASID Enterotoxicity
(Li et al, 2020, DMD)





CHIM

- Most complete in vitro enteric system
- Potent drug metabolizing enzyme activities
- Applications:
  - Fg; metabolite profiling
  - Regional variations in drug metabolism
  - DDI
  - Enterotoxicity

3D-Hepatic and Enteric Experimental Systems

- Hepatic System: 999Elite™ Human Hepatocyte Spheroids
  - Ready spheroid formation from multiple human hepatocyte lots
  - Long duration of culture: >21 days
  - In vitro human hepatocyte spheroid hepatotoxicity assay
- Enteric System: Cryopreserved Human Intestinal Mucosa (CHIM™)
  - Robust drug metabolizing enzyme activities
  - Applicable toward enteric drug metabolism, drug-drug interactions, and enterotoxicity evaluation

FAD and MCERSI Workshop
August 14, 2020

UNIVERSITY of MARYLAND
SCHOOL OF MEDICINE

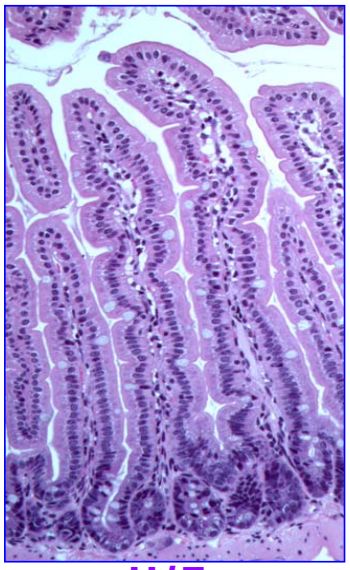
Intestinal Organoids: An excellent model for studying gut epithelium homeostasis

Jian-Ying Wang, MD, PhD

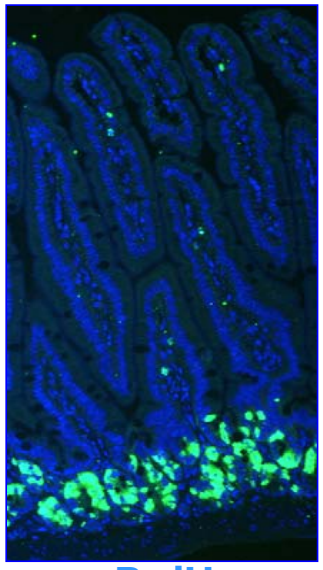
Joseph and Corinne Schwartz Professor

University of Maryland School of Medicine

Intestinal epithelial renewal

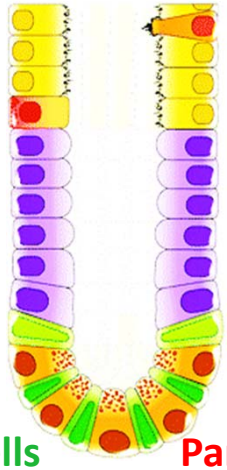


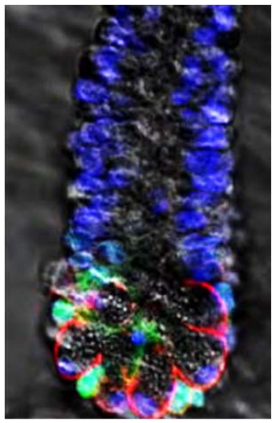
H/E



BrdU

Paneth/stem cell niche

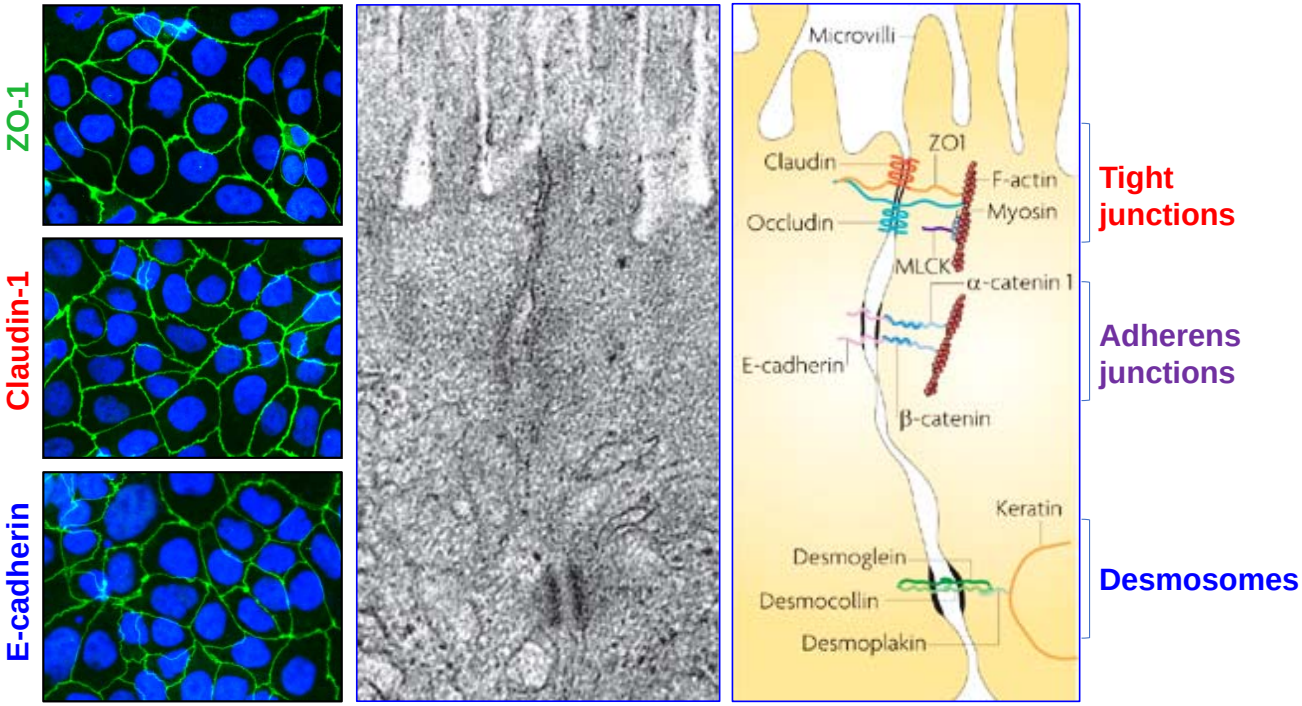




Stem cells

Paneth cells

Intestinal epithelial barrier



Isolation of primary enterocytes in mice

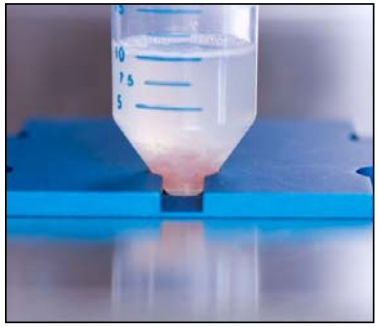
To remove external membrane and fat

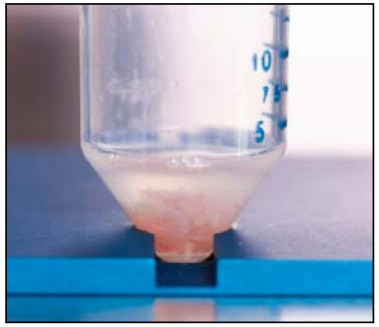
To harvest intestinal segment

To cut and open

To rinse the segments

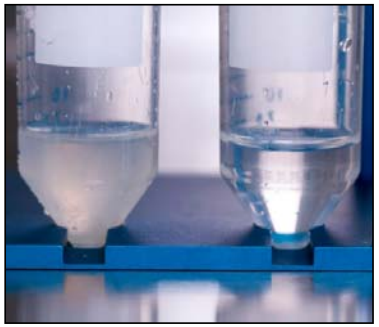
To wash mouse intestinal pieces



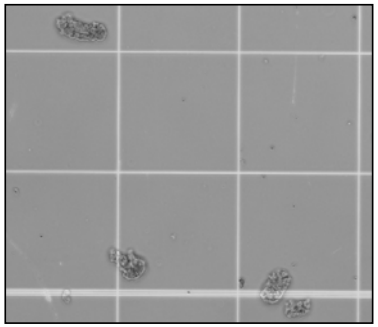


Culture of intestinal organoids

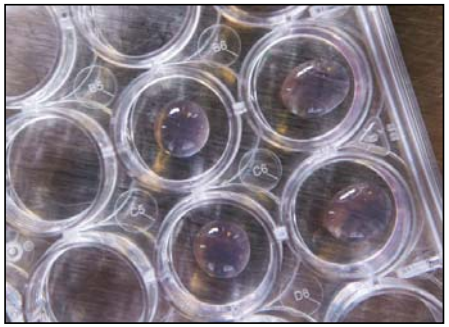
To wash supernatants first (left) and 20th



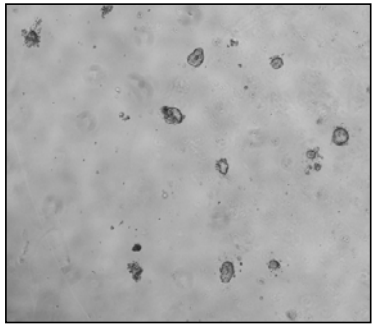
To fractionize intestinal crypts



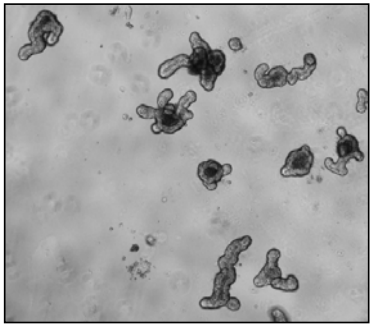
To suspend organoids in mixed matrigel dome



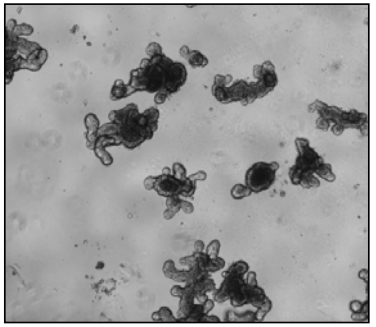
day 1



day 3



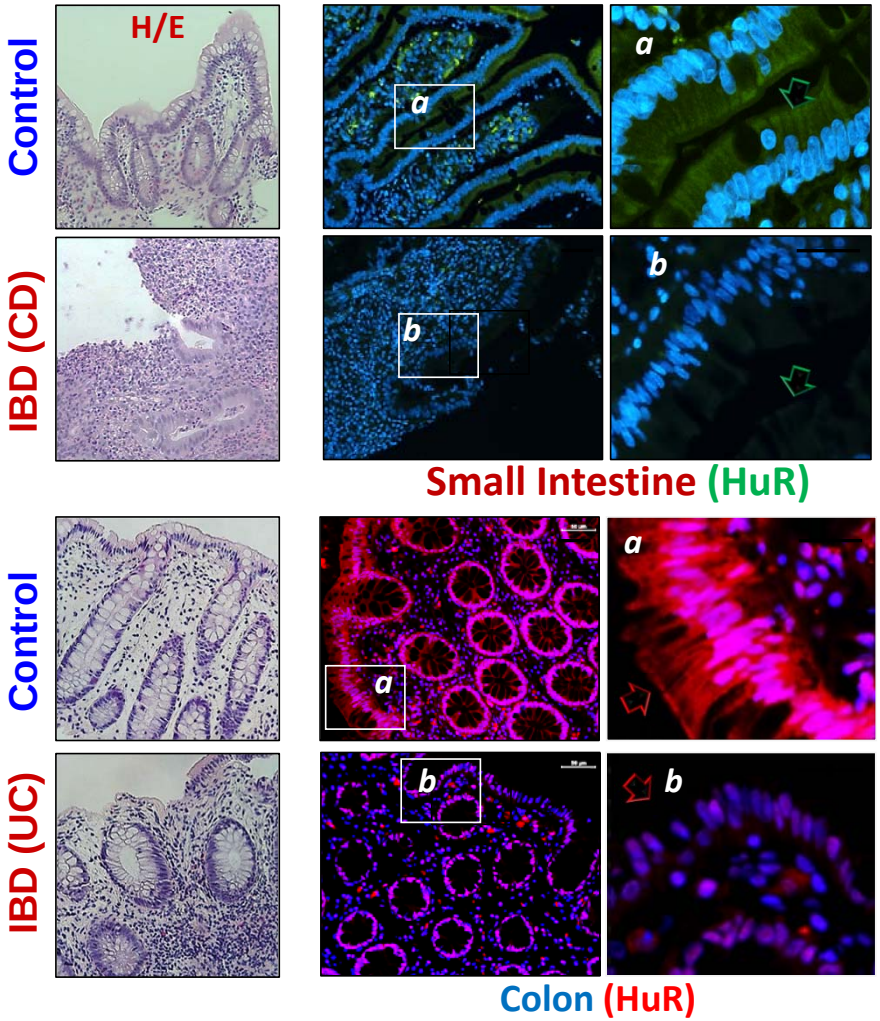
day 5



RNA-binding proteins (RBPs)

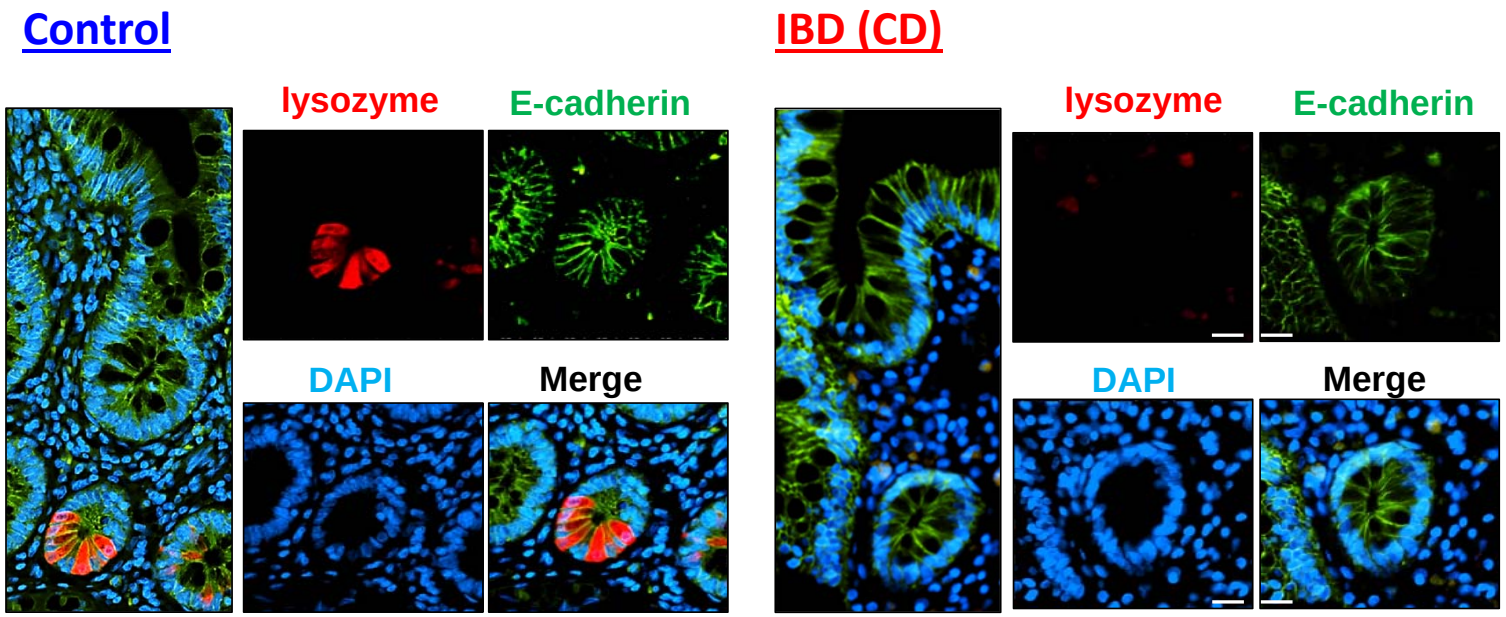
- RBPs directly interact with mRNAs via A/AU-rich elements (AREs) or GU-rich elements (GREs)
- AREs and GREs are commonly located at 3'-untranslated regions (UTRs) of target mRNAs
- RBP/mRNA interactions alter the stability and translation of target mRNAs, thus regulating gene expression
- RBPs implicated in intestinal epithelial homeostasis:
  - HuR
  - CUGBP1
  - AUF1
  - TIAR

HuR in intestinal mucosa of patients with IBD



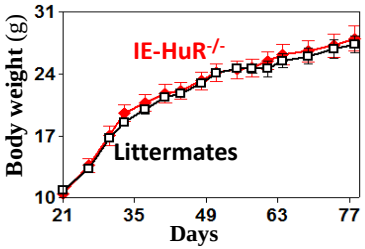
Gastroenterology 157: 731-743, 2019

Defective Paneth cells in patients with IBD

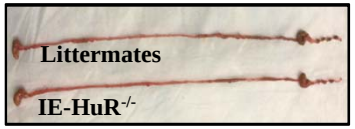


HuR deletion inhibits growth of small intestinal mucosa

A) Body weights

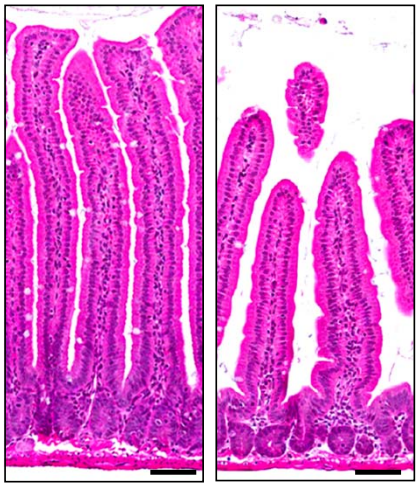


B) GI gross morphology



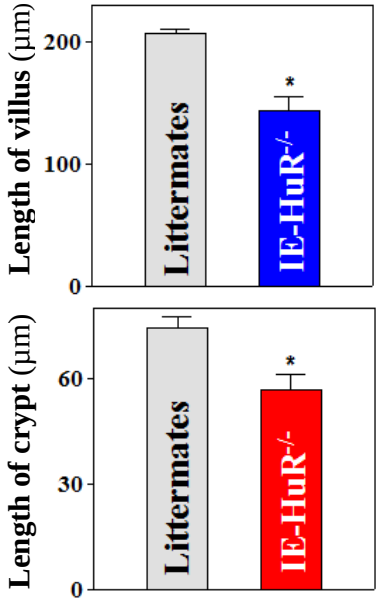
HuR	S. Intestine	Colon
+/+	30.6 ± 3.7	6.26 ± 0.55
-/-	31 ± 2.9	6.41 ± 0.67

C) H/E staining

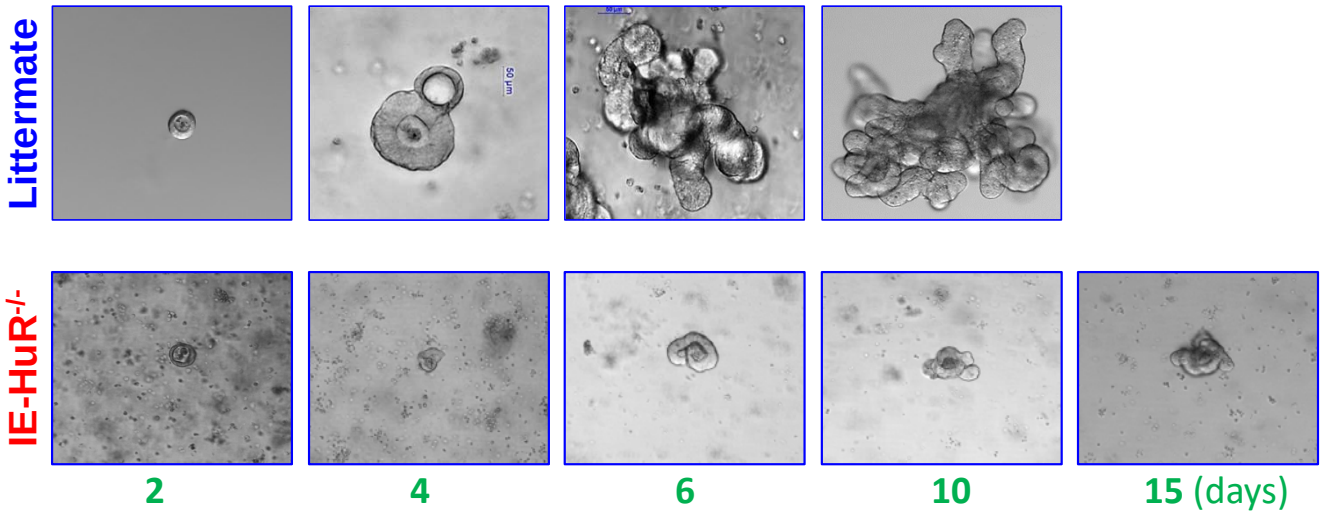


Littermates IE-HuR-/-

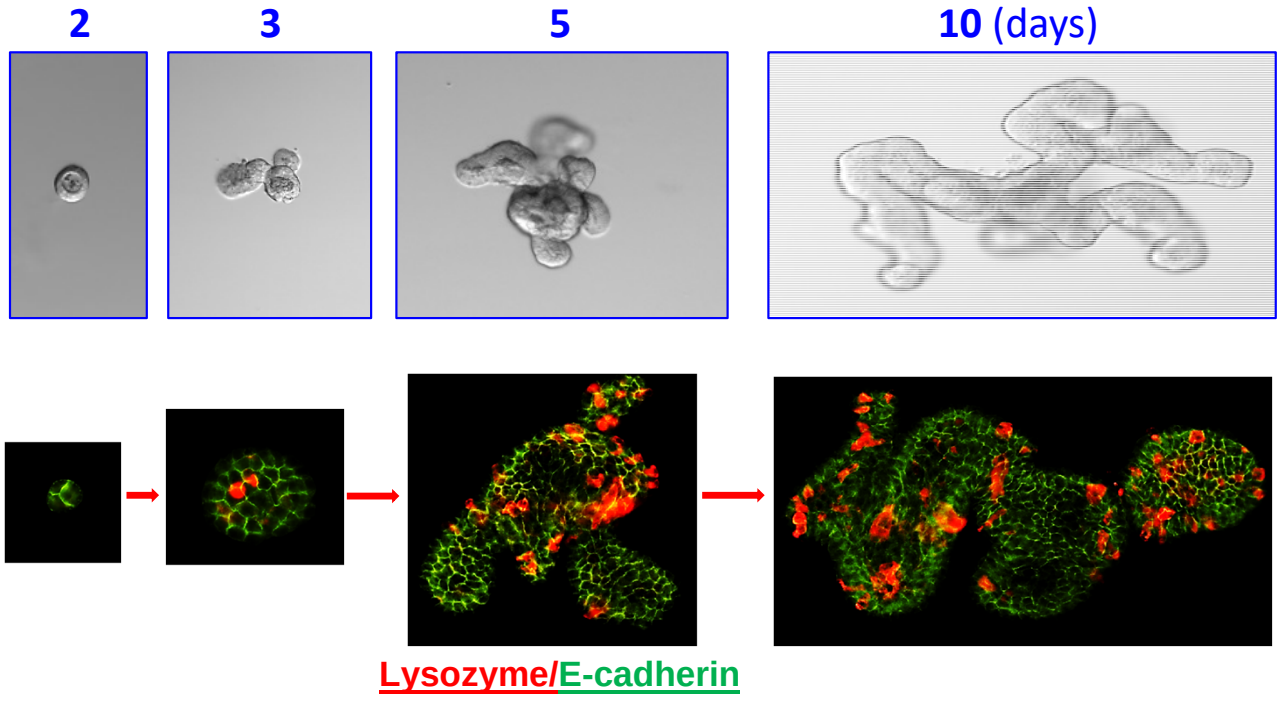
D) Lengths of villi and crypts



Growth of intestinal organoids isolated from littermate and IE-HuR$^{-/-}$ mice

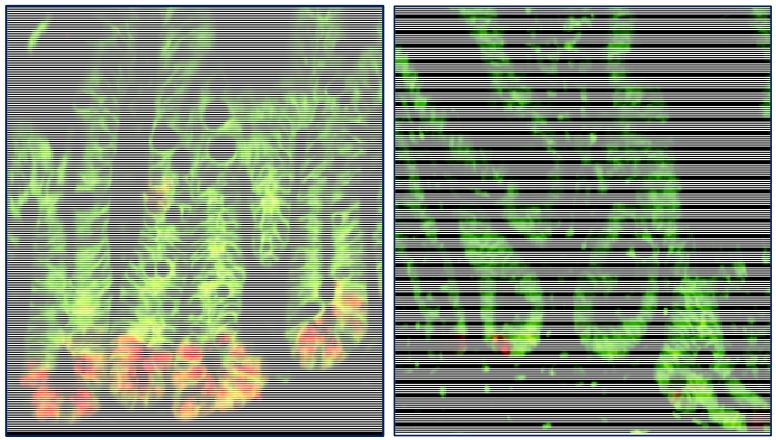


Paneth cells in intestinal organoids



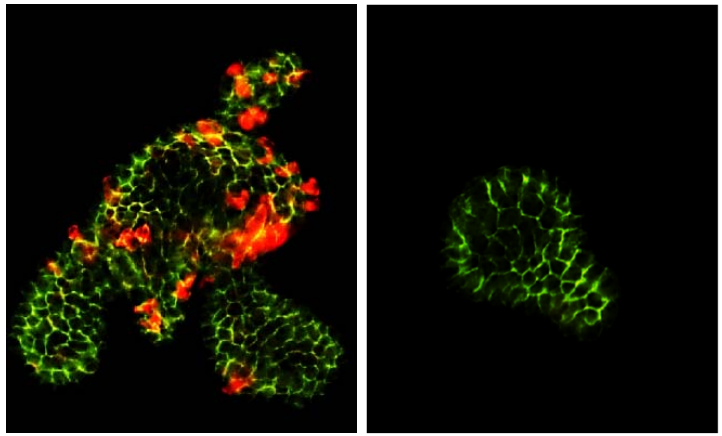
HuR deletion disrupts Paneth cell function

Littermates IE-HuR$^{-/-}$



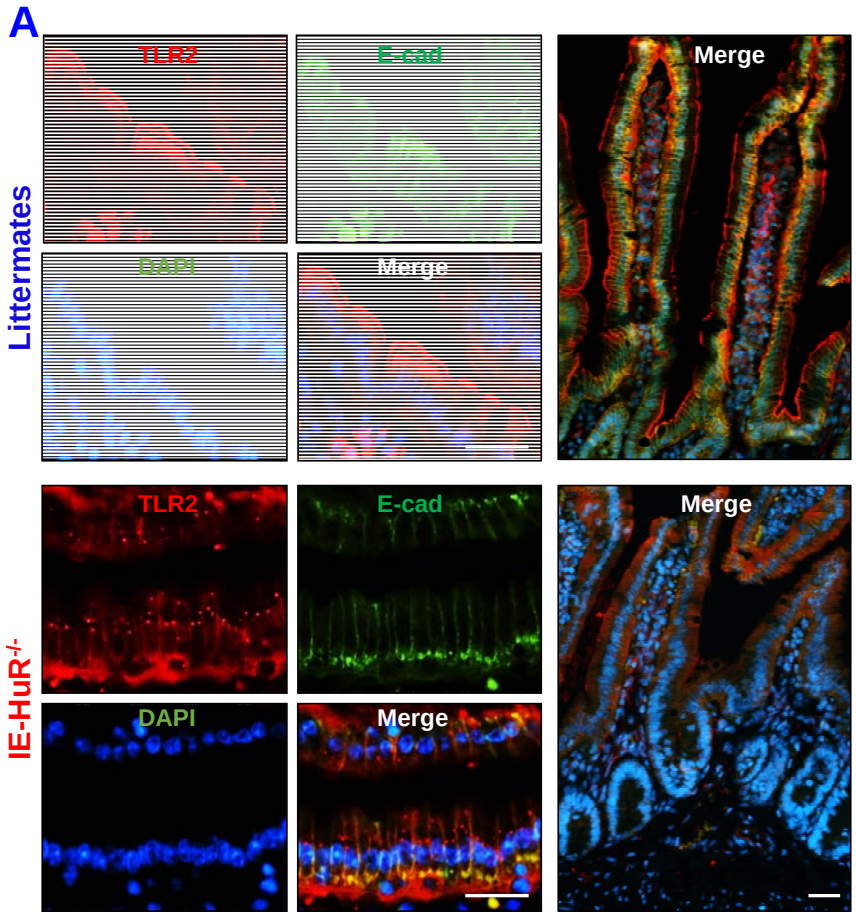
Lysozyme/E-cadherin

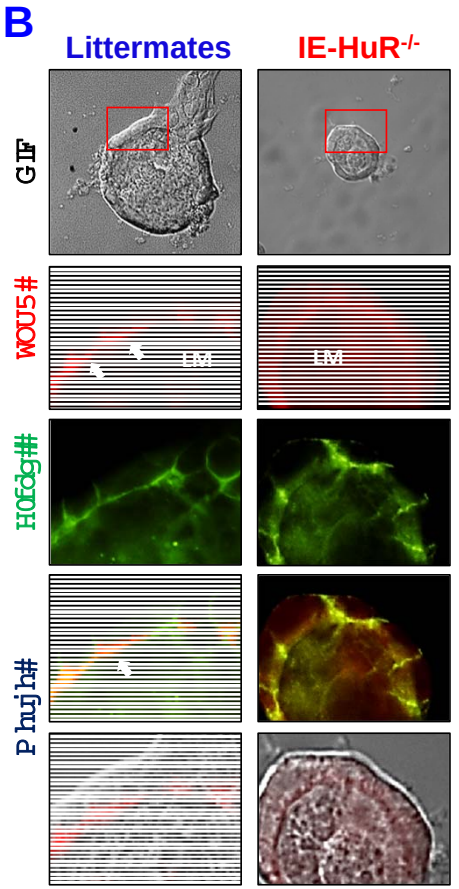
Littermates IE-HuR$^{-/-}$ (day 5)



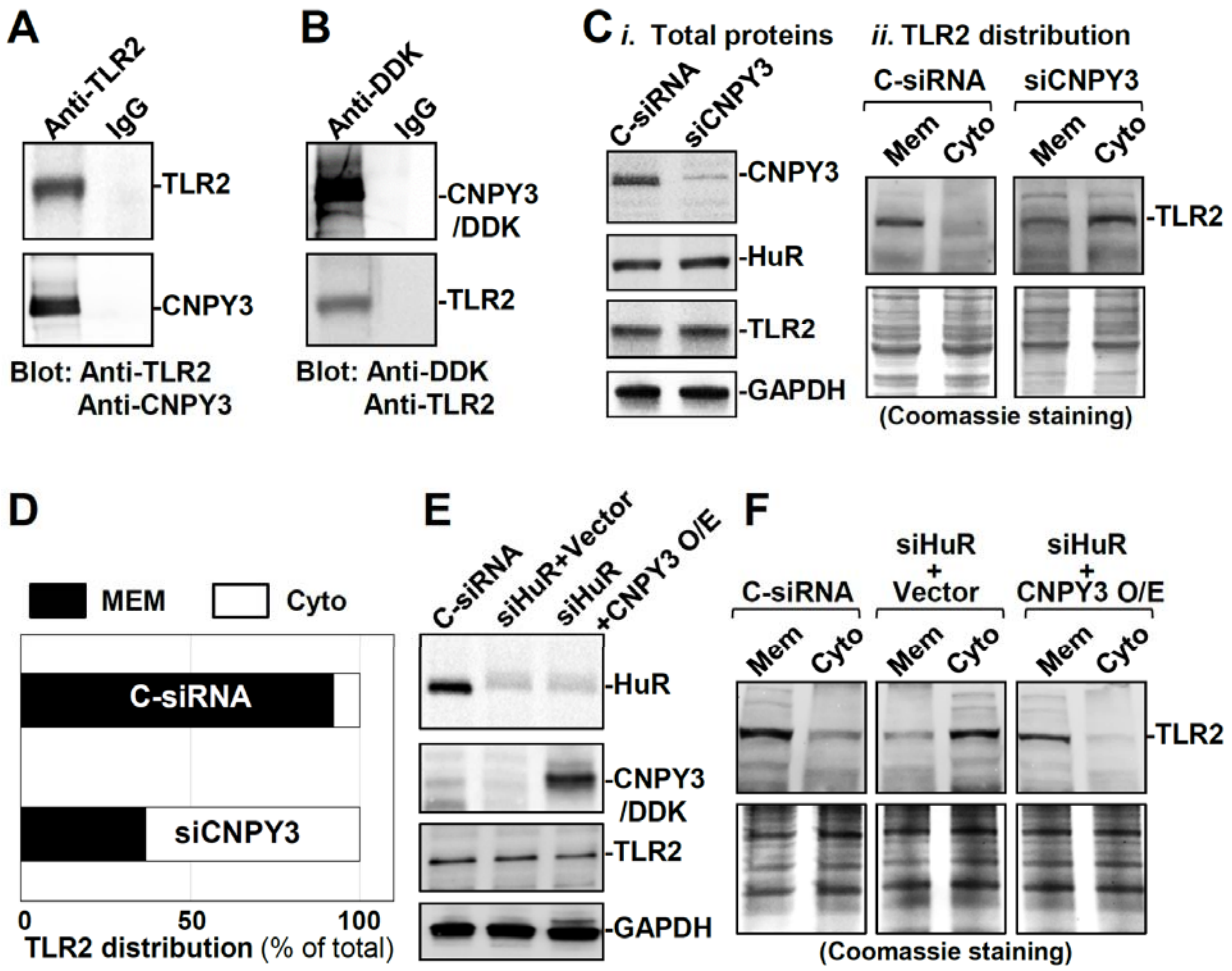
Lysozyme/E-cadherin

HuR knockout alters TLR2 subcellular distribution

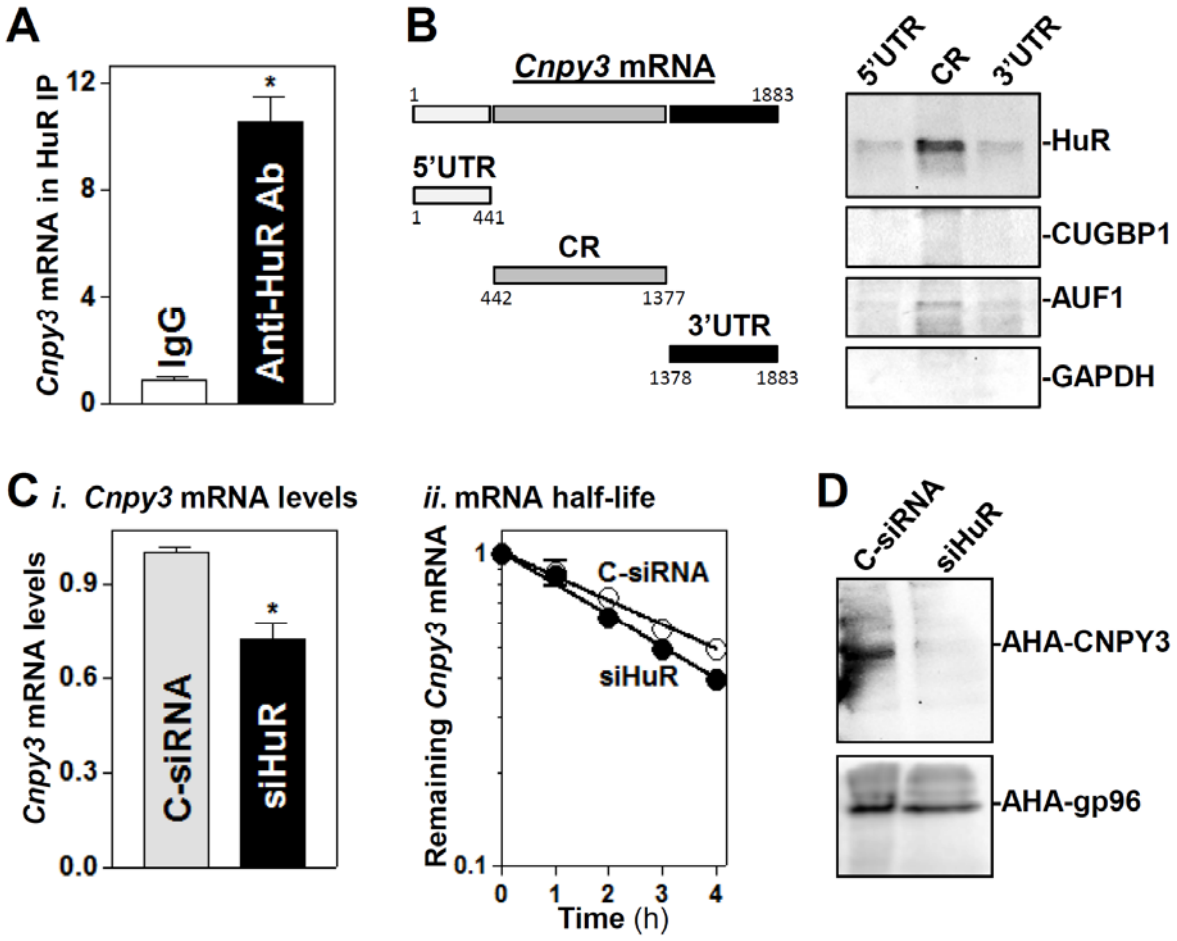




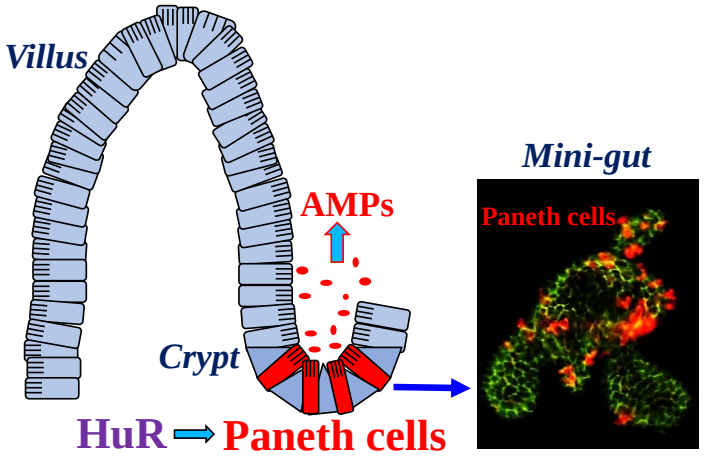
HuR is required for TLR2 membrane distribution via CNPY3

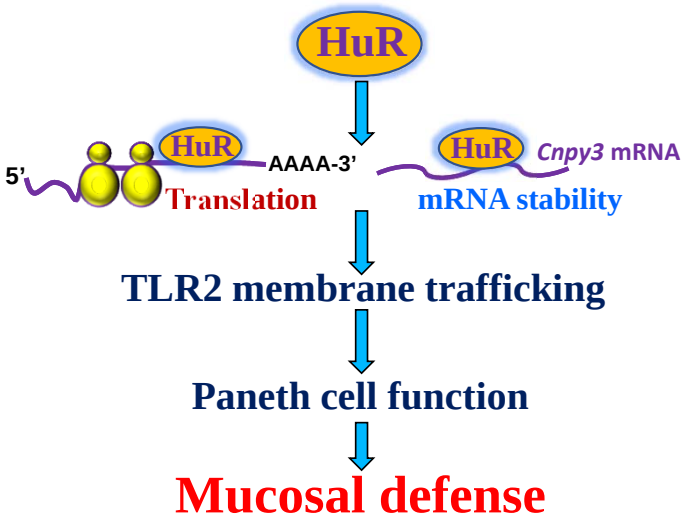


HuR interacts with *Cnpy3* mRNA and increases its stability and translation



HuR is essential for Paneth cell function



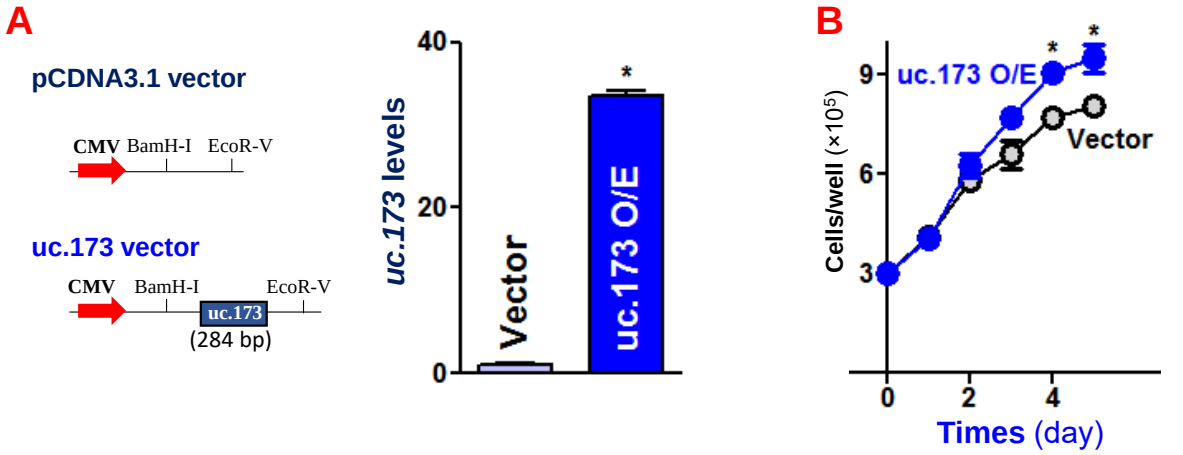


Long noncoding RNAs (LncRNAs)

LncRNAs are the transcribed RNAs spanning >200 nucleotides

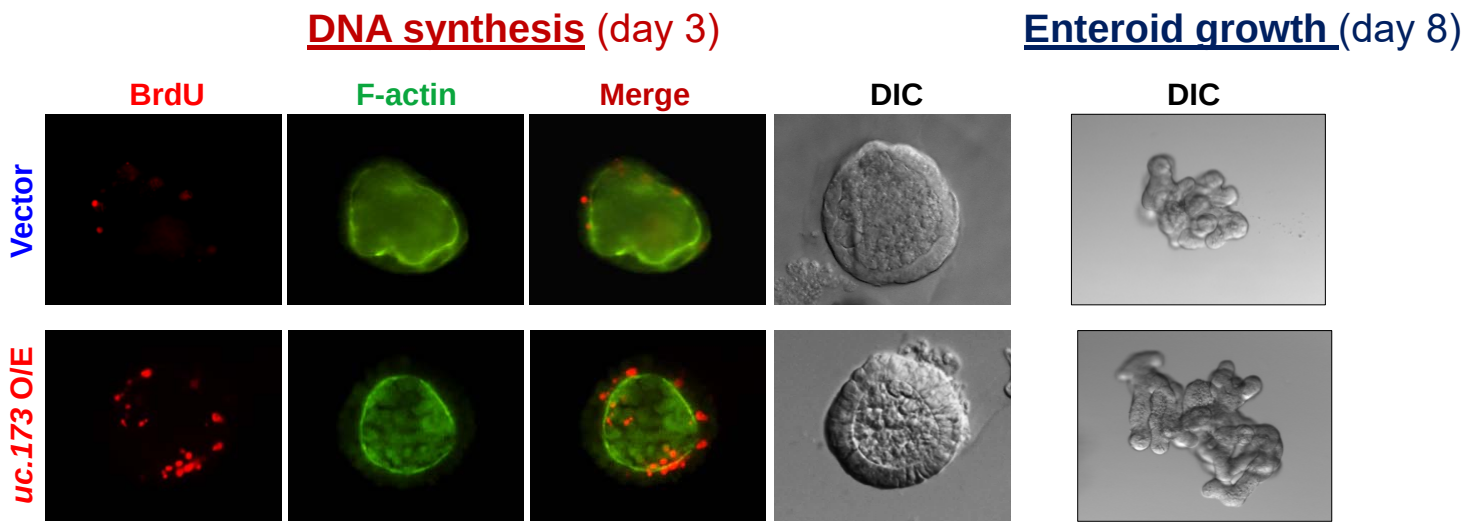
LncRNAs lack protein-coding capacity but are involved in multiple biological functions

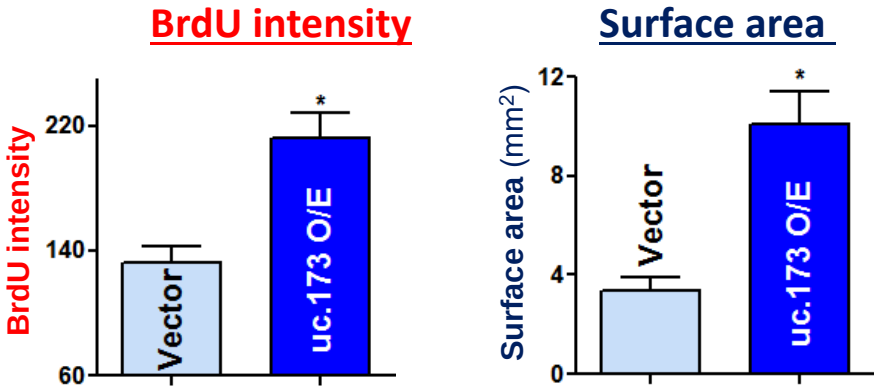
LncRNA *uc.173* are differentially expressed in gut mucosa in response to stress



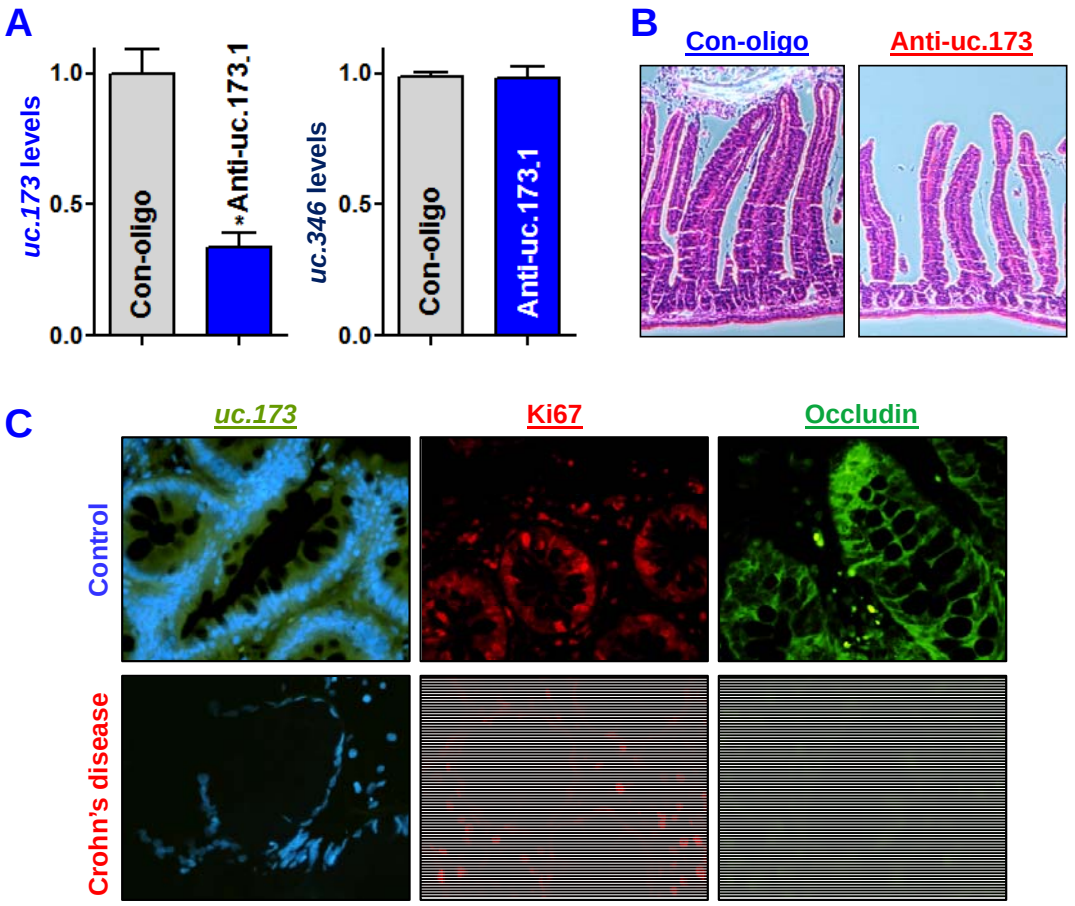
Gastroenterology 154:599-611, 2018

LncRNA *uc.173* enhances organoid growth





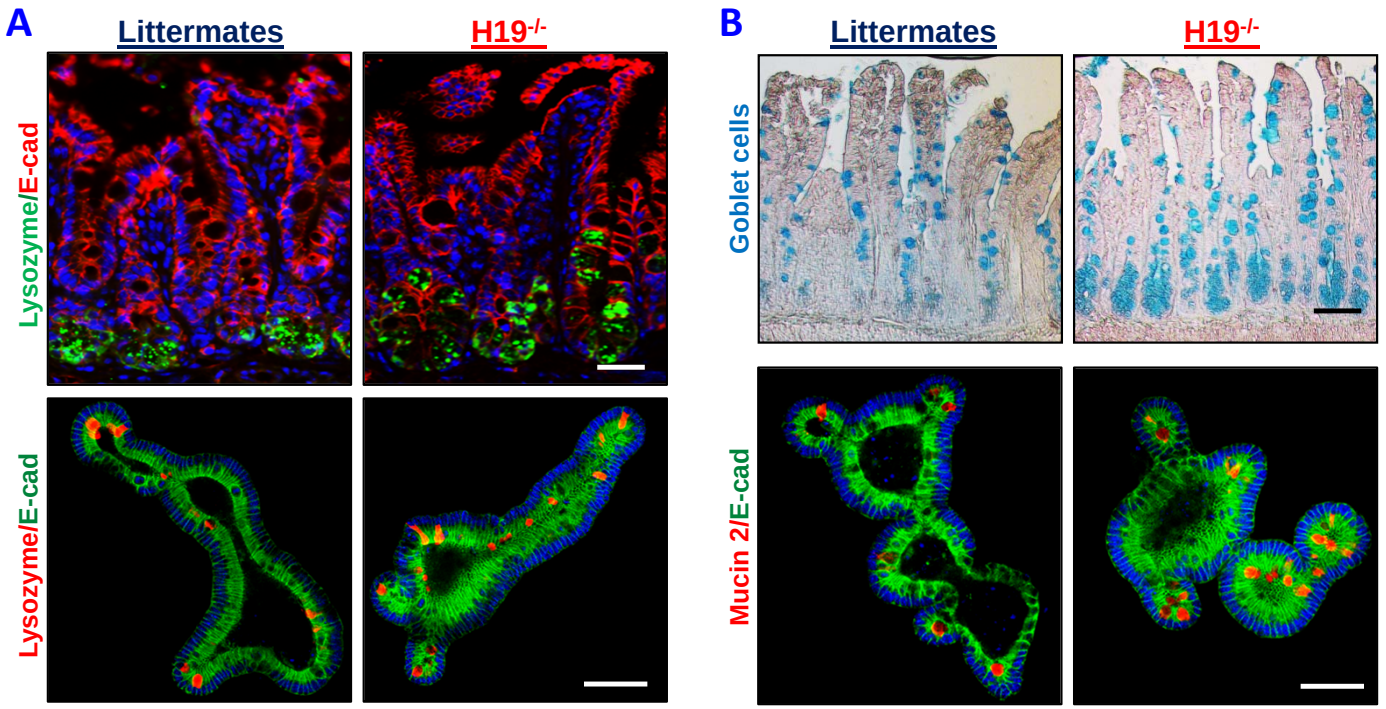
LNA-mediated *uc.173* silencing inhibits intestinal mucosal renewal



LncRNA *H19*

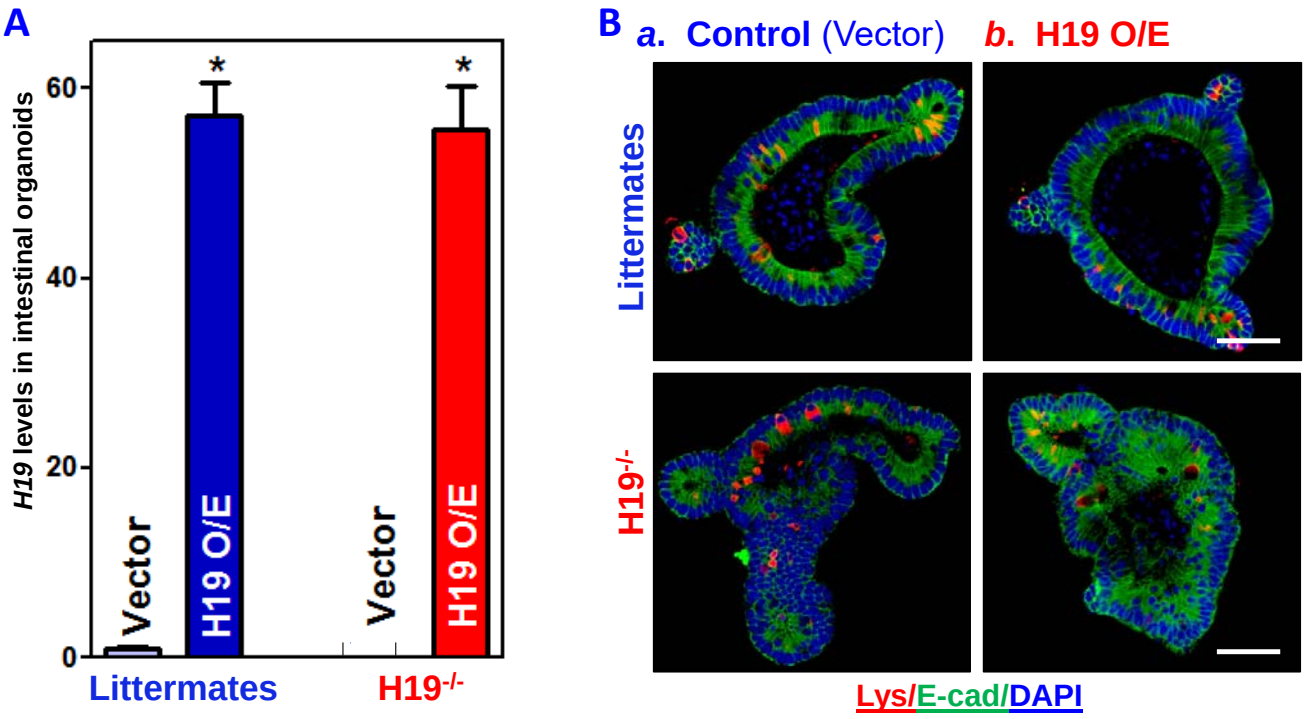
- ***H19*** **is a 2.3-kb long, capped, spliced, and polyadenylated noncoding RNA**
- ***H19*** **is transcribed from the conserved imprinted *H19/igf2* gene cluster**
- ***H19*** **is highly expressed during embryogenesis but is down-regulated after birth**
- **Increased expression of *H19* is commonly detected in various pathologies**

Targeted deletion of *H19* in mice increases Paneth and goblet cells

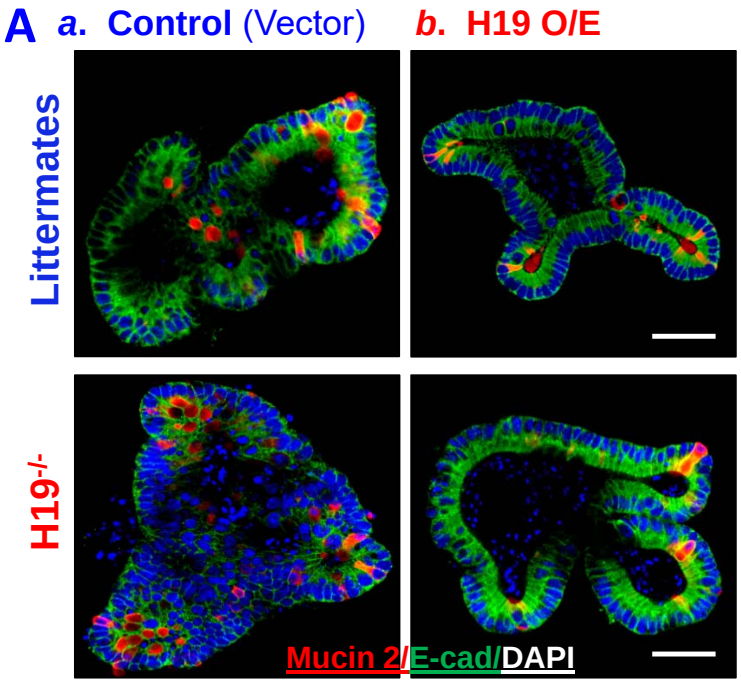


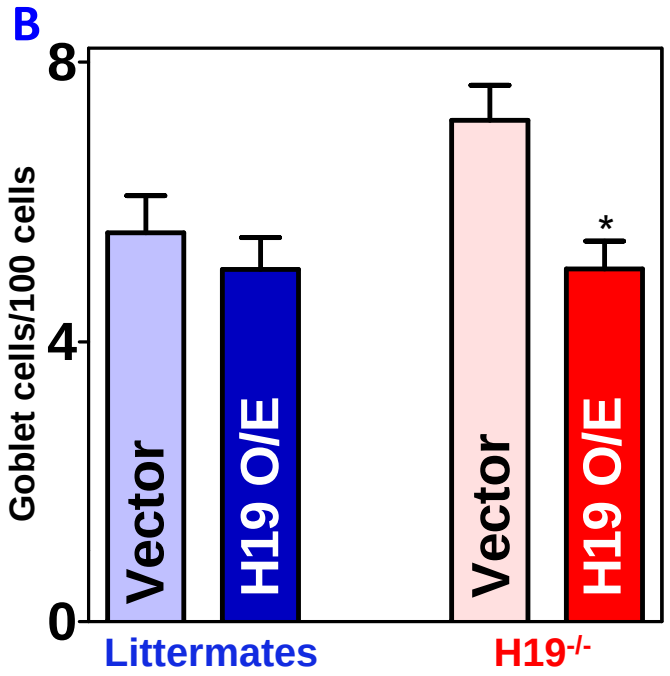
Cell Mol Gastroenterol Hepatol 9:611-625, 2020

Ectopic overexpression of *H19* prevents an increase in Paneth cells *ex vivo*

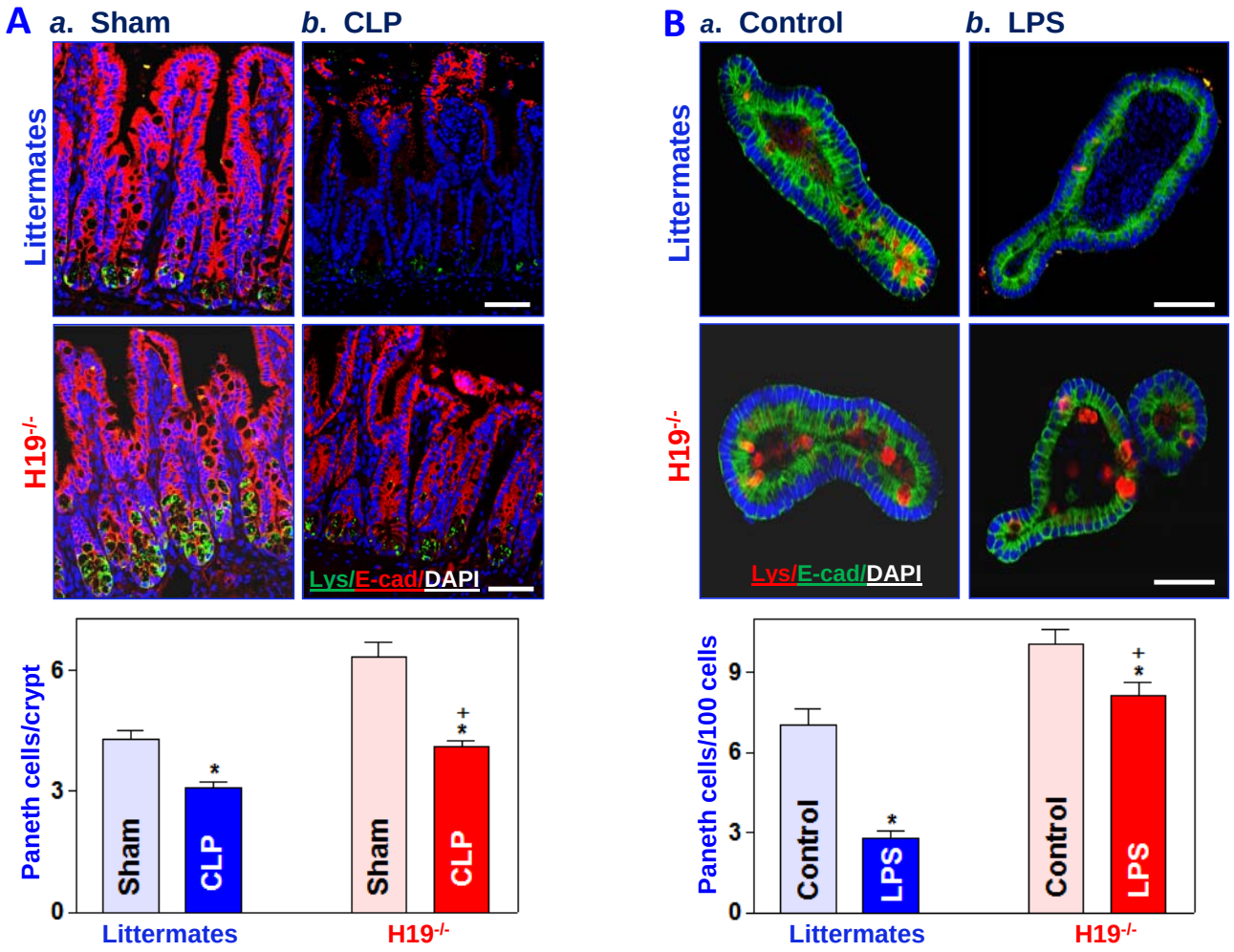


H19 overexpression reduces goblet cells in intestinal organoids from H19$^{-/-}$ mice

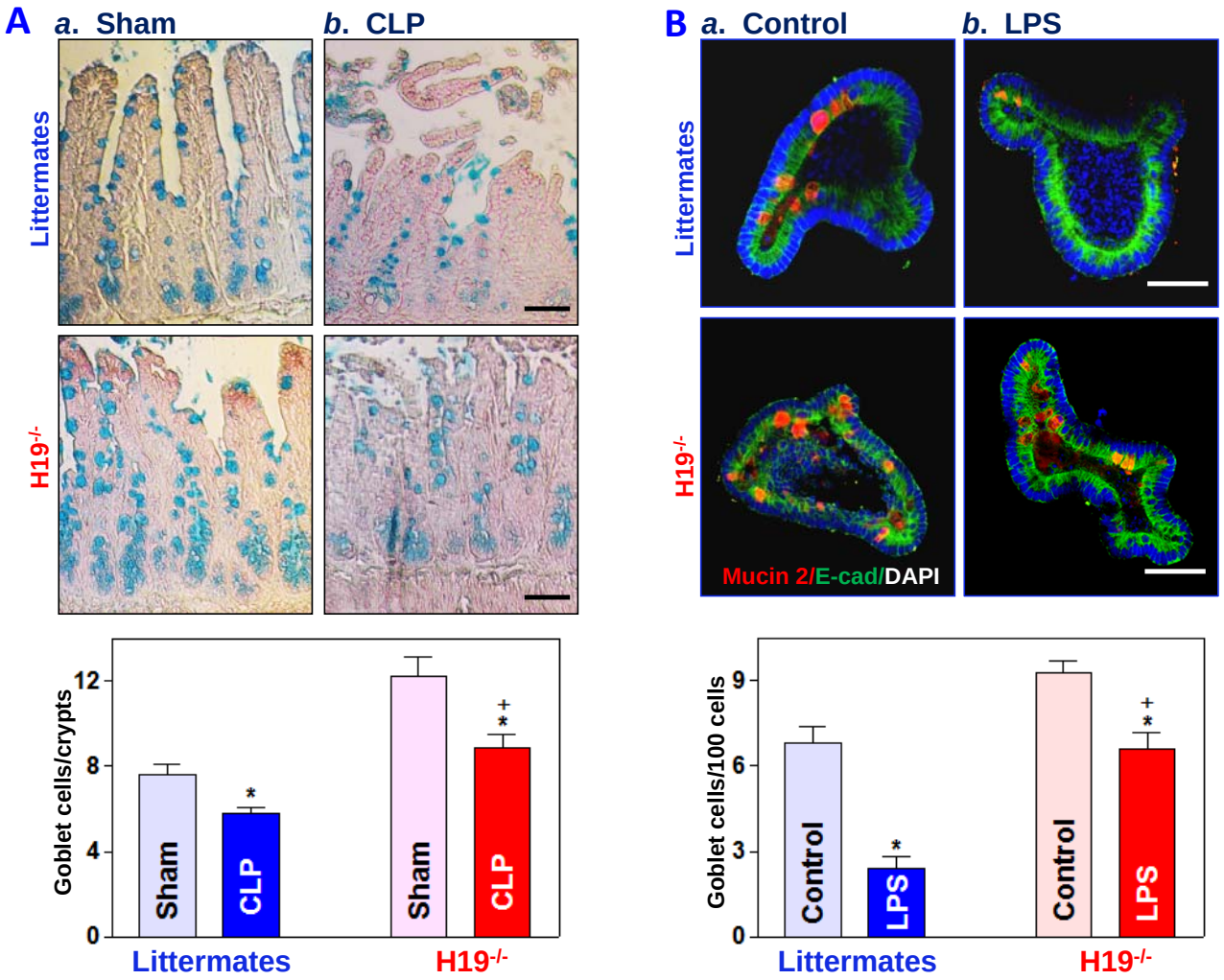


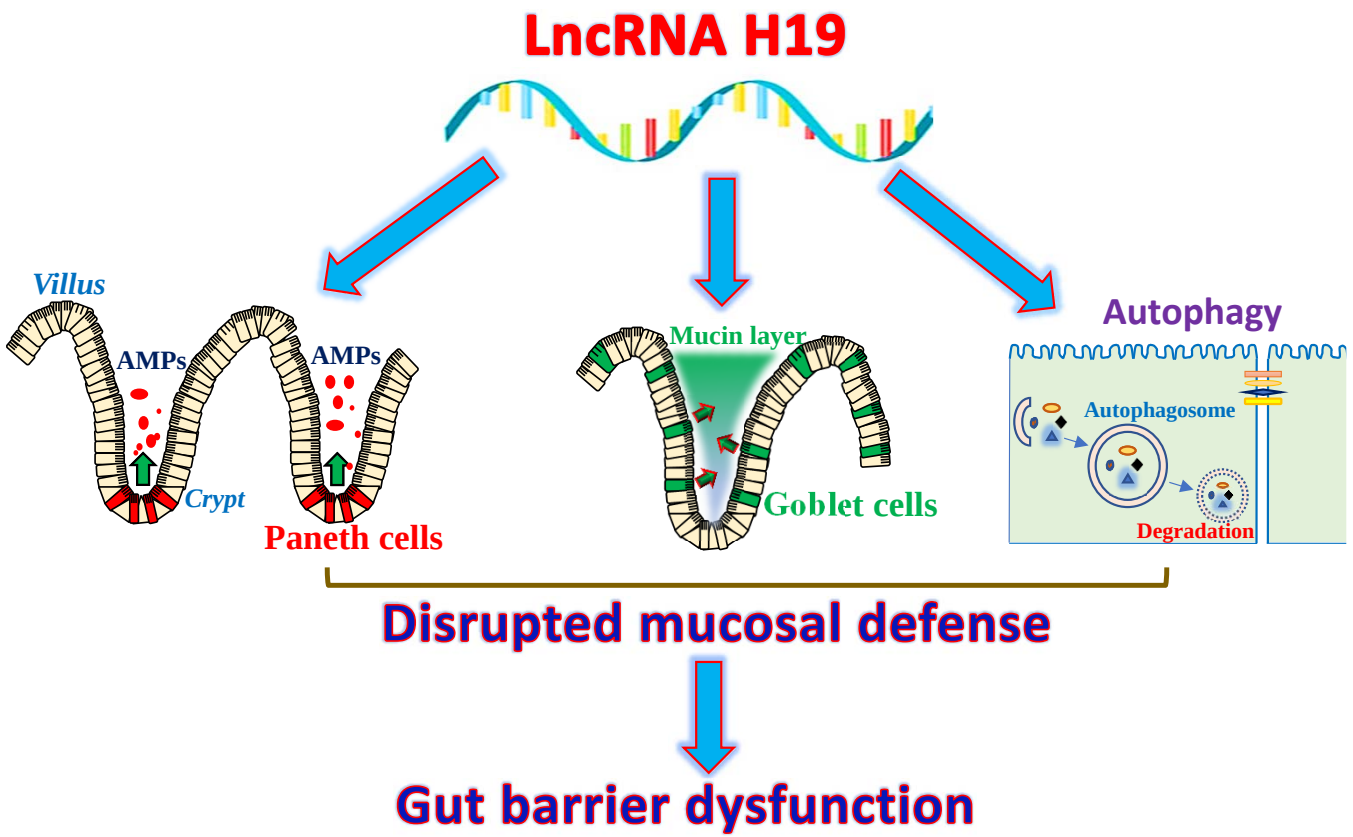


H19 deletion promotes Paneth cell function



H19 deletion protects goblet cells from stress




LncRNA H19
Villus
AMPs
AMPs
Crypt
Paneth cells
Mucin layer
Goblet cells
Autophagy
Autophagosome
Degradation
Disrupted mucosal defense
Gut barrier dysfunction

America's Oldest Public Medical School –

University of Maryland School of Medicine (1807-present)

53

SCHOOL OF PHARMACY
UNIVERSITY of WASHINGTON

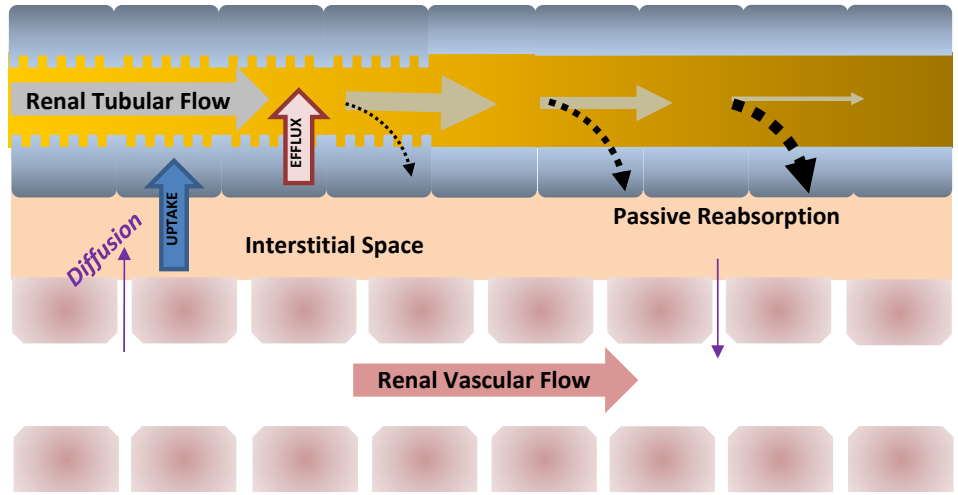
Use of Vascularized Human Kidney Proximal Tubule Microphysiological System and PBPK Modeling to Predict Renal Clearance in Subjects with Variable Kidney Function

Nina Isoherranen, Tomoki Imaoka, Weize Huang, Sara Shum, Shirley Chang, Catherine K Yeung, Jonathan Himmelfarb and Edward Kelly

Department of Pharmaceutics
University of Washington

Predicting Renal Clearance of Drugs is Very Challenging due to Dynamic and Sequential Processes

GFR= 120mL/min



Urine flow = 1 mL/min

Tubular flow decreases and drug concentration increases along the tubule

$$CL_r = (1-F_R)(f_u \bullet GFR + CL_{secretion})$$

? ?

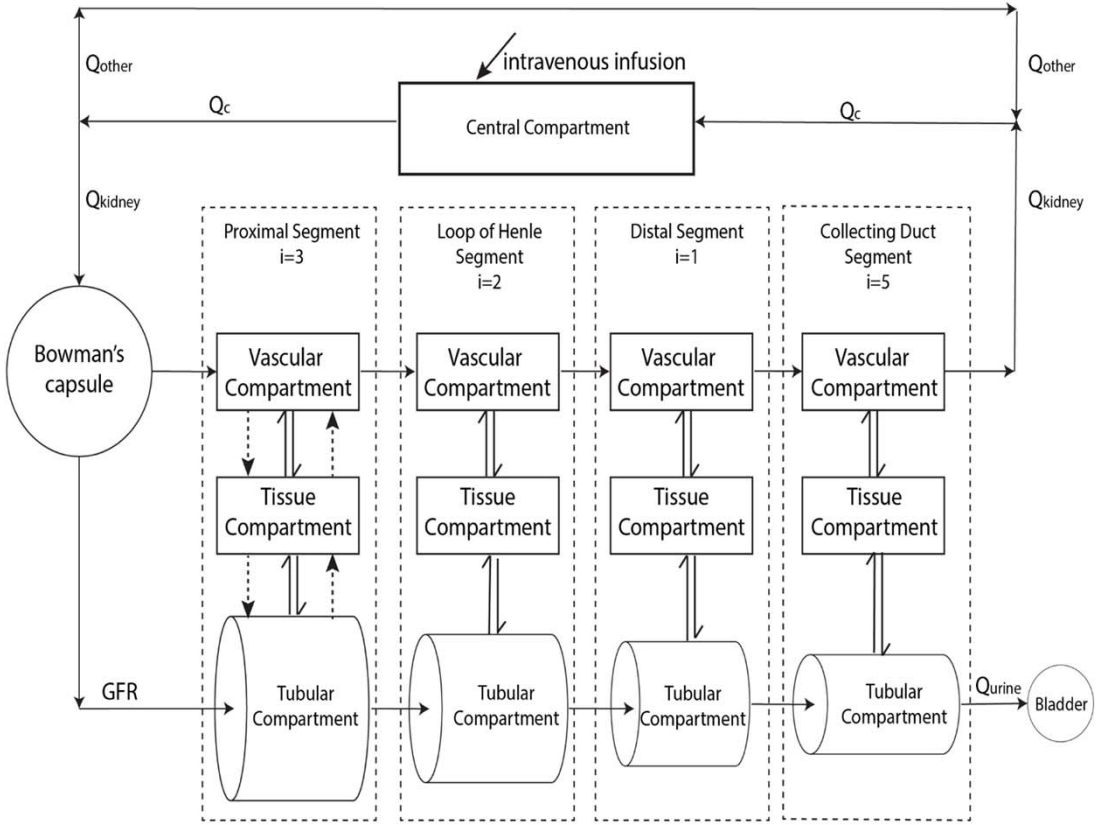
Passive reabsorption is driven by permeability, surface area, and concentration gradient:

$CL_{R,PD}$ = permeability across cell × renal tubular surface area

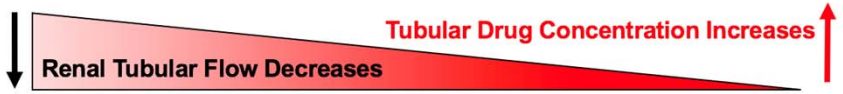
Rate of drug reabsorption $(\frac{dA}{dt})$ = $CL_{R,PD} \times \Delta C$

Can $CL_{R,PD}$ and $CL_{secretion}$ be determined using kidney proximal tubule chips?
Can this data be used to predict *in vivo* CL_R ?

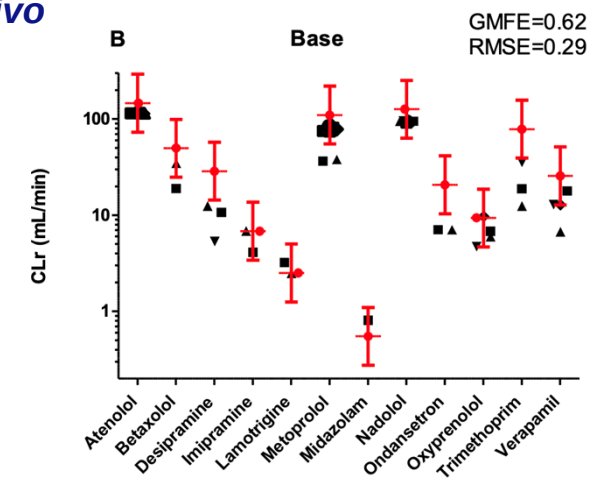
Physiologically-Based Kidney Model to Predict CL_r



Tubular surface area (dm^2):	6107	61	156	6.7
Tubular volume (mL):	30.5	2.7	19.4	237
Tubular flow (mL/min)	120→94→68→43	24	11	9 →7→5→3→1 (urine flow)
Tubular fluid pH value:	7.4→7.2→7.1→7	7	6.9	6.8→6.7→6.6→6.5→6.5 (urine pH)

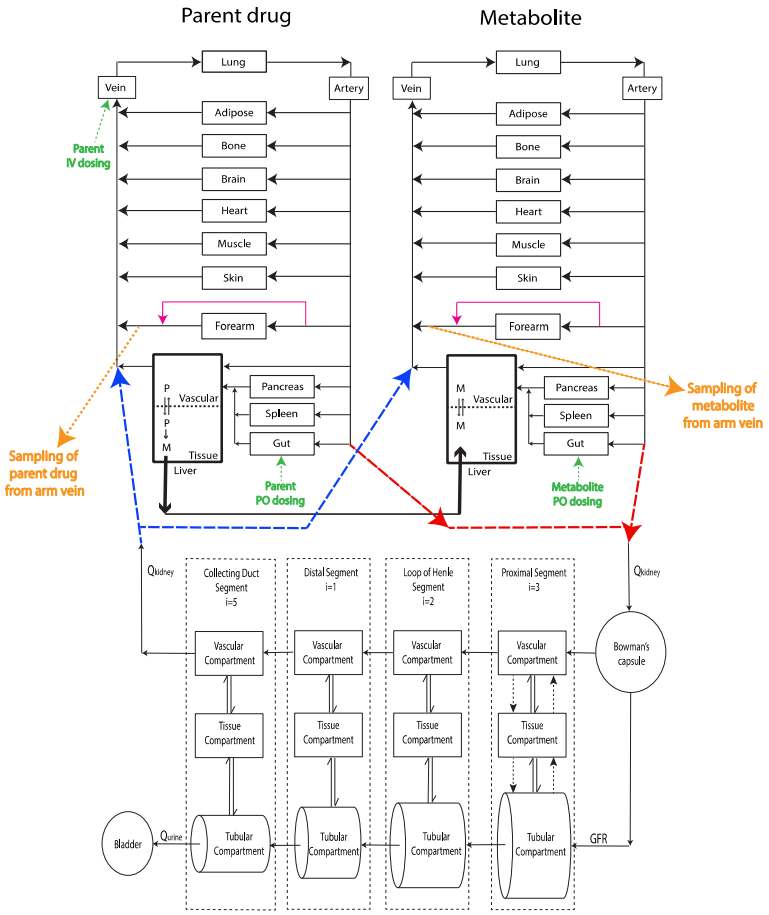


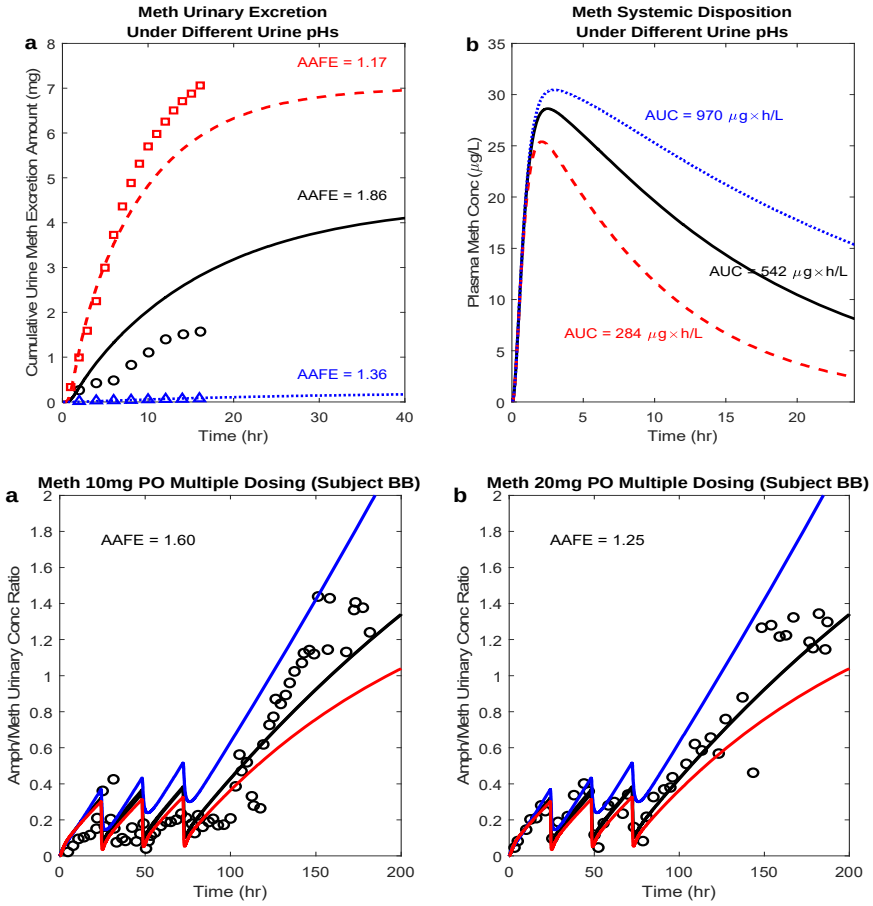
- ✓ All model compartments (n=37) are based on documented kidney physiology, known surface areas and content of microvilli
- ✓ Tubular filtrate pH gradient is estimated based on known reabsorption of ions and pH in the glomerulus and urine
- ✓ Simulated 87% of renal clearances (46 test compounds) within 2-fold based on Caco-2/MDCK permeability data
- ✓ **Major challenge is how to model drugs with active secretion and scale from *in vitro* to *in vivo***



Huang and Isoherranen *CPT:PSP* 2018

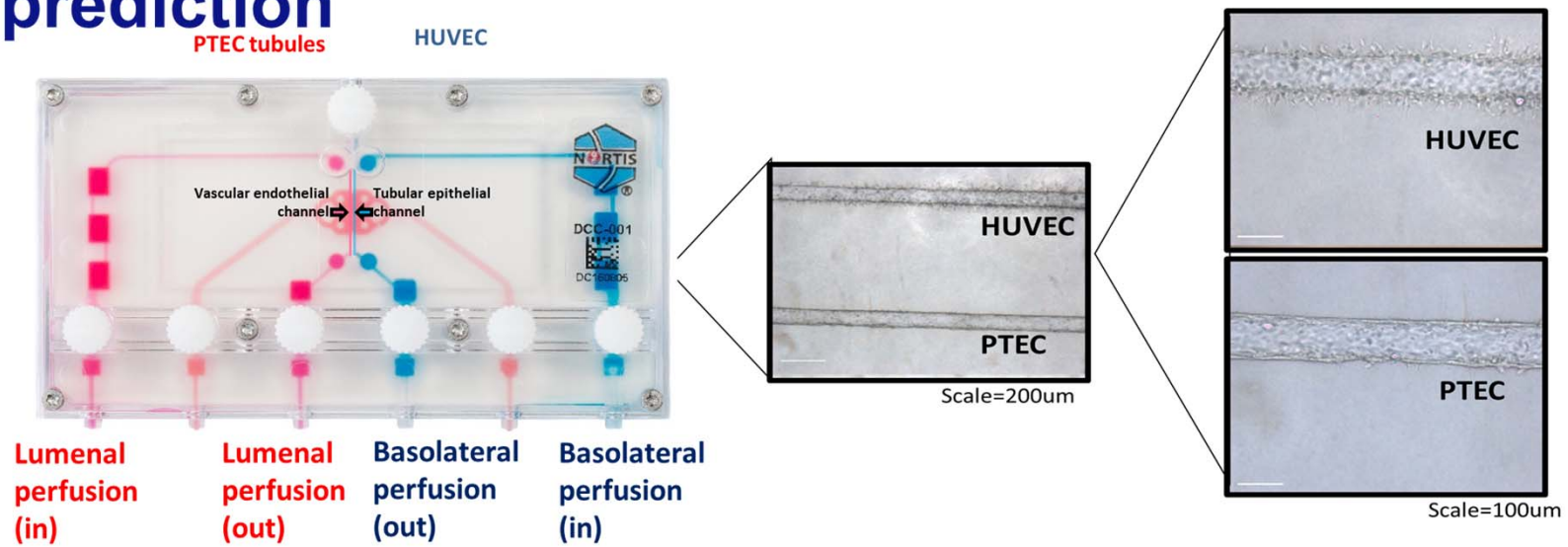
Kidney model can be incorporated into full PBPK models and used to simulate complex changes in CL_r and systemic disposition

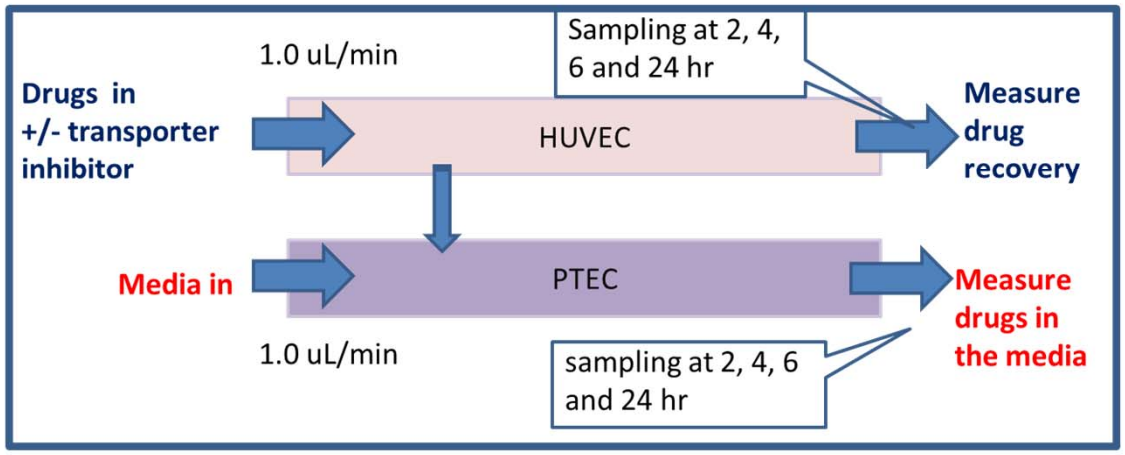




Huang, Czuba and Isoherranen *JPET* 2020

Vascularized Human Kidney Proximal Tubule MPS for CL_r prediction



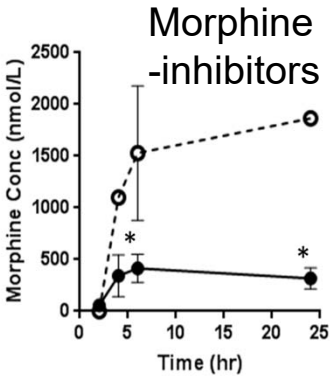


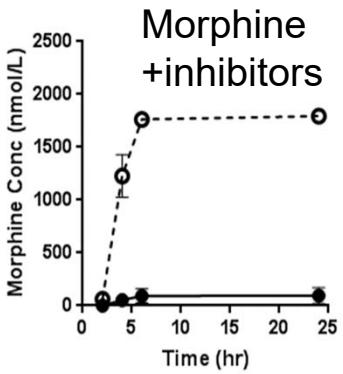
Passive permeability clearance and total clearance calculated from

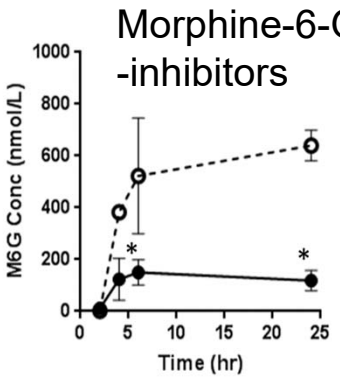
$$CL_r = \frac{dA_{tubule}/dt}{C_{HUVEC}}$$

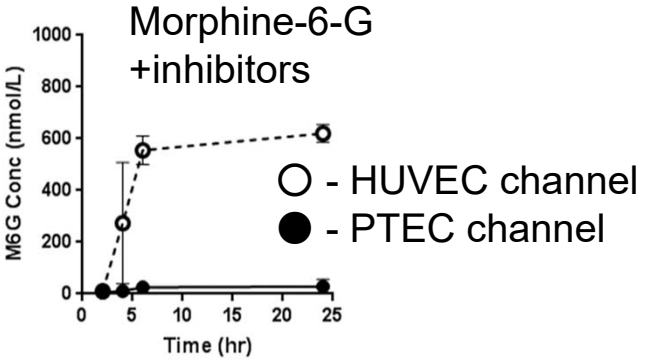
Active transport calculated as the ΔCL between +/- inhibition

Prediction of Morphine and Morphine-6-Glucuronide renal clearance from kidney tubule MPS data





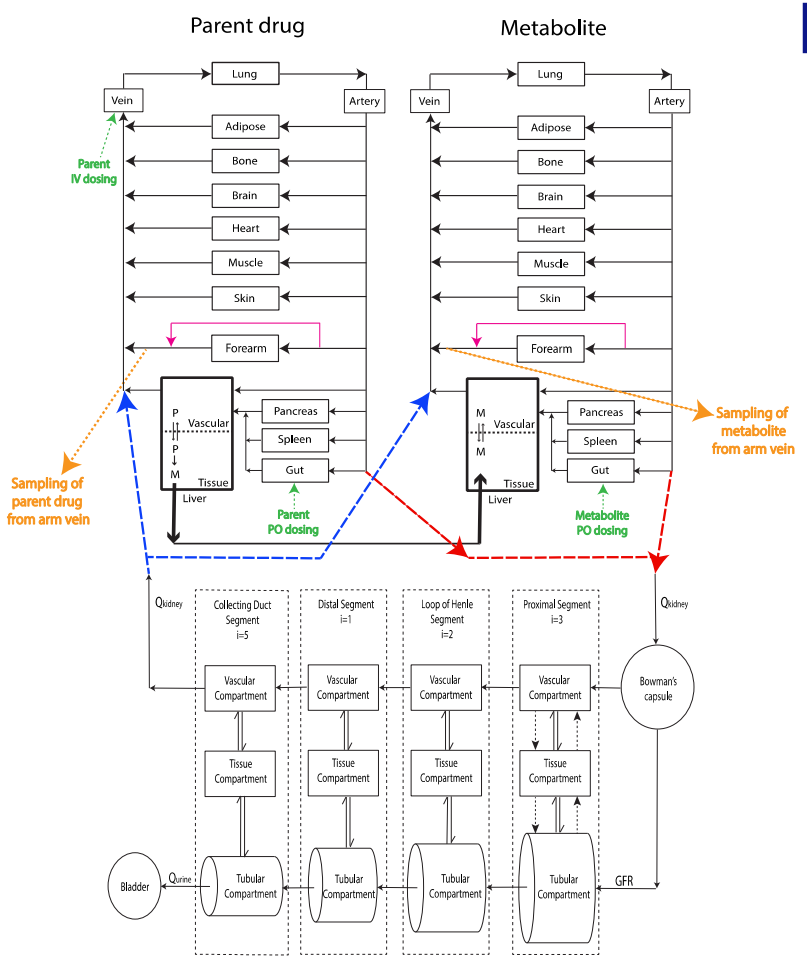


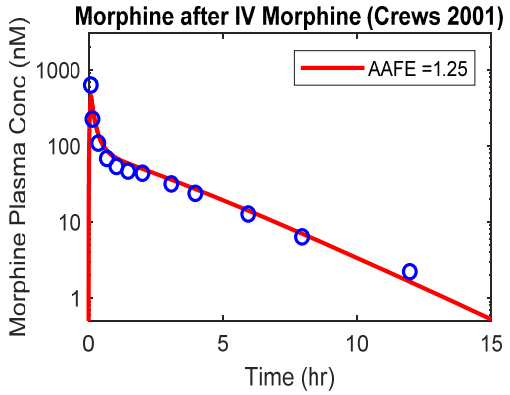


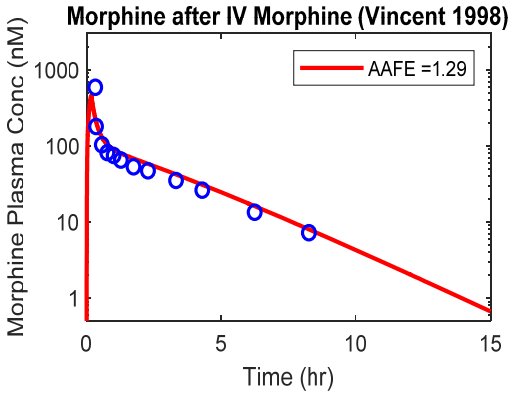
	Morphine			M6G		
	Donor 1	Donor 2	Donor 3	Donor 1	Donor 2	Donor 3
$CL_{int,sec}$ (µL/hr/MPS)	14	1.3	21	16	1.3	23
Permeability (10^{-6} cm/s)	28	13.9	43	26	9.7	42
$CL_{r,predicted}$ (L/hr)	9.7	4.8	8.3	12	7.2	9.5
$CL_{r,observed}$ (L/hr)	6.8-9.6			9.2-14		

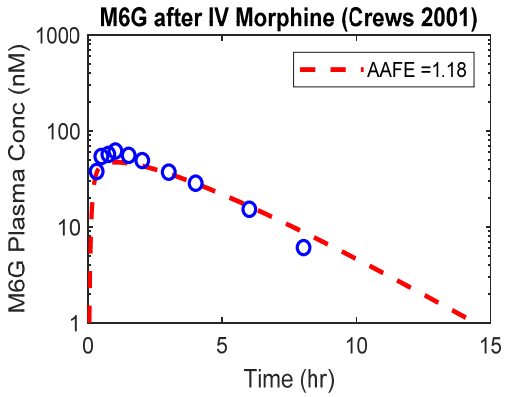
Observed CL data from Hasselstrom et al. 1993; Crews et al. 2001

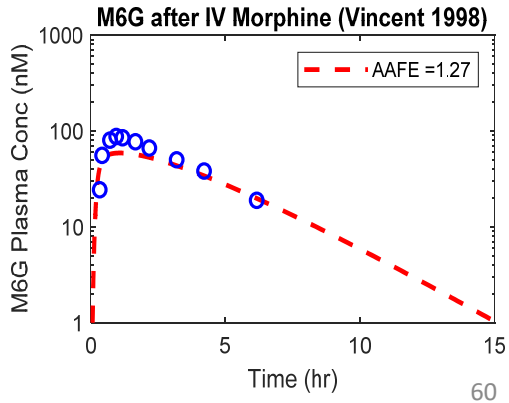
Morphine and morphine-6-glucuronide disposition can be predicted fusing MPS CL_r predictions and PBPK model



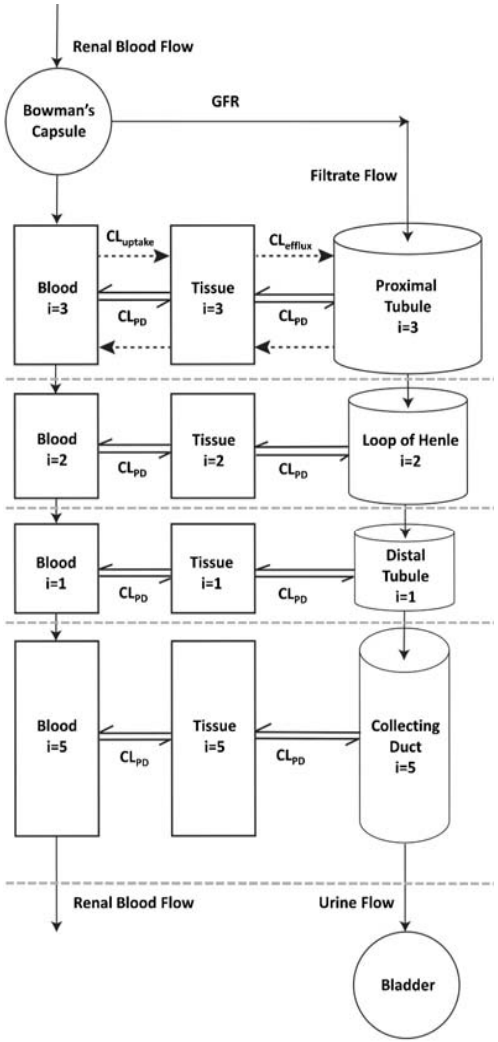


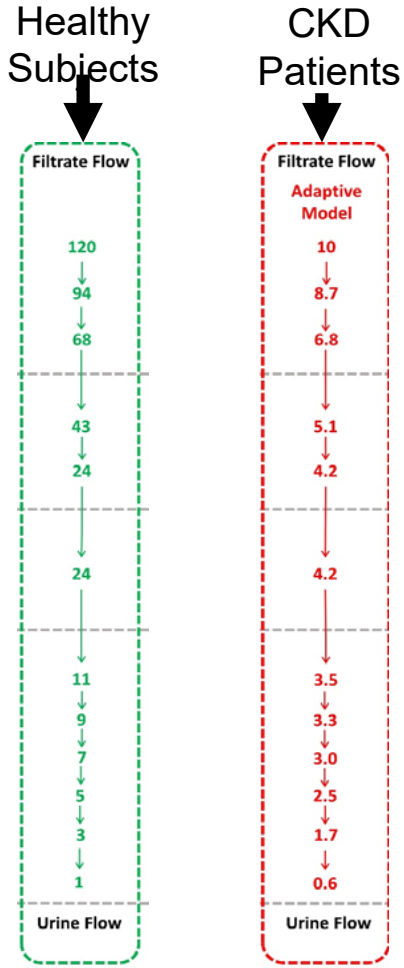




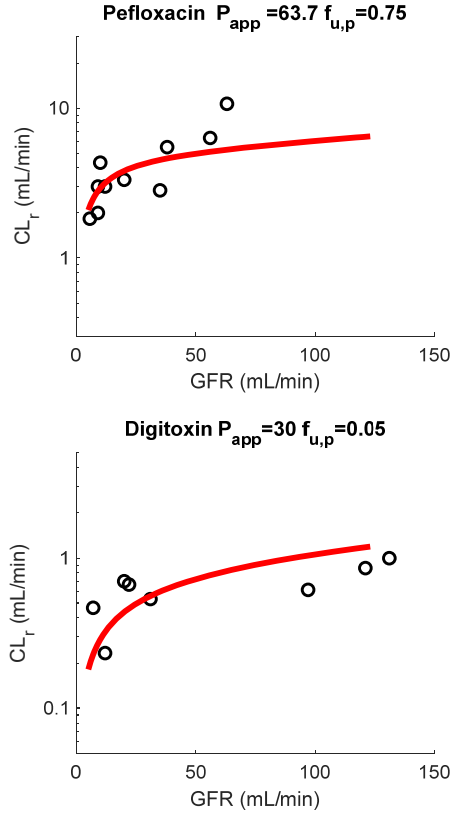


Mechanistic Kidney Model was Expanded to Model CKD



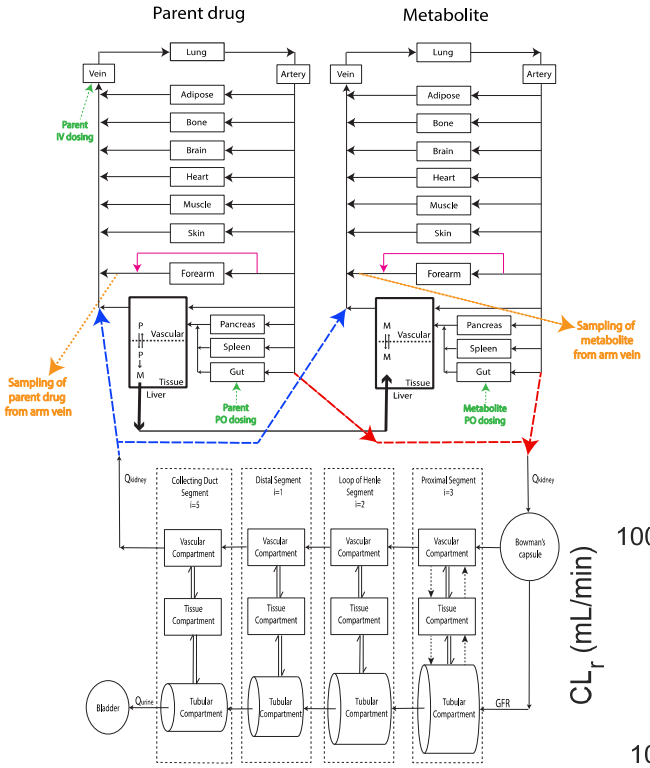


- A novel model to incorporate kidney adaptation of water reabsorption
- Accurately predicted impact of declining GFR on CL_r of 20 model compounds

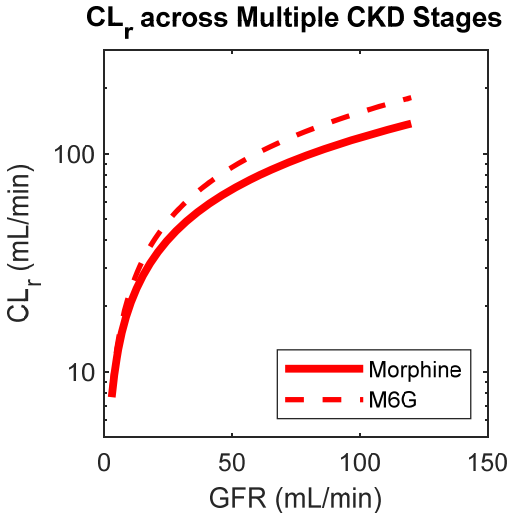


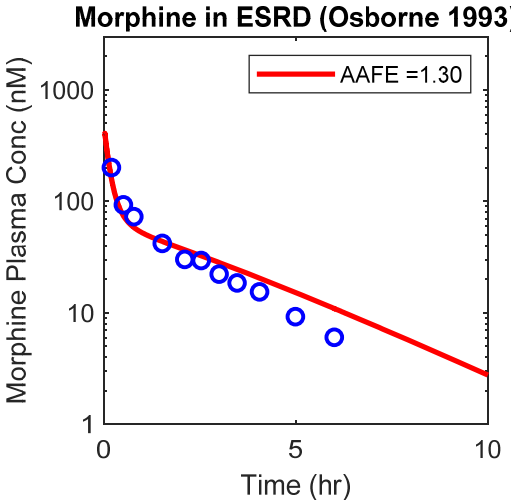
Huang and Isoherranen CPT:PSP 2020

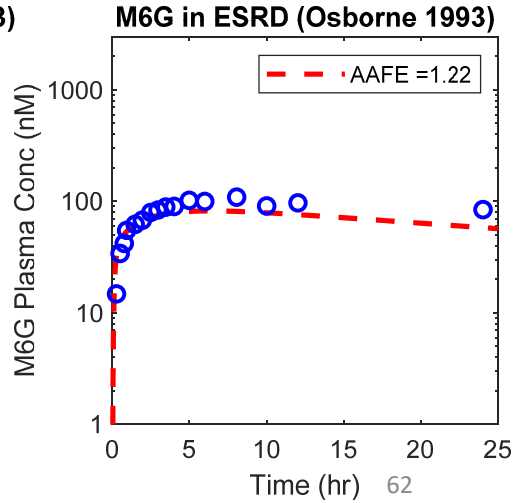
Based on the MPS system and PBPK model we can predict Morphine and Morphine-M-6-G disposition in CKD



- Adaptive kidney model for CKD was incorporated for parent and metabolite PBPK models
- Renal clearance modeled based on MPS data (IVIVE)
- Transporter expression assumed to decline according to intact nephron hypothesis







Conclusions

- ✓ The physiologically based mechanistic kidney model allows prediction of renal clearance from *in vitro* data.
- ✓ MPS system is useful in generating *in vitro* data for predicting renal clearance of drugs, has a unique role in predicting passive permeability together with transport
  - ✓ Application of the workflow will assist in predicting the sensitivity of renally cleared drugs to DDIs and to assess DDI risk
- ✓ The kidney model can be incorporated into complex PBPK models to simulate plasma concentration-time curves
  - ✓ Can be useful in designing clinical studies, in mechanistic interpretation of data and in identifying sensitive populations
- ✓ PBPK modeling coupled with *in vitro* experiments and MPS data offers potential for exploring how renal clearance changes in CKD.
  - ✓ This can aid in identifying sensitive drugs and in extrapolating clinical study findings with one drug to other clinically relevant scenarios that cannot/have not been studied

Session Four:

3D Spheroids/Organoids for Disease Modeling

Session Chair: William Hedrich (BMS)

Senior Scientist
Bristol-Myers Squibb Company, Pharmaceutical Candidate Optimization, Metabolism and Pharmacokinetics, Rt. 206 and Province Line Road, Princeton, NJ 08543; William.Hedrich@bms.com

Kidney Organoids for Disease Modeling and HTS

Benjamin “Beno” Freedman

M-CERSI Symposium on 3D Cell Culture Models

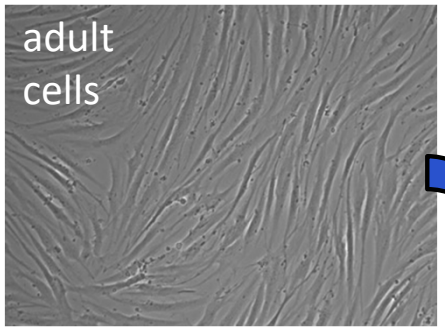
August 14, 2020

The need for new kidney therapies

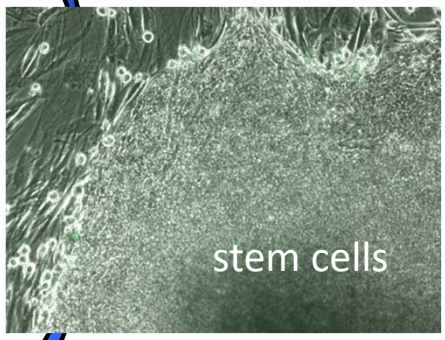
- ~10 % of population affected
- Kidney tissue is complex, cannot naturally regenerate
- Dialysis & transplantation are limited, have side effects
- Few therapies to treat kidney disease exist
- Therapies for other organs can damage the kidneys

Generating kidney structures from patient stem cells

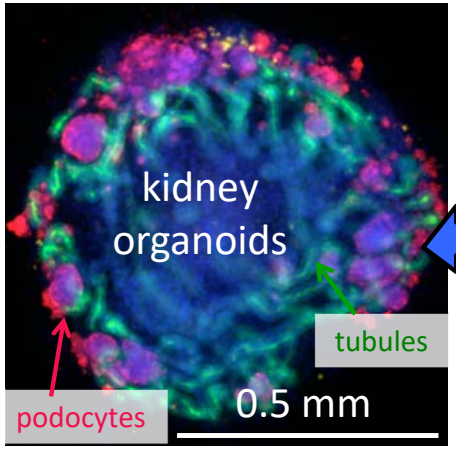
(e.g. urine sample)



Stem cell genes and growth signals



CHIR99021



disease modeling
drug discovery

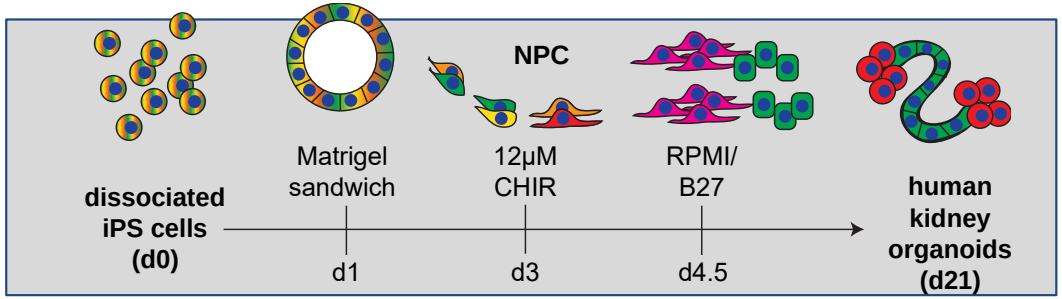
1. Genetic correction
2. Self-transplant
(100 % match)

Time frame from cell donation to transplant ~ 1 year

Taguchi et al., *Cell Stem Cell,* 2013
Takasato et al., *Nat. Cell Biol.,* 2014
Freedman et al., *Nat. Commun.,* 2015

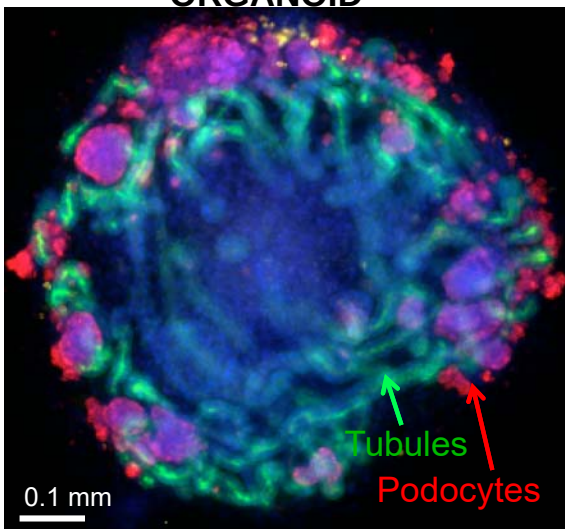
Reconstitution of human nephrogenesis

Protocol commercialized by STEMCELL Technologies

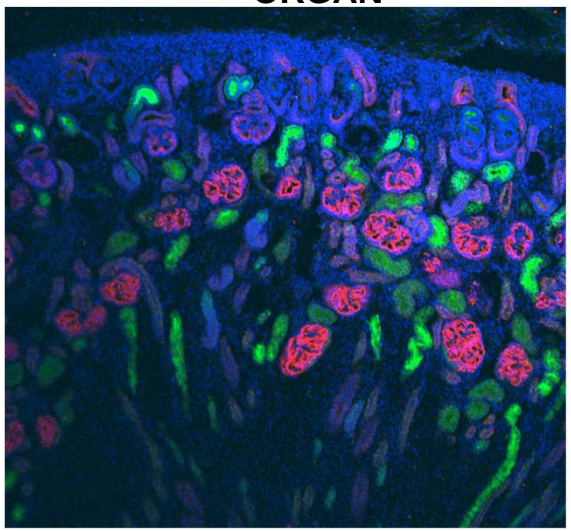


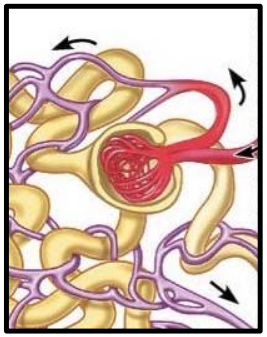
Freedman et al., Nat. Commun., 2015

ORGANOID



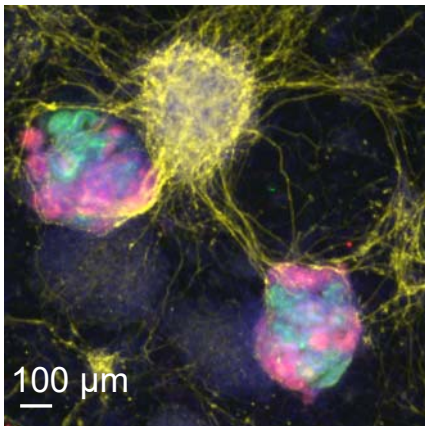
ORGAN



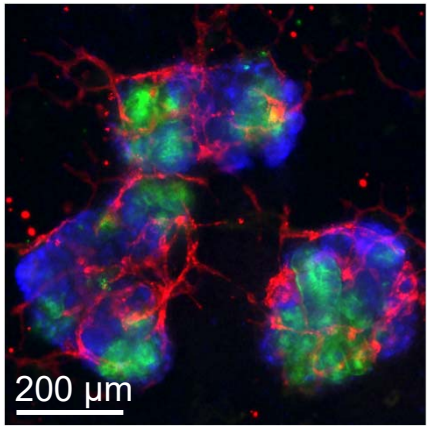


Organoids contain many different cell types

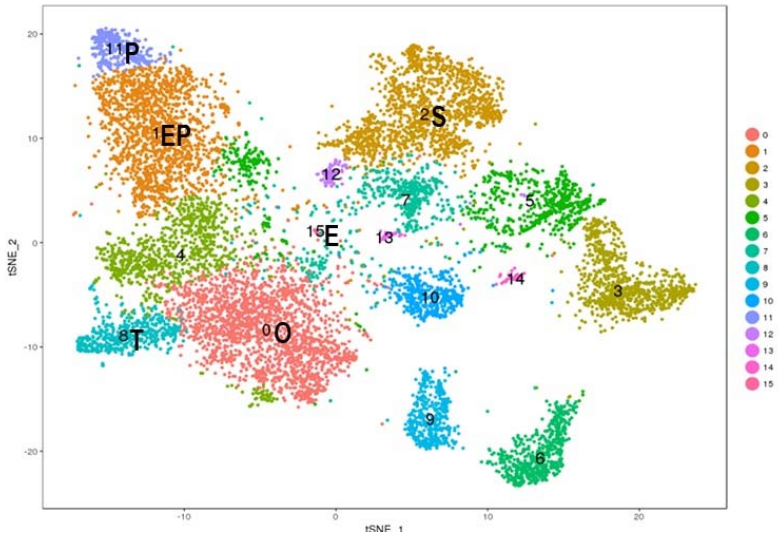
podocyte/tubule/neuron/DNA

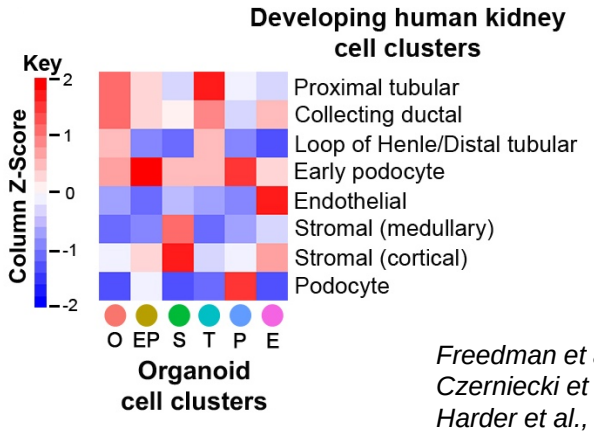


endothelial/tubule/podocyte



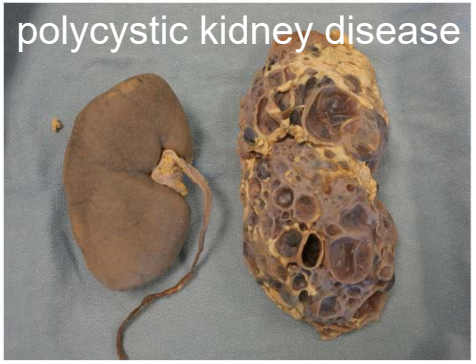
Freedman et al., *Nat. Comm.*, 2015

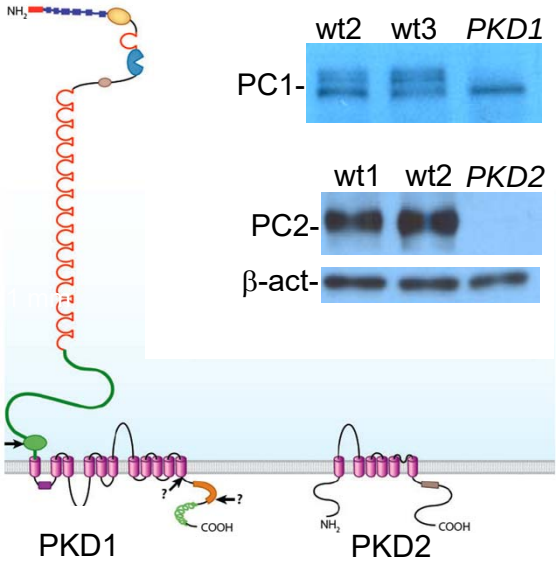


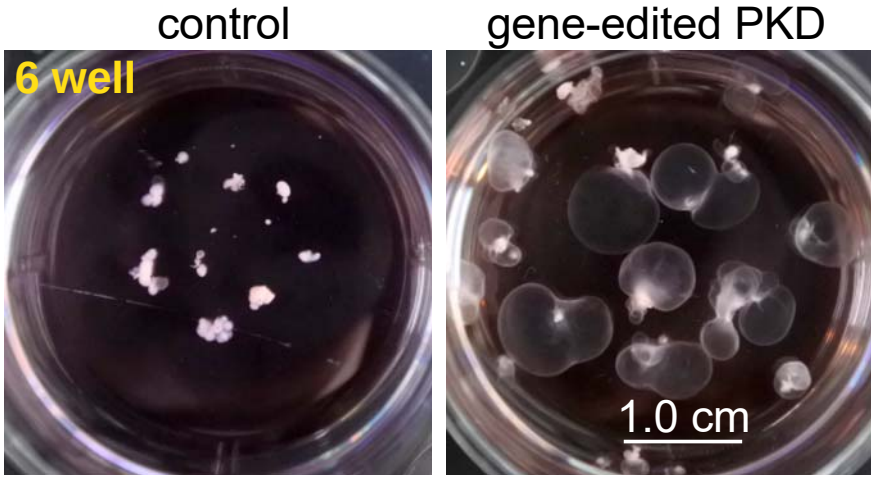


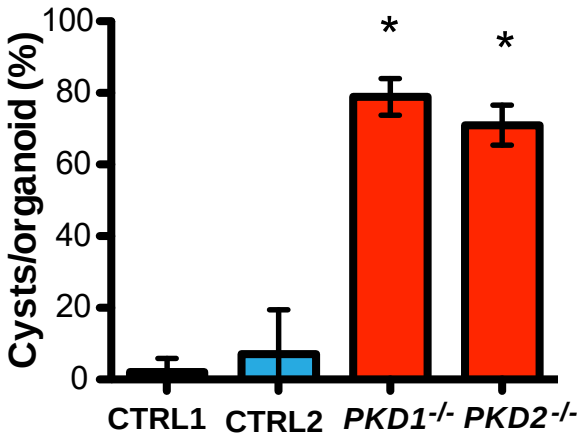
Freedman et al., Nat. Commun., 2015
Czerniecki et al., Cell Stem Cell, 2018
Harder et al., JCI Insight, 2019

PKD organoids form cysts from tubules



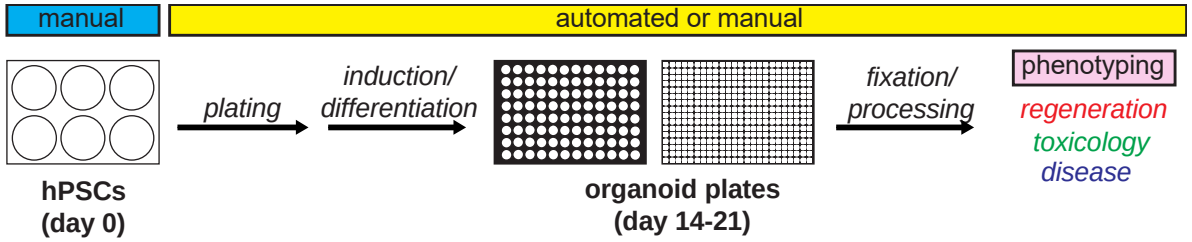




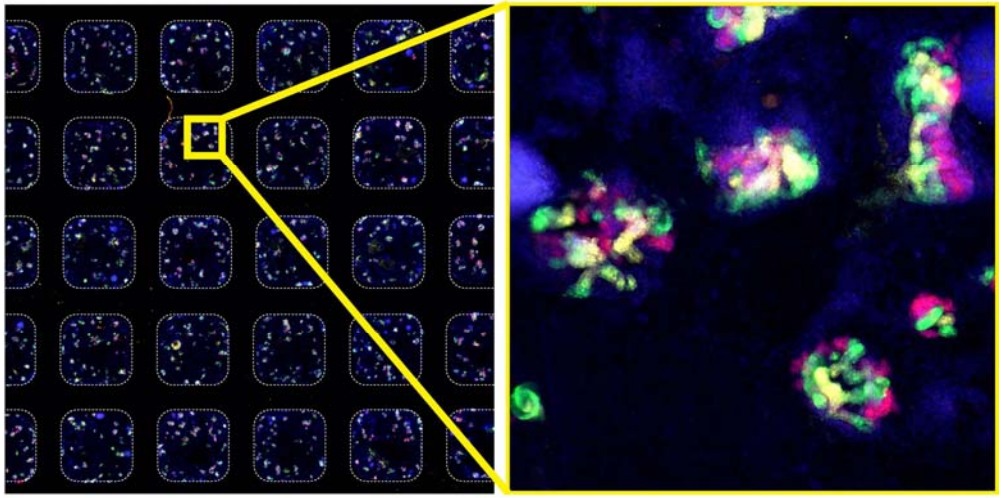


Freedman et al., Nature Communications, 2015
Cruz et al., Nature Materials, 2017

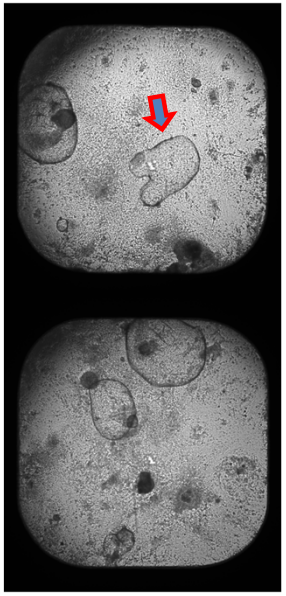
Organoids in high throughput screening formats



podocytes/proximal/distal tubules/DNA

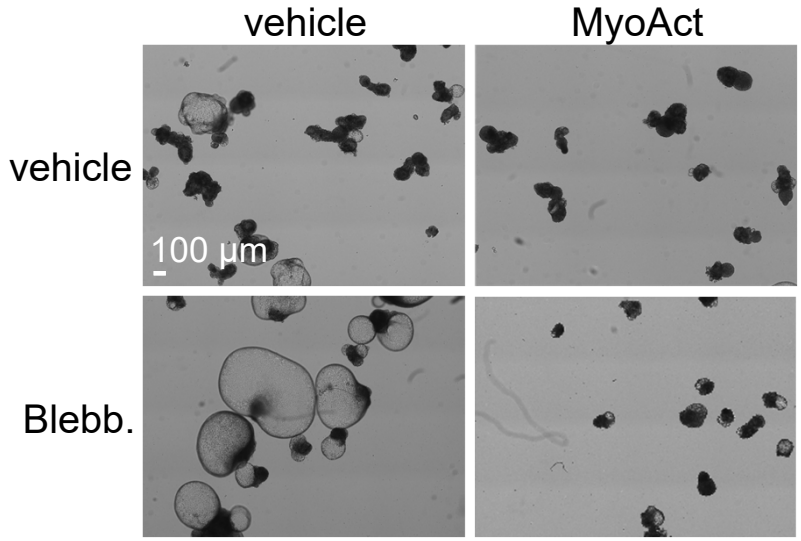


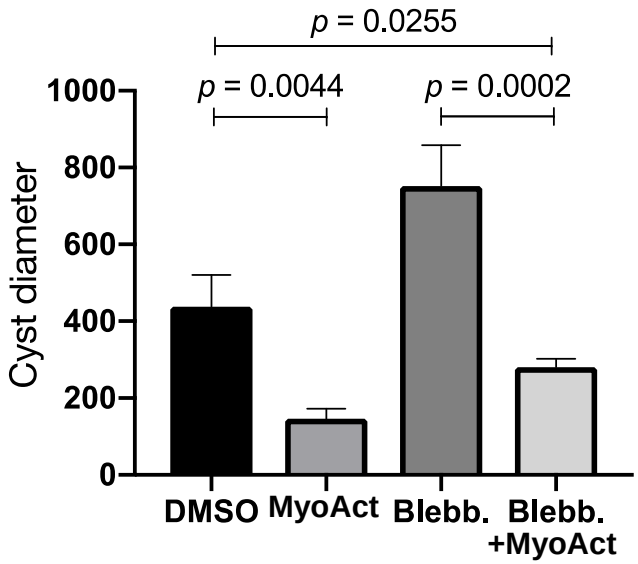
PKD



Czerniecki, Cruz, Harder et al., Cell Stem Cell, 2018

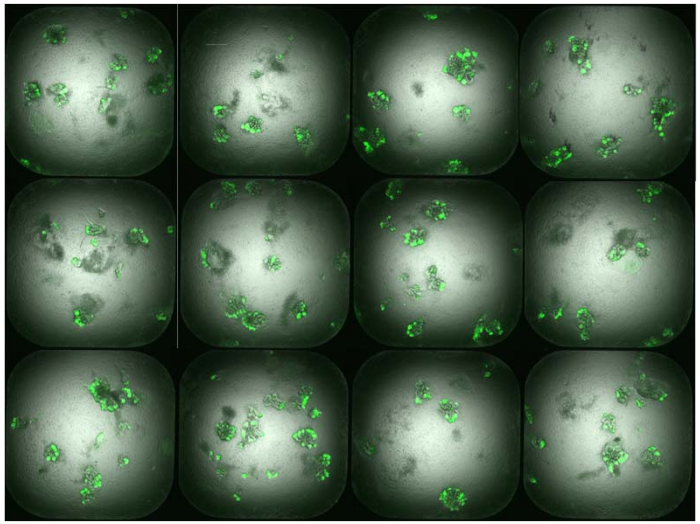
Discovery of a novel candidate therapeutic for PKD

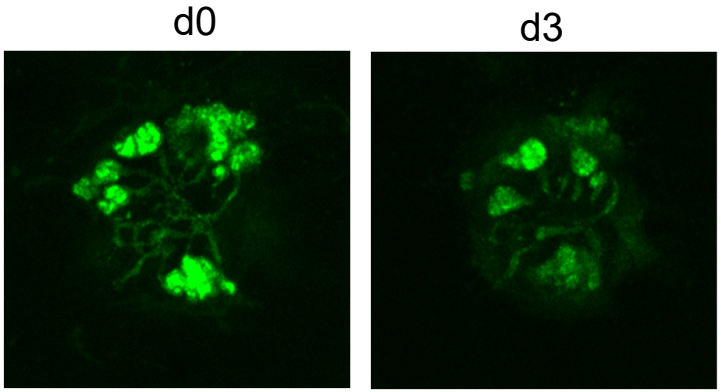


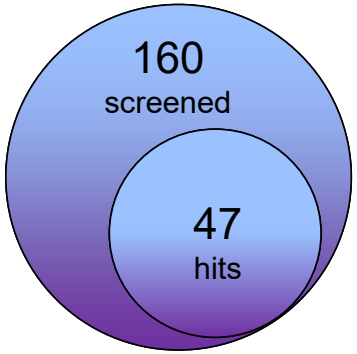


Unpublished

HTS for human nephrotoxicity



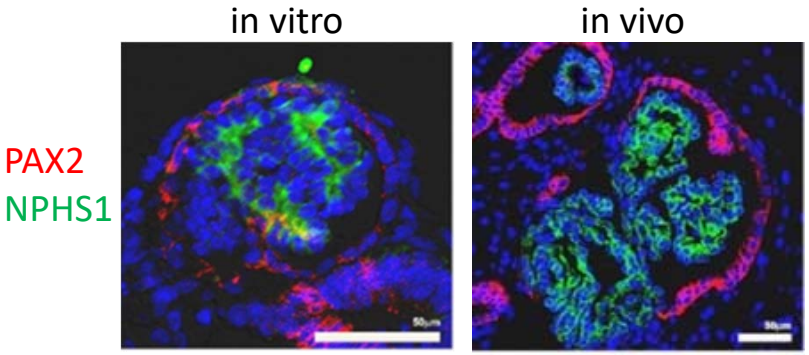


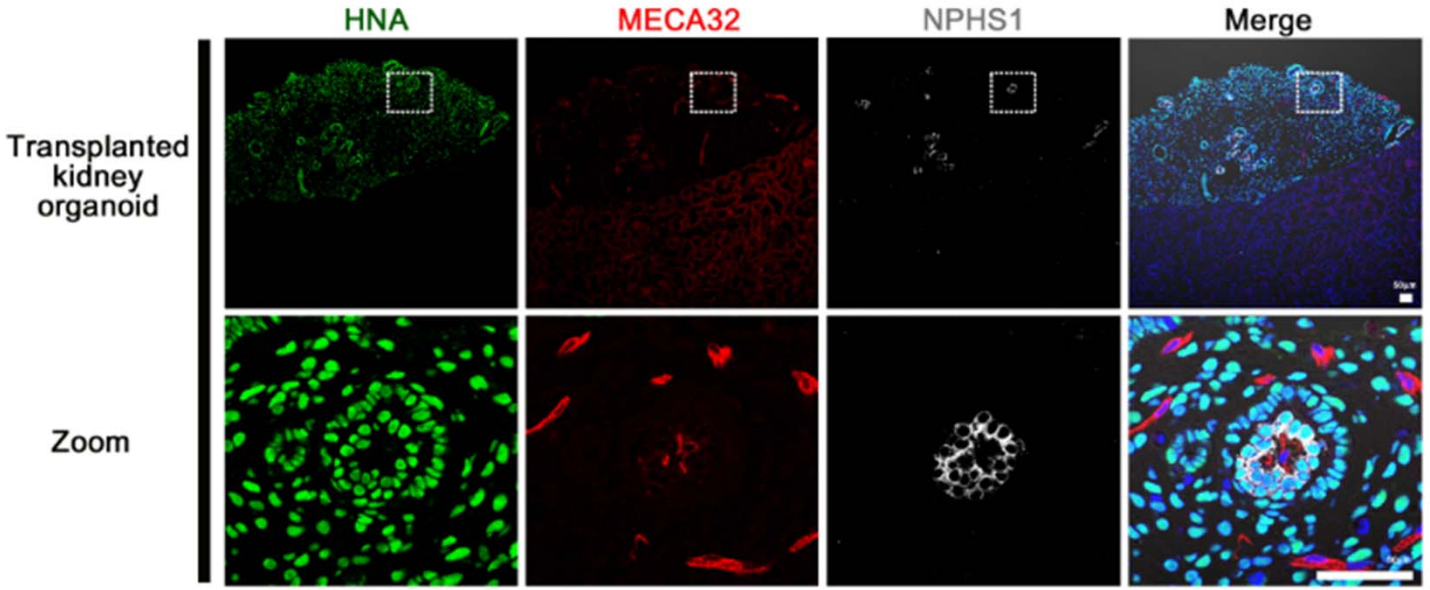


- 2 doses for each drug, 2 replica plates
- Automated analysis to assess organoid GFP
- Good reproducibility, several known nephrotoxicant ‘hits’

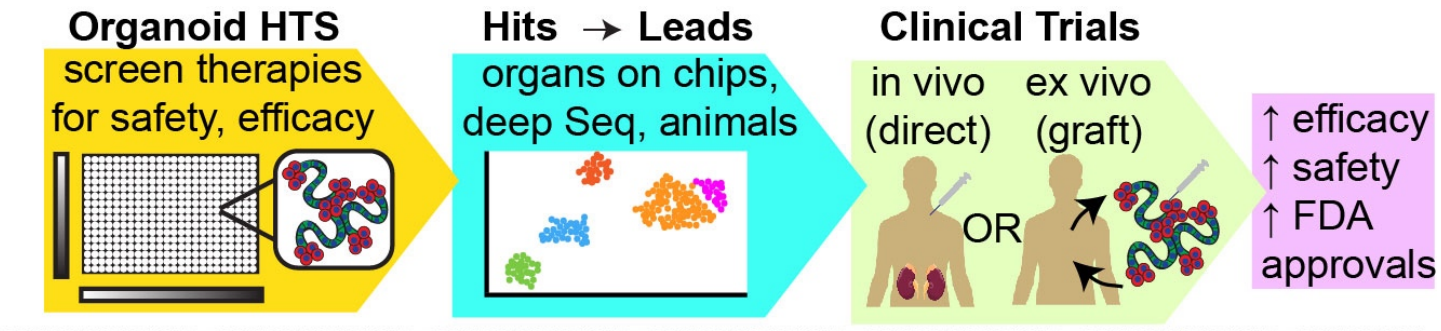
Unpublished

Kidney organoids engraft and vascularize





Conclusions and Future Work



- iPS cells can be differentiated into kidney organoids that contain structures resembling primitive nephrons
- Organoids can be gene edited to reconstitute and reveal mechanisms of polycystic kidney disease and other disorders
- Automated manufacture + HTS + real time reporters enable the assessment and discovery of therapeutic entities
- Organoids can engraft into the kidneys and undergo partial maturation

Thanks to

Nelly Cruz
Yong-Kyun Kim
Stefan Czerniecki
Sienna Li
Linh Tran
Raghava Reddy
Jonathan Himmelfarb
Stuart Shankland
Hongxia Fu

FreedmanLab
regenerating the kidney

benofreedman

NIDDK

cystinosis RESEARCH FOUNDATION | RESEARCH HOPE CURE

NIH National Center for Advancing Translational Sciences

ISCRM Institute for Stem Cell and Regenerative Medicine UNIVERSITY OF WASHINGTON

NORTHWEST Kidney Centers Live. Learn. Hope.

ALLEN INSTITUTE for CELL SCIENCE

KIDNEY RESEARCH INSTITUTE
A collaboration between Northwest Kidney Centers and UW Medicine

Division of Nephrology UNIVERSITY OF WASHINGTON

Neuronal Multi-organ-on-Chip Models for Disease Modeling and Risk Assessment

James J Hickman

Professor of Nanoscience Technology,
Chemistry, Biomolecular Science, Physics, and Electrical Engineering
Head, Hybrid Systems Laboratory
University of Central Florid and Chief Scientist, Hesperos, Inc.

3D Cell Culture Models for Drug PK, Safety, and Efficacy Assessment
August 14, 2020

Conflict of Interest - Hesperos

- Over 20 US patents have been licensed by Hesperos thereby documenting the innovation and novelty of this platform. This also provides full freedom to operate in this space & a strong defendable IP position.
- Winner of the 2015 London-based *Lush Prize* for creating an alternative to animal testing for industry.
- Have won multiple SBIR grants including a $2M Phase II and recently a $4M phase IIB award to Bridge the "valley of death".
- Established R&D contracts with multiple national and international Pharma companies.
- Currently a member of UCF's Incubator program but moving to new 14,100 sq. ft. state of the art facility in August 2019.
- Have recruited excellent staff for company, 27 at present.
- No products will be offered at this time, only services based on compounds sent to our Orlando facility.

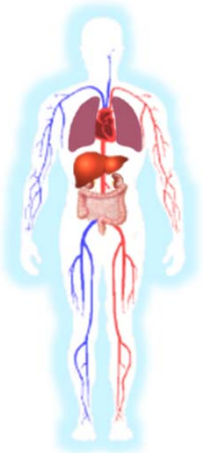
THE 2015 LUSH PRIZE

SUPPORTING ANIMAL-FREE TESTING

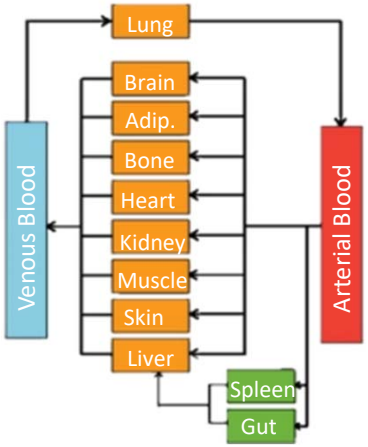
Human-on-a-Chip Systems for Disease Modeling

HESPEROS™

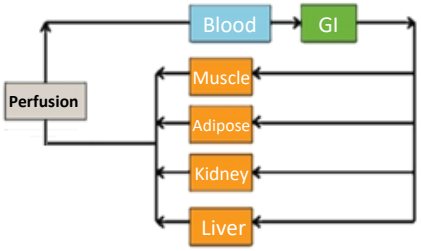
Human Body



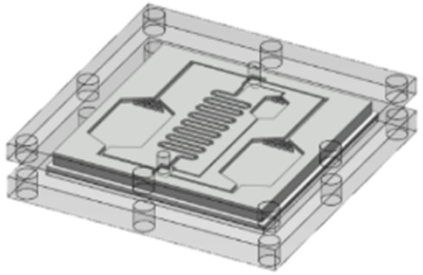
PBPK



Chip PBPK

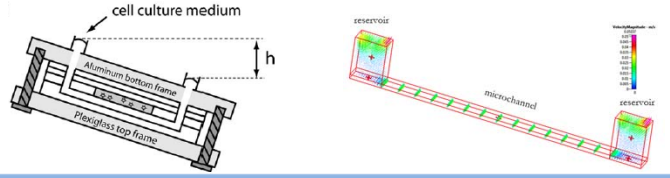


Serum-Free Human-on-a-Chip



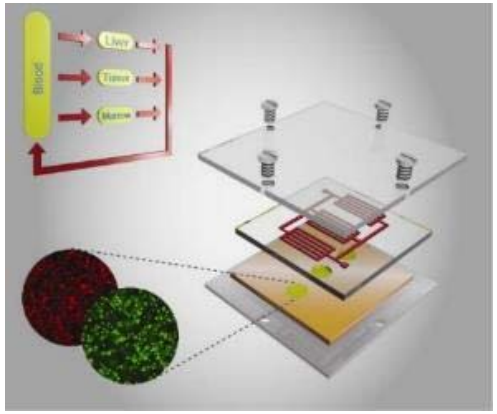
Gravity-induced flow through a microfluidic device on a rocking platform

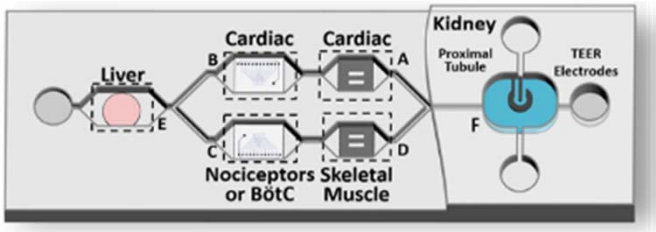
- Pumpless operation
- Minimizes bubble formation



Medium recirculation with gravity-induced flow. Tilting of the device causes liquid to flow between the wells. In a timed manner, the rocking platform changes the angle and medium flows in the opposite direction. [1]

OVER 20 PUBLICATIONS USING THE PUMPLESS SYSTEM





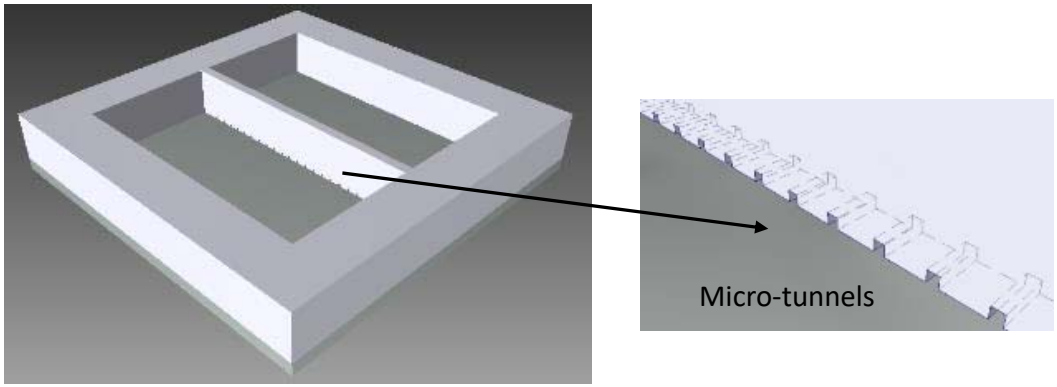
References:

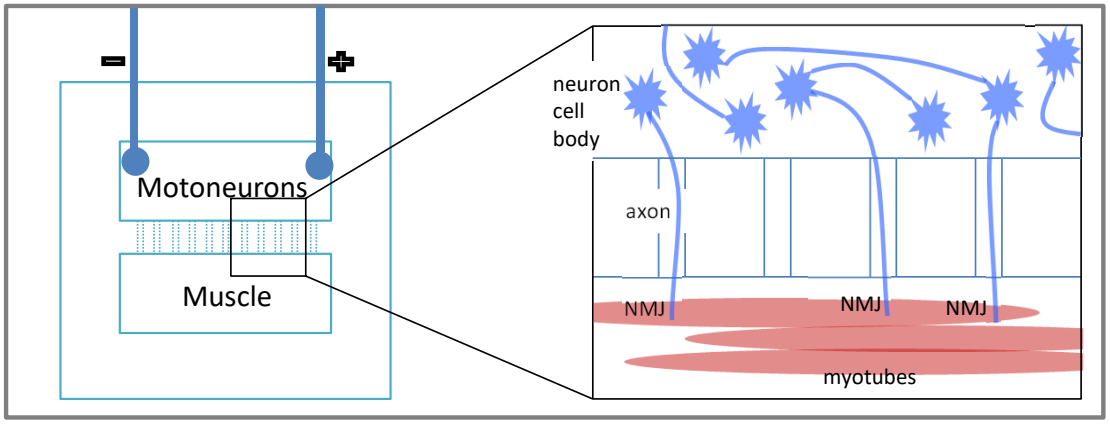
Oleaga C., et al., *Biomaterials*. 182:176-90 (2018)
Oleaga C., et al., *Sci Rep*. 6:20030 (2016)
Chen, H.J., et al., *Nat Biotechnol*, 34:845-851 (2016)
JH Sung, C Kam, ML Shuler, Lab on a chip,10: 446 (2010)
Castellanos M, et al., *Proc Natl Acad Sci U S A*. 101(17):6681-6 (2004)
Sweeney LM, et al., *Toxicol In Vitro*. 9(3):307-16 (1995)

What are Functional Readouts?

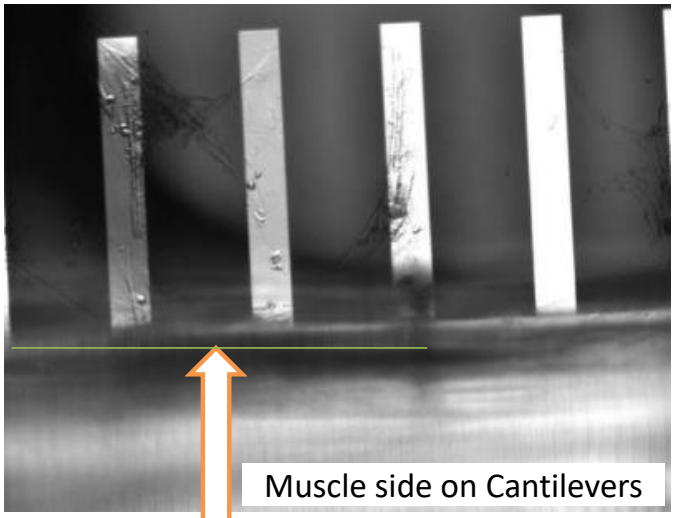
- Mechanical or electrical readouts of cellular functions such as:
  - ***muscle contraction***
  - ***electrical activity*** from neurons and cardiac cells
  - Motoneuron → muscle: ***NMJ physiology*** and other combinations
- Allows ***functional*** analysis of cellular health non-invasively for acute, but more importantly, for ***chronic*** monitoring of human-on-a-chip systems
- ***Reduces substantially***, if not eliminates, the need for measuring ***biomarkers*** in these systems for certain organ mimics. Normally need to measure multiple biomarkers by molecular techniques and put them together ***to extrapolate functional*** activity, with these systems can ***measure directly***.
- Allows ***mechanistic*** determination of toxicity and for target identification for efficacy.
- Facilitates ***physiological*** determination of drug efficacy and safety

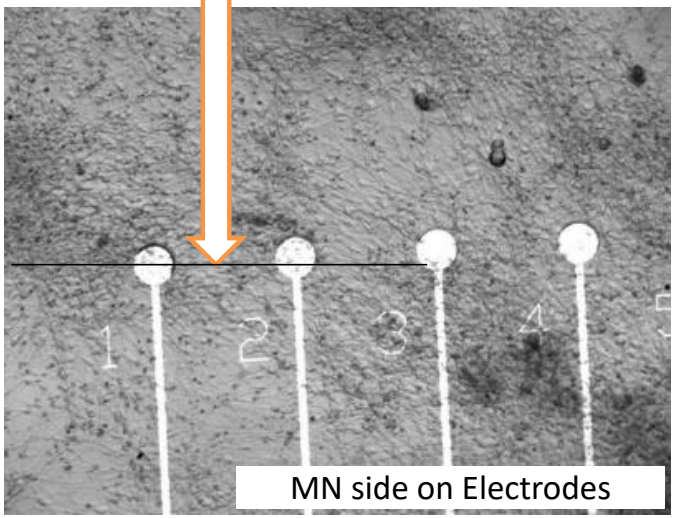
Neuromuscular Junction (NMJ) Platform

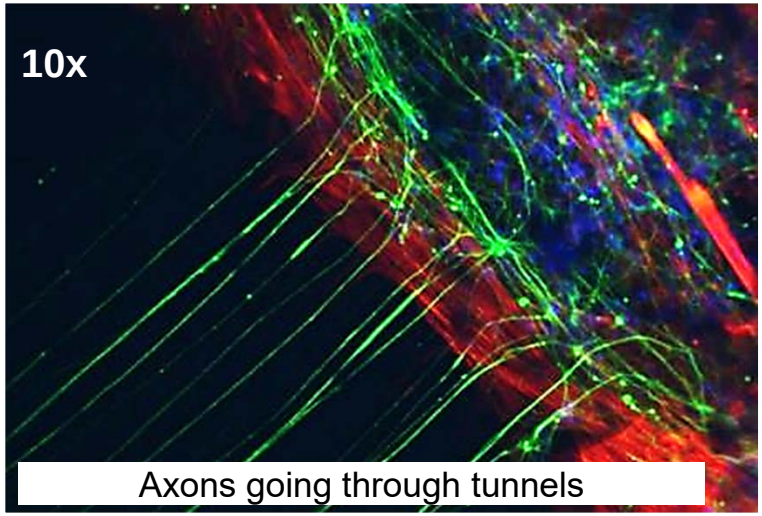


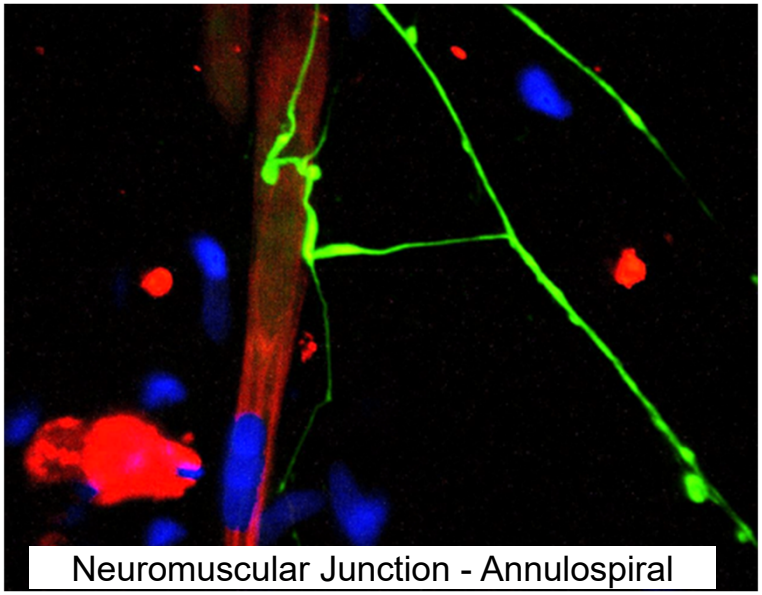


- PDMS molded chambers bonded to glass coverslips
- Two chambers separated by micro-tunnels
- Motoneurons send axons through tunnels and form NMJs
- Electrical stimulation and drugs partitioned by barrier

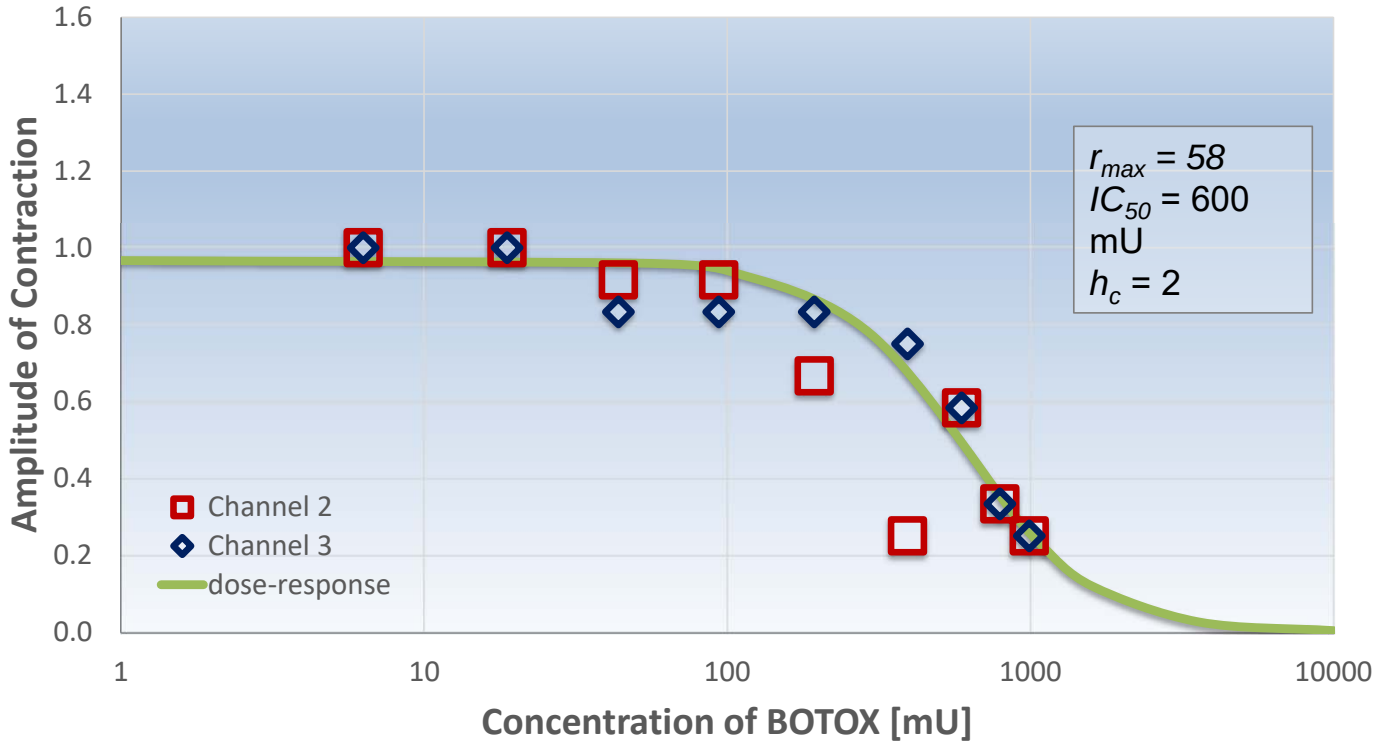

Muscle side on Cantilevers


1
2
3
4
MN side on Electrodes

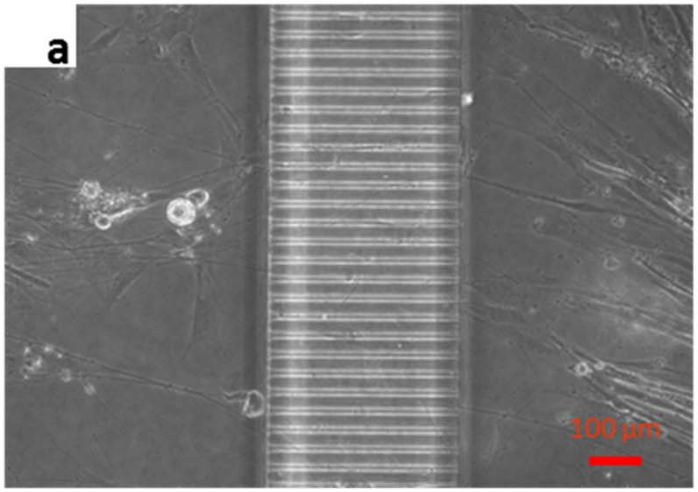

10x
Axons going through tunnels

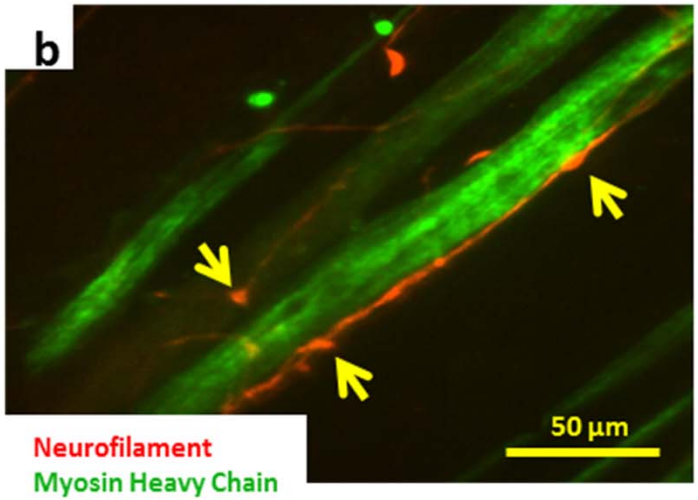

Neuromuscular Junction - Annulospiral

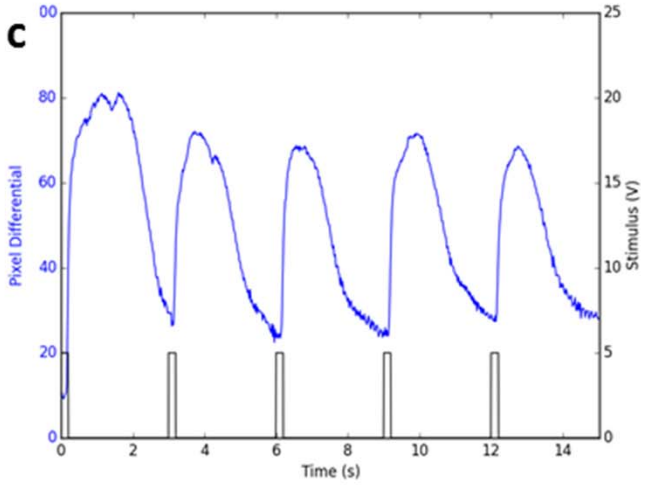
Monophasic Dose-Response for BOTOX at 0.33 Hz



Santhanam et al, ***Biomaterials***, 166:64-78 (2018)





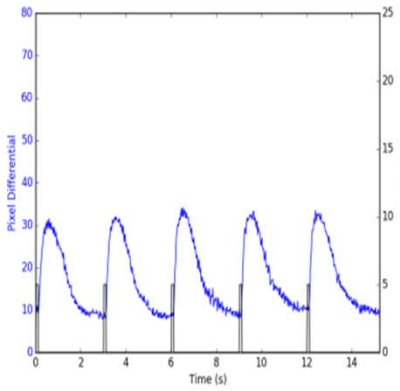


Induced pluripotent stem cell derived motoneuron from ALS patients in the system

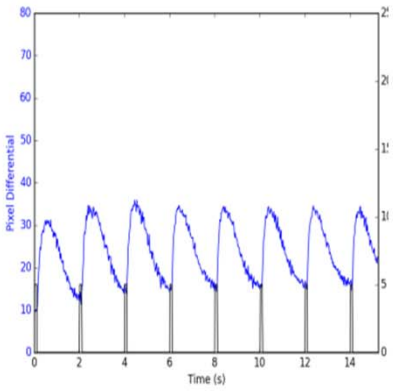
Parameters Analyzed

- Number of functional NMJs/chamber (before and after extensive stimulation)
- NMJ stability (post-NMJ/pre-NMJ)
- NMJ function under different stimulation frequencies (0.33 Hz, 0.5 Hz, 1 Hz, 2 Hz)
  --- NMJ fidelity (number of muscle contractions induced by MN stimulation/total number of stimulations)

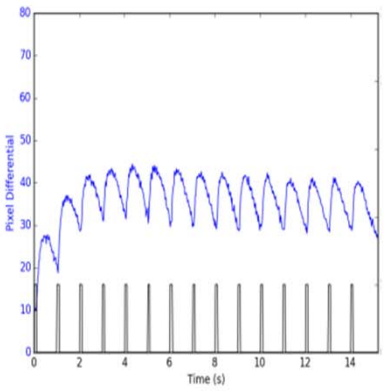
0.33 Hz



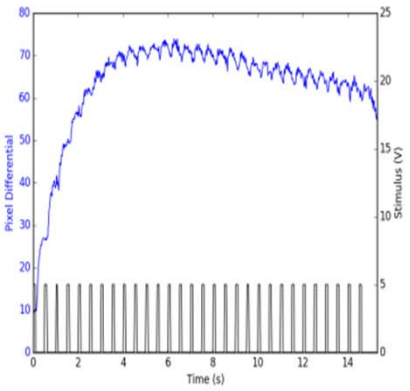
0.5 Hz



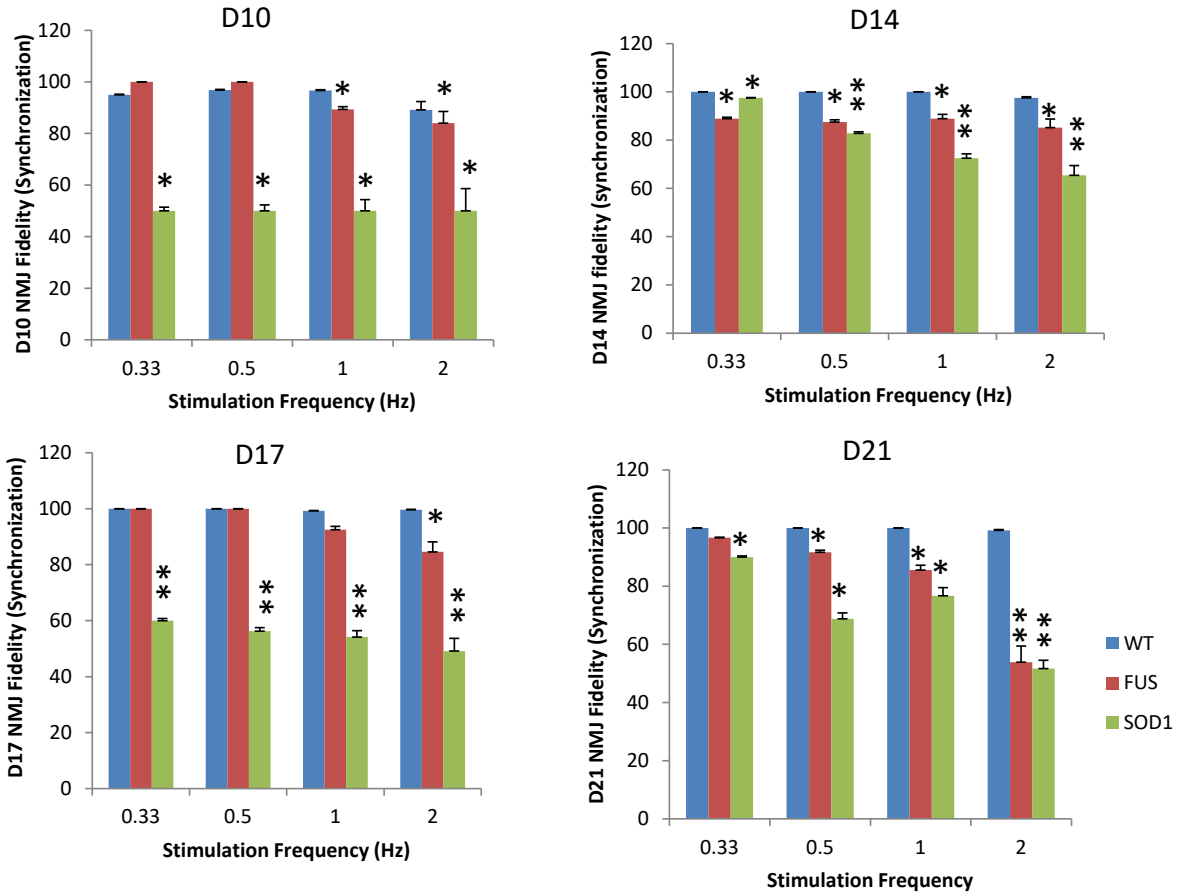
1 Hz



2 Hz



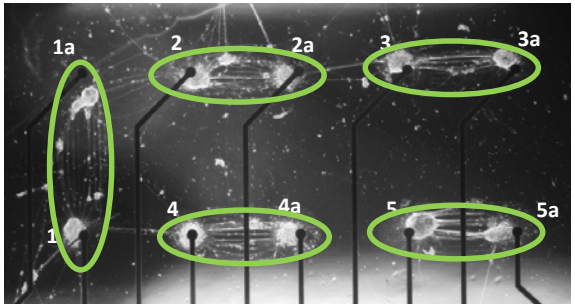
Comparison of NMJ fidelity (synchronized release)

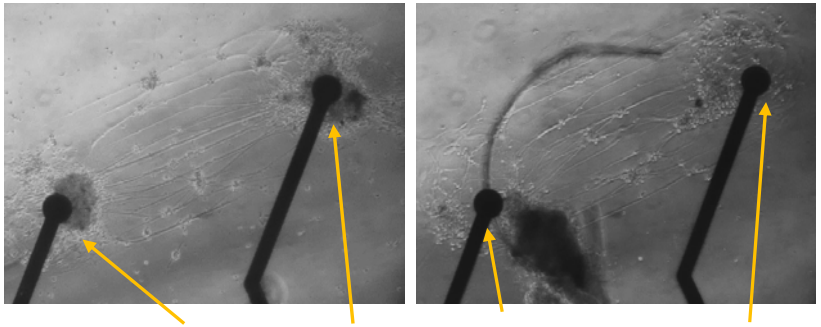


Guo et.al, ***Advanced Therapeutics***, in press (2020)

Patterned Neural Networks on MEAs - Long Term Potentiation (LTP)

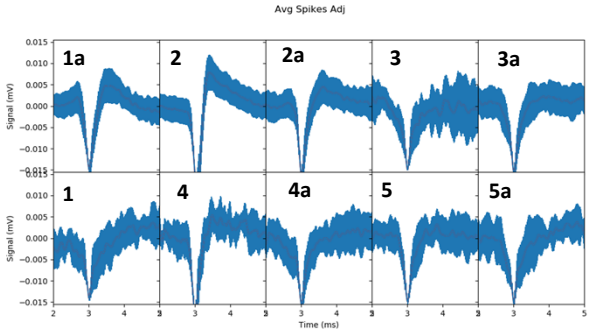
Whole-MEA View





- High magnification phase images indicating long-term pattern conformity and network formation

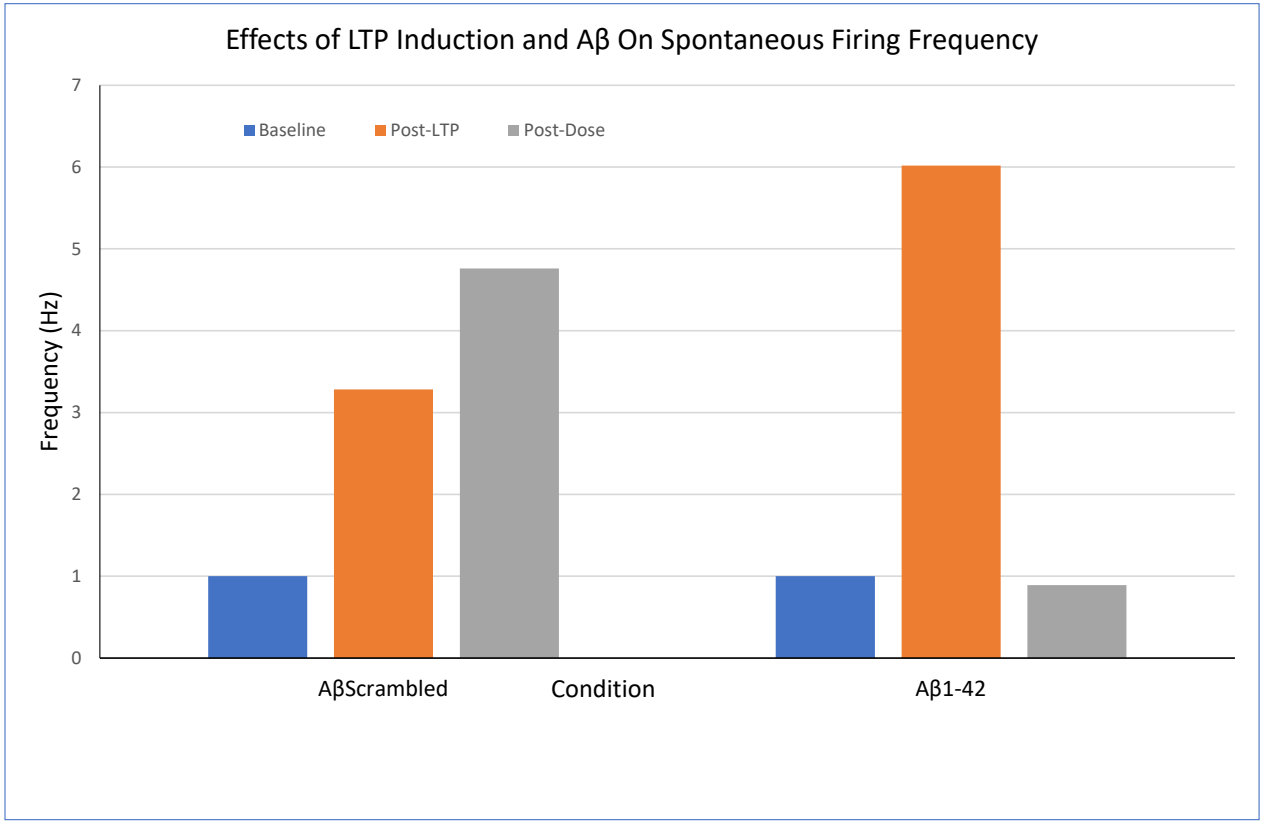
Ave. Electrode Signal Recordings



Depolarizing Potentials Recorded from MEAs

- 5 network pairs per MEA
- 45 days *in vitro*
- Spontaneous action potentials recorded on electrodes from paired neural circuits

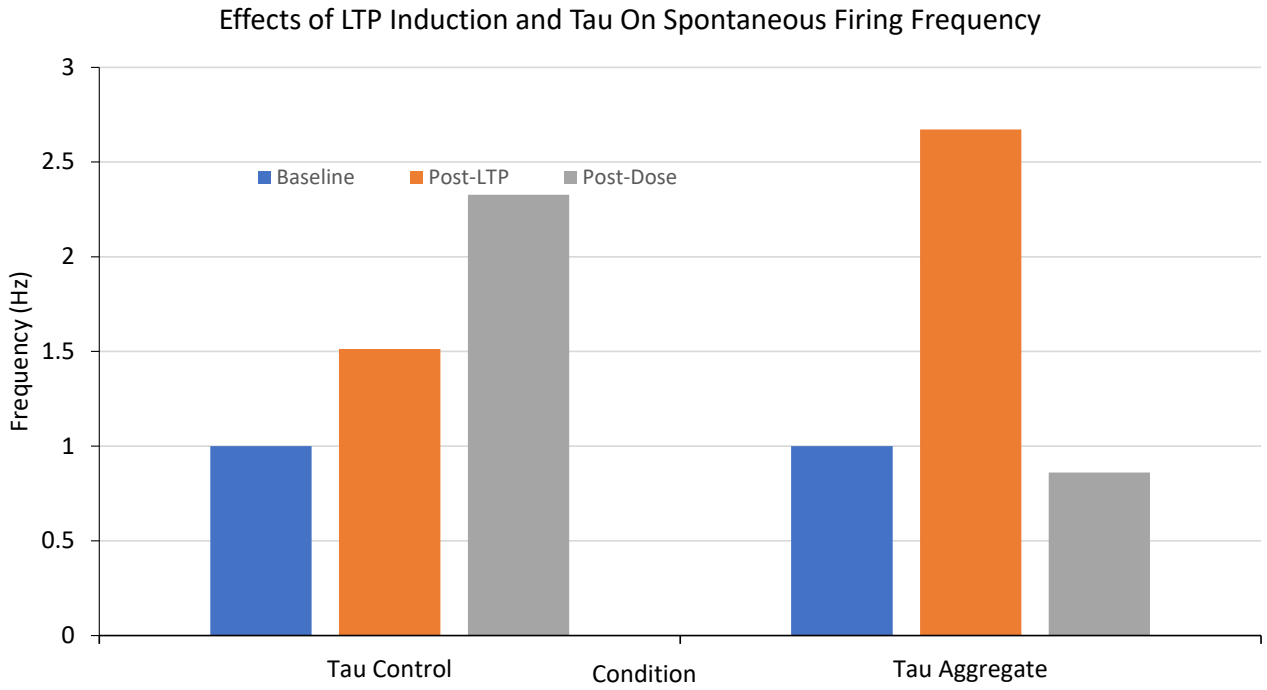
Effects of $A\beta_{1-42}$ on Cortical Neuron Spontaneous Firing Frequency



- LTP can efficiently be induced in cortical neurons grown on MEAs
- Dosing MEAs for one hour with $A\beta_{1-42}$ after LTP induction abolishes the LTP effects compared to $A\beta_{scrambled}$ treated MEAs

N=4, nested replicates

Effects of Tau Aggregate on Cortical Neuron Spontaneous Firing Frequency

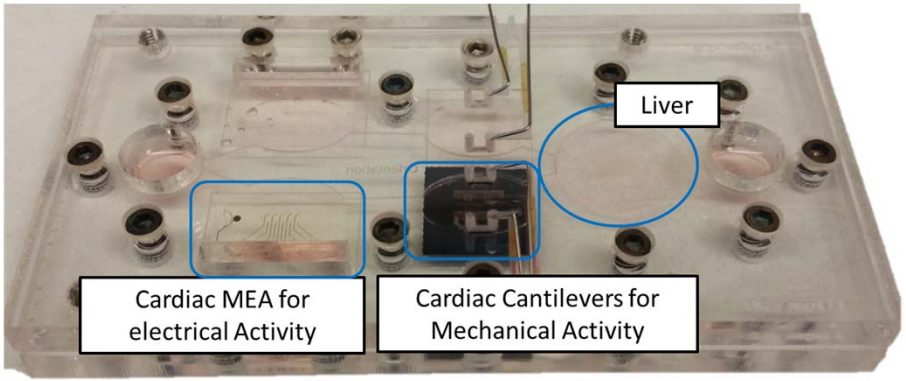


- Dosing MEAs for one hour with tau aggregates after LTP induction abolishes the LTP effects compared to tau buffer control MEAs

N=4, nested replicates

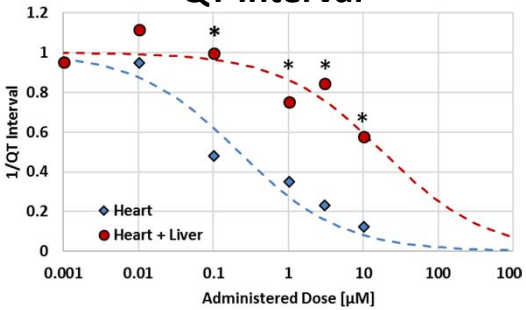
J. Caneus, etal., "A Human Induced Pluripotent Stem Cell-Derived Cortical Neuron Human-on-a chip System to Study Aβ42 and Tau-induced Pathophysiological Effects on Long-Term Potentiation," ***Alzheimer's & Dementia: Translational Research & Clinical Interventions***, Online First: May 28: (2020) DOI: 10.1002/trc2.12029.

Heart-Liver Systems with Recirculating Serum-Free Medium



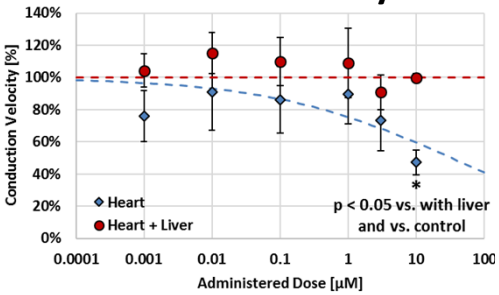
Heart + liver system derived from 4-organ system with similar flow characteristics

QT Interval



Terfenadine Addition Results in Elongation of QT Interval in the Absence of a Liver Component

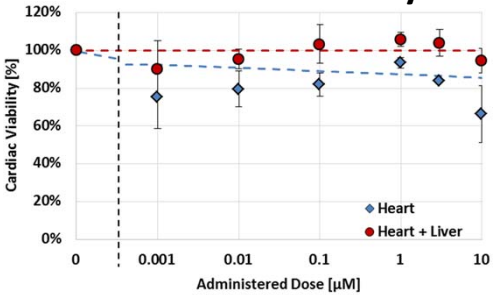
Conduction Velocity



Terfenadine Addition Reduces Conduction Velocity in the Absence of a Liver Component

IC_{50} = 33 µM (Heart)
IC_{50} = NA (Heart + Liver)

Cardiac Viability



Without liver, terfenadine slightly reduces cardiac viability at 10 µM terfenadine

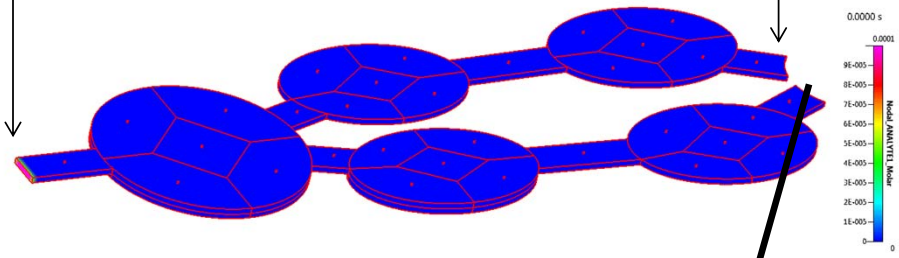
$IC_{50,1}$ > 10 µM (Heart)
IC_{50} > 10 µM (Heart + Liver)

HESPEROS INC

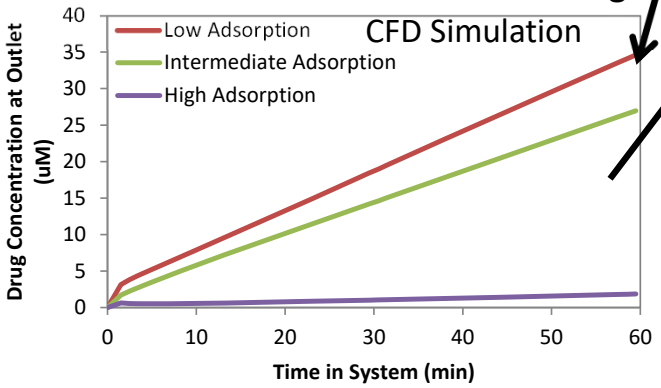
Lipophilic Compound Concentration in Systems 5 min to 24 hours

Compound (330 uM) added to this reservoir for 100 uM final concentration

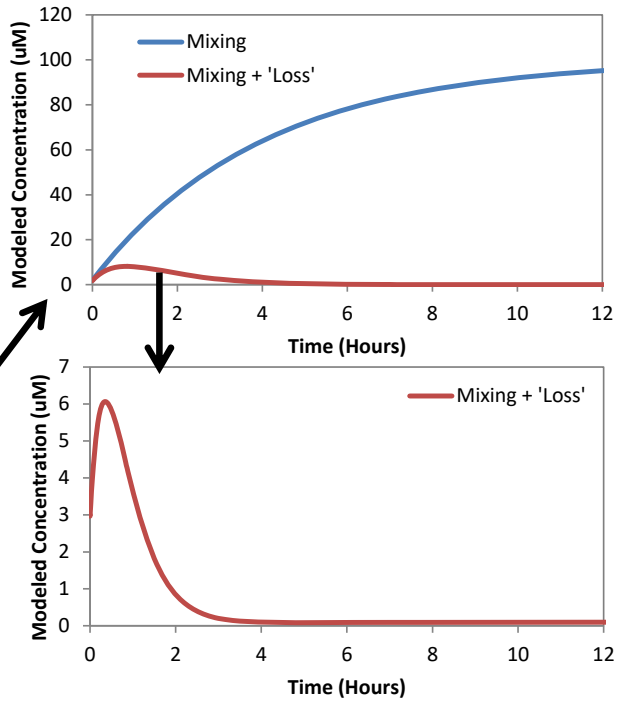
Withdrawal from this reservoir



Simulation of Drug addition to one side with initial perfusion due to volume difference + Rocking

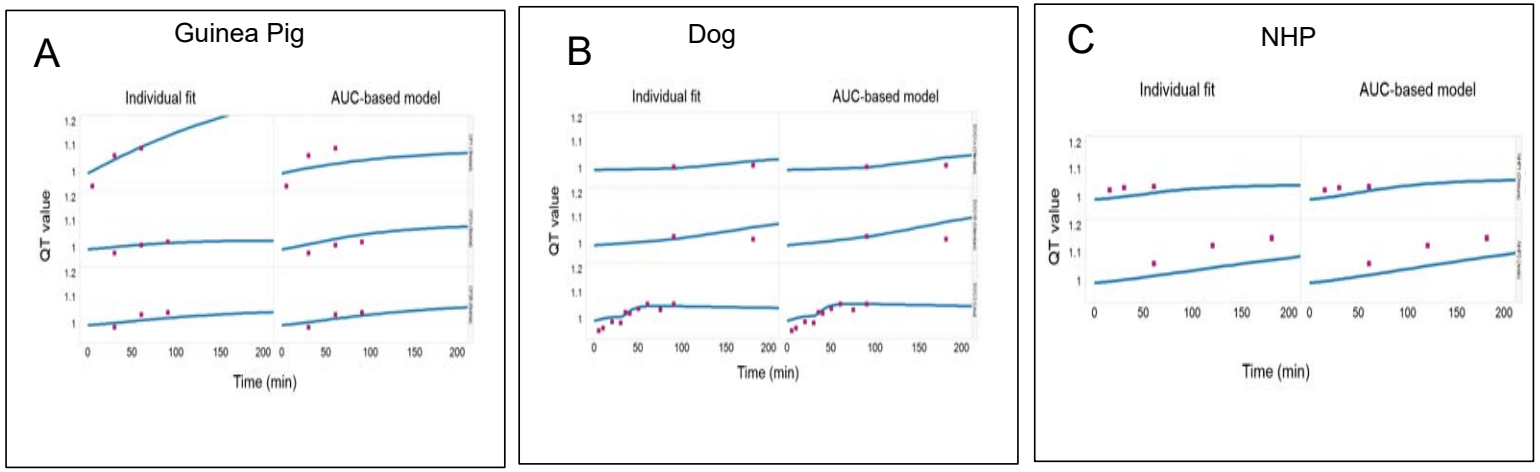


Numerical Simulation



Concentration at reservoir opposite liver dependent on mixing along with adsorption, metabolism, and bioaccumulation (“loss”)

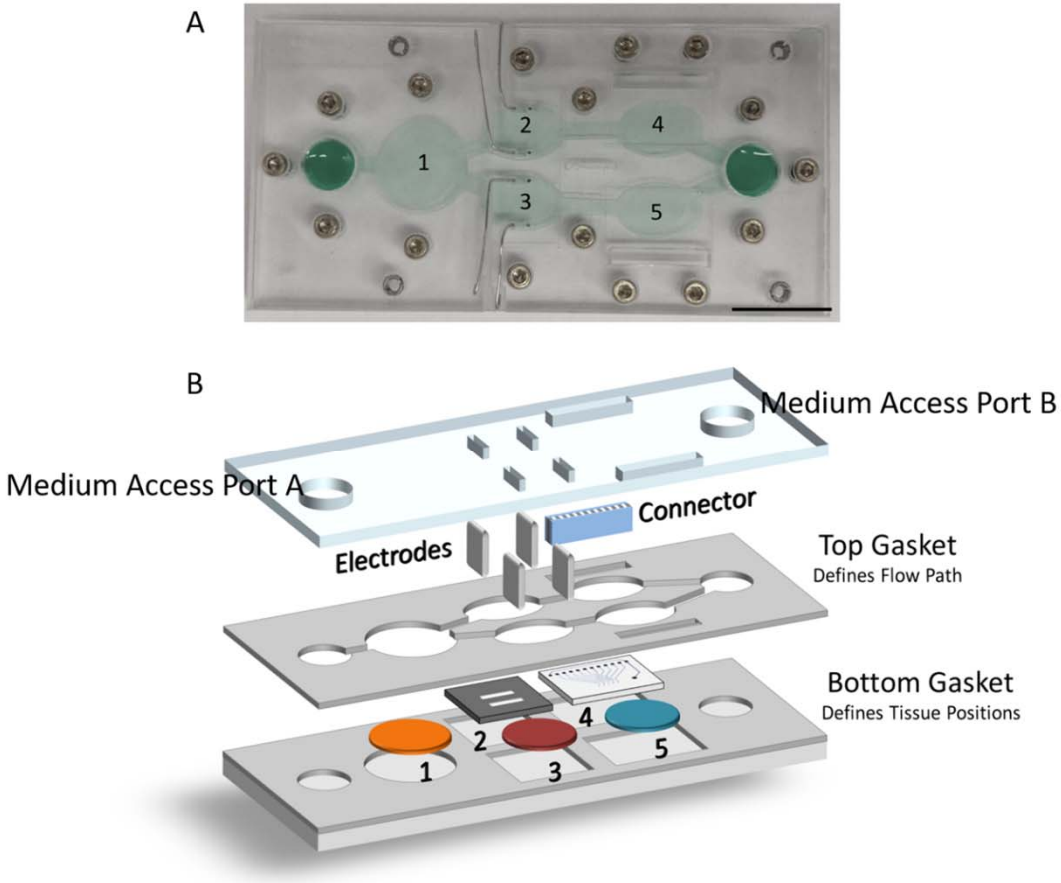
Correlation of In Vitro PKPD Model with In Vivo Animal PKPD Models



Since in vivo animal data has been correlated with clinical data we should be able to correlate our in vitro models with clinical data as well both retrospectively and prospectively

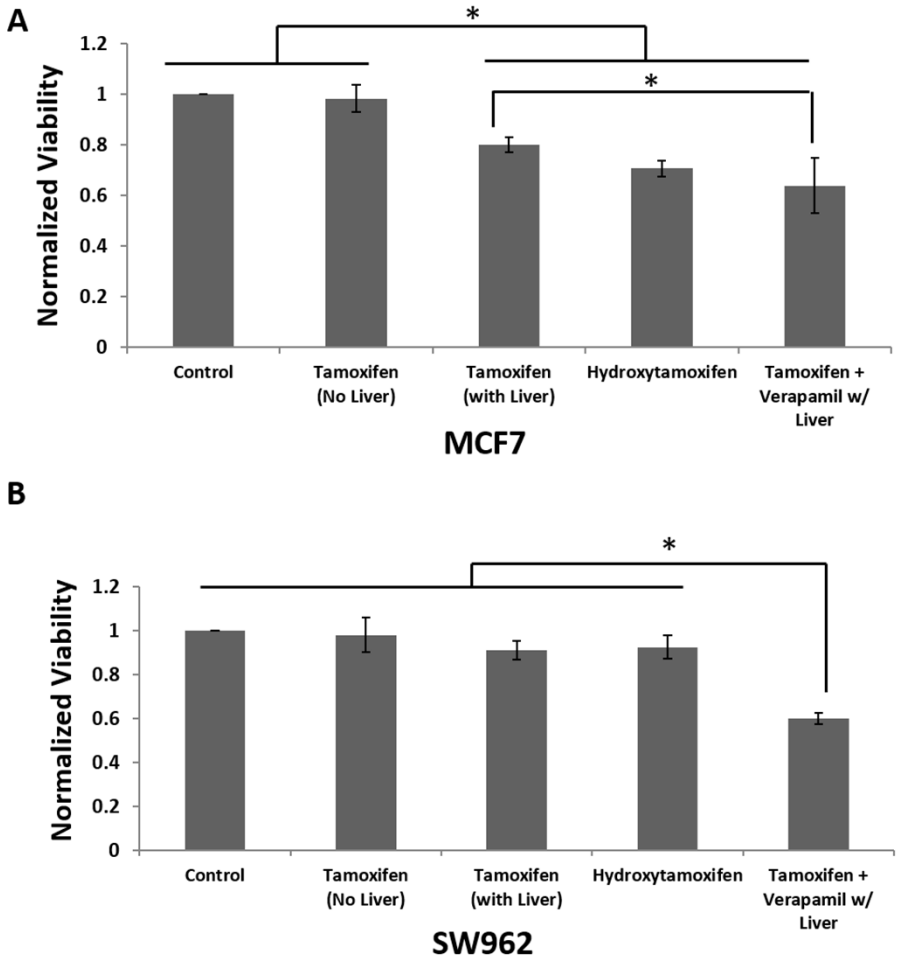
McAleer et al., ***Nature Scientific Reports***, 9:9619, (2019)

Cancer, Cardiac and Liver System for Efficacy and Toxicity



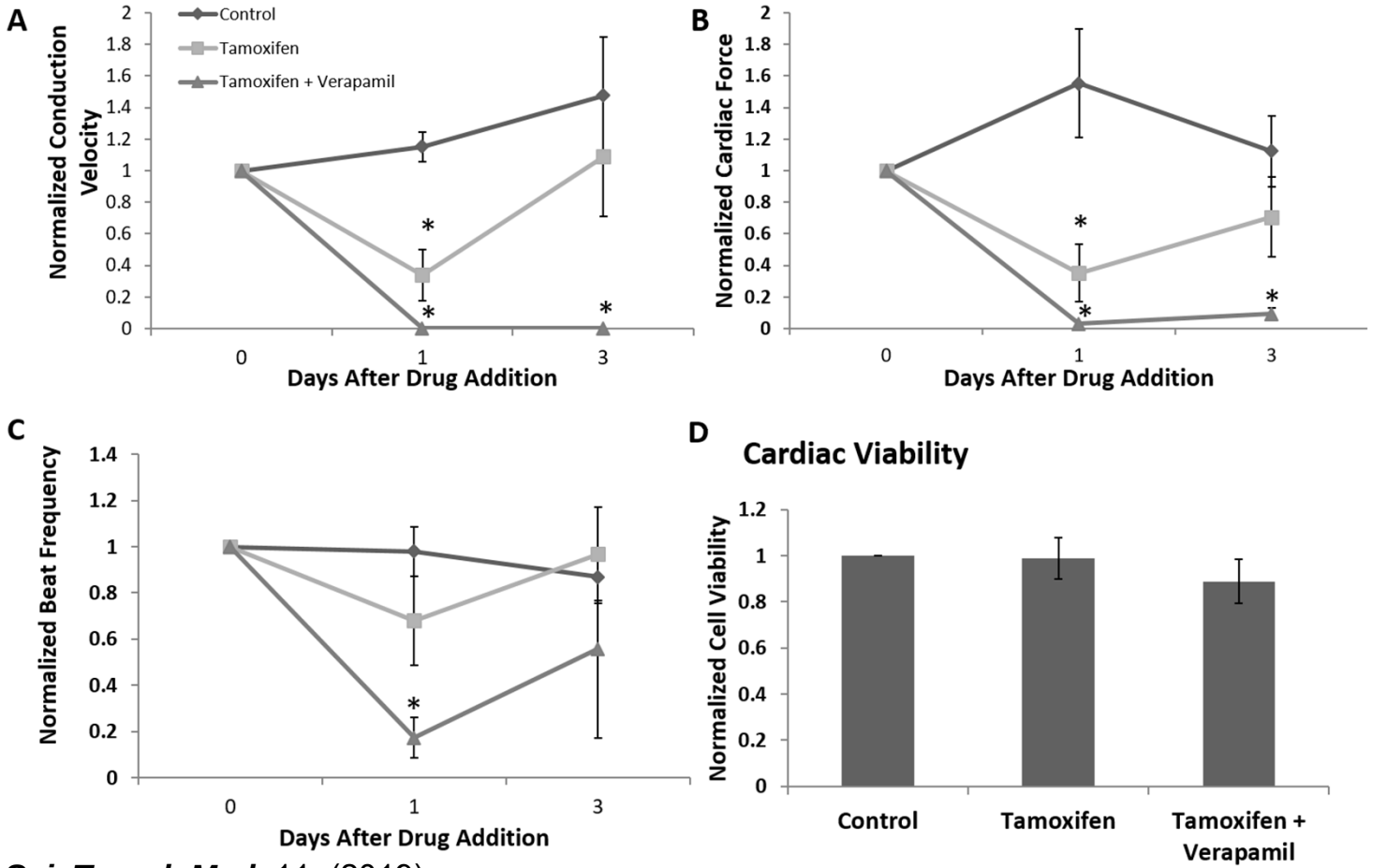
Five chamber reconfigurable multi-organ system. Scale bar is 2 cm. B) Schematic representation of the MPS assembly and design used in the system 2 study of tamoxifen. Chamber 1 houses hepatocytes on coverslips Chambers 2 and 4 are cardiac cantilevers and MEAs respectively. Chambers 3 and 5 are for cancer cells SW962 and MCF7. Drugs were applied to Medium Access Port A and initially pass over the liver to mimic aspects of first pass metabolism. Electrodes are embedded for the option of using broadfield stimulation to elicit contraction.

Tamoxifen, with and without metabolism, and the effect on cancer viability



Error bars are represented as SEM. *p < 0.02.

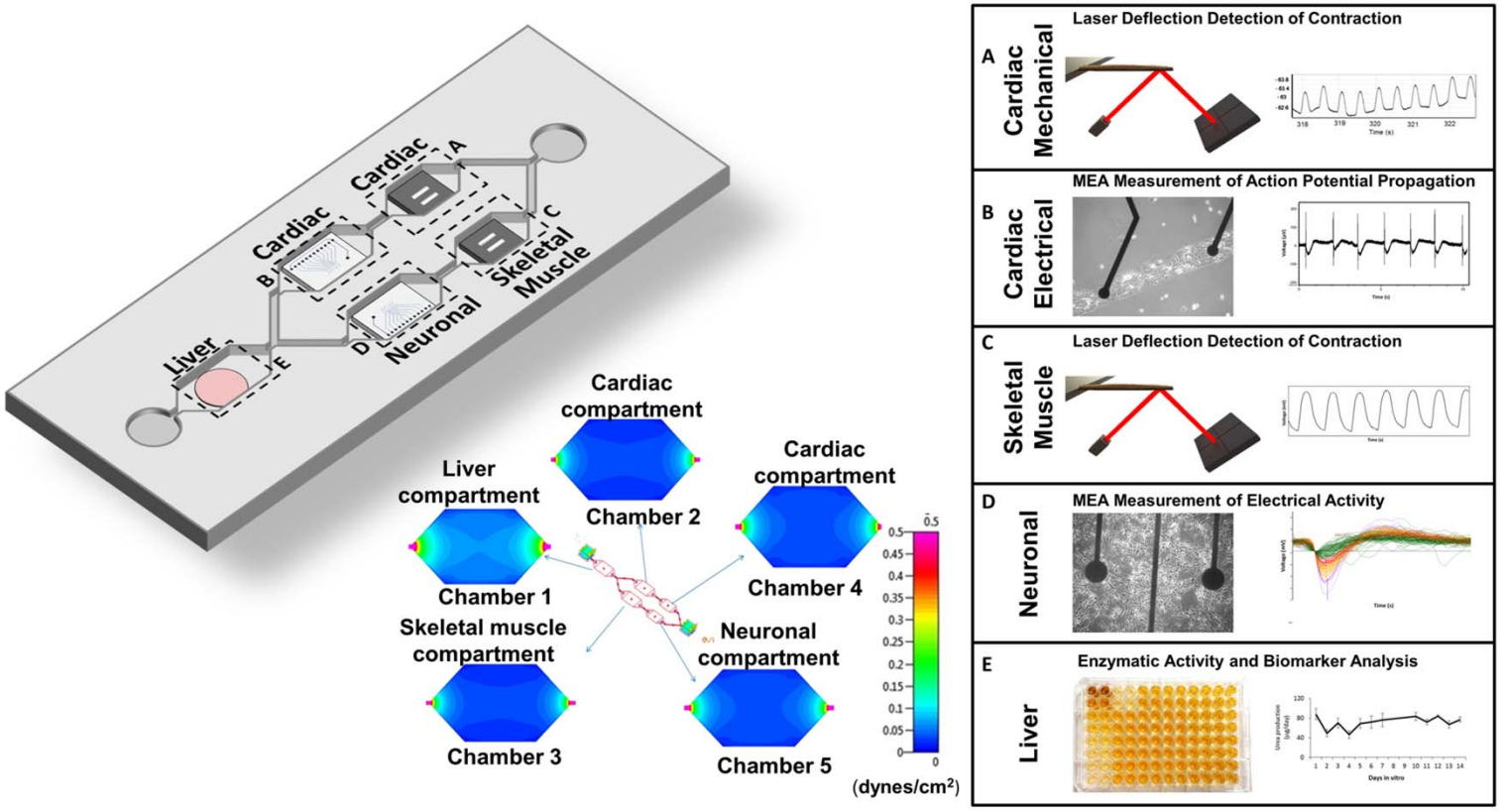
Sentinel Monitoring of Cardiac Function
Cardiac, Cancer, Liver Systems



McAleer et al., ***Sci. Transl. Med***. 11, (2019)

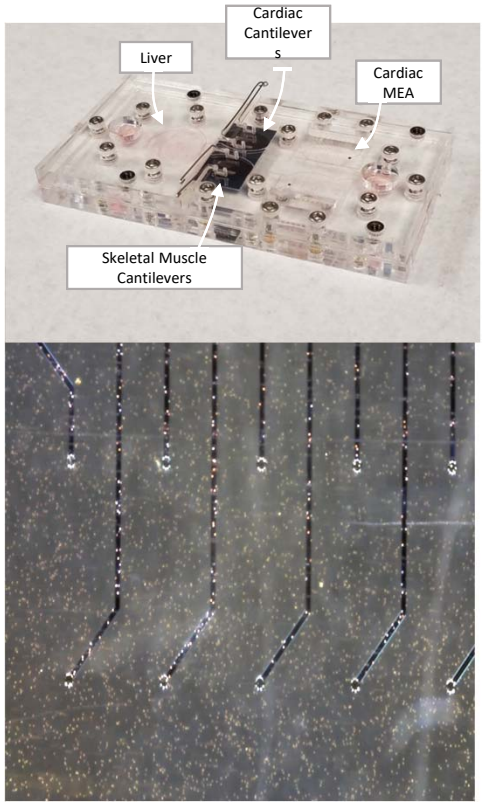
Error bars are represented as SEM. *p < 0.02

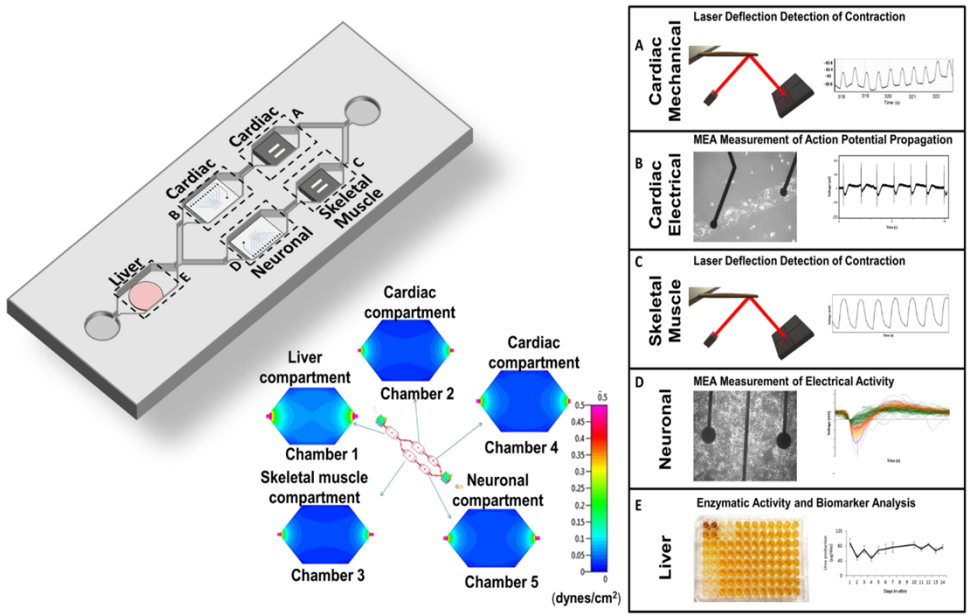
Functional Measurements in 4-Organ Systems



Oleaga et al. 2016. ***Nature Scientific Reports*** "Multi-Organ toxicity demonstration in a functional human *in vitro* system composed of four organs"
Oeaga, et al. 2019. ***Advanced Functional Materials*** "Long-Term Electrical and Mechanical Function Monitoring of a Human-on-a-Chip System"

Our Multi-Organ MPS Device Supports Recirculating Immune System Cells



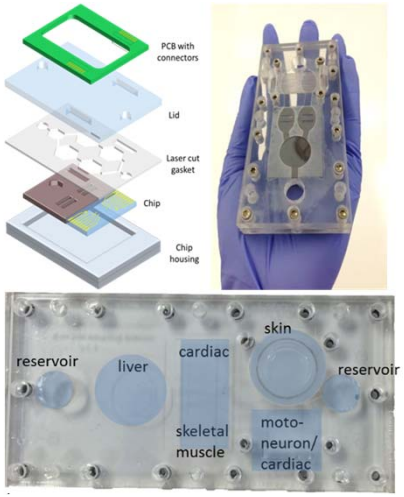


T. Sasserath, "Differential Monocyte Actuation in a Three-Organ Functional Innate Immune System-on-a-Chip," ***Advanced Science***, In Press (2020)

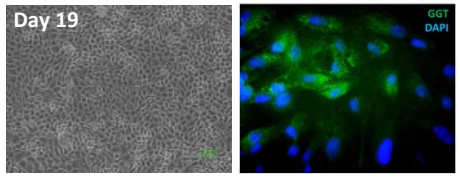
Barrier Tissue Organ Systems

Cornell University

Skin in 4-Organ System

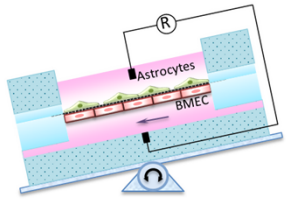


Proximal Tubule System

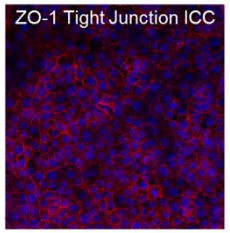


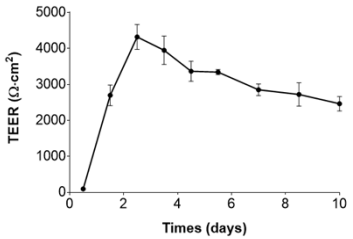
- Human proximal tubule cells grown on membranes under continuous flow maintain conformal monolayer
- Cells stain for kidney cell marker **Gamma-glutamyl transpeptidase (GGT)**

Human Blood-Brain Barrier System



Rocker platform

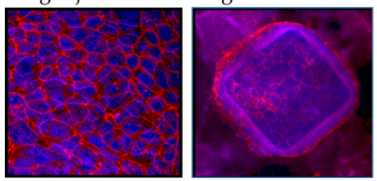


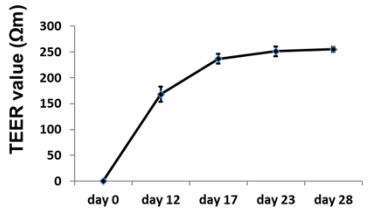


Gastrointestinal Tract Barrier System

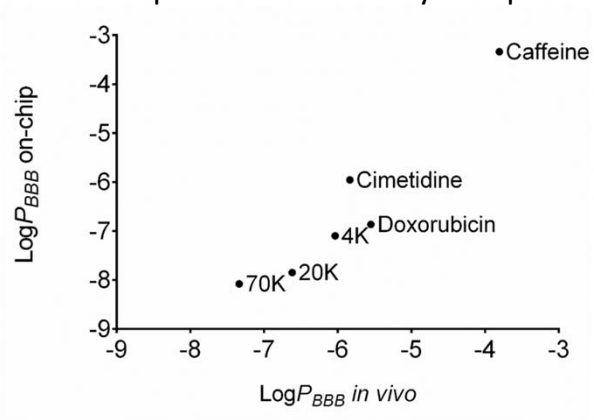
Primary Human Colon Epithelial Cells, by knock-in of telomerase reverse transcriptase (TERT), co-cultured with myofibroblasts and 5 nM GSK-3beta inhibitor

Tight junction staining in 2D and 3D





Compound Permeability Comparison



Five minutes Break!

Panel Discussion II

Chair: Hongbing Wang (UMB)

Panelists:

N. Isoherranen

G. Guo

J-Y Wang

H. Wang

S. Heyward

A. Li

W. Hedrich

B. Freedman

J. Hickman

Conclusion Remarks

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