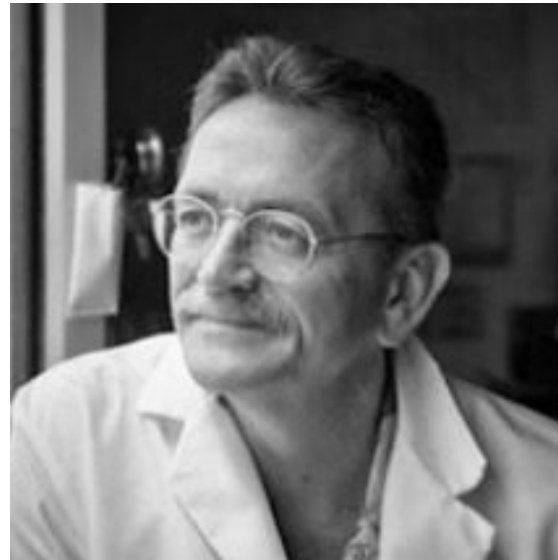




Human Neuromuscular Junction Platform to Evaluate  
Botulinum Toxin Potency  
***Accepted into the I STAND Program***

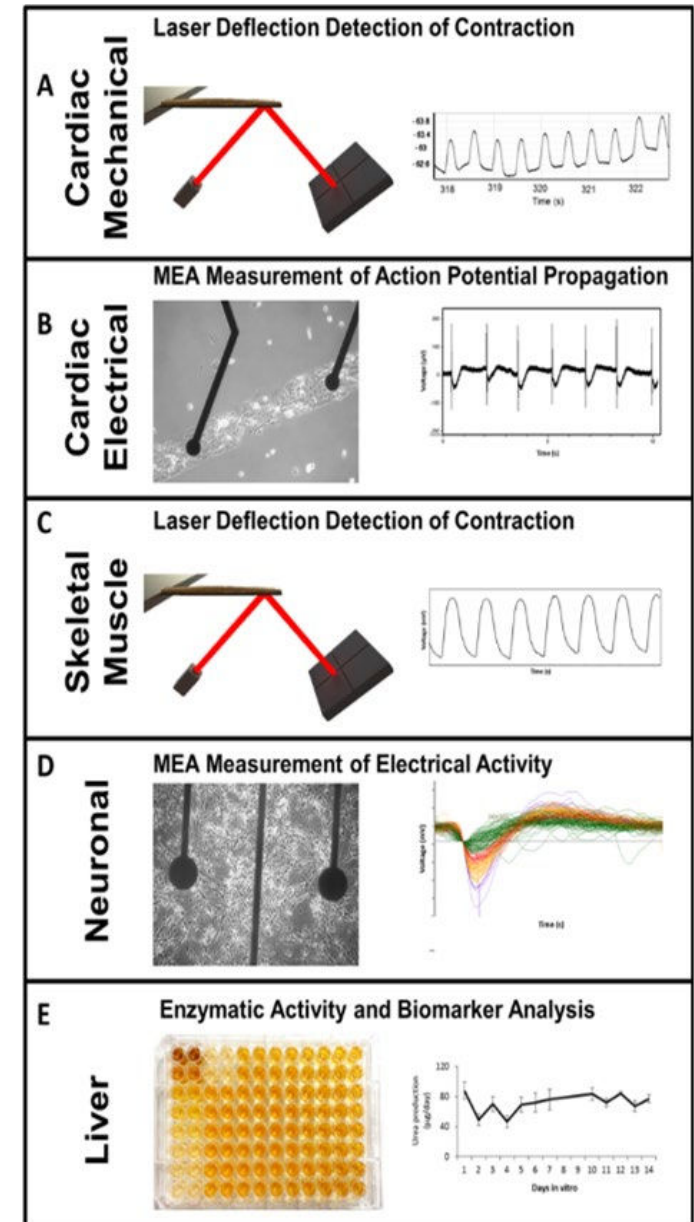
**PI: James J. Hickman, Ph.D.**  
Hesperos, Inc.  
12501 Research Parkway, Suite 100,  
Orlando, FL 32826



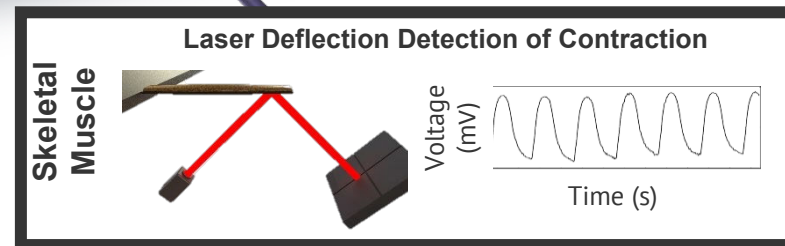
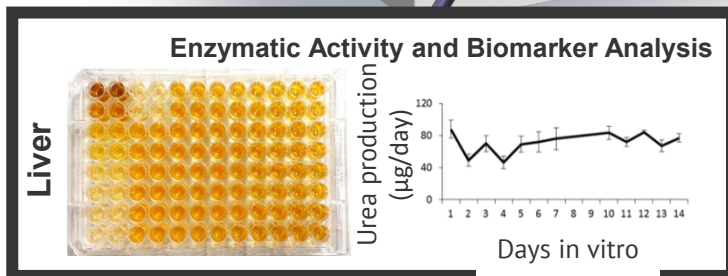
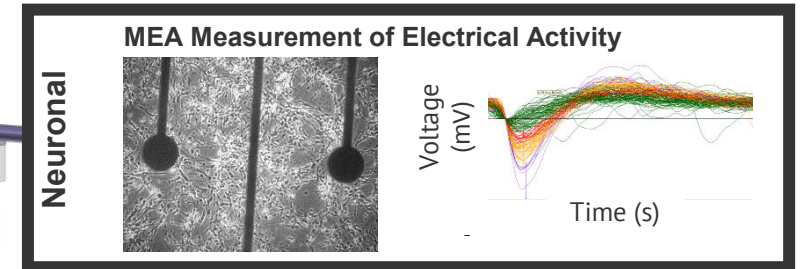
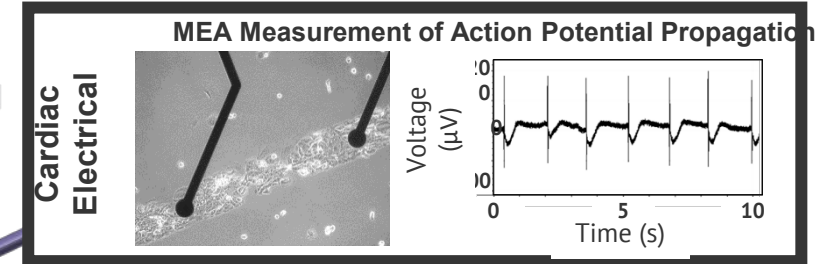
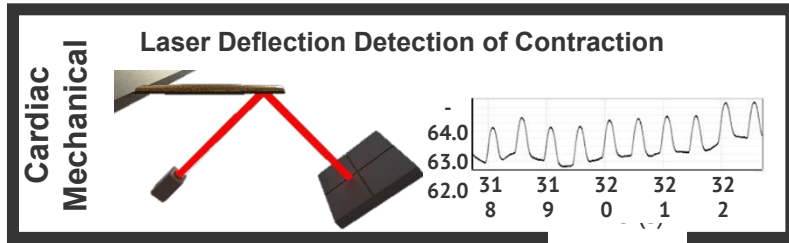
**PI: James Kronauge, Ph.D.**  
Hesperos, Inc.  
12501 Research Parkway, Suite  
100, Orlando, FL 32826

# Clinically Relevant Functional Readouts

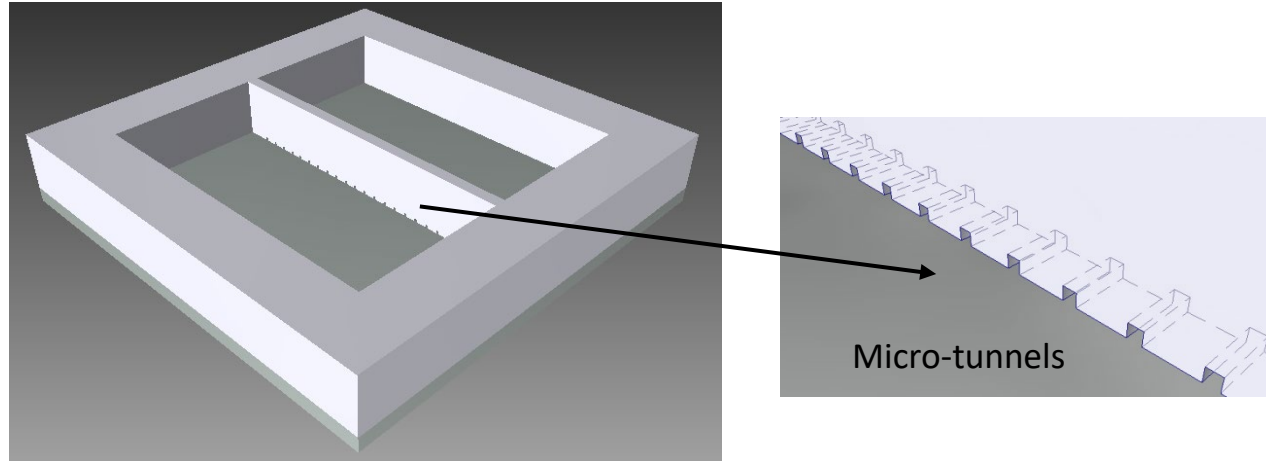
- Mechanical or electrical readouts of cellular functions such as:
  - **muscle contraction**
  - **electrical activity** from neurons and cardiac cells
  - motoneuron → muscle: **NMJ physiology** and other combinations
  - barrier integrity (TEER) and active transport for barrier tissues
- Allows **functional** analysis of cellular health non-invasively for acute, but more importantly, for **chronic** monitoring of human-on-a-chip systems, **BUT WITHOUT CELL DEATH**
- Allows **mechanistic** determination of toxicity and for target identification.



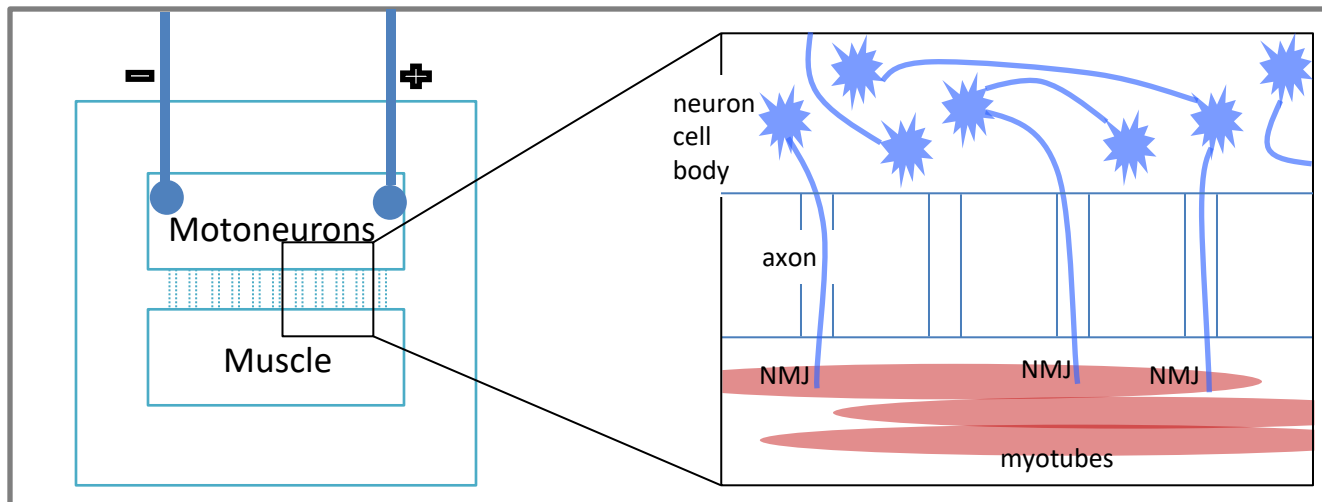
# HOW IT WORKS



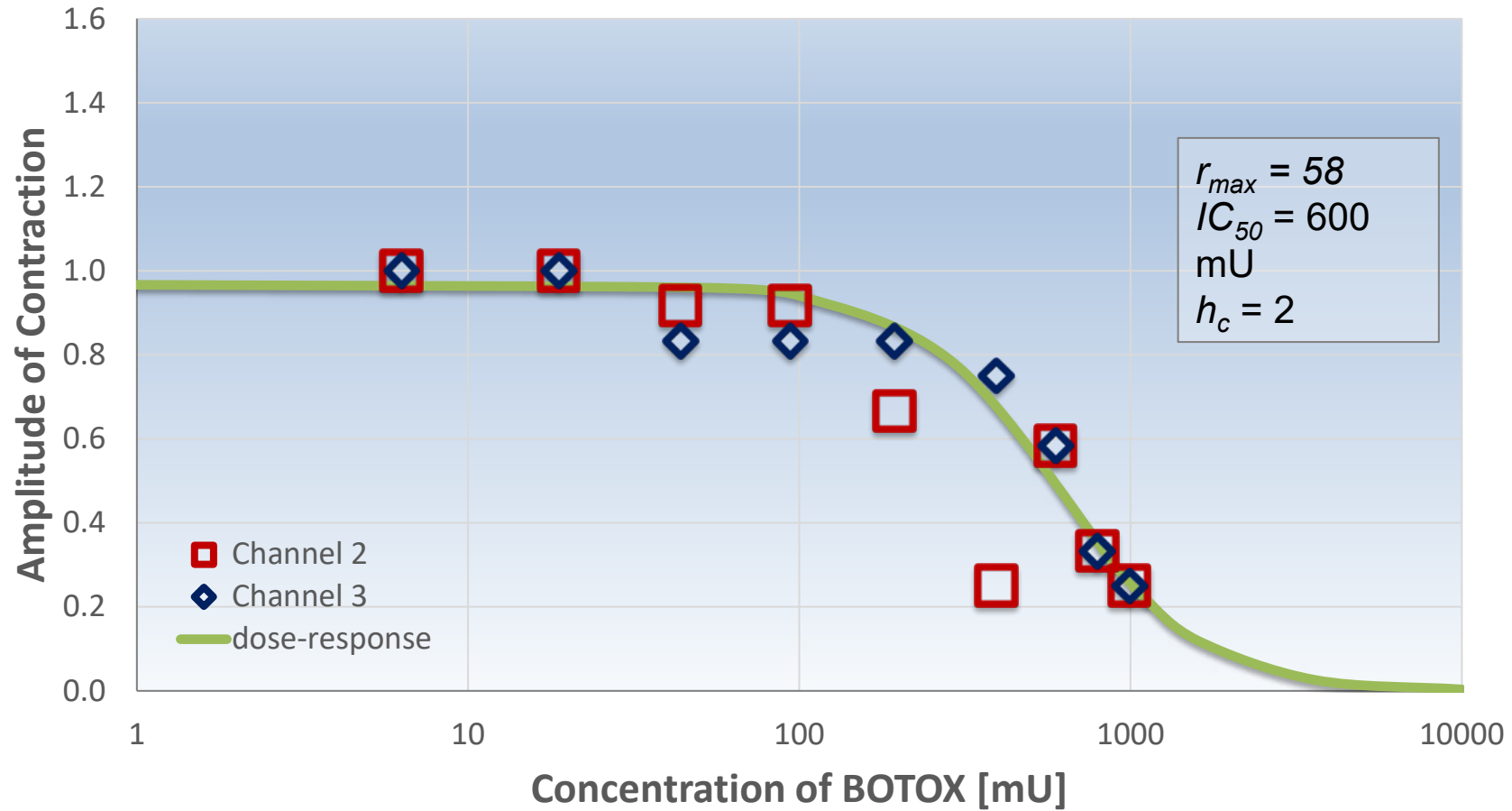
# Neuromuscular Junction (NMJ) Platform



- 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

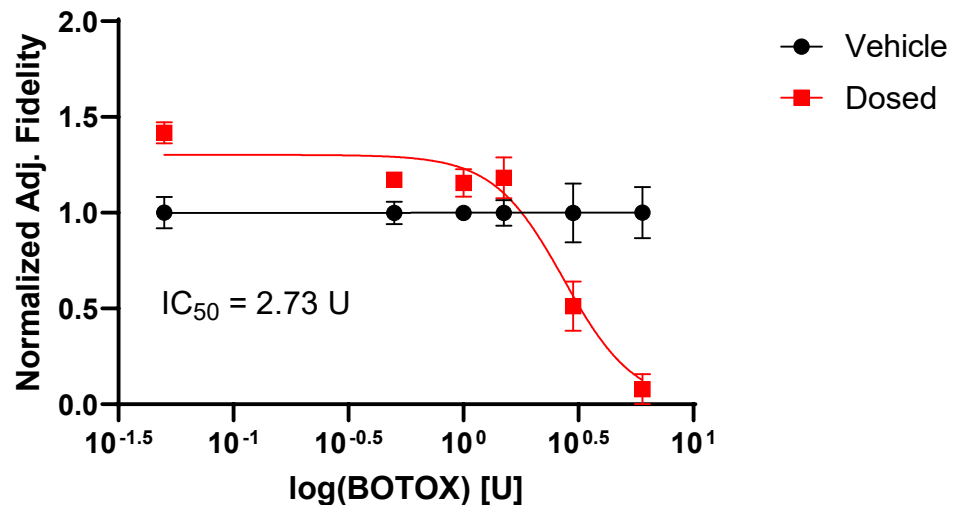


# Monophasic Dose-Response for BOTOX at 0.33 Hz

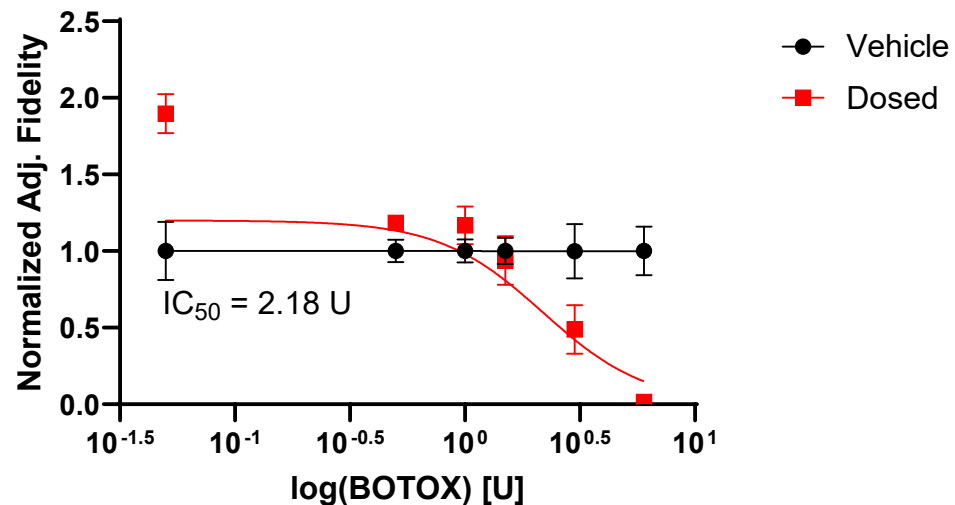


# Three Hour Data Collection by Frequency

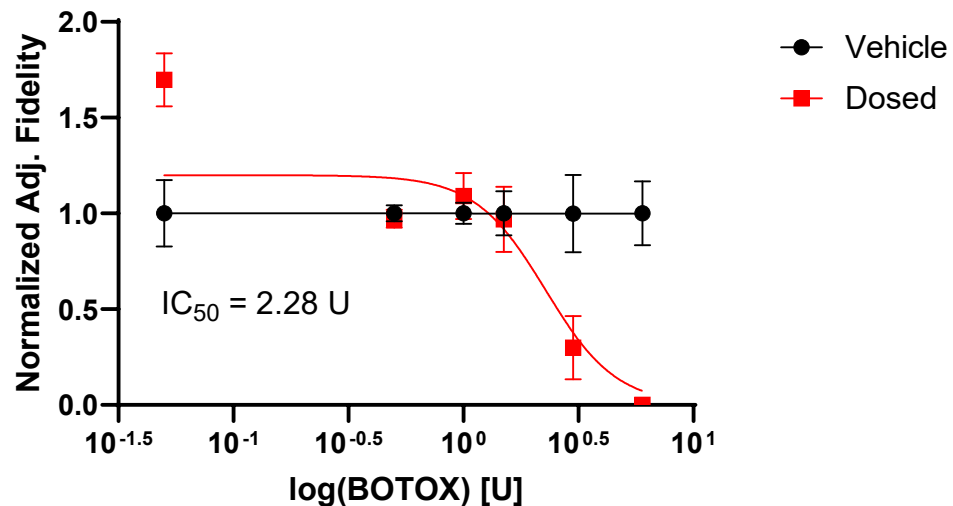
0.33 Hz



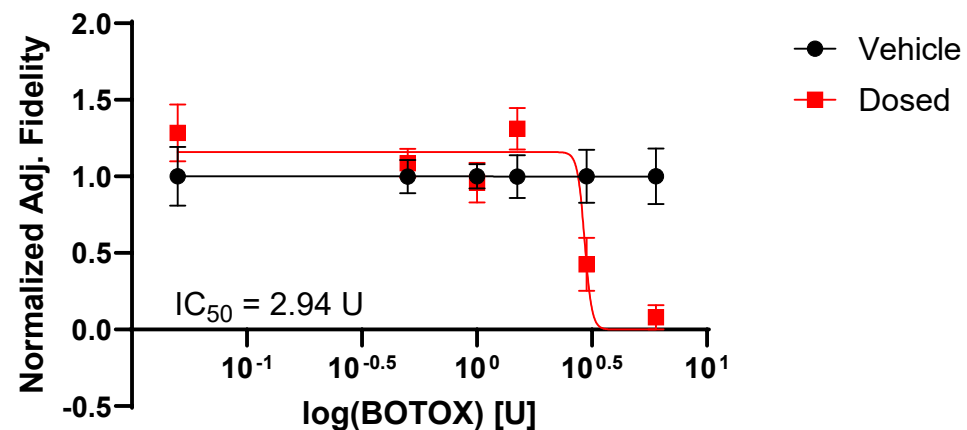
0.50 Hz



1.0 Hz

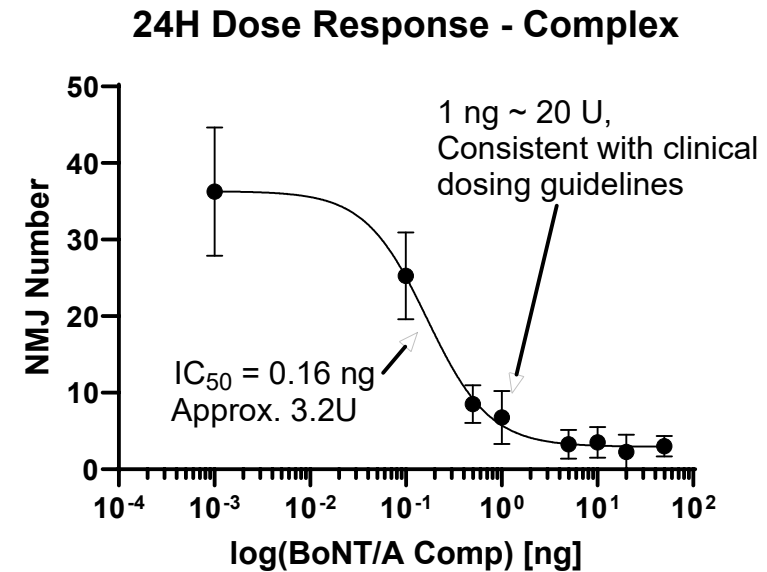
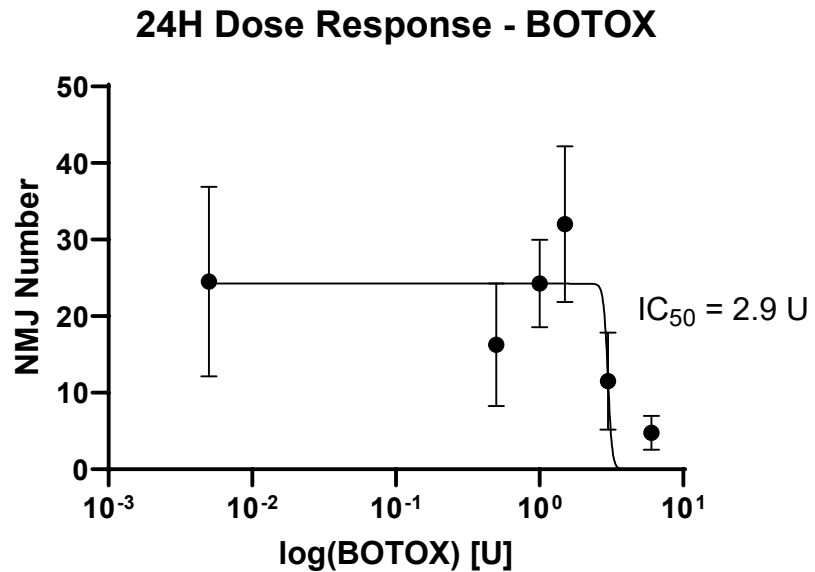


2.0 Hz



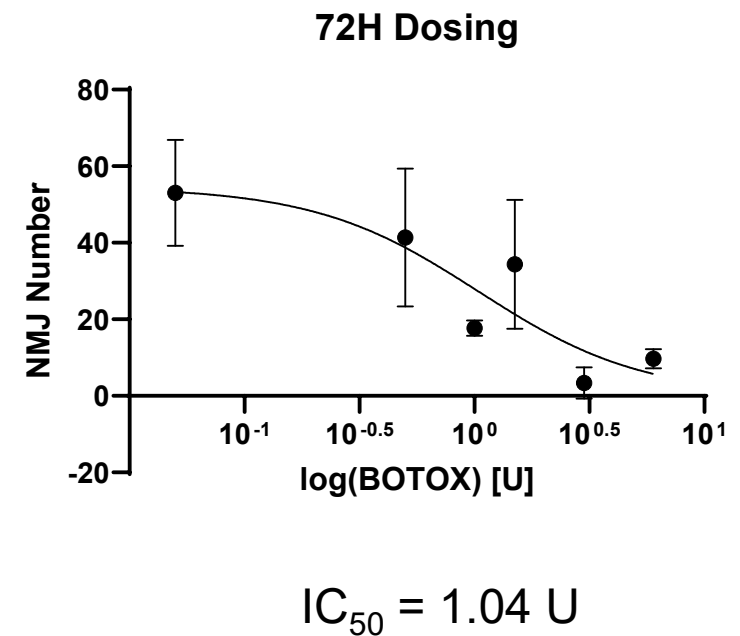
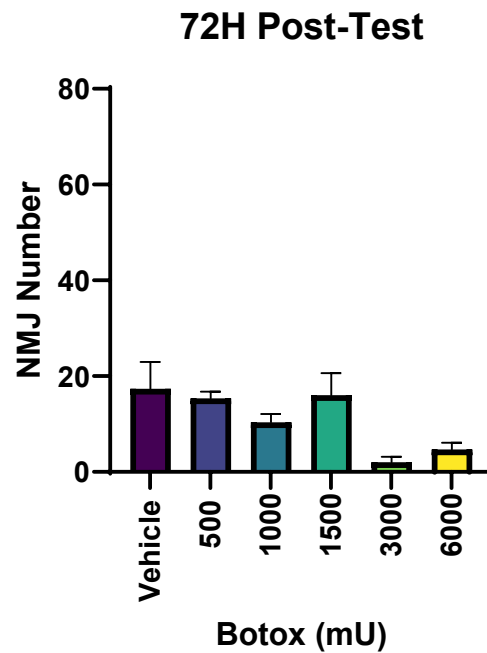
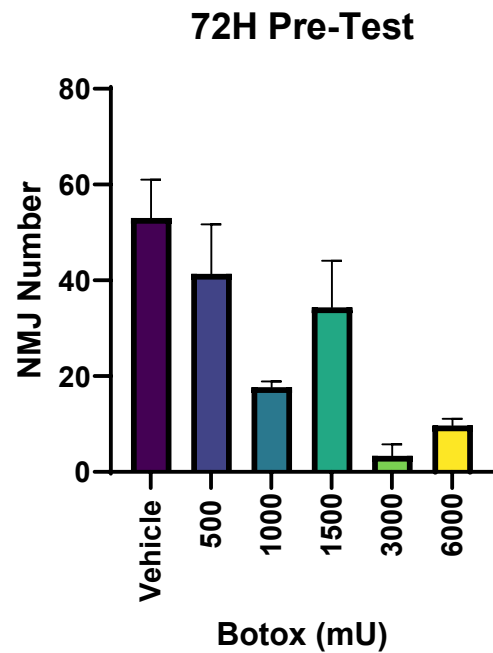
# 24 Hour Data Collection by NMJ Number

- 24 Hour Incubation
- NMJ Counts Only, dosed with Purified BoNT/A Complex and commercial BOTOX



# 72 Hour Data Collection by NMJ Number

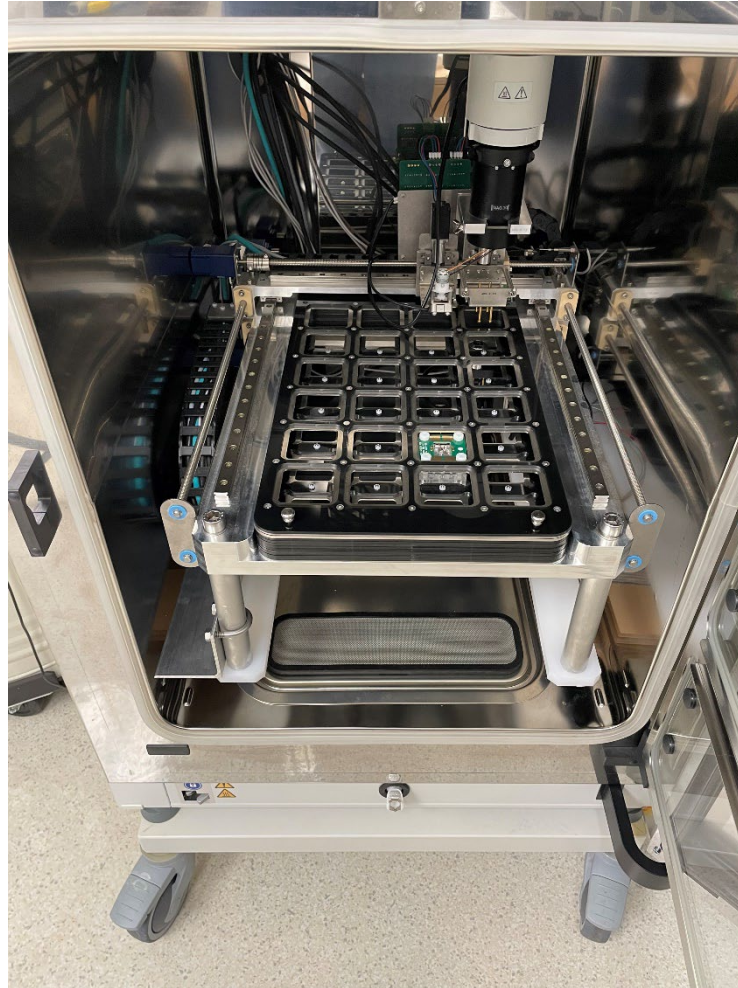
- Different Chambers, 72 Hour Incubation
- Incorporated depletion (10 Hz, 2 seconds before each frequency)



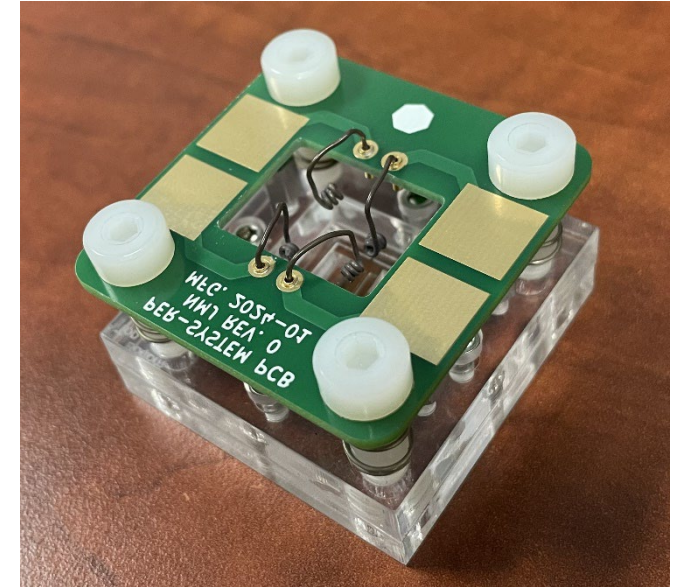
# Robotic NMJ Measurement System

- Functional automated system inside incubator.
- Modified NMJ system can be seen in the images (placed in system rack and closeup).

Complete system in incubator

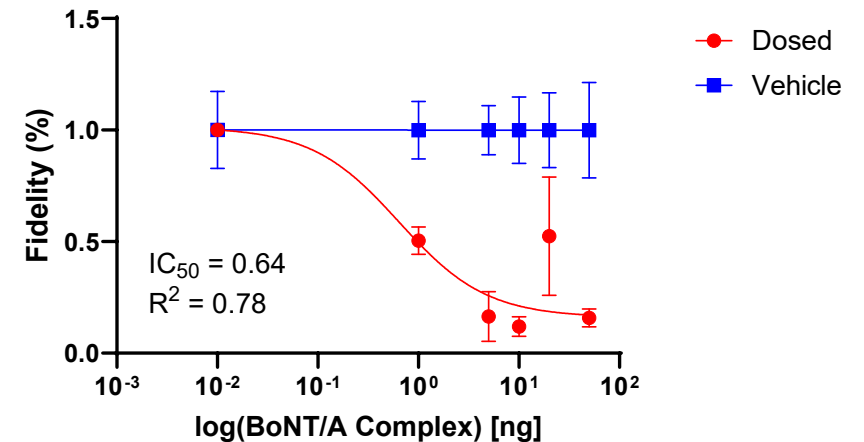
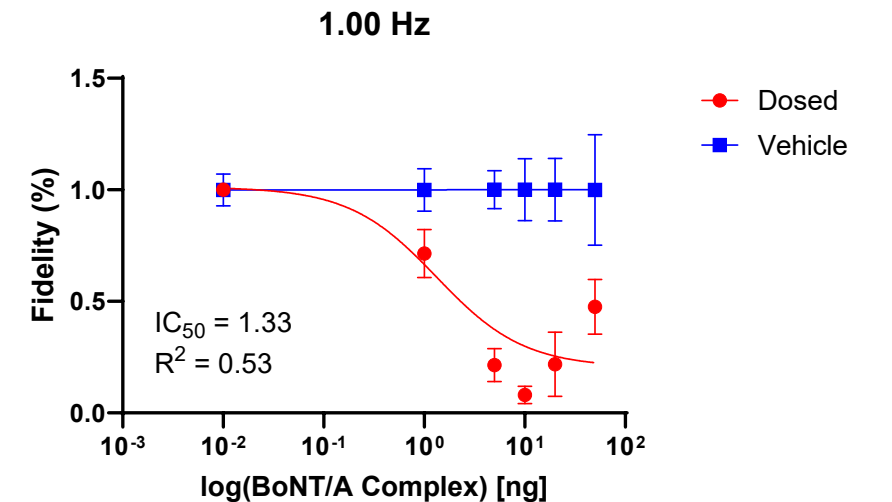
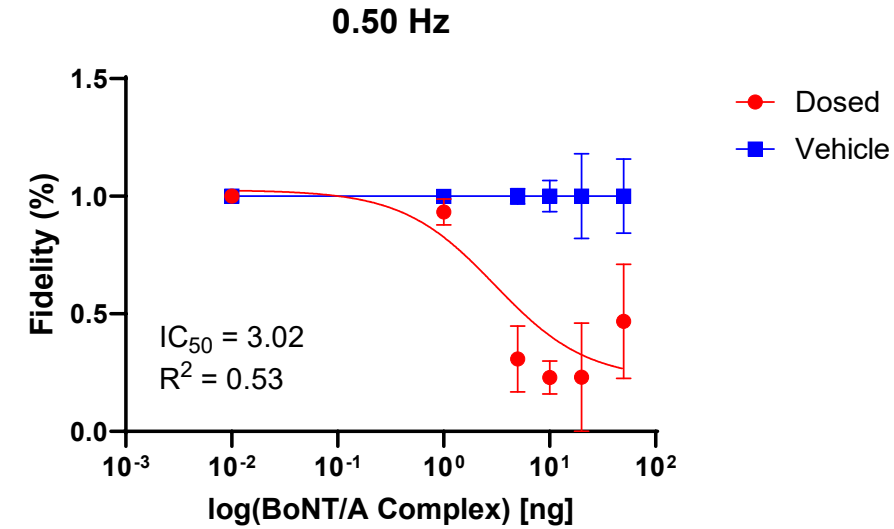
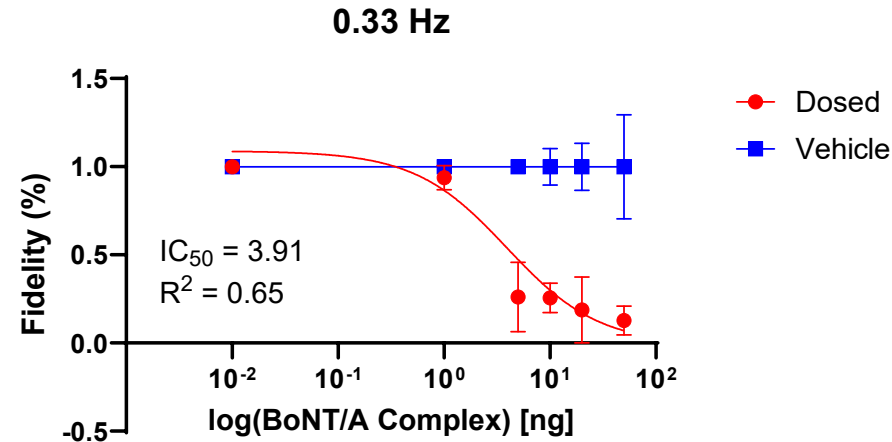
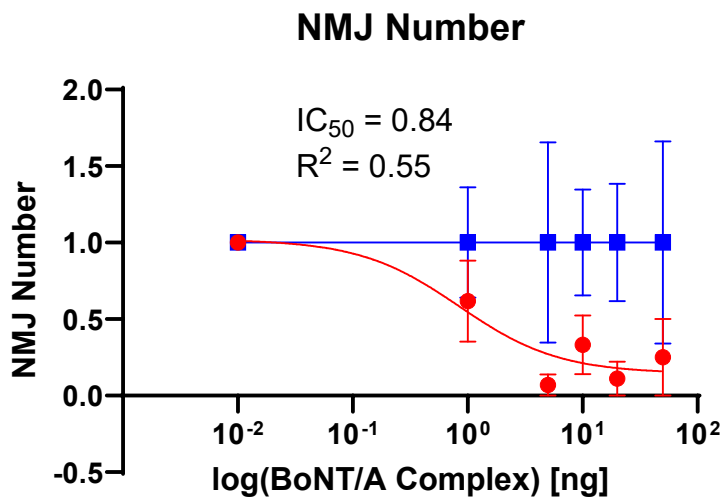


Tester compatible modified NMJ system













# Testing Systems within Automated Tester

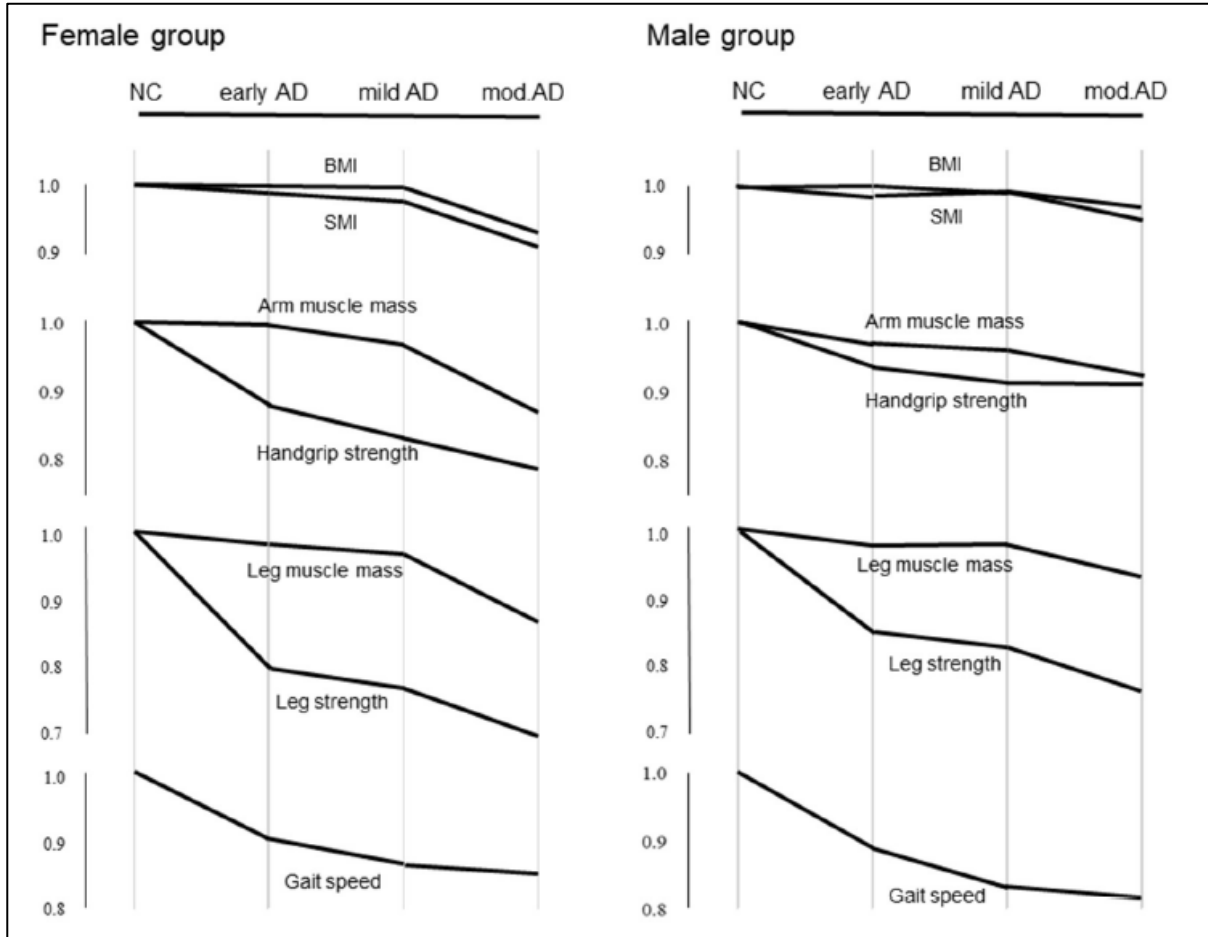
- Tested multiple systems with BoNT/A Complex
- Results comparable to manual testing and expected ranges



# Clinical Trials Supported by Human-on-a-Chip® data

Phase II/III  	Trial	Indication	Asset	Sponsor	Hesperos Contribution
ODD	Orphan Drug Designation	Charcot-Marie-Tooth (CMT)	VCA-894A	Vanda Pharmaceuticals	Effects confirmed in neuromuscular junction (NMJ) model with patient-derived cells where VCA-894A demonstrated significant improvements in neuromuscular function, including reduced muscle fatigue and improved synaptic transmission between motor neurons and skeletal muscle myotubes.
	MERIDIAN <a href="#">[NCT04579666]</a>	Amyotrophic lateral sclerosis (ALS)	Pegcetacoplan	Apellis Pharmaceuticals	Efficacy of pegcetacoplan (APL2) was determined using our NMJ-ALS neuroinflammation system to evaluate the most common amyotrophic lateral sclerosis (ALS) mutation (aka 'sporadic' ALS) involving TDP43 and one of the most common rarer types that involves SOD1—a form of 'familial' ALS.
	ReALiSe <a href="#">[NCT06441682]</a>	Amyotrophic lateral sclerosis (ALS)	ARGX-119	Argenx	Efficacy of ARGX-119 to ameliorate neuromuscular deficits in sporadic and familial types of ALS associated with TDP43 and SOD1 phenotypes, respectively, was confirmed using our neuromuscular junction (NMJ) model.
	MAGIC <a href="#">[NCT06282159]</a>	Myasthenia Gravis (MG)	DNTH-103	Dianthus Therapeutics	Induced MG phenotype using patient sera in our healthy NMJ model and determined that DNTH103 anti-active C1s antibody ameliorated deficits in neuromuscular junction functionality and complement deposition comprised of healthy iPSC-derived motoneurons (MN) and skeletal muscle (SKM) myotubes.
	MOMENTUM <a href="#">[NCT06537999]</a>	Multi-focal Motor Neuropathy (MMN)	DNTH-103	Dianthus Therapeutics	Induced a dysfunctional neuronal phenotype using patient sera in healthy co-cultures of iPSC derived motoneurons and primary Schwann cells mediated by autoantibodies to determine efficacy of DNTH103 and complement deposition in the Hesperos conduction velocity (CV) model.
	CAPTIVATE <a href="#">[NCT06858579]</a>	Chronic Inflammatory Demyelinating Polyneuropathy (CIDP)	DNTH-103	Dianthus Therapeutics	The purpose of this Phase III study is to demonstrate the efficacy of DNTH103 as compared to placebo in participants with chronic inflammatory demyelinating polyneuropathy (CIDP).
	Proof-of-Concept <a href="#">[NCT04658472]</a>	Chronic Inflammatory Demyelinating Polyneuropathy (CIDP)	TNT005	Sanofi	Using patient sera, our CV model confirmed the ability of TNT005 (riliprubart) a C1s inhibitor to hinder complement activation and downstream membrane attack complex (MAC) formation.
	MOBILIZE <a href="#">[NCT06290128]</a>	Chronic Inflammatory Demyelinating Polyneuropathy (CIDP)	Riliprubart	Sanofi	Successful Phase II enabled Phase III to investigate the efficacy of riliprubart compared to placebo in adult participants with CIDP whose disease is refractory to standard of care.
	VITALIZE <a href="#">[NCT06290141]</a>	Chronic Inflammatory Demyelinating Polyneuropathy (CIDP)	Riliprubart	Sanofi	Successful Phase II enabled Phase III to evaluate efficacy of riliprubart compared to IVIg in adult participants with CIDP who are receiving maintenance treatment with IVIg.

# ***AD in PNS – negatively affecting BMI, strength and gait speed***

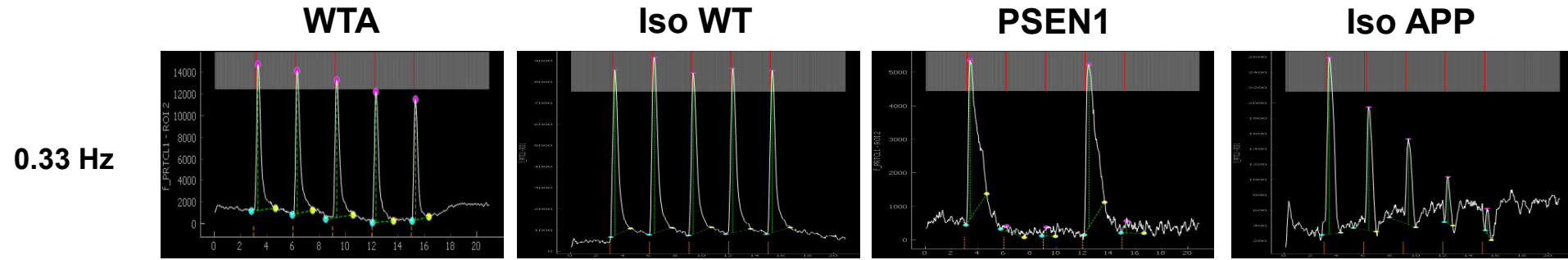


**AD changes to BMI and etc (Ogawa et al., 2018)**

- AD patients also typically show deterioration in muscle mass and strength (Burns et al., 2010);
- In certain patients that loss of body weight occurred before typical clinical manifestations of AD such as cognitive impairment (Johnson et al., 2006);
- Reduction in body weight is typically associated with faster clinical progression of AD and generally body mass index and AD pathology are negatively correlated (Buchman et al., 2006);
- Postmortem examination of AD patients indicated accumulation of  $A\beta_{42}$  in peripheral tissues including skeletal muscle (Kuo et al., 2000).

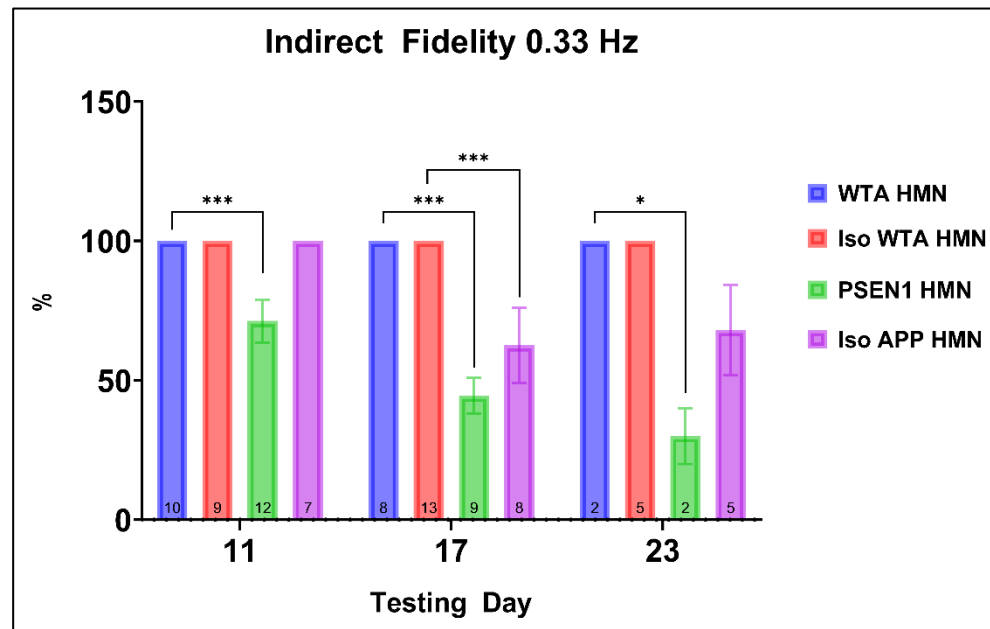
# AD NMJ functional analysis – fidelity at low frequency

A

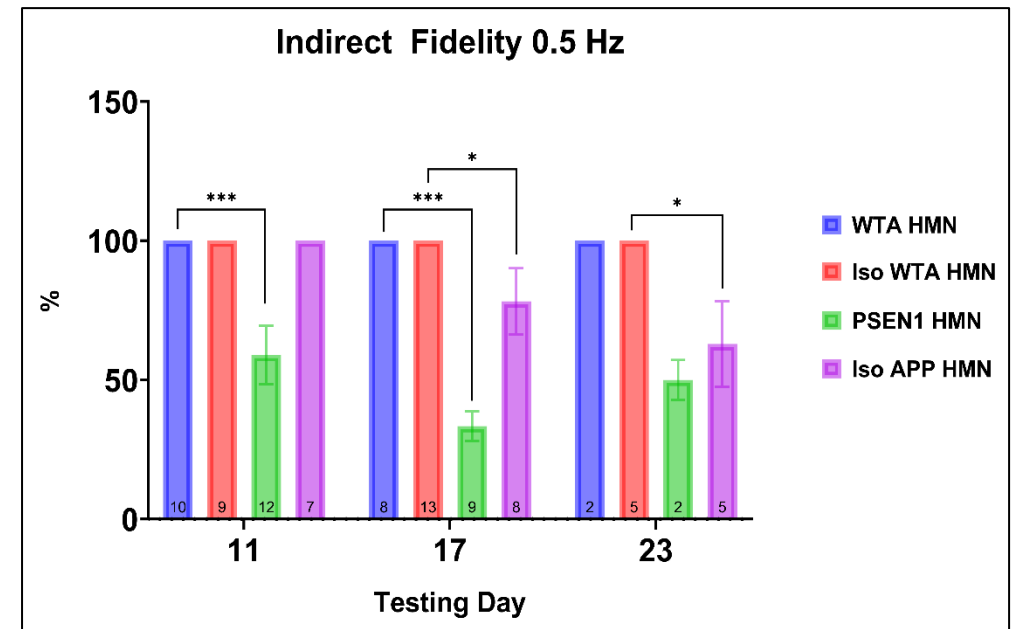


B

i)



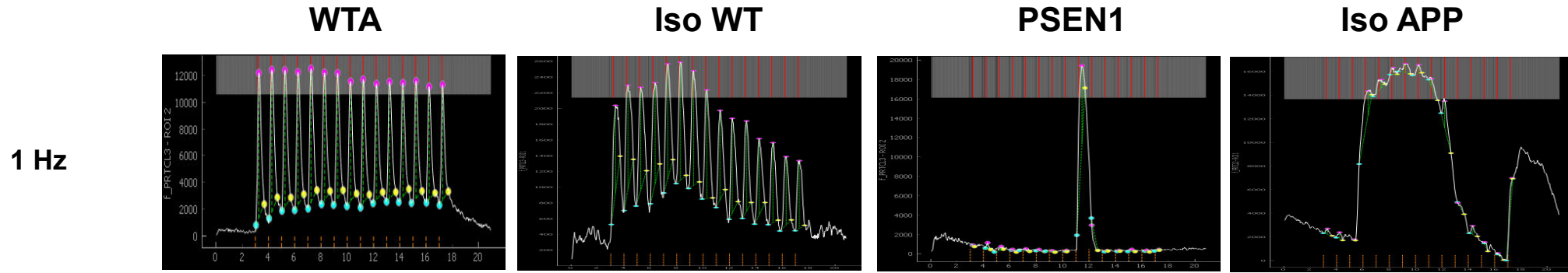
ii)



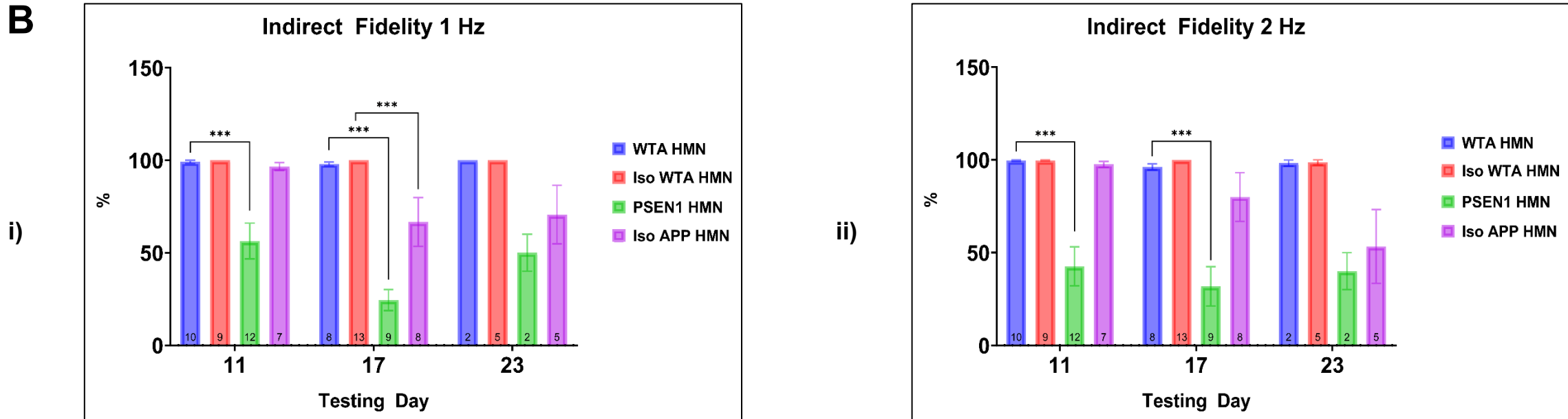
One-way ANOVA, Sidak Multiple comparison ( $P \leq 0.05$  (\*),  $P \leq 0.01$  (\*\*),  $P \leq 0.001$  (\*\*\*)).

# AD NMJ functional analysis - fidelity at high frequency

**A**

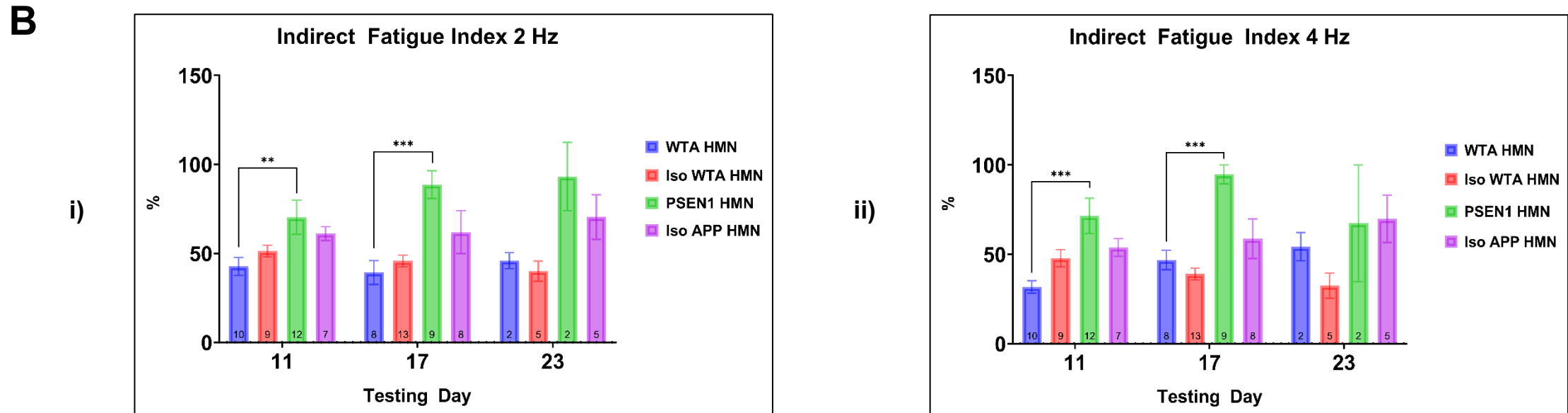
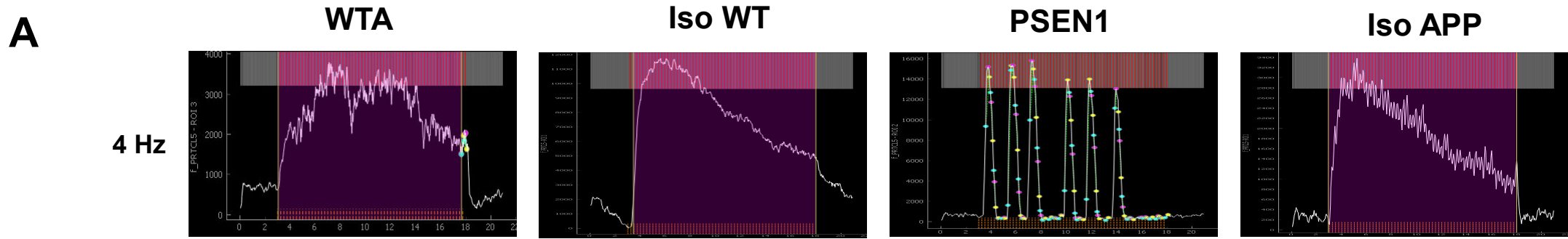


**B**



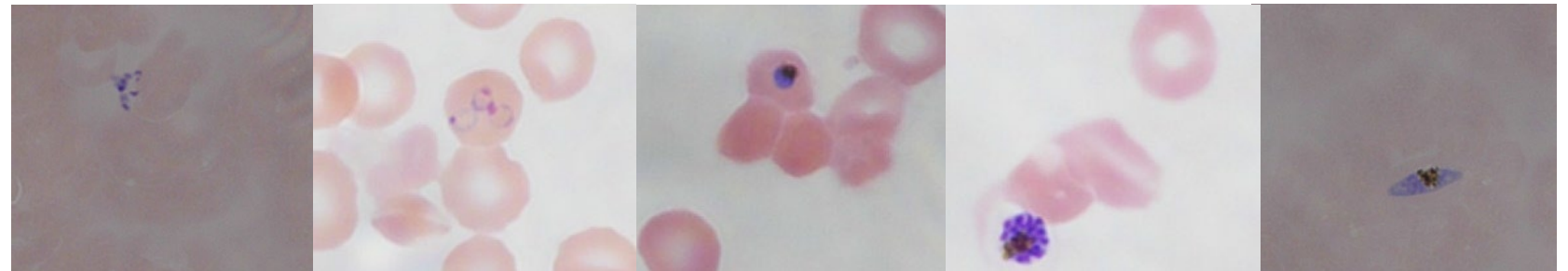
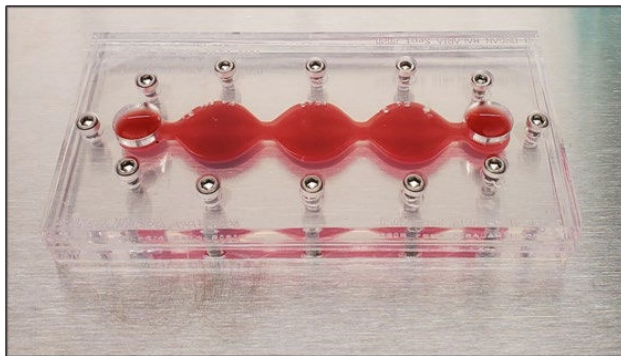
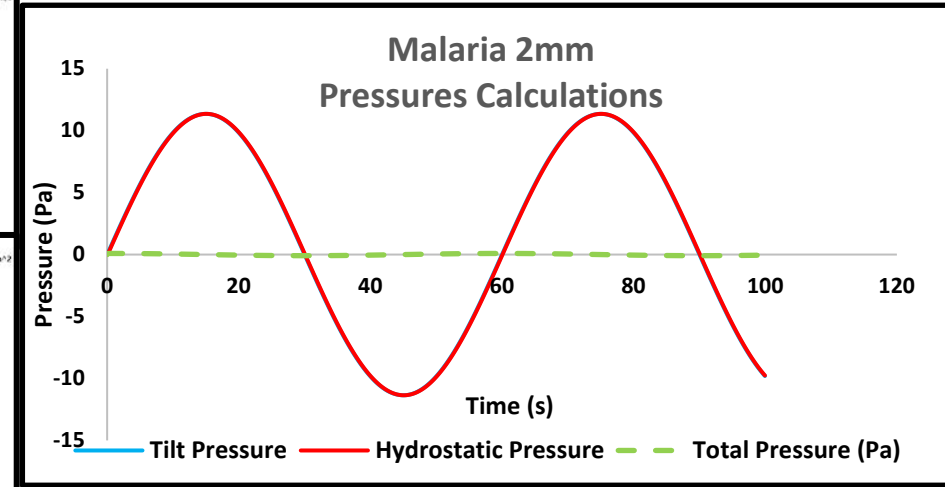
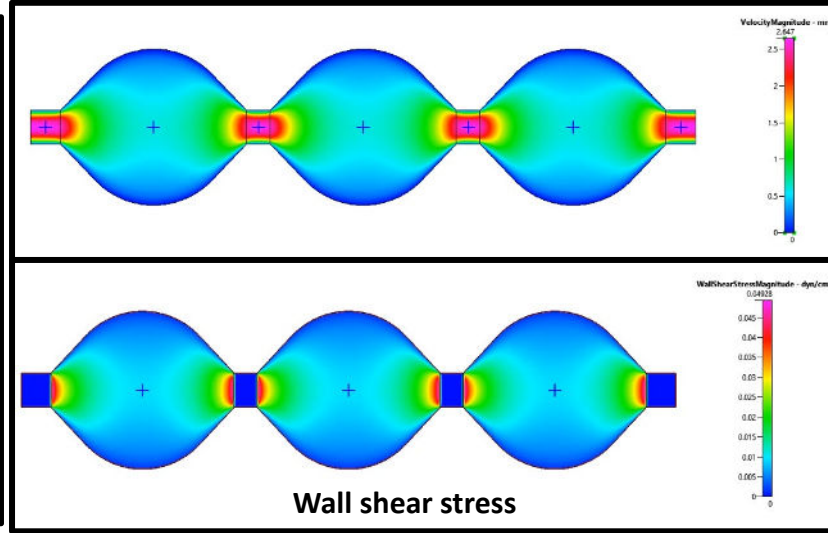
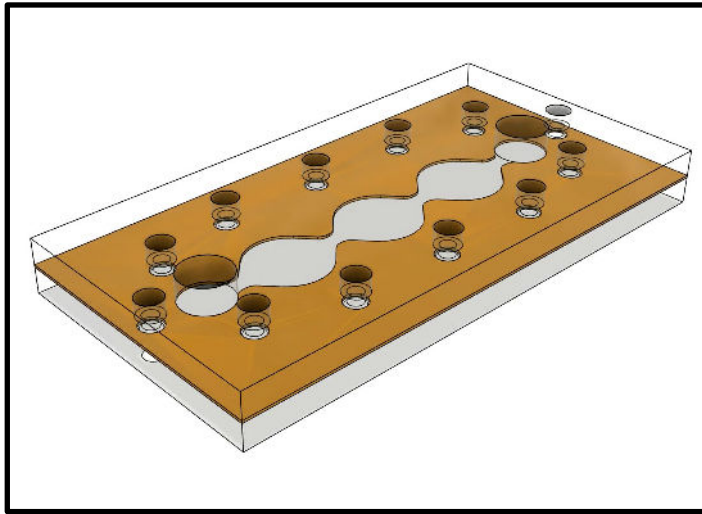
One-way ANOVA, Sidak Multiple comparison ( $P \leq 0.05$  (\*),  $P \leq 0.01$  (\*\*),  $P \leq 0.001$  (\*\*\*)).

# AD NMJ functional analysis - Fatigue Index



One-way ANOVA, Sidak Multiple comparison ( $P \leq 0.05$  (\*),  $P \leq 0.01$  (\*\*),  $P \leq 0.001$  (\*\*\*)).

# Malaria-on-a-chip Phenotypic Digital Twin Model

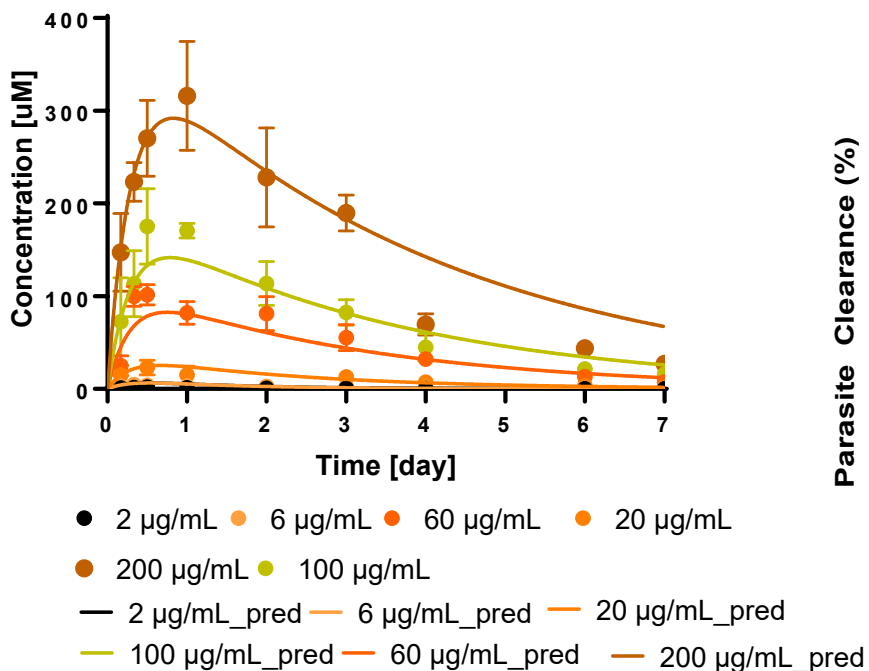


## Pharmacokinetic Modeling

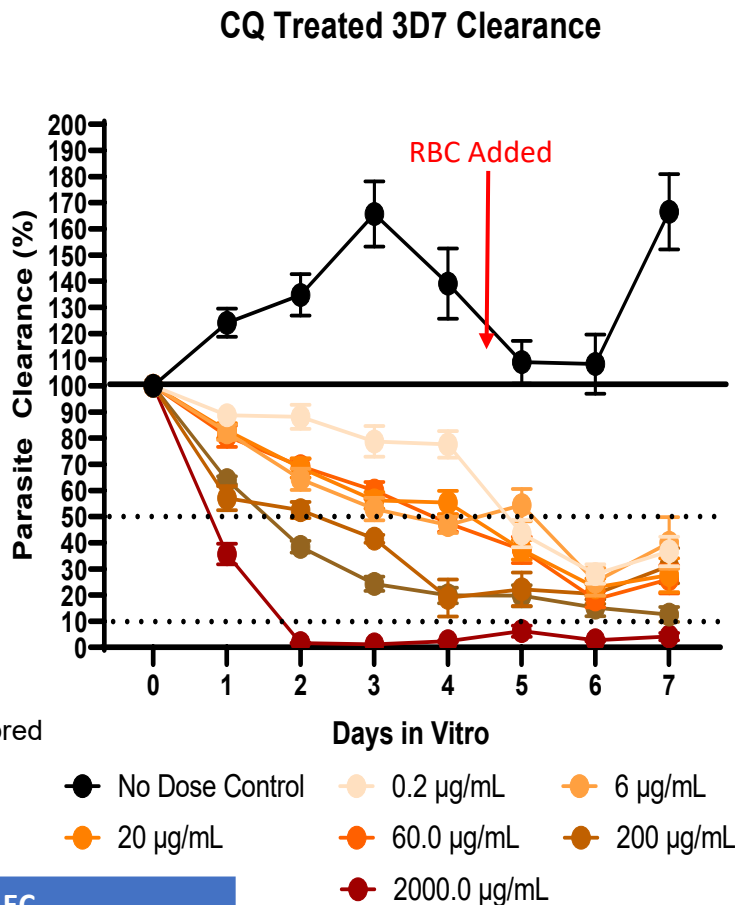
$$\text{Active Concentration } C_{active}(t) = f \left( \begin{array}{l} \text{Convective mixing } C_{convective}(t) \\ \text{Adsorption to housing materials } C_{ads}(t) \\ \text{Protein binding (Fractional unbound) } f_u(t) \\ \text{Media change} \\ \text{Metabolism } K_m, V_{max} \end{array} \right)$$

# In Vivo Prediction of Chloroquine treatment for 3D7 from IVIVE

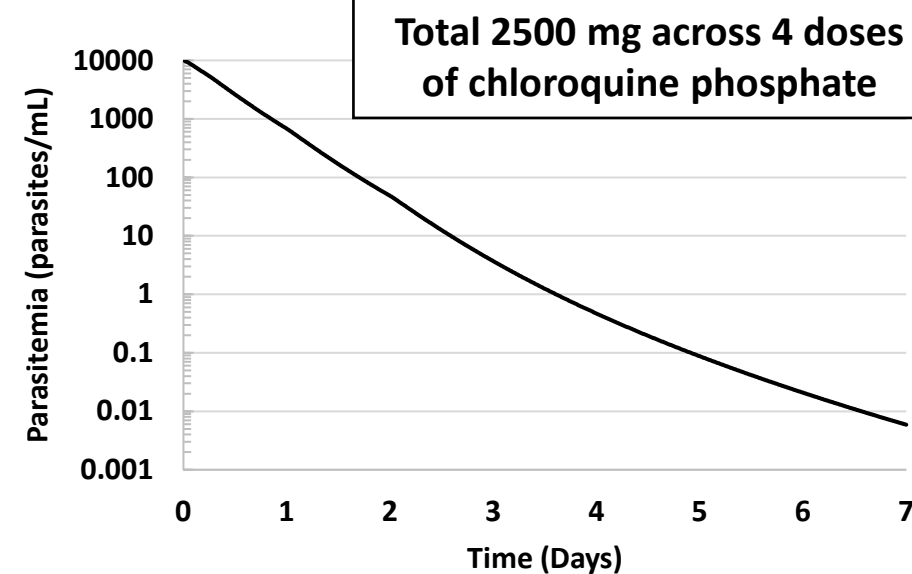
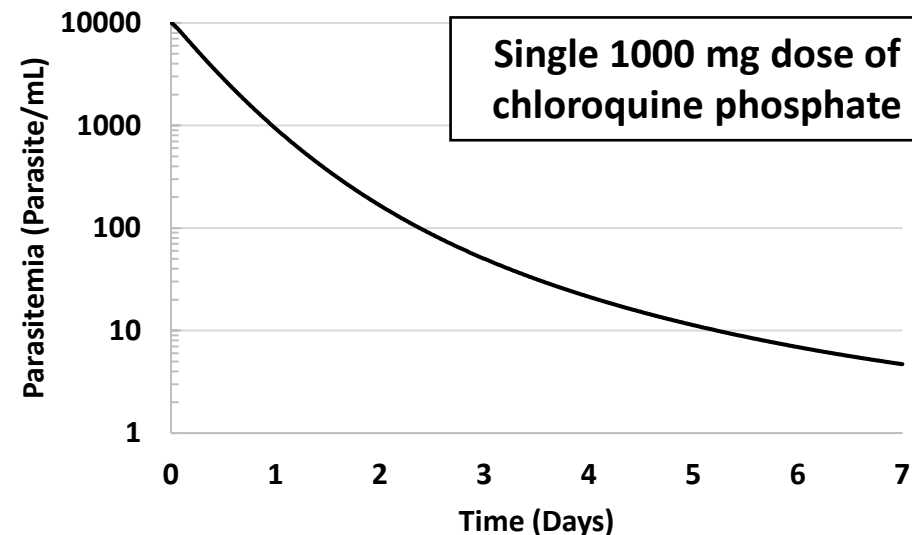
PK Profile



PD Profile



In Vivo Prediction



MTD	NOAEL	EC <sub>50</sub> Day 7 (in vitro)	EC <sub>50</sub> (in vivo, Uncomplicated P. falciparum)
6 µg/mL	1 µg/mL	0.1 µg/mL	0.051 µg/mL

# HESPEROS

THE HUMAN-ON-A-CHIP COMPANY

## A Platform for toxicity testing and *every* Human Disease

### Human-on-a-Chip®

- Functional, **multi-organ**, in vitro platforms
- Determine **therapeutic index**
- **Reducing** the need for **animal testing**
- Focus is on building **custom models** for therapeutic development and toxicity evaluations



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