

emulate

## Development of Human, Rat and Dog Liver-Chips to Facilitate Safety Assessment of Candidate Drugs

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# Imagine the Scenario ...

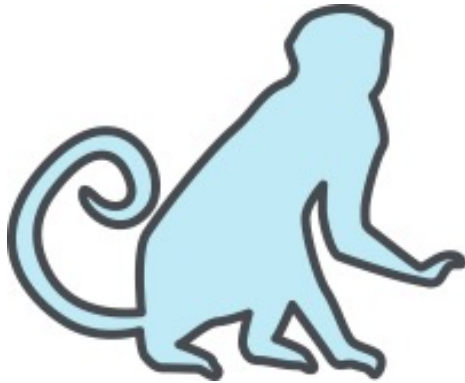
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- You are a project toxicologist on a biopharmaceutical company's highest priority project that is about to go into Phase I clinical trials
- The candidate drug shows a liver toxicity signal in the large animal species but not the small animal species
- The project team want to know the relevance for human and whether they have a margin to enable progression

# What are Your Options?

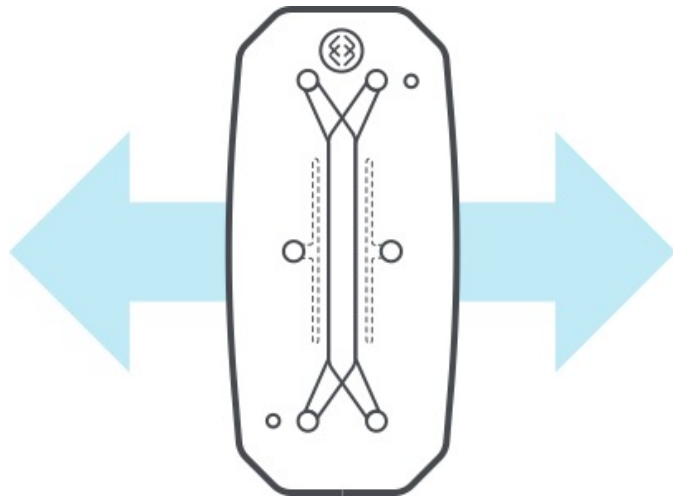
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- Perform further animal studies to learn about the mechanism of toxicity and thus determine whether this mechanism may translate to human
- Consult the literature for previous precedent of this mechanism in animals and humans
- Explore whether toxicity is on or off-target

# But What if There was an Alternative?

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- Organ-Chips can bridge between conventional in vitro and in vivo models by faithfully recreating physiology, function and disease phenotypes
- Cell viability and functionality can be maintained for extended durations
- Microfluidics enables recapitulation of clinical exposure profiles
- Agnostic to cell type (e.g., cell line, primary cell, induced pluripotent stem cell, fragmented organoids)
- Agnostic to cell source (e.g., animal or human)





RESEARCH ARTICLE | DRUG TESTING

## Reproducing human and cross-species drug toxicities using a Liver-Chip

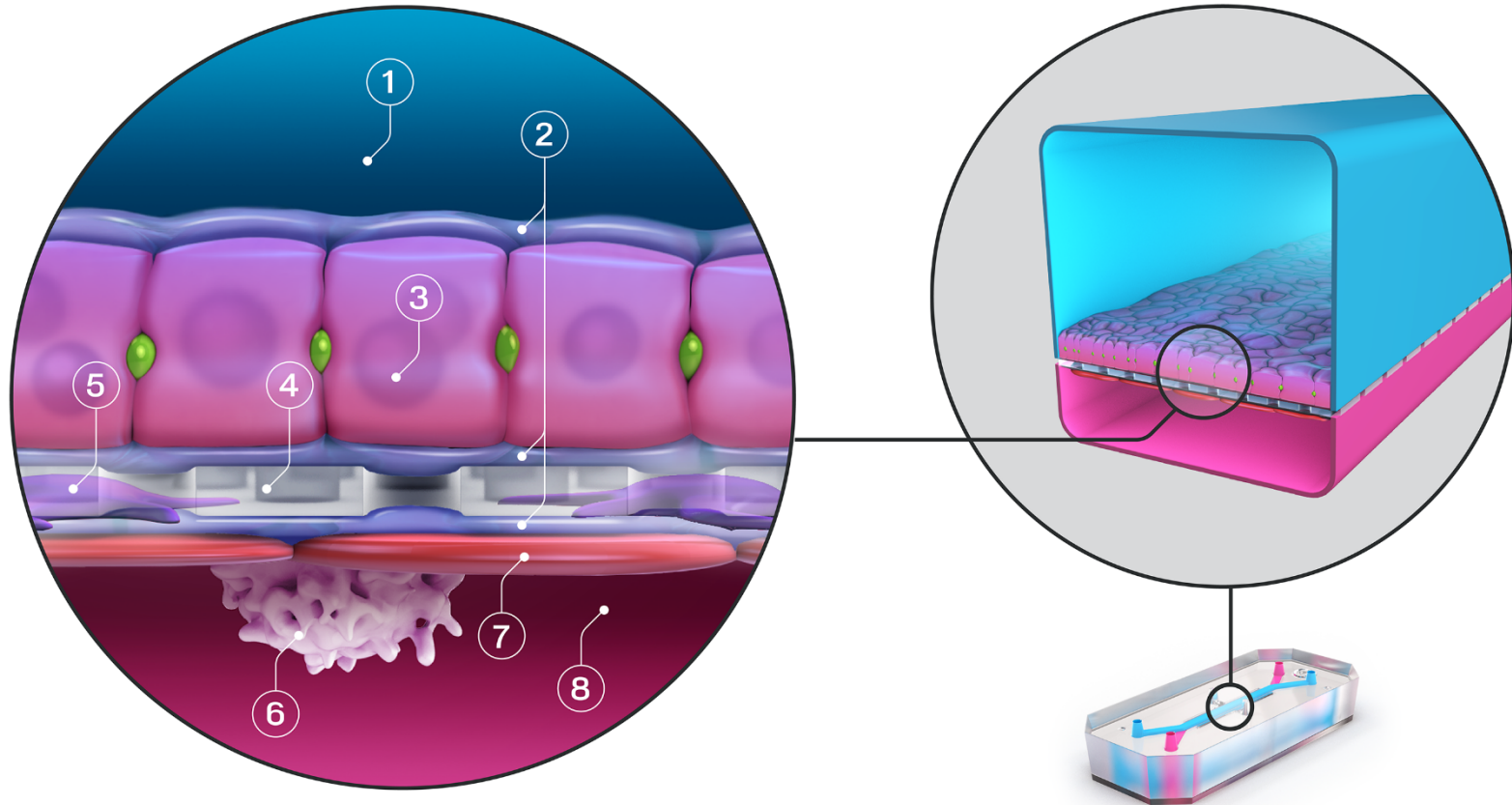
 Kyung-Jin Jang<sup>1,\*</sup>,  Monicah A. Otieno<sup>2,\*†</sup>,  Janey Ronxhi<sup>1</sup>,  Heng-Keang Lim<sup>3</sup>,  Lorna Ewart<sup>4</sup>,  Konstantia R. ...

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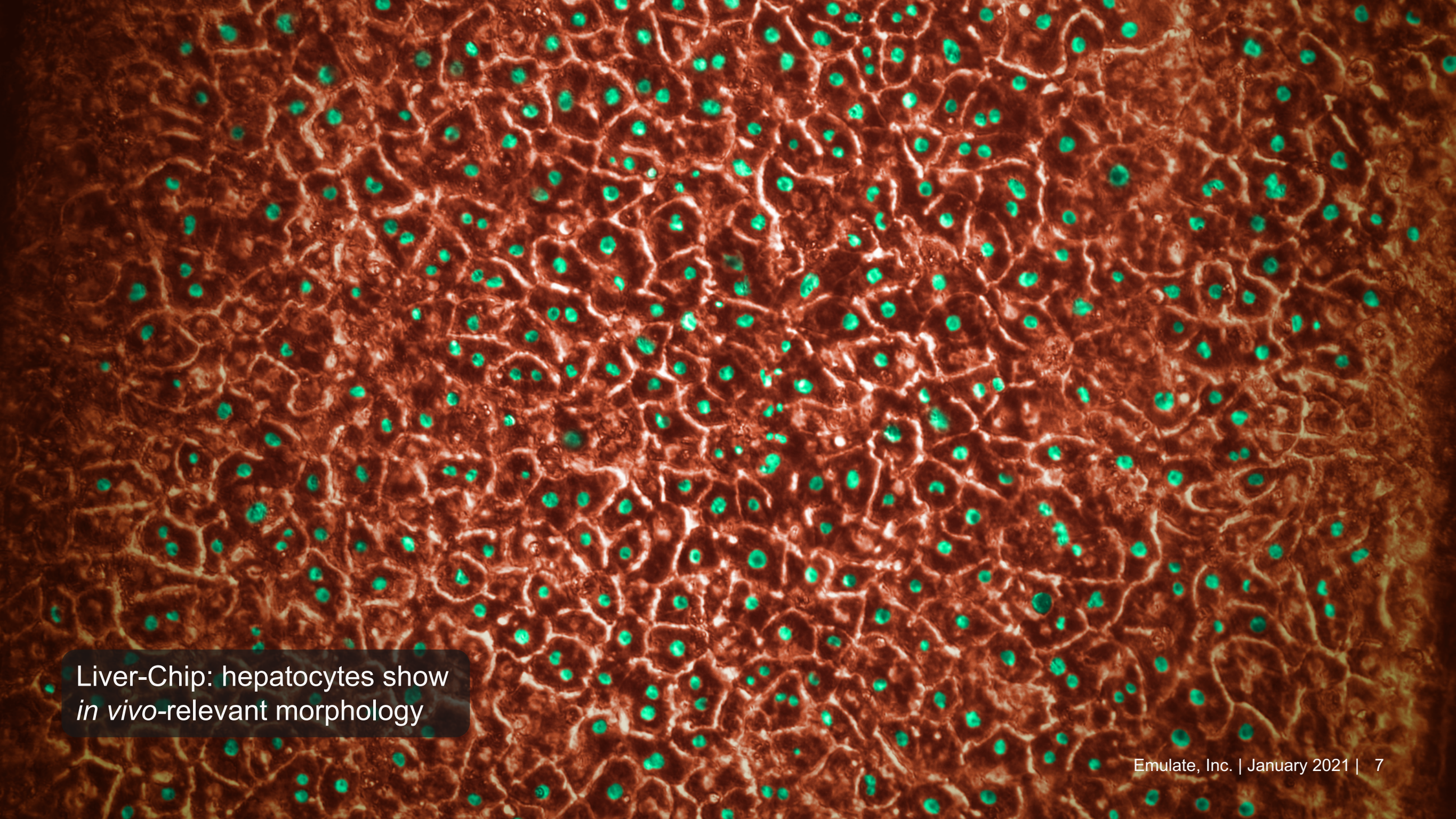
*Science Translational Medicine* 06 Nov 2019:  
Vol. 11, Issue 517, eaax5516  
DOI: 10.1126/scitranslmed.aax5516

# Liver-Chip Configuration

1. Top Channel
2. Extracellular Matrix
3. Hepatocytes
4. Porous Membrane
5. Stellate Cells
6. Kupffer Cells
7. Endothelial Cells
8. Bottom Channel





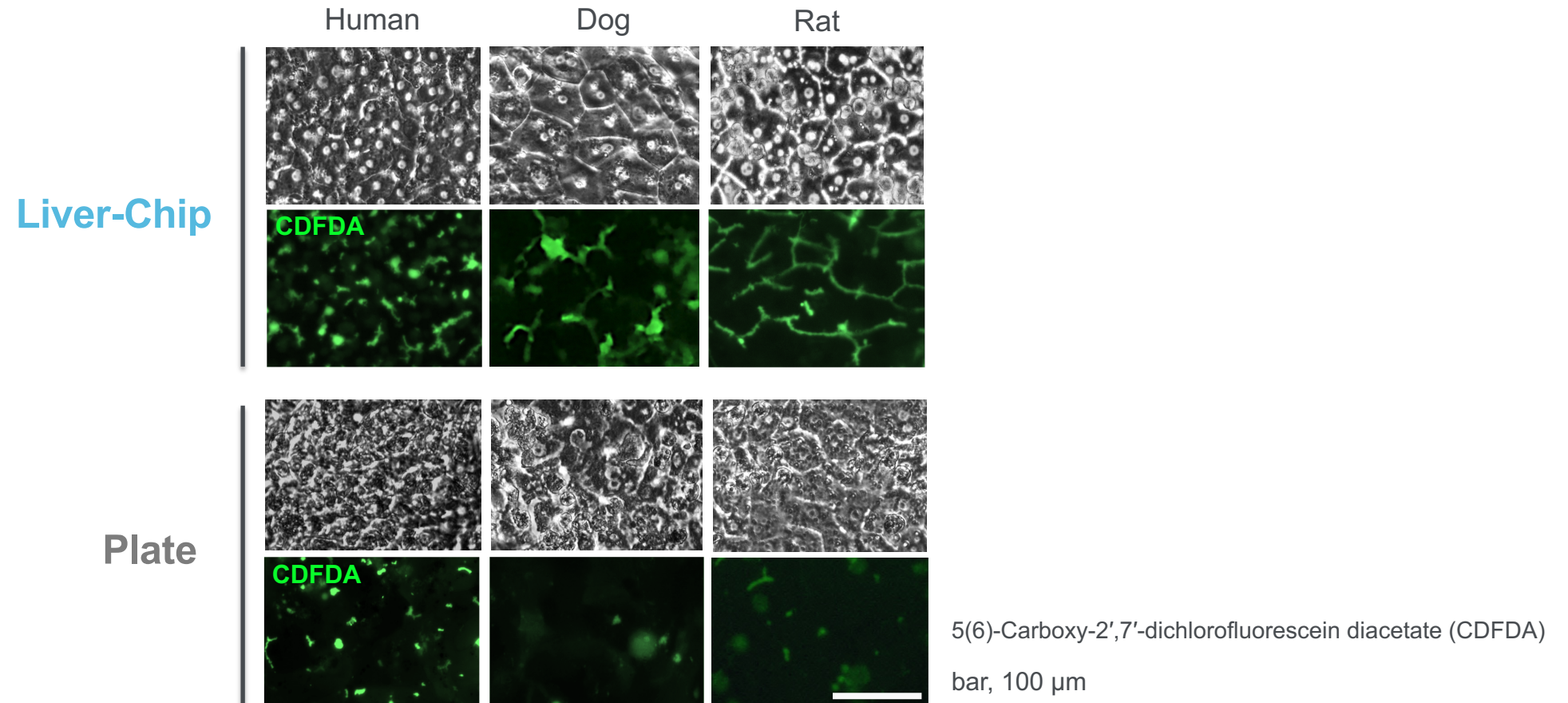
A high-magnification micrograph of a Liver-Chip. The image shows a dense, interconnected network of cells, likely hepatocytes, which are stained with a green fluorescent dye. The cells are arranged in a honeycomb-like pattern, with bright green nuclei visible within the cells. The overall color of the image is a warm, brownish-orange, suggesting the use of a specific filter or lighting condition.

Liver-Chip: hepatocytes show  
*in vivo*-relevant morphology



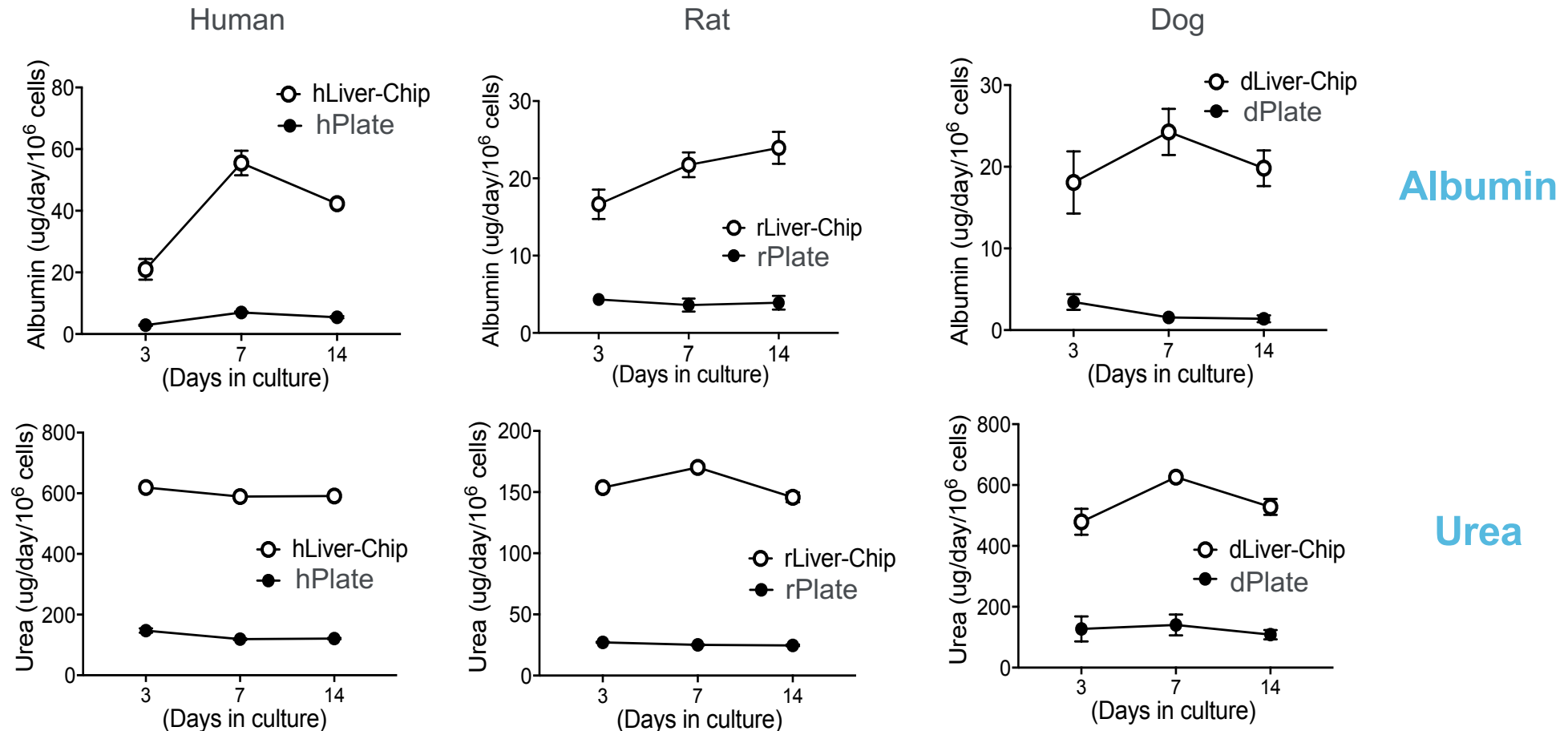
# Morphology in Liver-Chip vs. Plate Sandwich Culture

Day 14 in Culture



Hepatocytes from three different species maintained their stereotypical *in vivo*-like morphologies and formed improved bile canalicular networks compared to plate sandwich culture over 2 weeks in culture

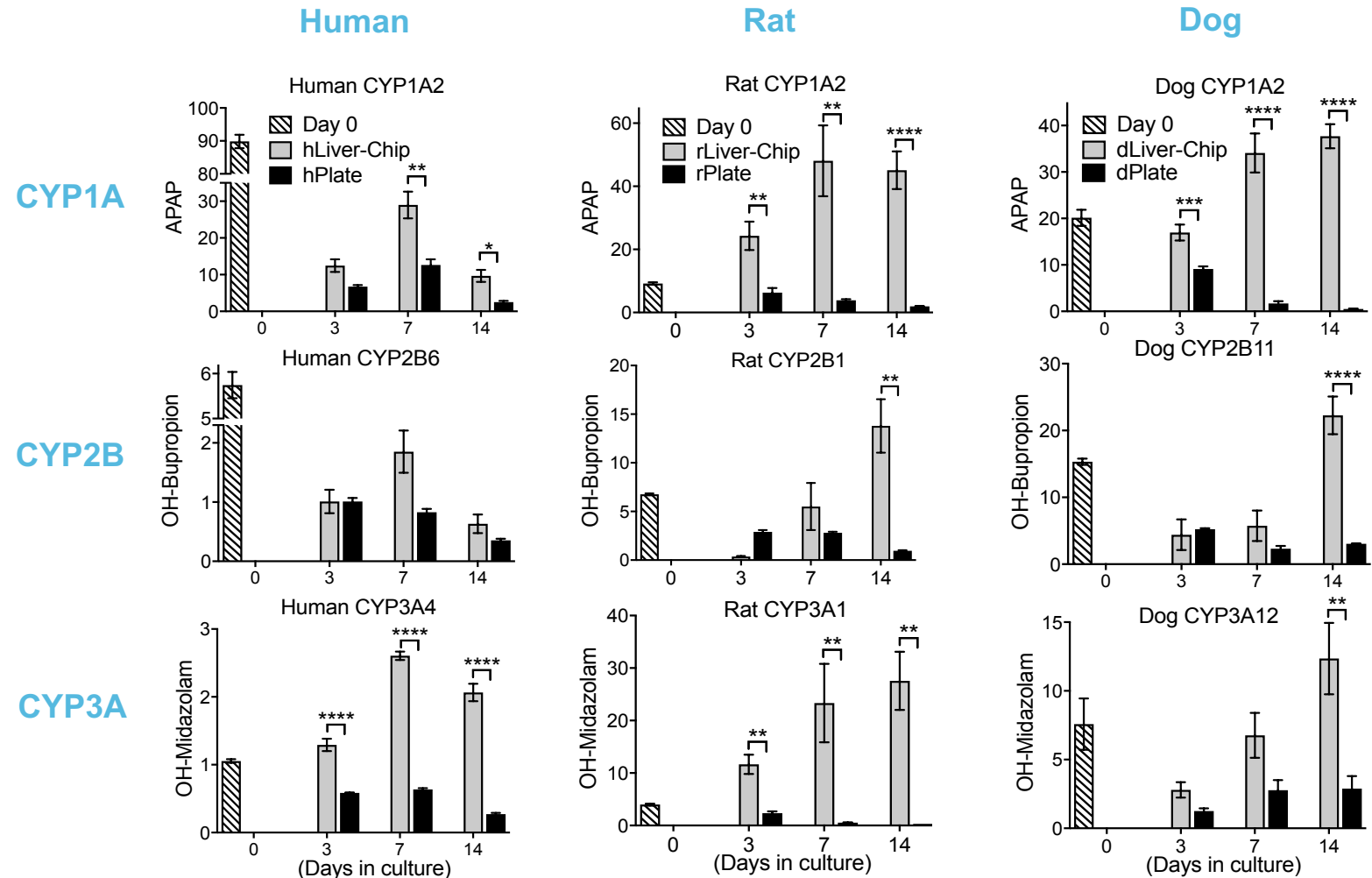
# Liver-Chip: Albumin and Urea Secretion



Human, Rat and Dog Liver-Chips have higher levels of albumin and urea secretion compared to Sandwich Plate Culture over a 2-week period

# Liver-Chip: Major P450 Enzyme Activities

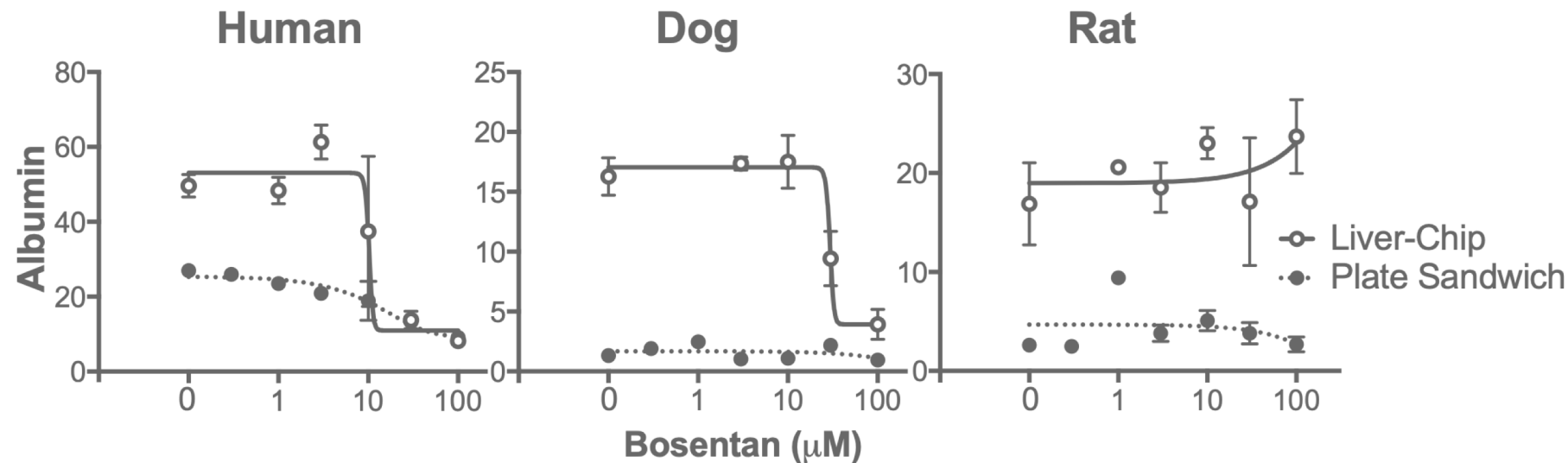
Human, Rat and Dog Liver-Chips maintain higher levels of Cytochrome P450 activity compared to Sandwich Plate Culture over 2 weeks



Unit : pmol/min/10<sup>6</sup> cells  
 \* p<0.1, \*\* p<0.01, \*\*\* p<0.001, \*\*\*\* p<0.0001

# Species Differences in Drug Transporters Drive Liver Toxicity

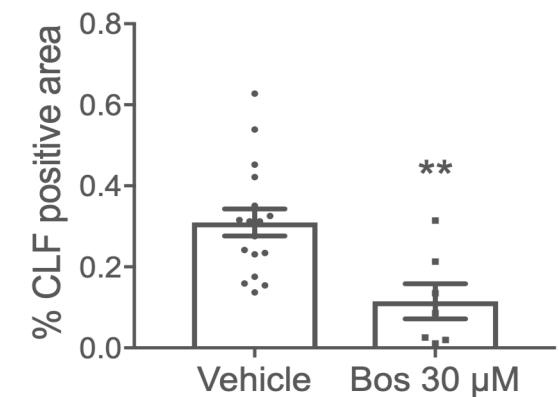
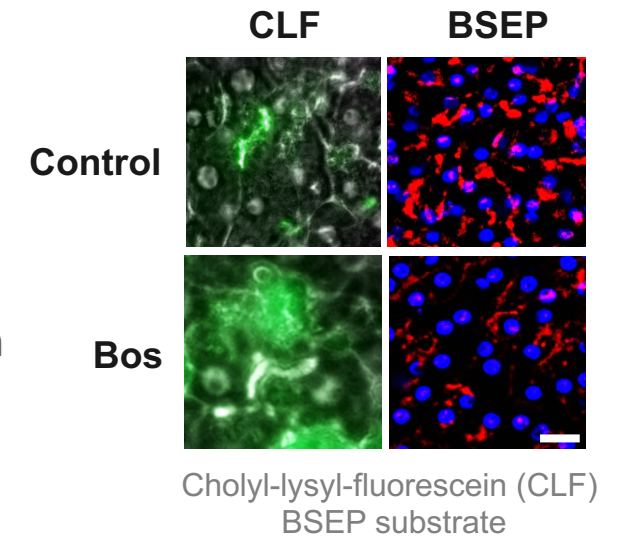
Effect of Bosentan on Albumin Secretion



Demonstrated species differences in the hepatotoxicity  
at *in vivo* relevant doses of Bosentan

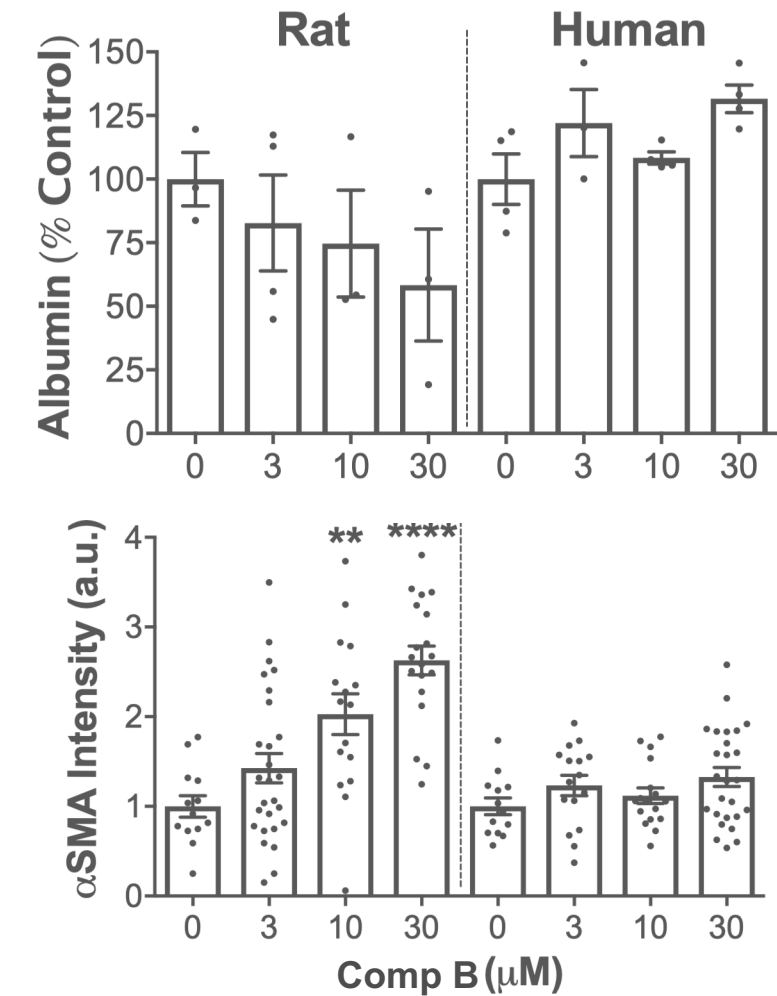
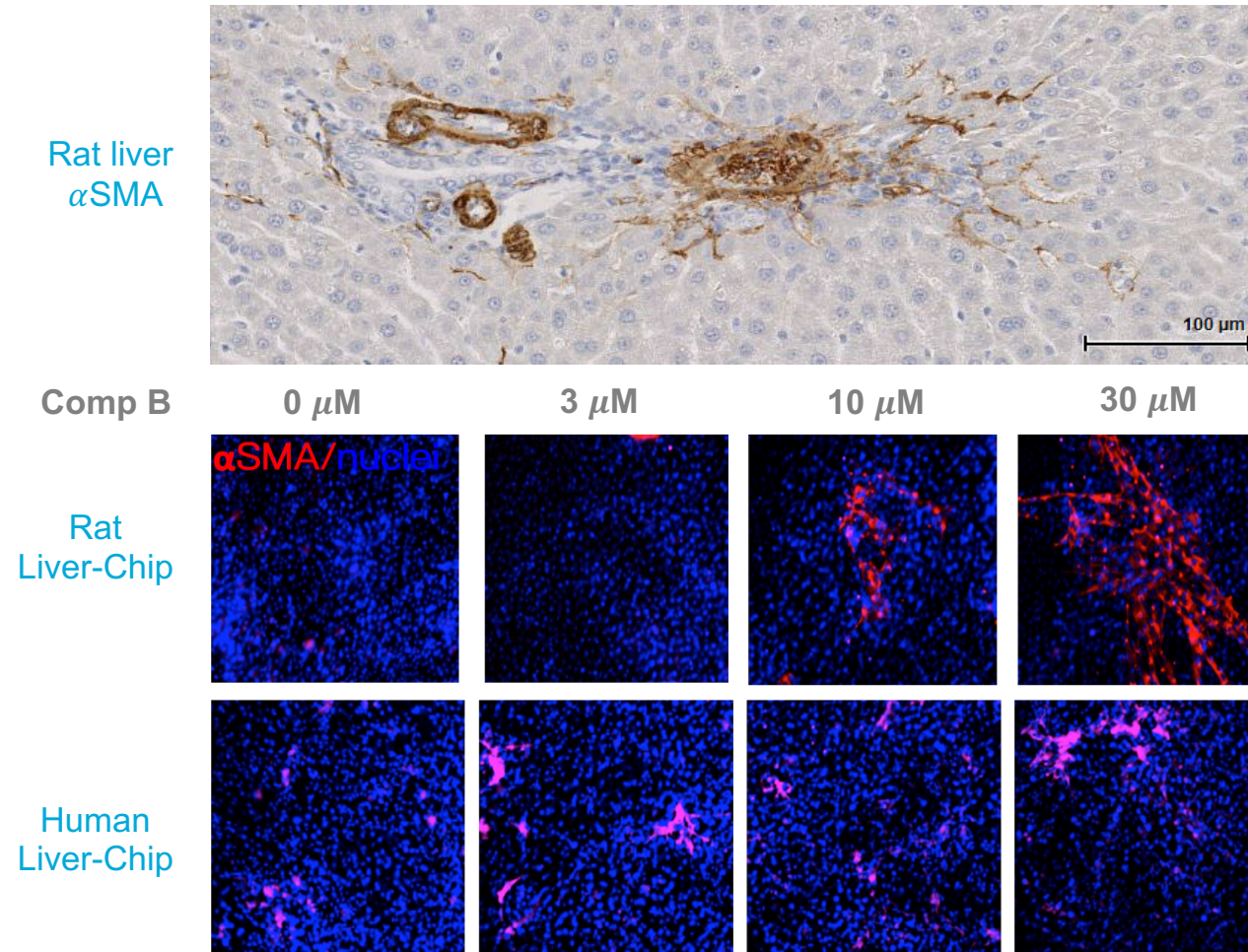
Albumin data shows concentration response effects for dog and human,  
but not for rat, which correlates with *in vivo* data: human > dog > rat

Inhibition of BSEP Transporter





# Modeling Rat *In Vivo* using the Rat Liver-Chip



Rat Liver-Chip data correlated with rat *in vivo* data and human Liver-Chip indicates low risk for toxicity

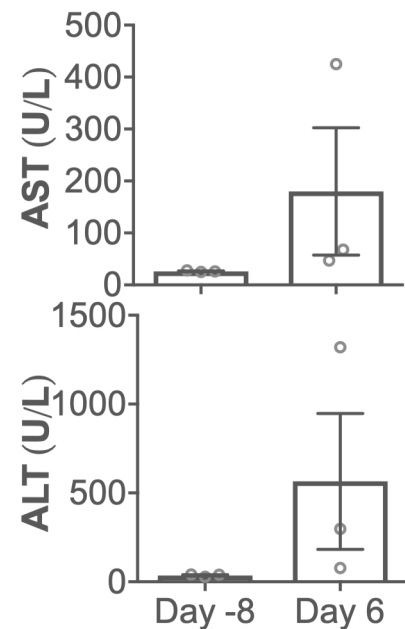
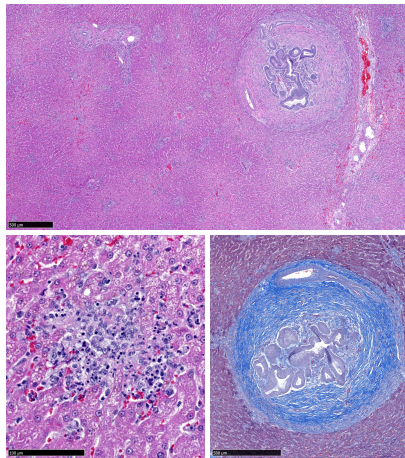


# Modeling Dog *in vivo* using the Dog Liver-Chip

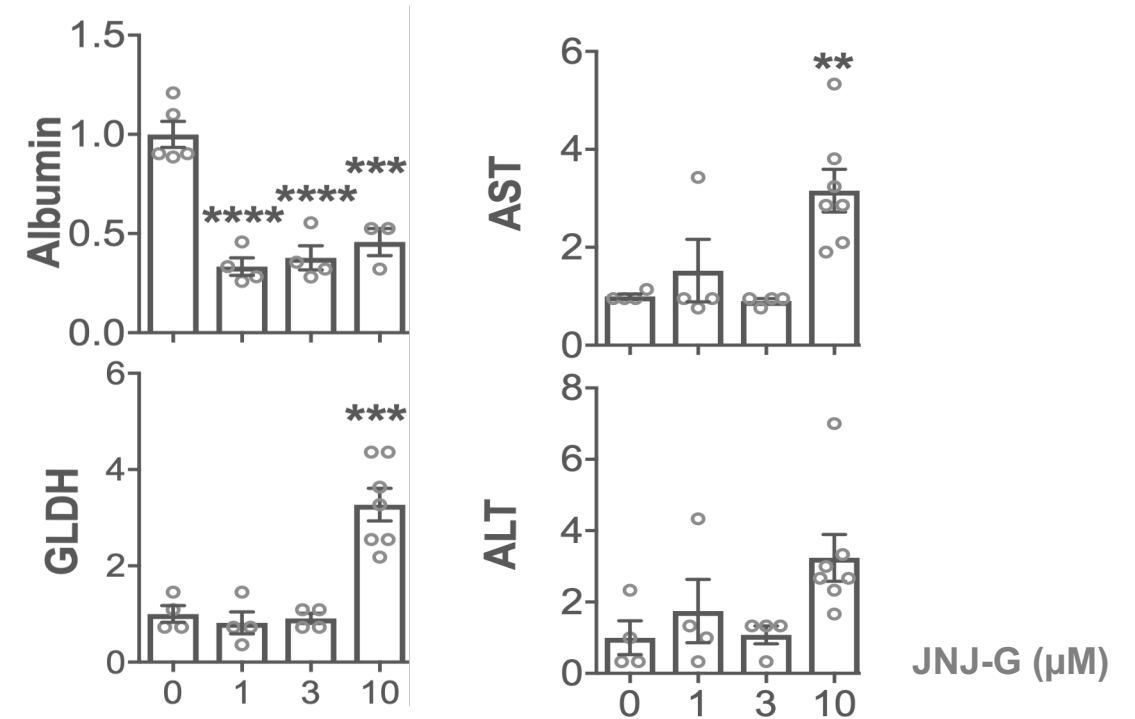
## Dog In Vivo

Hepatocellular necrosis, portal fibrosis, biliary hyperplasia associated and significant elevations of ALT and AST after daily dosing at 15 and 65 mg/kg in dogs (C<sub>max</sub> plasma 12.7 and 19.4 μM, respectively)

JNJ-G



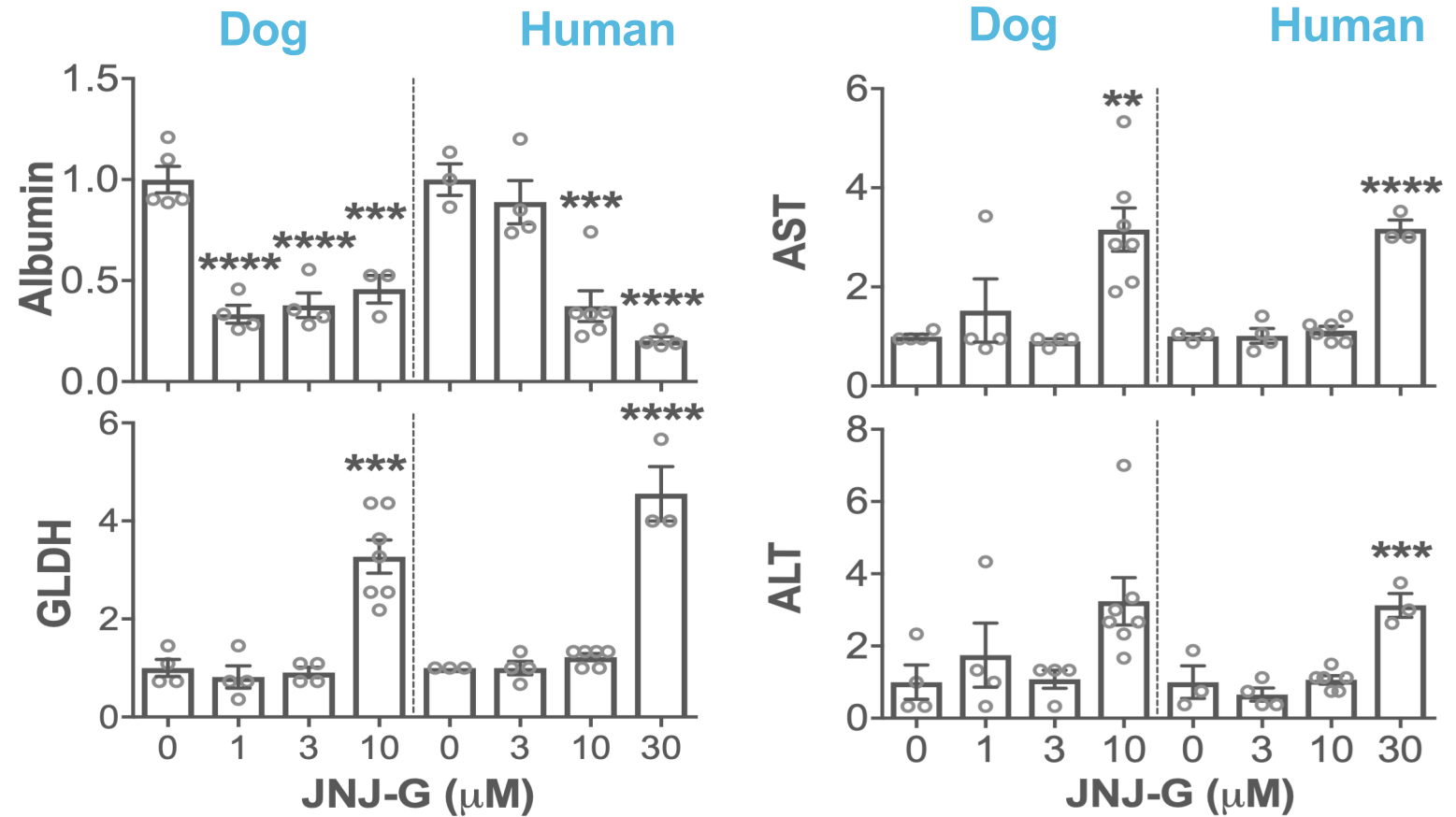
## Dog Liver-Chip



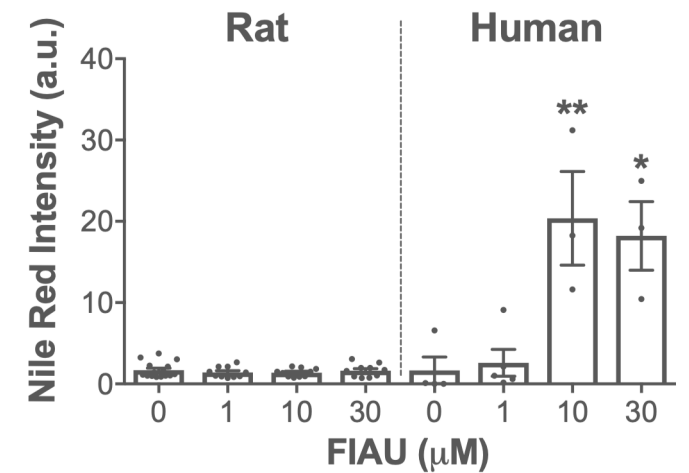
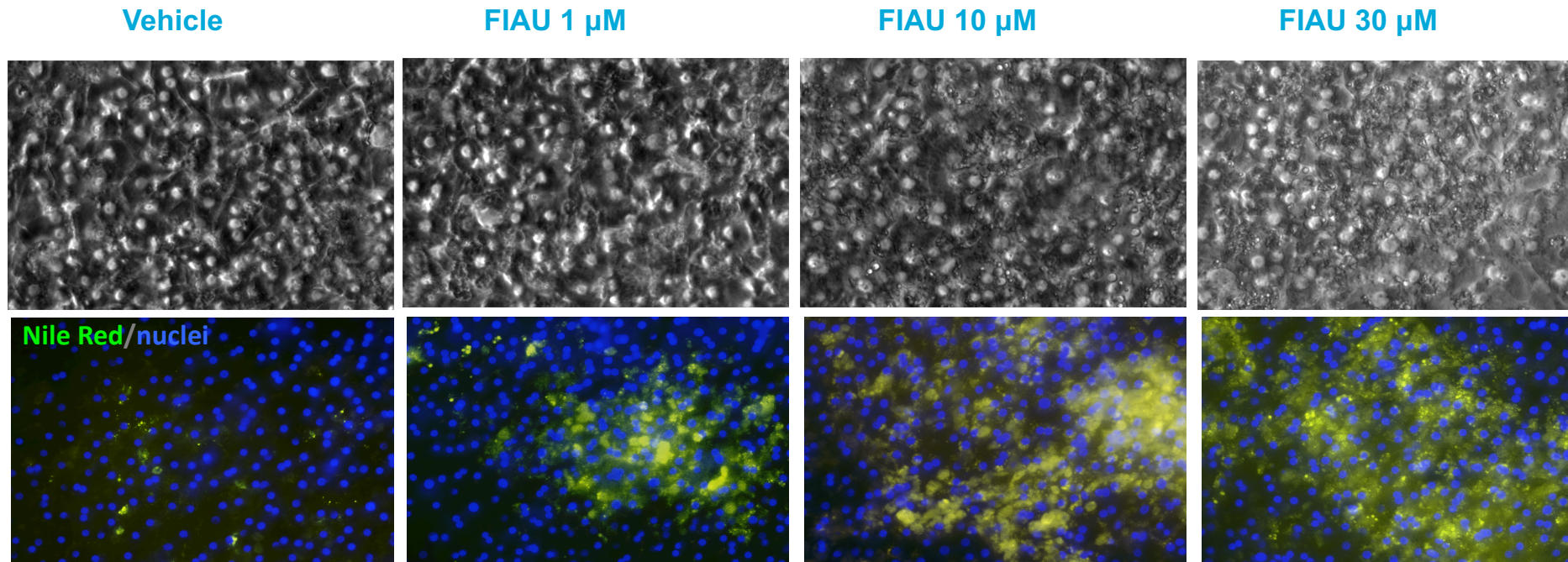
JNJ-G caused similar changes in Dog Liver-Chip data compared to *in vivo* data.  
For this compound albumin was a sensitive marker of hepatocyte dysfunction in the Liver-Chip

# Use of Liver-Chip to Query Human Relevance of Dog Toxicity

Dog Liver-Chips were more sensitive to JNJ-G than human Liver-Chips but the response in the human Liver-Chip indicates that JNJ-G could induce liver toxicity in human subjects



# Predicting Human Safety Risk with Liver-Chip



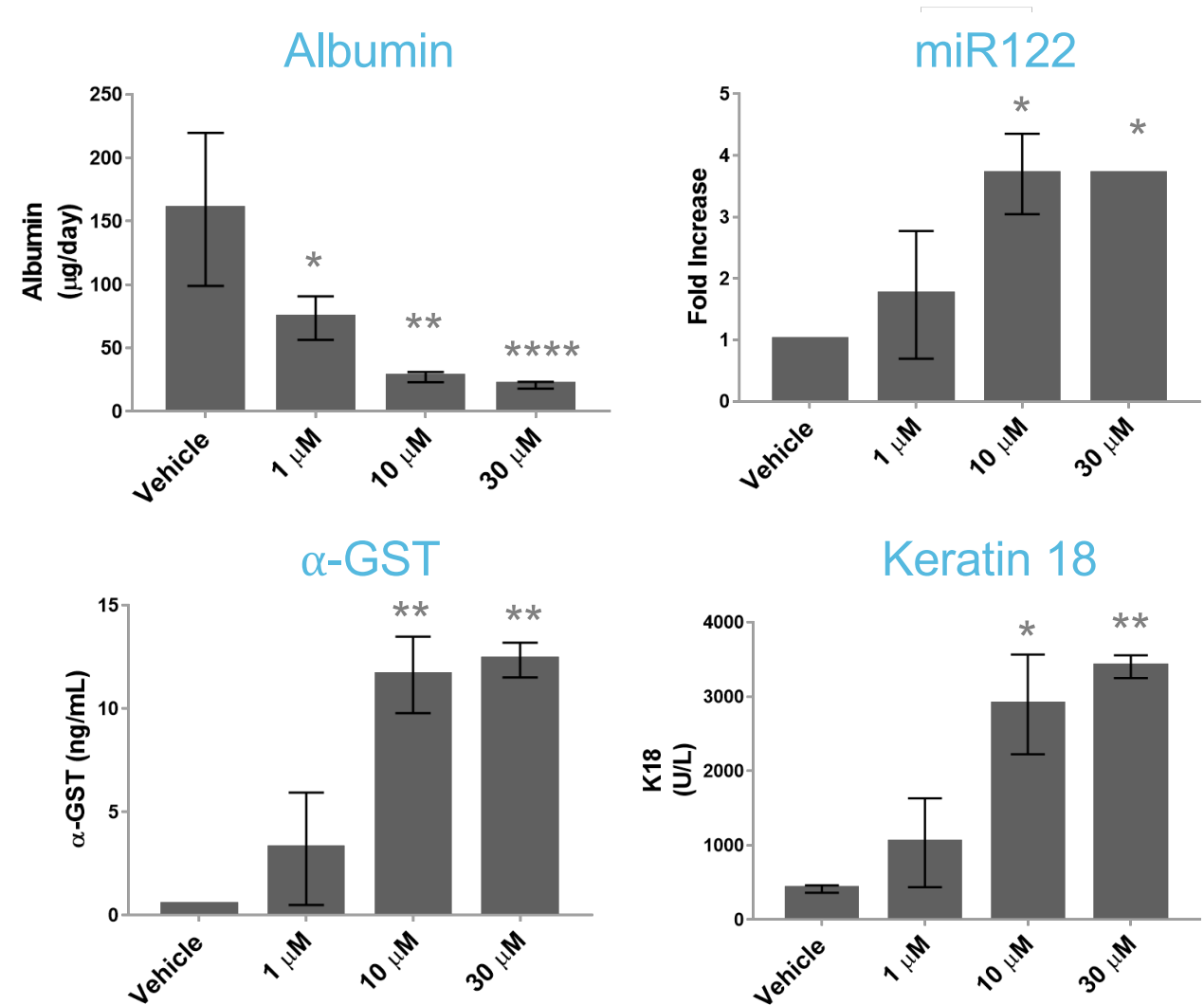
Fialuridine (FIAU) caused toxicity in clinical trial and patient death from liver failure (microvascular steatosis)  
Toxicity not predicted in pre-clinical animal safety studies

Liver-Chip demonstrated species differences in progression to steatosis  
Human Liver-Chip data correlated with clinical findings



# Predicting Human Safety Risk with Liver-Chip

Liver-Chip predicted human liver toxicity of Fialuridine that was observed in clinical trial, and provides mechanistic data



# Summary

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- The Emulate Liver-Chip has been characterized using primary cells from human, rat or dog
- Chips display morphological markers and functional activities characteristic of hepatocytes
- Rat Liver-Chips were able to recreate rat *in vivo* outcome and dog Liver-Chips were able to recreate dog *in vivo* outcome
- Human Liver-Chips and rat Liver-Chips correctly identified the steatosis induced by fialuridine
- Taken together, these results suggest that Liver-Chips play a valuable role in risk assessment of new candidate drugs and can delineate species-specific toxicities and may contribute to a reduction in animal usage associated with strengthening a weight of evidence argument
- Looking forward, continued investment in access to fresh and cryopreserved animal cells will enable additional animal chips to be developed

