

HEPATIC STEATOSIS SHIFTS PHASE I METABOLISM & ALTERS SUSCEPTIBILITY TO TOXICANTS IN VITRO

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WHAT IS “HEPATIC STEATOSIS”?

Human ‘foie gras’

Fatty liver – an etiology

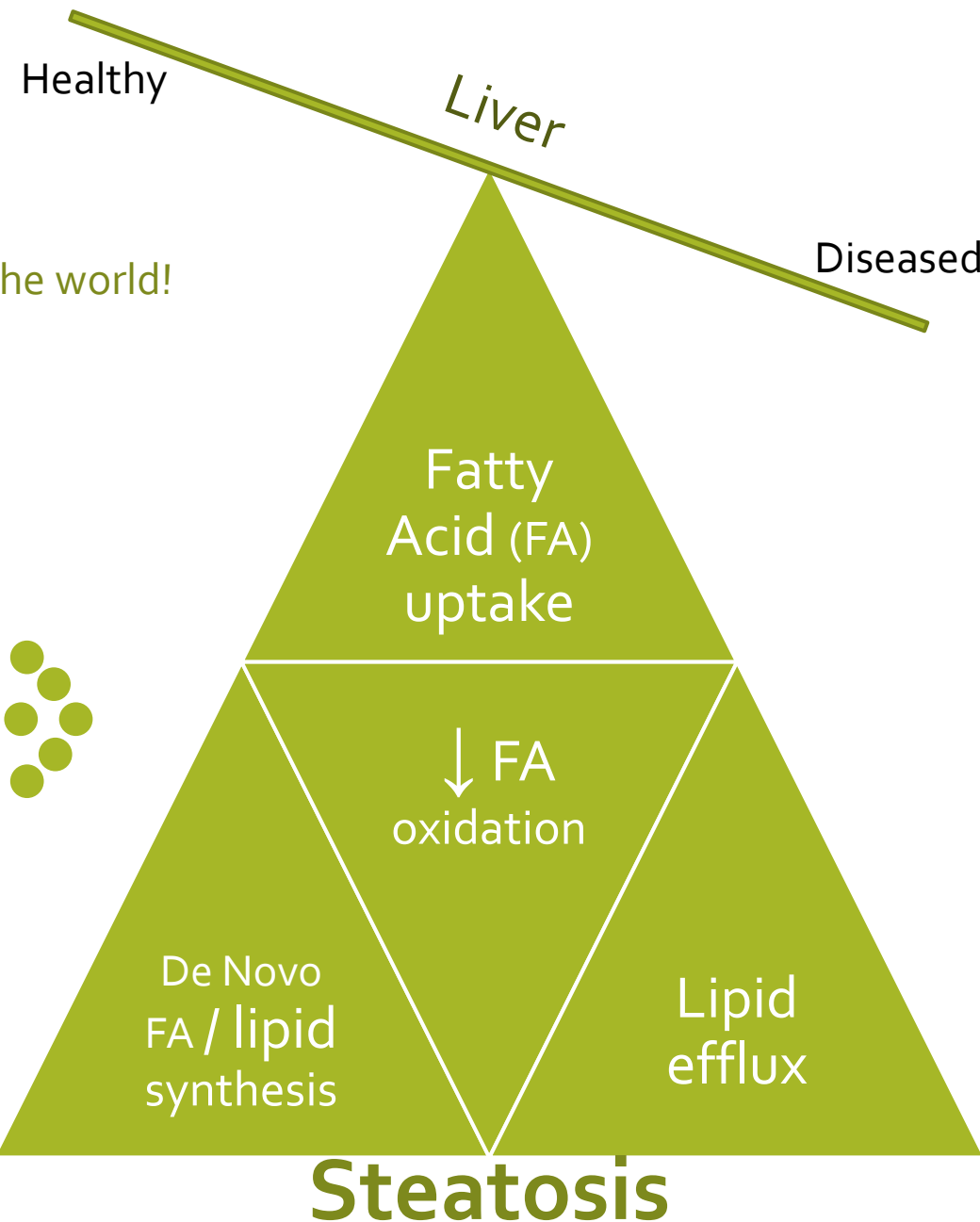
* WIDE SPREAD PREVALANCE * ~ 1/3rd of the world!

1. Alcohol

2A. Environmental Chemicals

2B. High-Fat Diet / Inactivity

Fatty
Liver



Disease spectrum



- Initially reversible,
- Progressive condition:
- 2 hit theory.



Reversible!

"Danger Zone"



Normal Healthy Liver

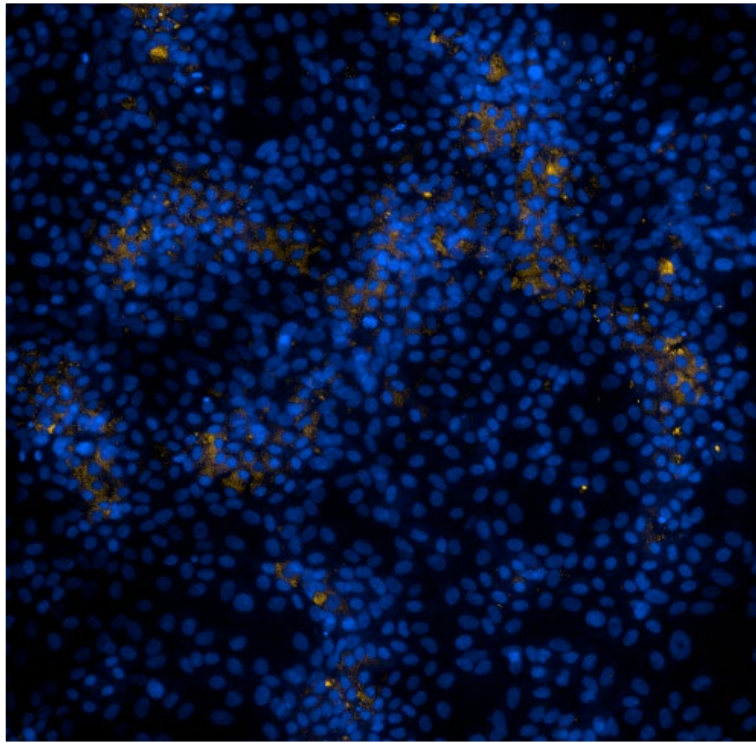
Fatty Liver

Steatohepatitis

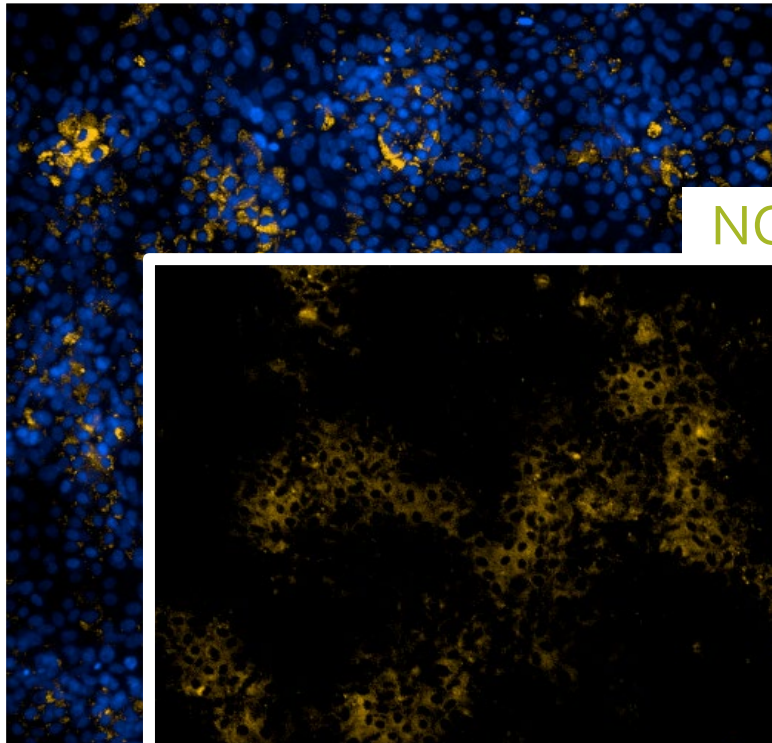
Cirrhosis

Cancer

With Phenotypic Change Comes Metabolic Change

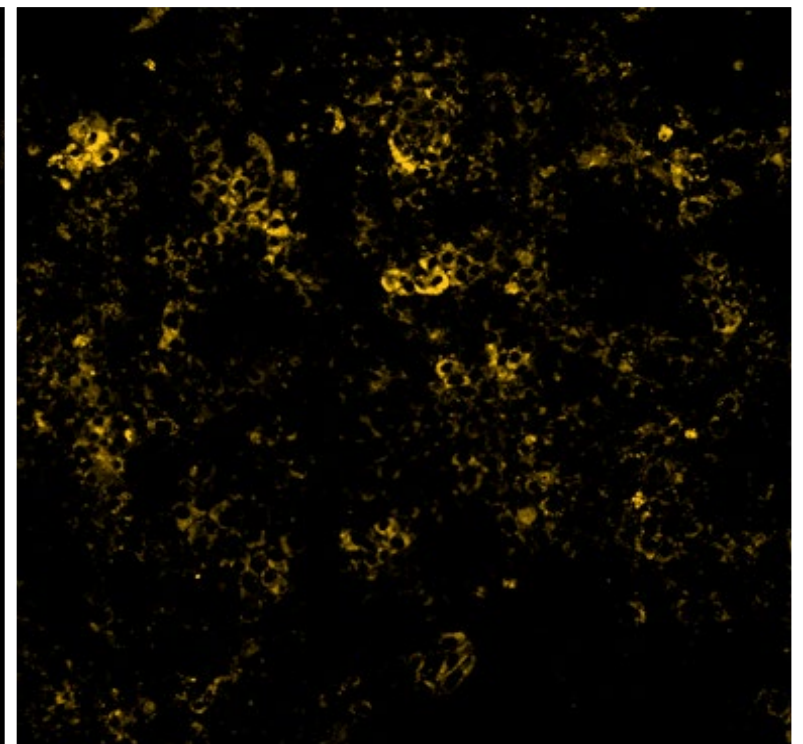
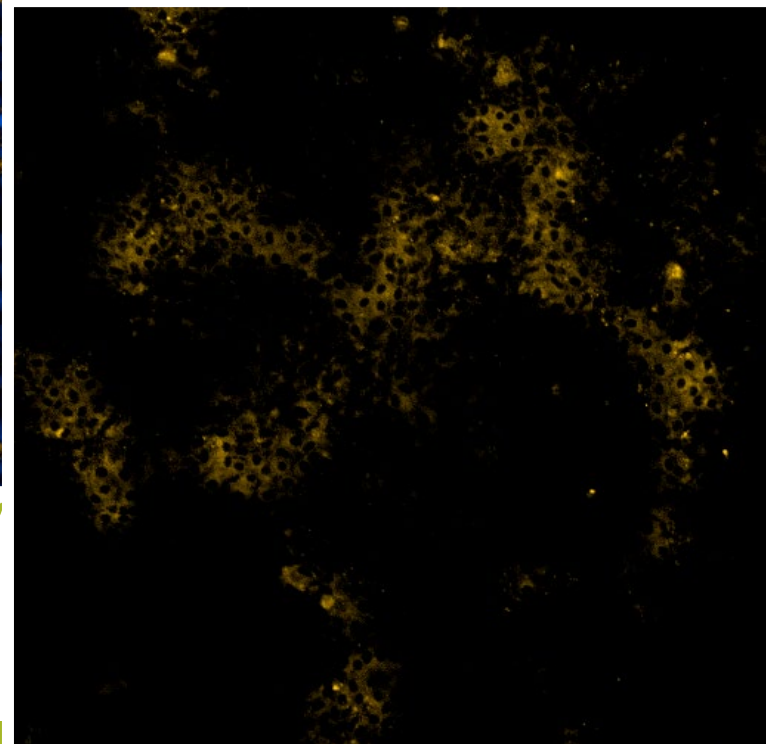


NO FFA



NO FFA

1 mM



1 mM FFA

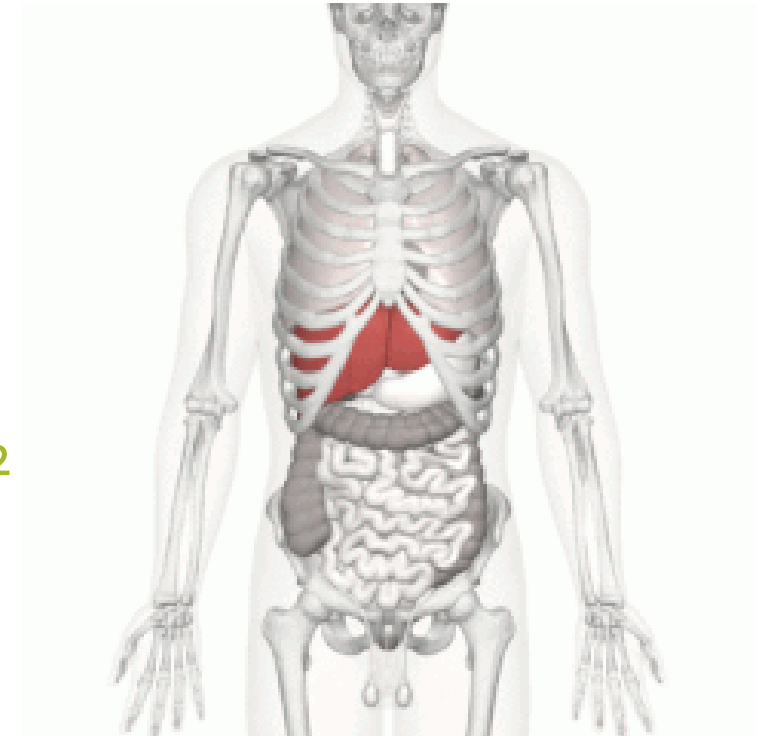
MODEL DEVELOPMENT

Evaluating a hidden susceptibility

Model System

HepaRG – human hepatoma cell line

- **Genetic homogeneity**
 - + Reproducible
 - Captures only effect on individual
- **Metabolic competency:**
 - Primary Human Hepatocytes > HepaRG > HepG2
 - Xenobiotic metabolites as an additional variable in assessing toxicity



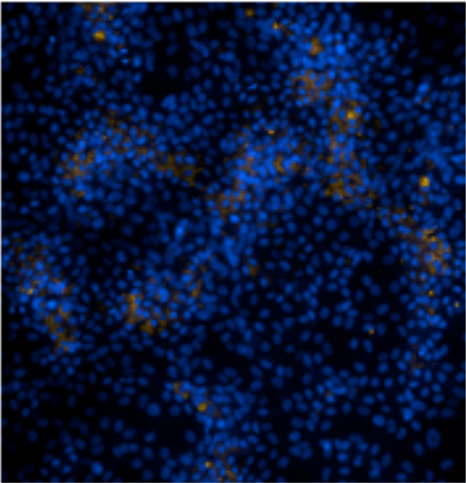
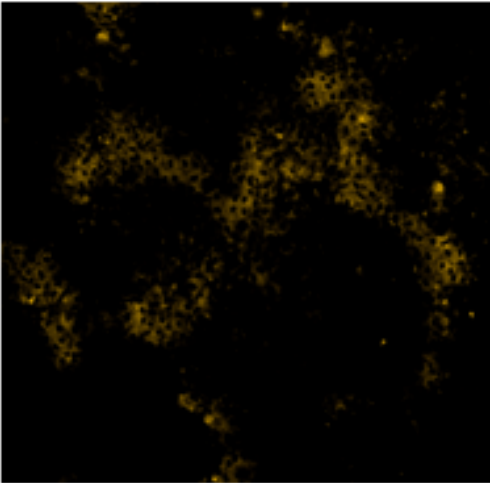
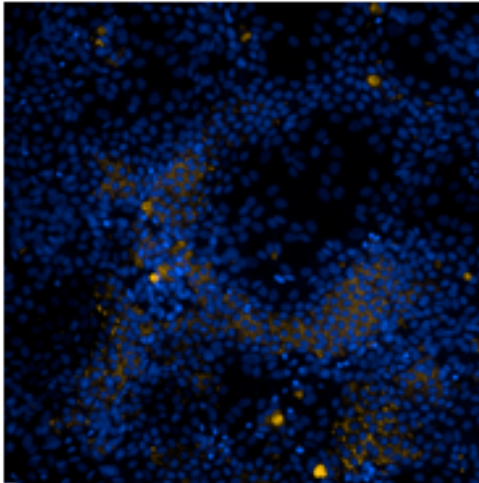
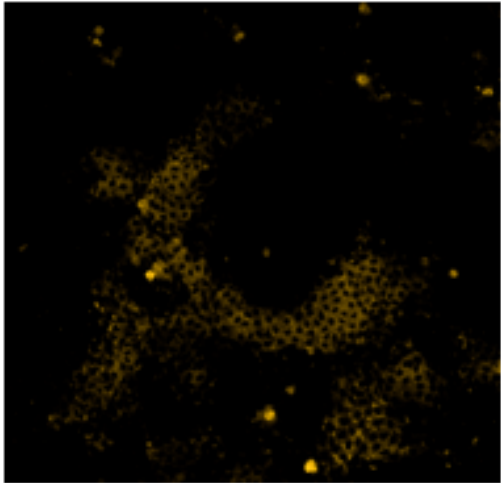
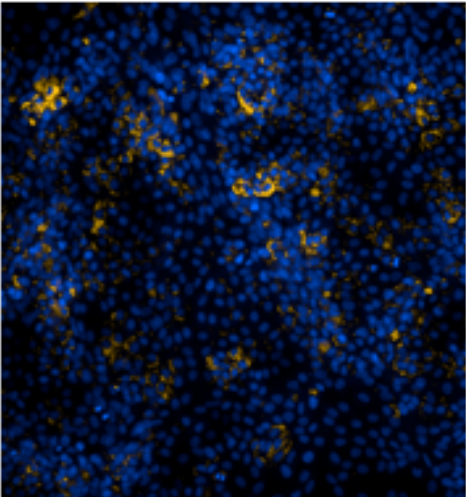
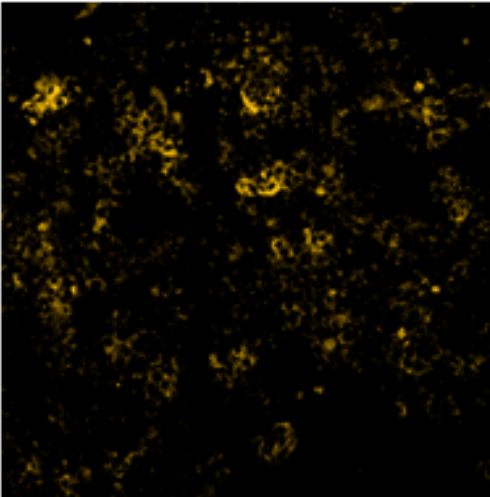
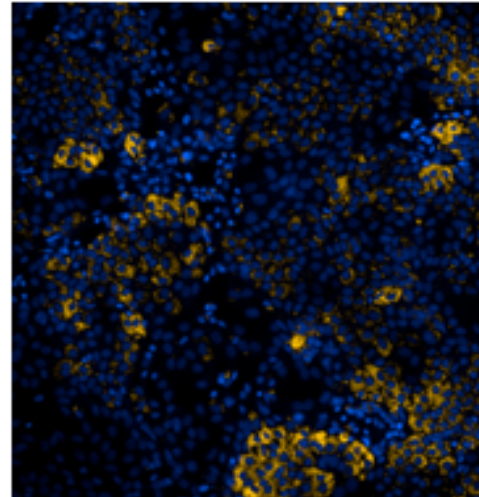
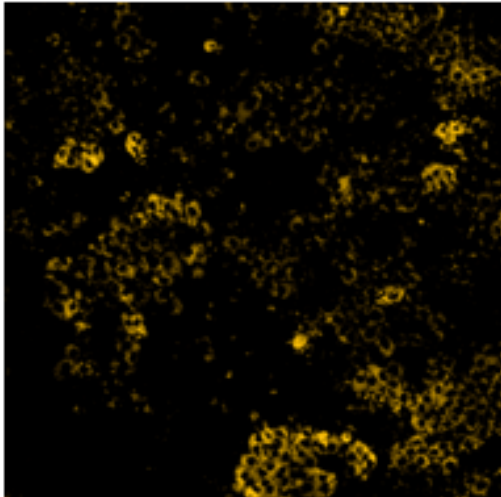
Find your liver! (Wikipedia)

Optimization Factors

Free-fatty acid (FFA): concentration, ratio, & incubation time.

Ultimately selected for model:

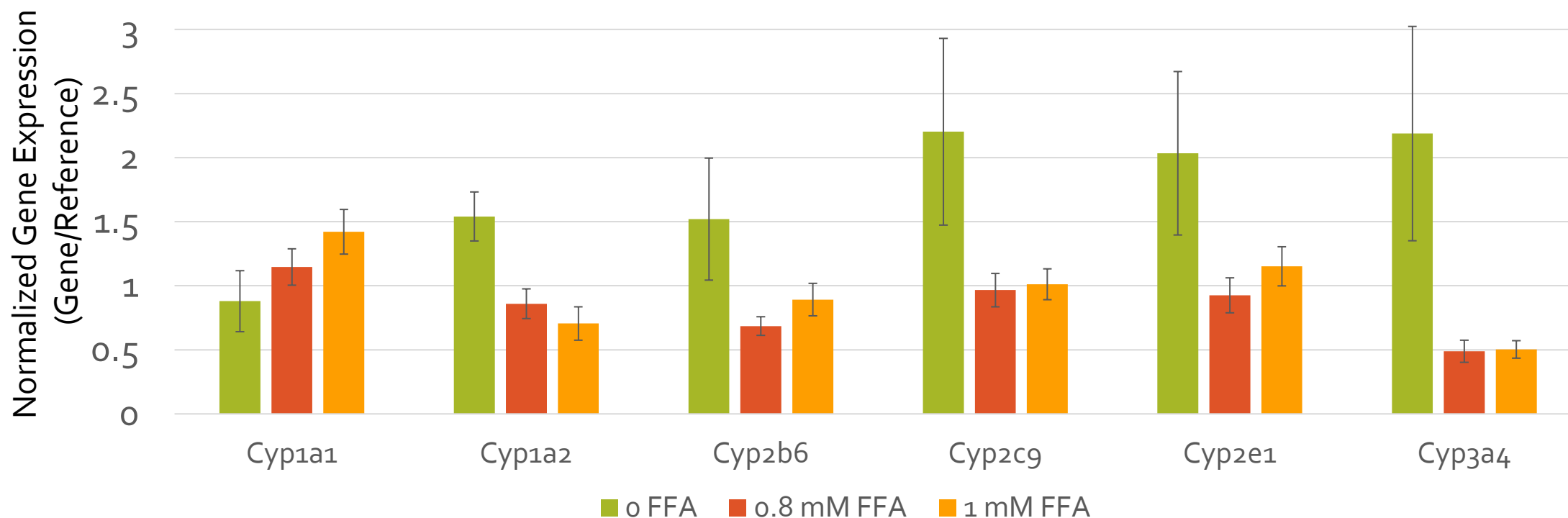
- 7 day incubation in free-fatty acid (FFA)
 - 1 mM FFA
 - 1:2 Oleate : Palmitate
- Chemical treatment
(24- / 48- hr treatment)

	No Chem		Rotenone (0.2 uM, 48 hr)	
	Composite	Nile Red	Composite	Nile Red
0 FFA				
1 FFA (chem exposure concurrent)				

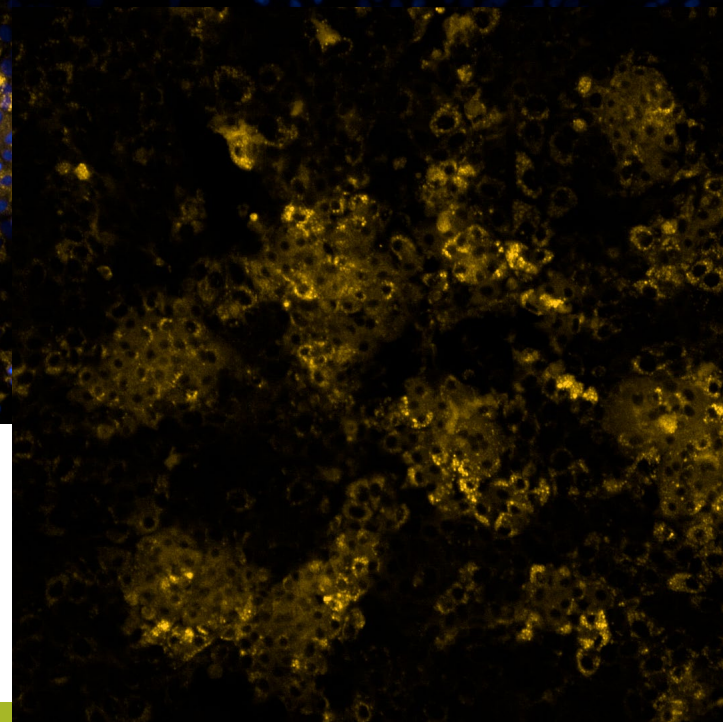
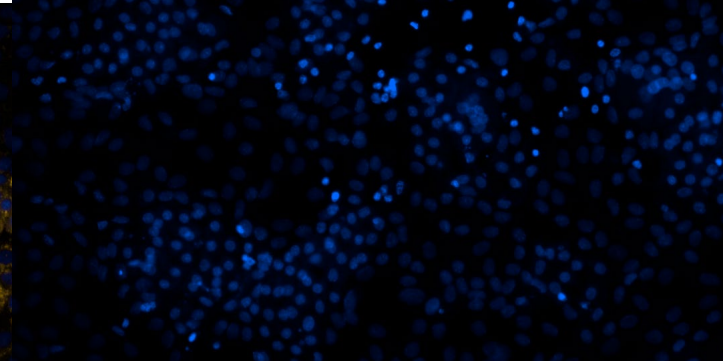
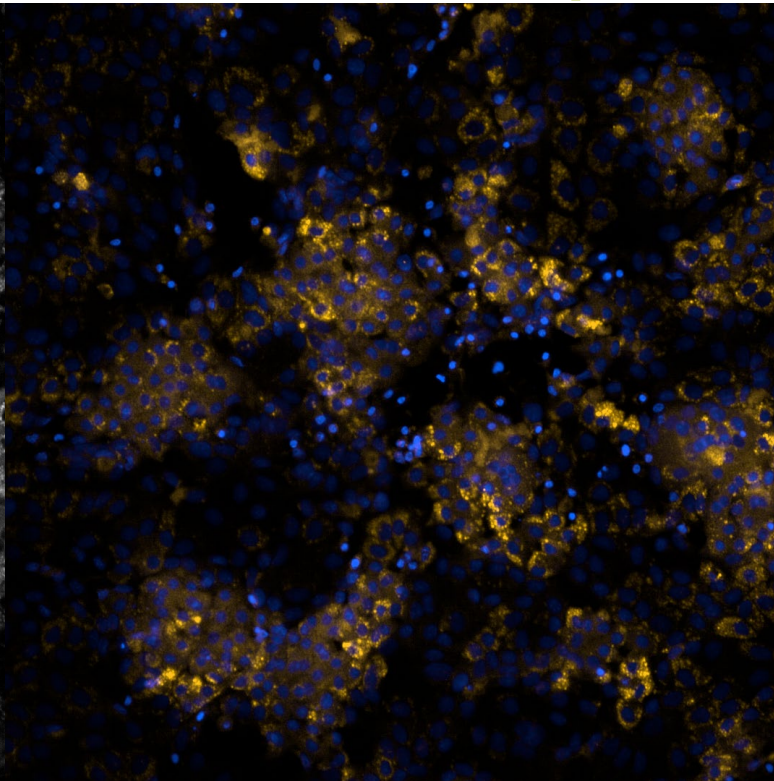
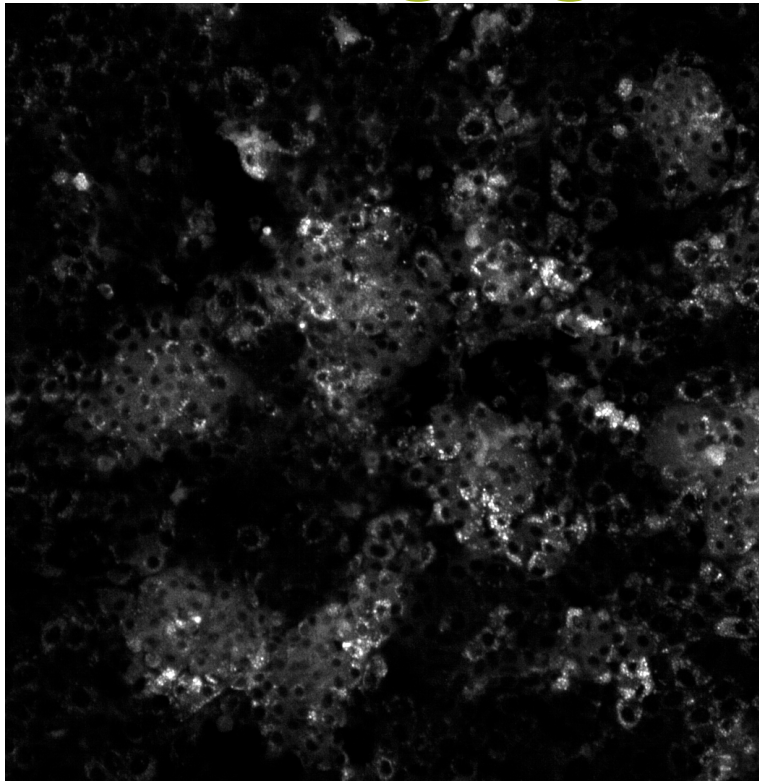
Cytochrome P₄₅₀s – a selection

One week incubation in zero, 0.8 mM, or 1 mM FFA

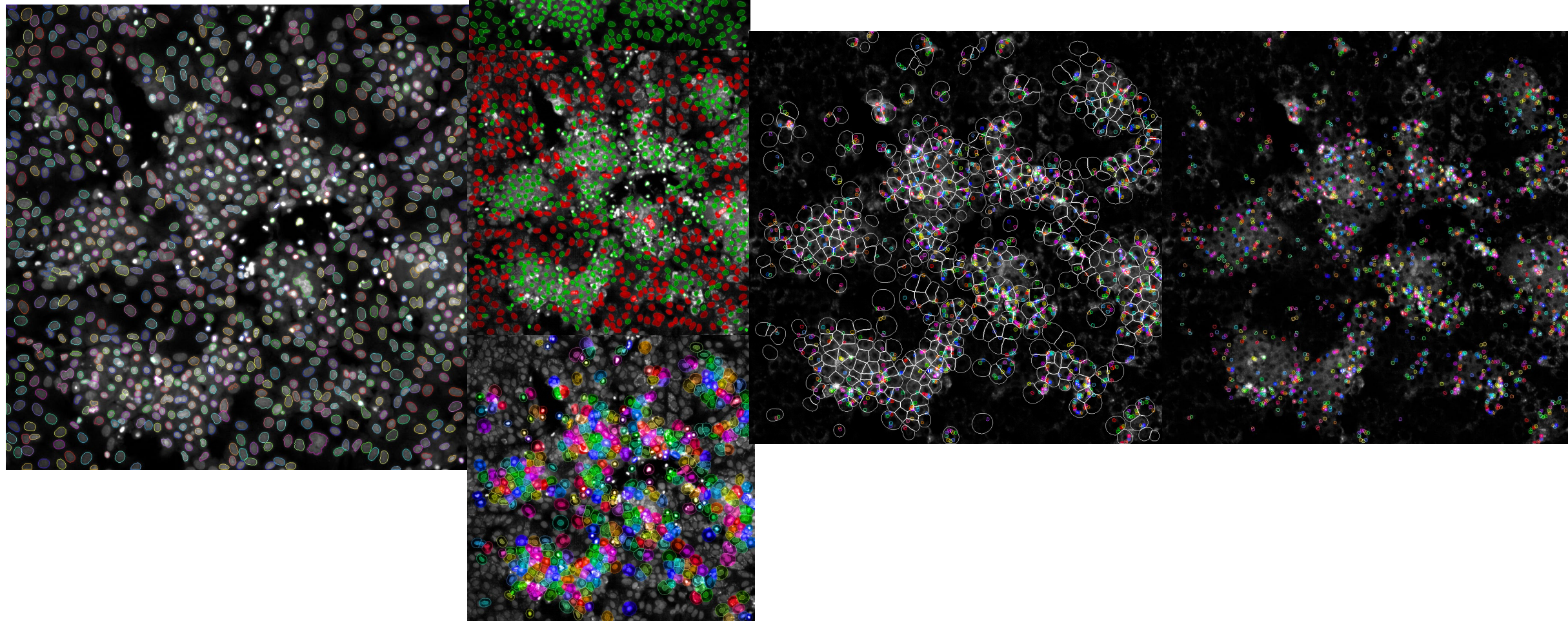
CYP Expression



Imaging Work Flow – Opera



Imaging W – Opera Phenix



RESULTS

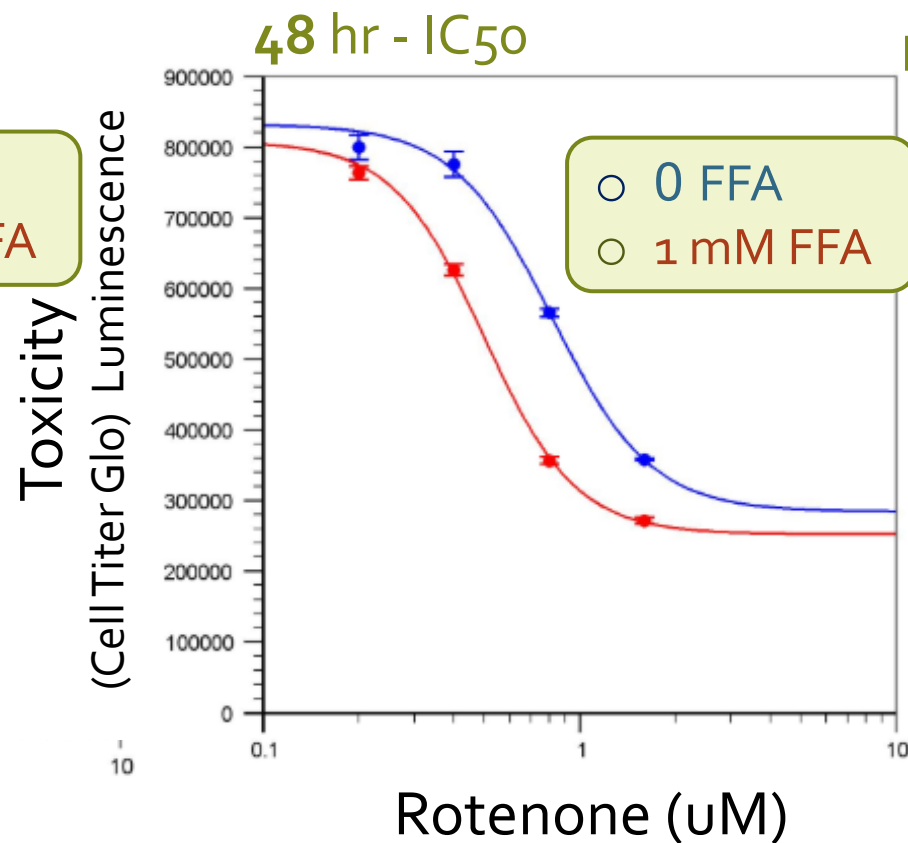
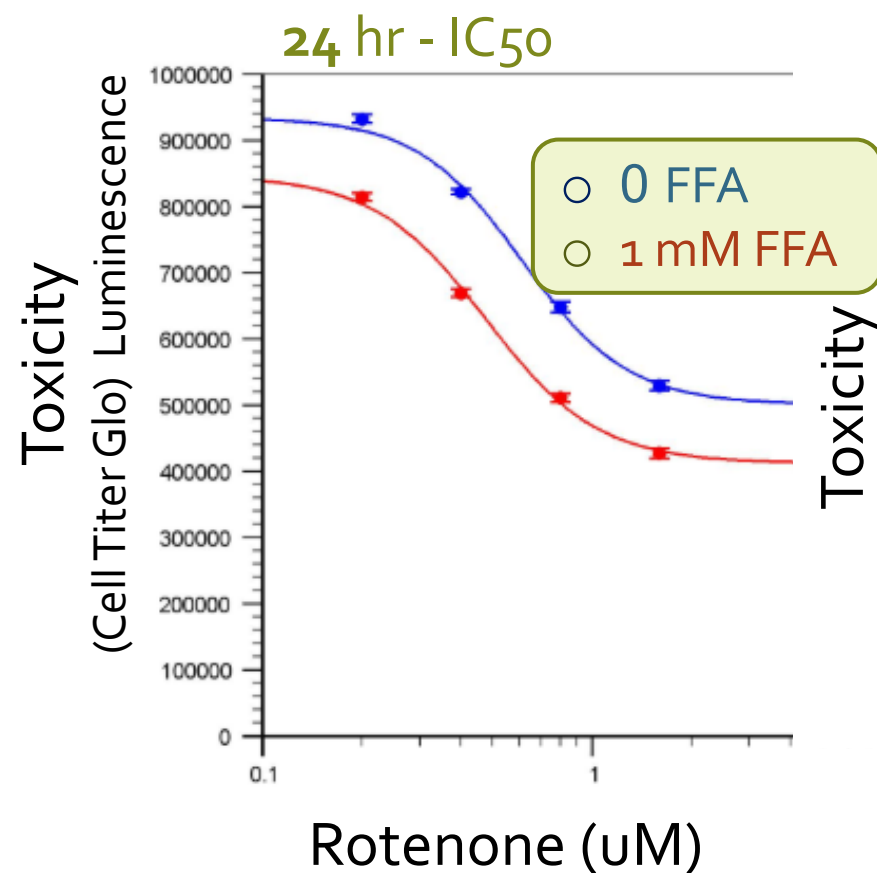
Endpoints

- **Toxicity** – Cell Titer Glo (ATP) – luminescence indicator – Spectramax
- **Toxicity** – Lactate dehydrogenase (LDH) – luminescence indicator – Spectramax (not shown)
- **Cell count** – Hoechst – fluorescence – Opera Phenix
- **Lipid accumulation** – Nile Red – fluorescence – Opera Phenix
- **Metabolism** – Cytochrome P₄₅₀ gene expression Δ – PCR
- **Multi-plex probes** for mechanistic info on toxicity*
 - Cellular membrane potential
 - Mitochondrial membrane potential
 - Reactive oxygen species generation
 - Glutathione characterization

*very soon

Toxicity shift - Timecourse

Rotenone (uM) Exposure - 24 vs 48 hr - IC₅₀



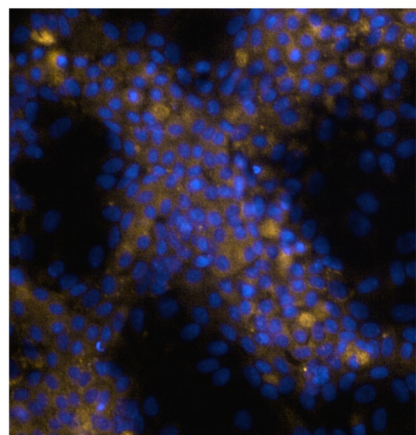
Rotenone (uM) Exposure - IC₅₀

Dose	24 hr	48 hr
0 mM FFA	0.62	0.82
1 mM FFA	0.48	0.50

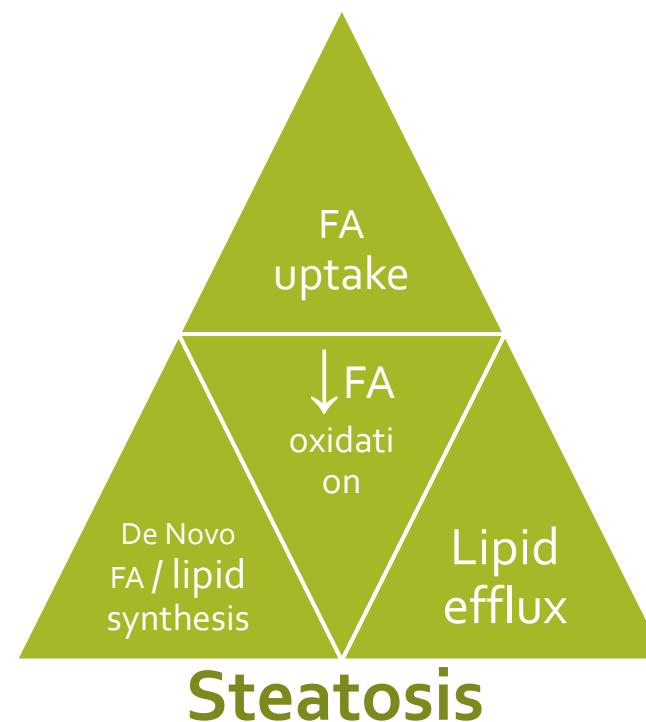
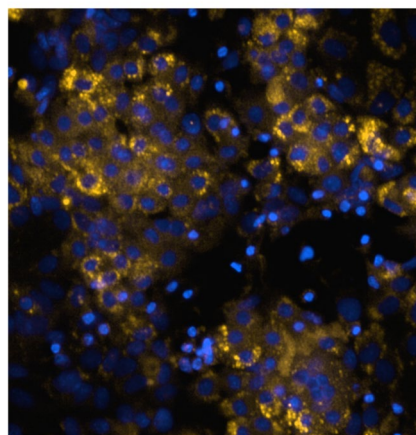
Conclusions

- Fatty buildup in the liver
- A silent disease effecting 30% of the world
- Changes metabolic gene expression
- Shifts toxicity of xenobiotics

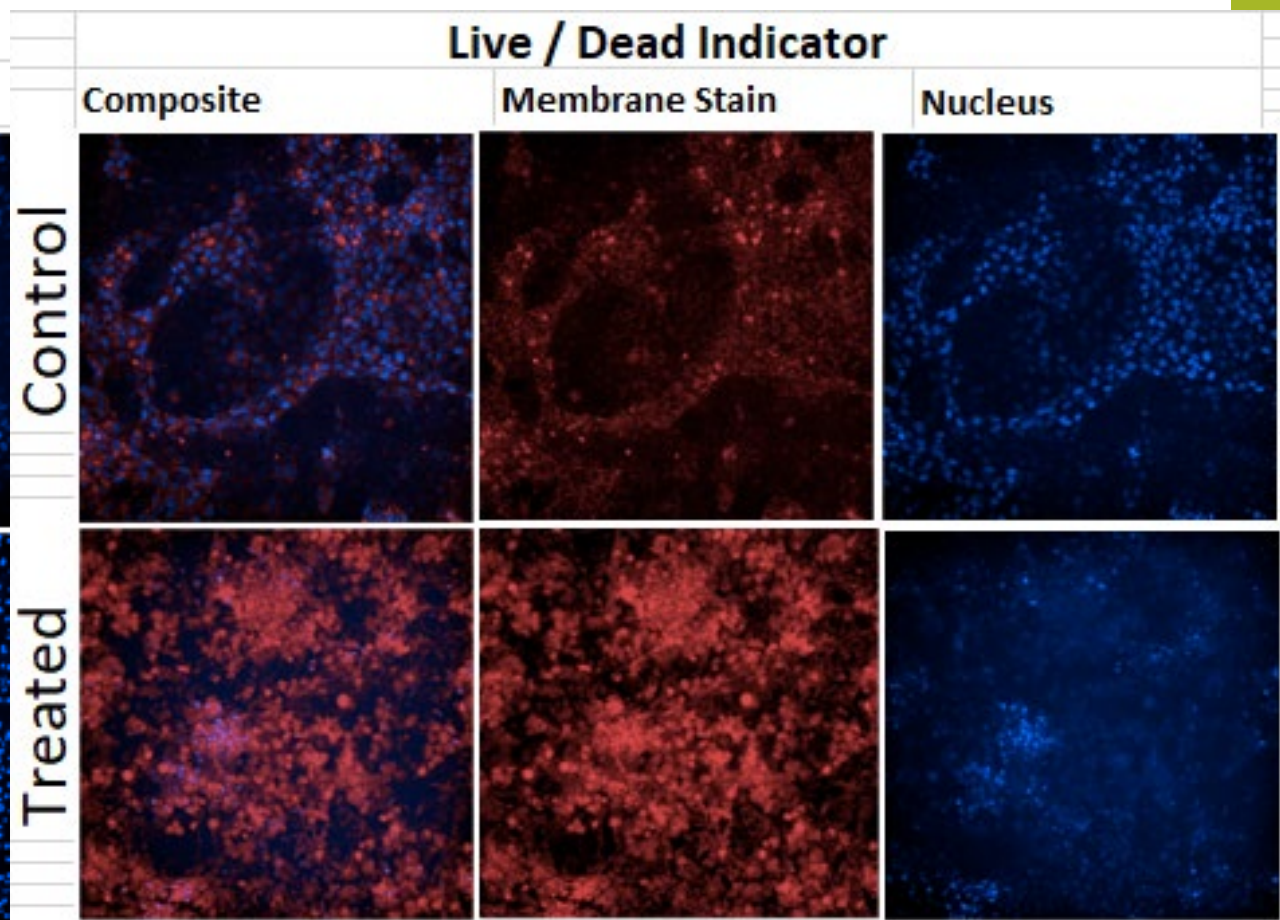
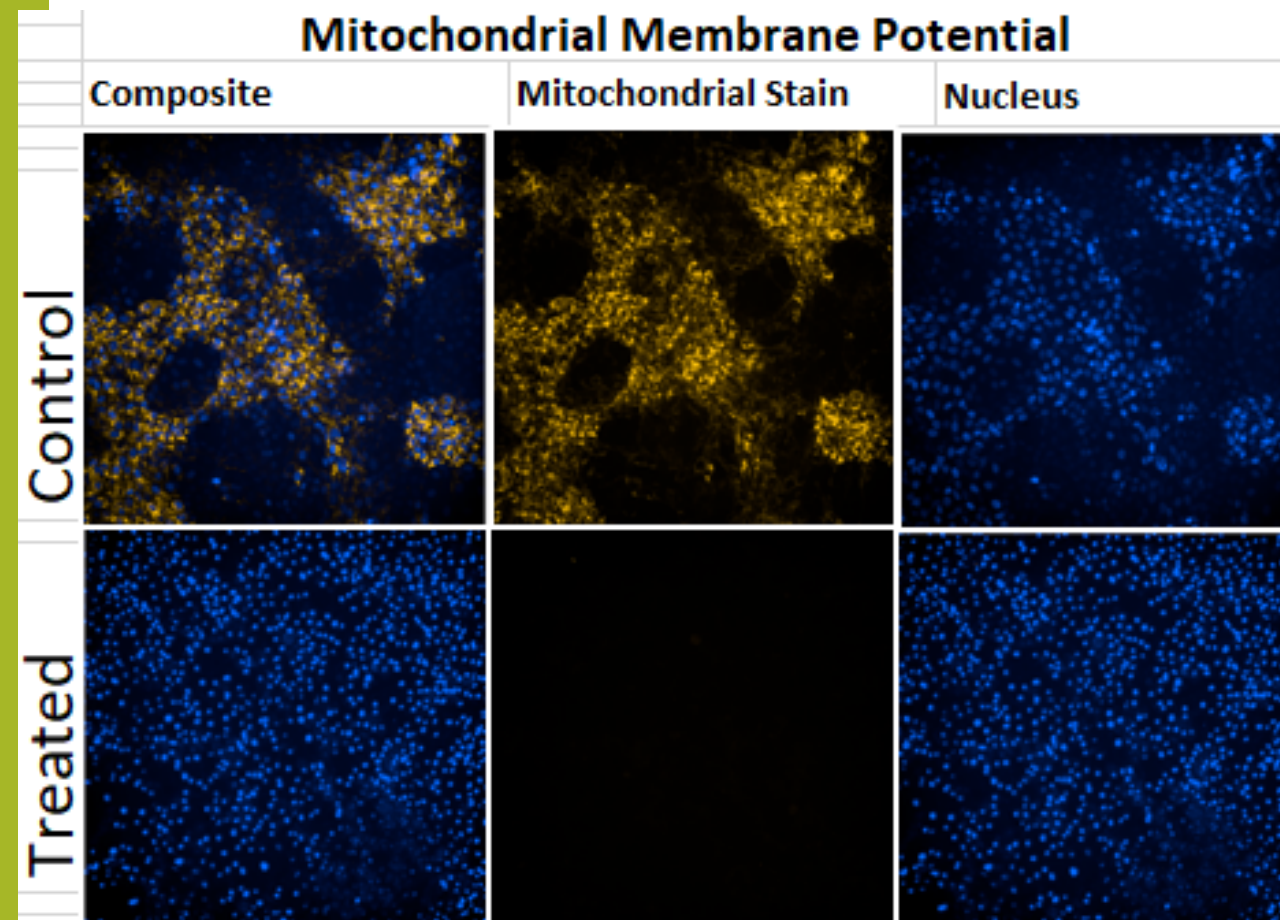
Control



Treated



Mechanisms of Tox



Future Directions

- Metabolomics as indicative of phenotype
- Test system with more chemicals –
 - CYP specific chemicals,
 - Explore mechanistic space, &
 - Assess biological response of steatotic hepatoma cells.
- Contribution of xenobiotic metabolism in:
 - generating toxic metabolites?
 - OR detoxifying parent chemicals?
 - OR prevents metabolism of chemical from toxic parent compound non-toxic metabolites?

Thank you!

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