Systems Bioengineering Group - NTUA

ΕΛΛΗΝΙΚΗ ΔΗΜΟΚΡΑΤΙΑ
Εθνικόν και Καποδιστριακόν Πανεπιστήμιον Αθηνών

Phosphoproteomic profiling of the NAFLD/NASH mechanism in a primary human hepatocyte model

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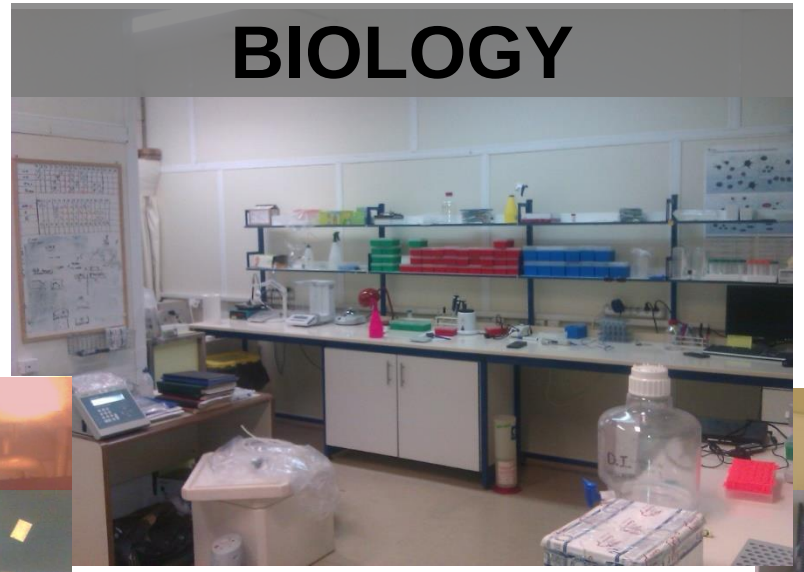
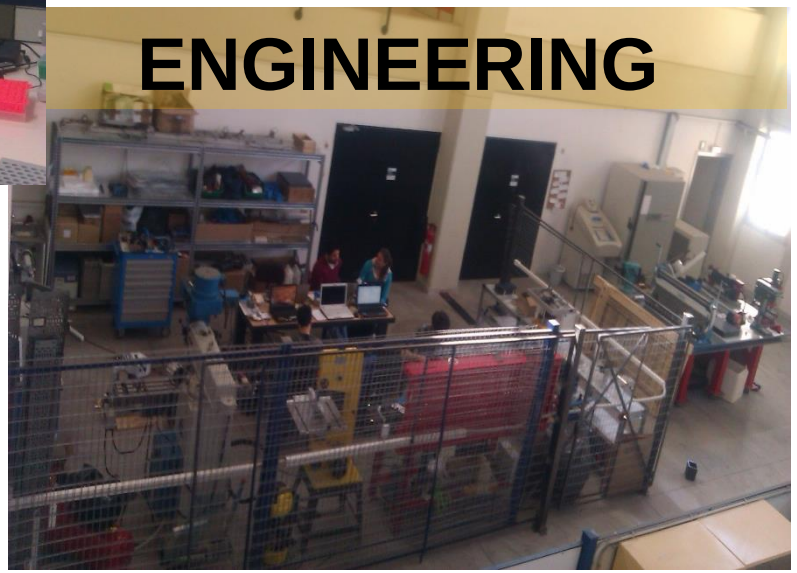
Interdisciplinary Research
between Engineering, Computer science and Biology





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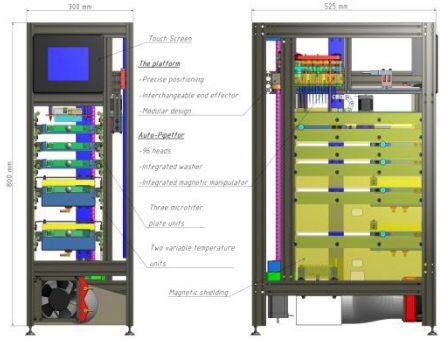
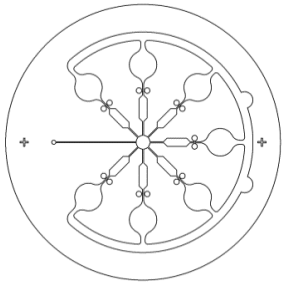
Medical Engineering Projects

Point of Care Diagnostics



Automated high-throughput multiplexed ELISA



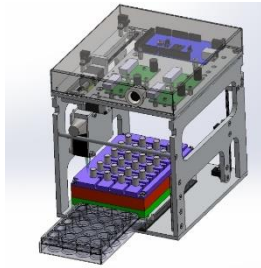
PoC multiplexed ELISA

Ultrasonic Cleaning

Cleaning device

Smartphone microscopy Mobile Health

Cartilage Testing



ECM degeneration

Automation

Automation of biological assays

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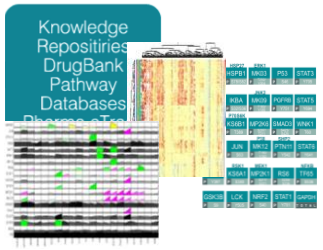
Systems Biology for Liver Disease

Multi-omics data



Gex, Seq, MS
HCS, databases
Phosphoproteomics
Clinical data

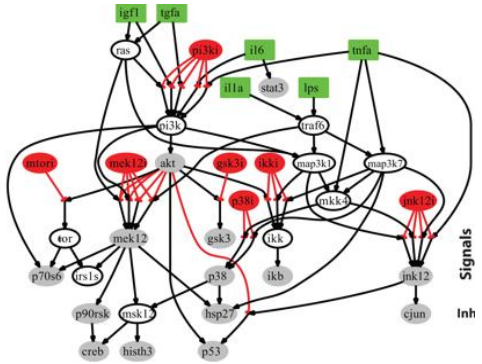
Liver Disease

- Drug Mode of Action
- Liver Toxicity
- Hepatocellular Carcinoma
- **NAFLD**

Collaborations

- Virtual Liver
- Hepatosys
- Aachen

Data analysis



Pathway analysis

Publications

Mitsos et al. **PLoS Comp Biol** (2009)
Saez-Rodriguez et al. **Mol Sys Biol** (2009)
Alexopoulos et al. **Mol Cell Prot** (2010)
Melas et al. **BMC *Sys Biology*** (2011)
Saez-Rodriguez et al. ***Science Sig*** (2011)
Melas et al. ***Mol Biosyst*** (2012)
Mitsos et al. ***PLoS ONE*** (2012)
Melas et al. ***Biopharm. & Drug disp.*** (2013)
Morris et al. ***Drug DiscToday*** (2013)
Melas et al. ***PLoS Comp Biol*** (2013)

NAFLD/NASH and research approaches

Non-alcoholic fatty liver disease (NAFLD): presence of hepatic steatosis in the absence of excess alcohol consumption and represents a spectrum of disease (simple steatosis, Non-Alcoholic Steatohepatitis (NASH),fibrosis, cirrhosis, hepatocellular carcinoma)

Limitations

- Cause and disease progression mechanisms still not completely understood → multi-omic approaches **but not on** signal transduction level
- No standard *in vitro* models

AIM: Profiling of proteins involved in the signal transduction mechanism of NAFLD in a new *in vitro* model of primary human hepatocytes

In vitro models - NAFLD/NASH induction

Primary Human Hepatocytes → The most relevant in vivo-like liver-based in vitro models

1) Free Fatty Acids (FFAs)

Mimics dietary FFA influx

2) Valproic acid sodium salt (VPA)

Causes microvesicular steatosis with no nuclear displacement.

- Impairs mitochondrial β-oxidation.
- Induces the mitochondrial permeability pore opening (lipoprotein secretion)

3) Amiodarone hydrochloride (AMI)

- Decreases the membrane potential
- Inhibits microsomal triglyceride transfer protein
- Inhibits electron chain and enzyme complexes

4) Tetracycline hydrochloride (TET)

- Inhibition of mitochondrial triglyceride transfer protein
- Impairment of β-oxidation
- Decreased evacuation of TGs

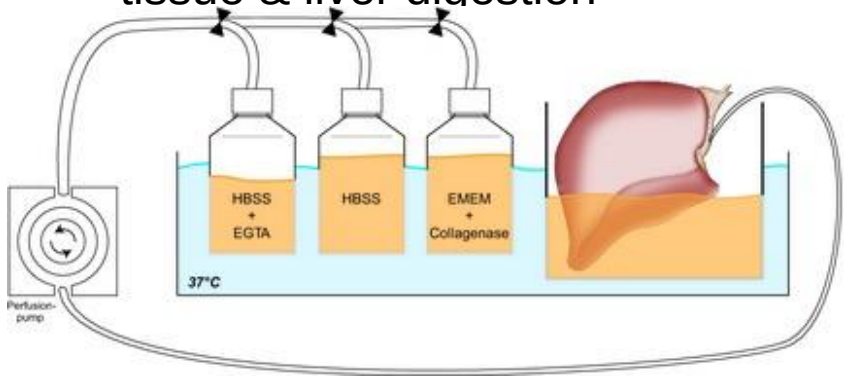
5) Tamoxifen citrate (TMX)

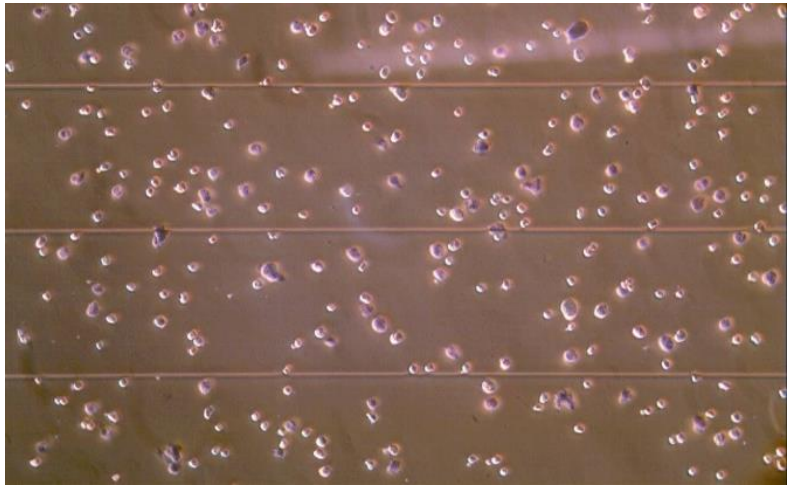
- Compromises the electron transport chain
- Decreases the regeneration of oxidized cofactors
- Upregulates fatty acid biosynthesis

Methods
Isolation of human hepatocytes

1st step: liver reception, observation and weight

2nd step: perfusion with buffers for flushing liver tissue & liver digestion



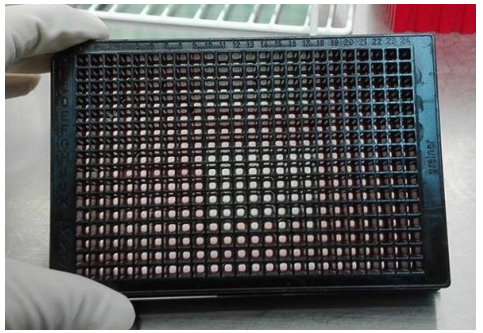


3rd step: isolation of fresh hepatocytes – cell counting & viability assessment by trypan blue exclusion

Source: Biopredic International

Methods
Experimental design

Primary Human Hepatocytes seeding



pHH culture on 384-well plate

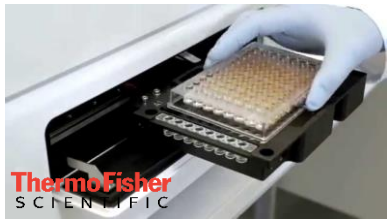
NAFLD induction with FFA and Drugs

High Content Screening

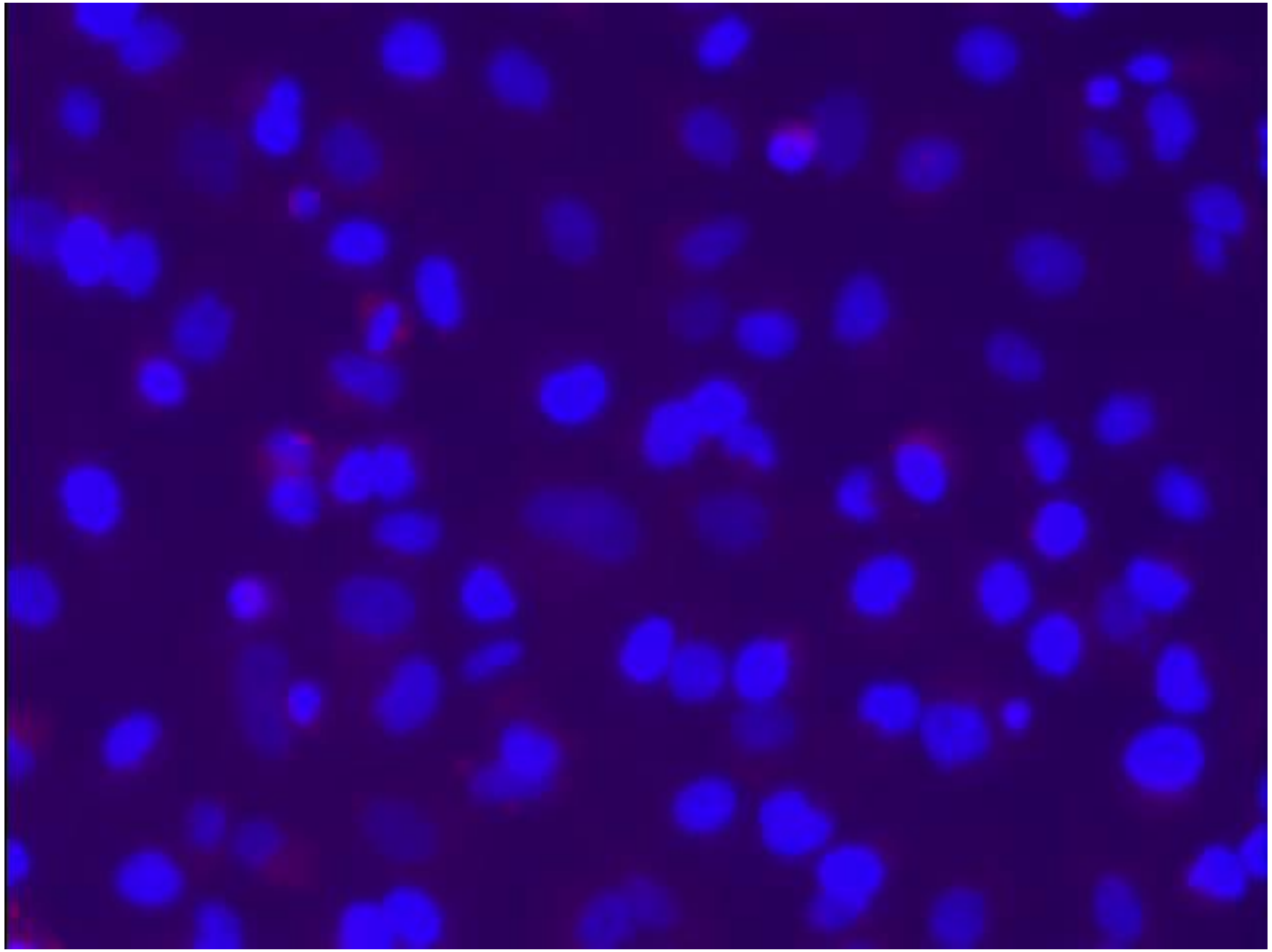
Cell Viability Assay using Resazurin

ROS Production

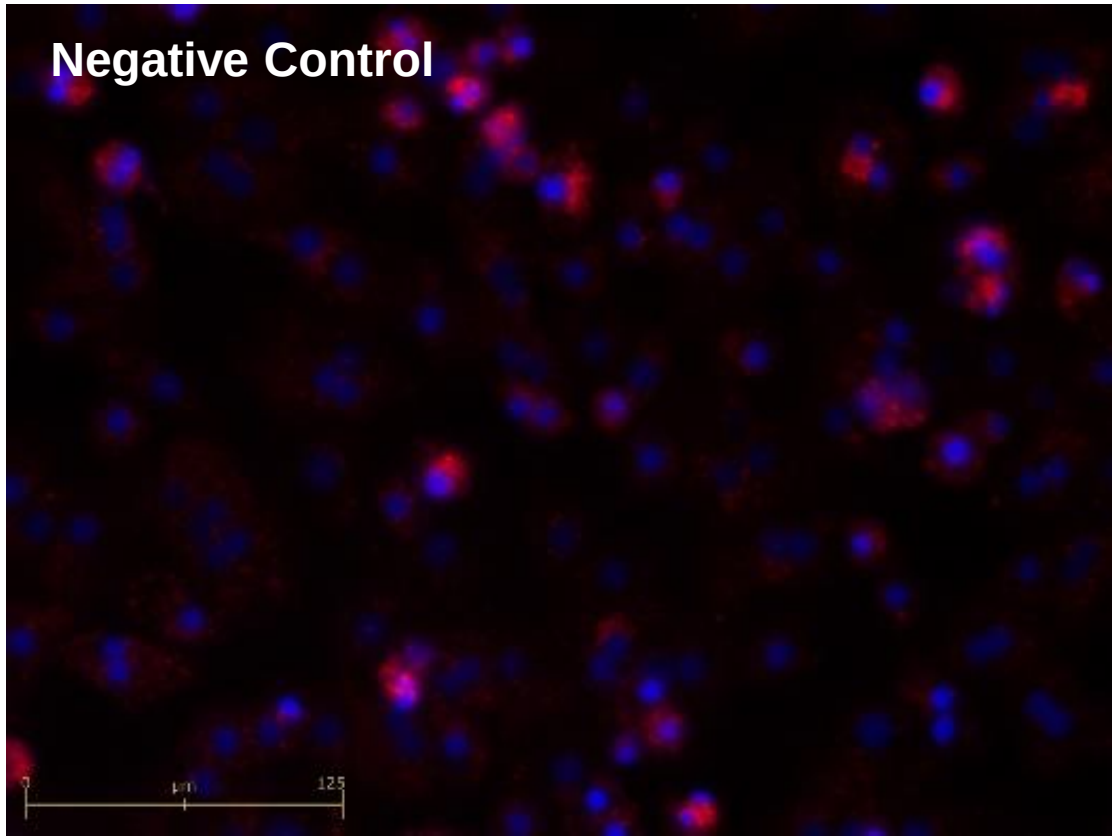
Multiplex Antibody-based ELISA

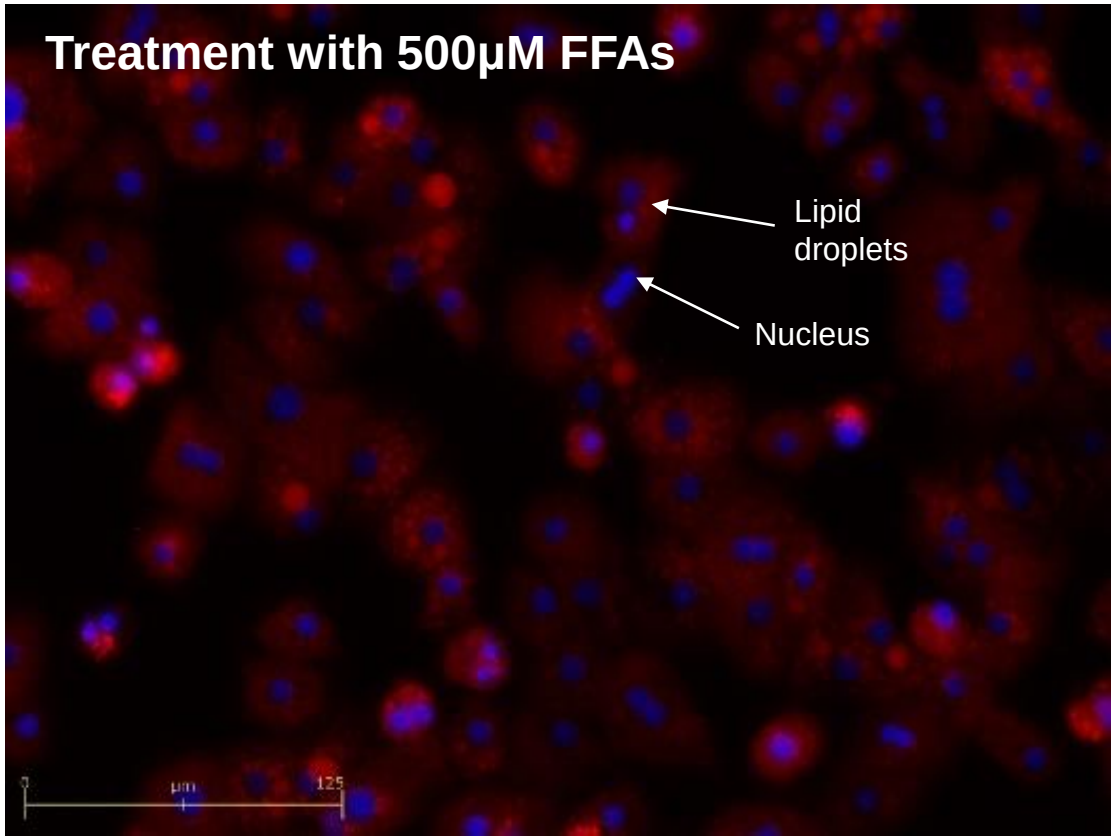


NAFLD induction - High content screening



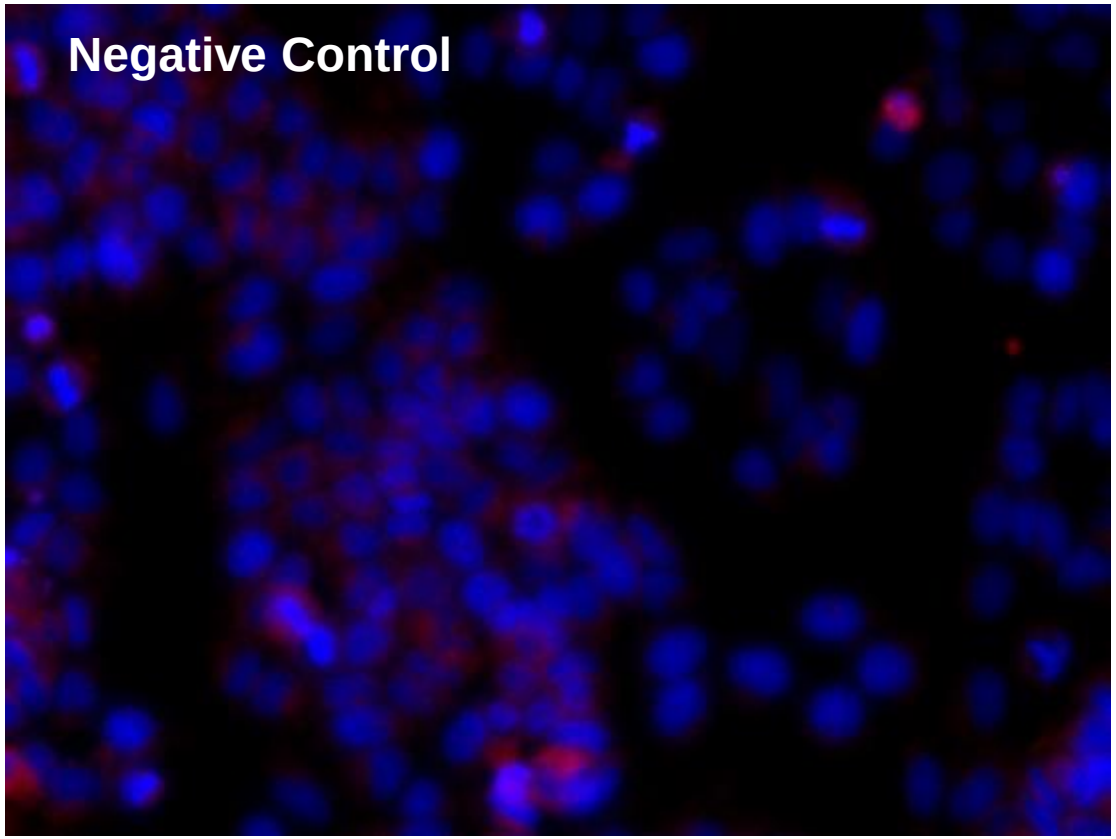
NAFLD induction with FFAs

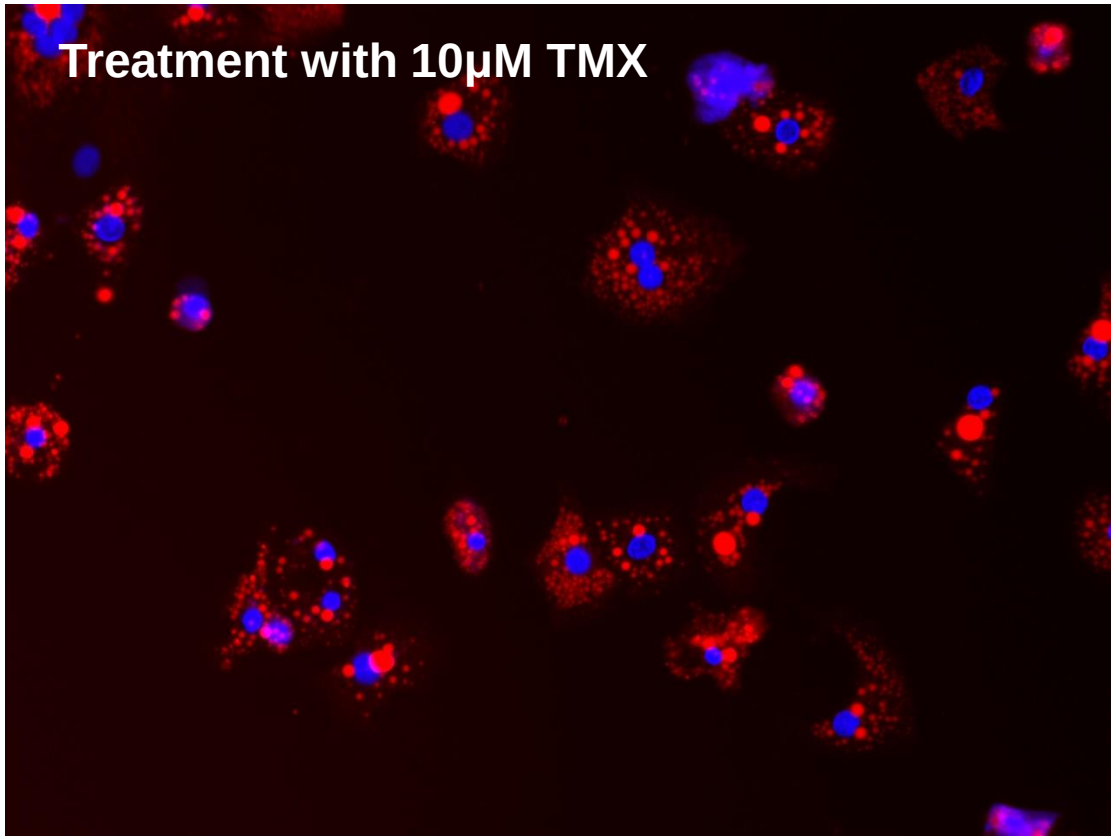




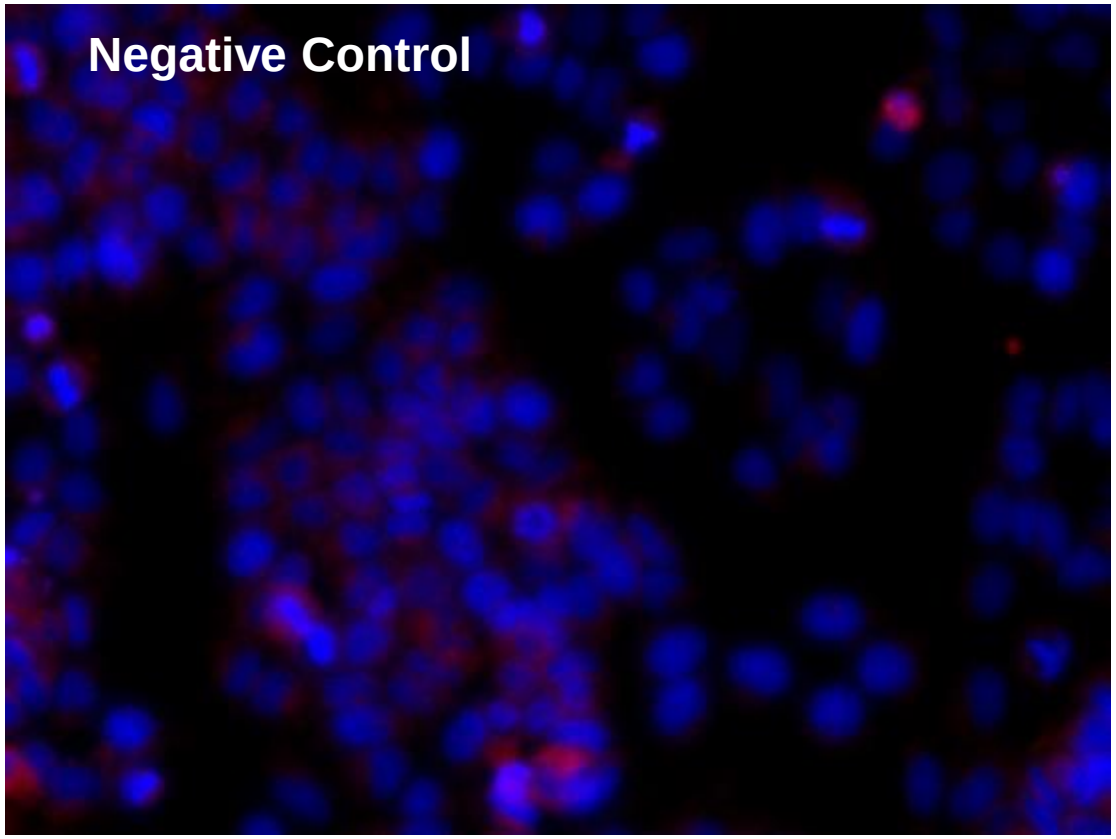
Lipid droplets were stained with Nile Red fluorescent probe and Hoechst 33342 was used for counterstaining cell nucleus.

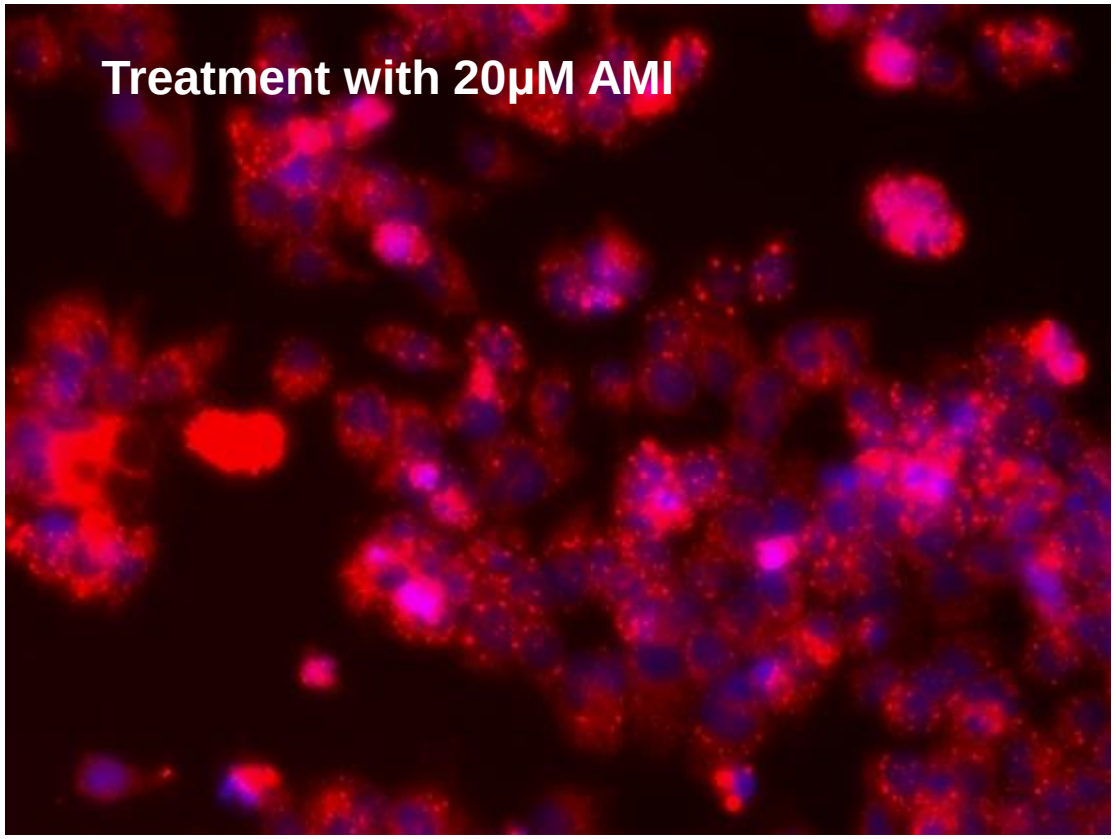
NAFLD induction with Tamoxifen



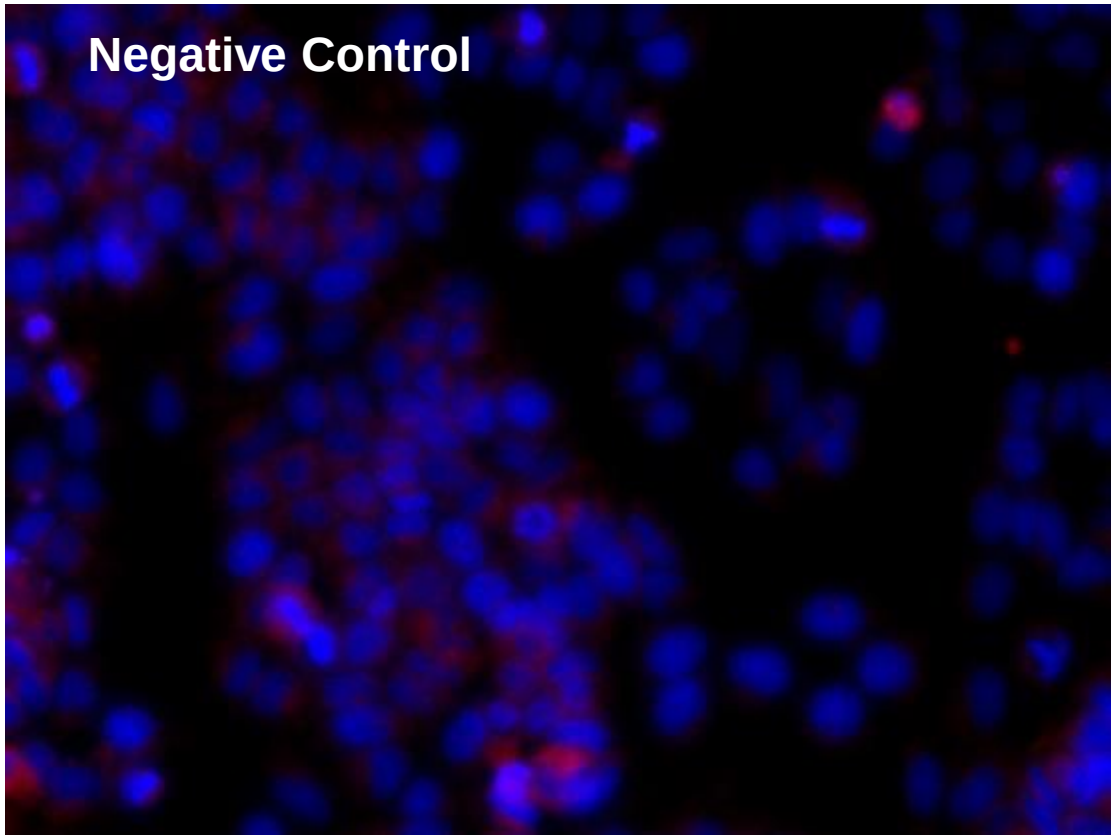


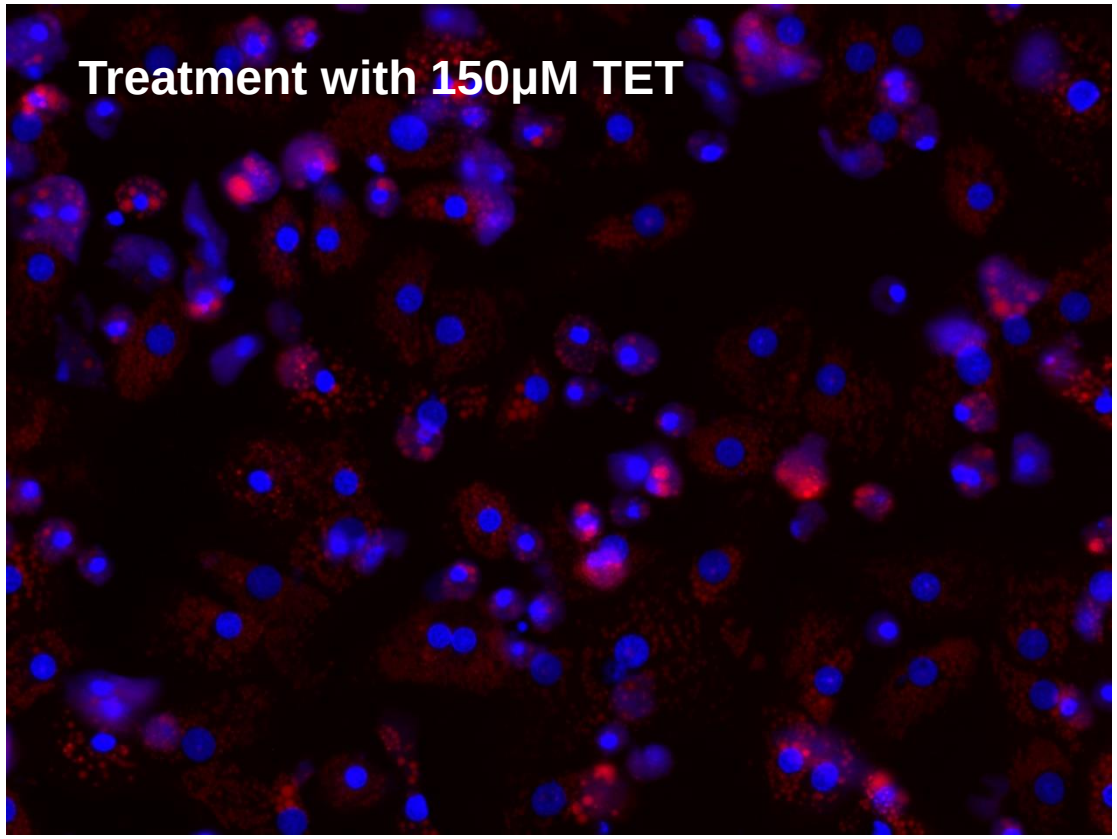
NAFLD induction with Amiodarone



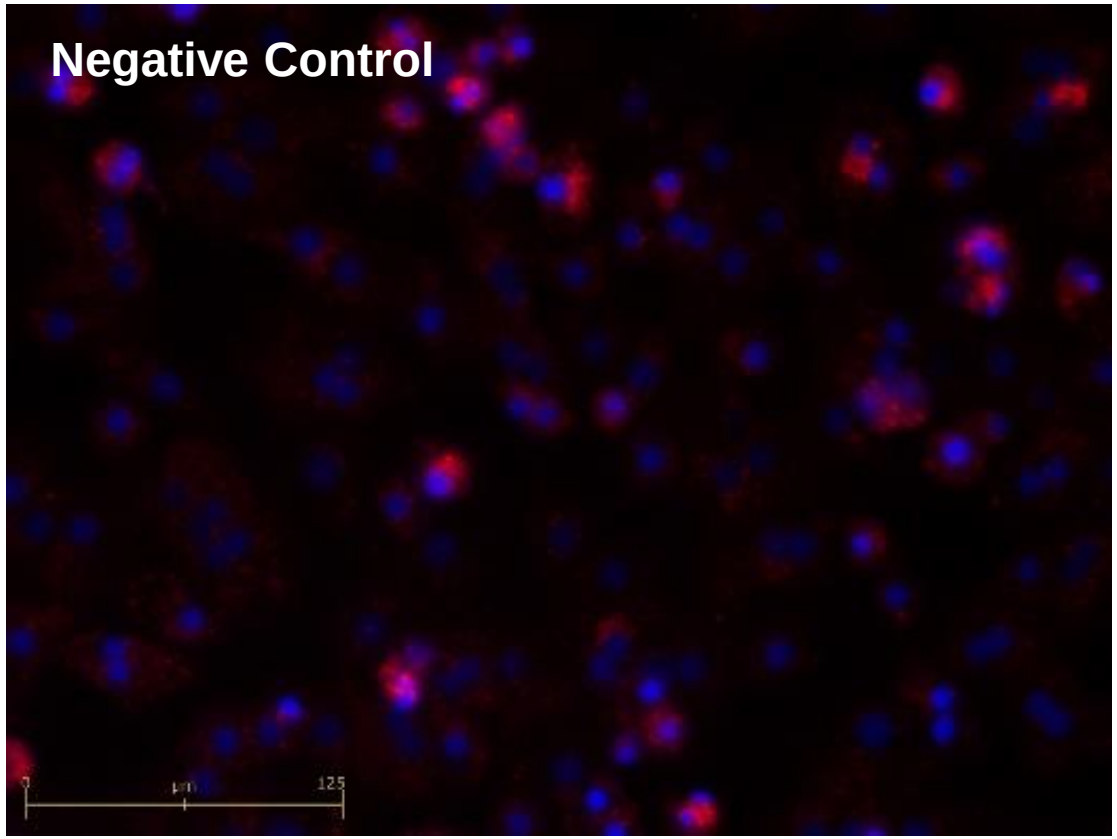


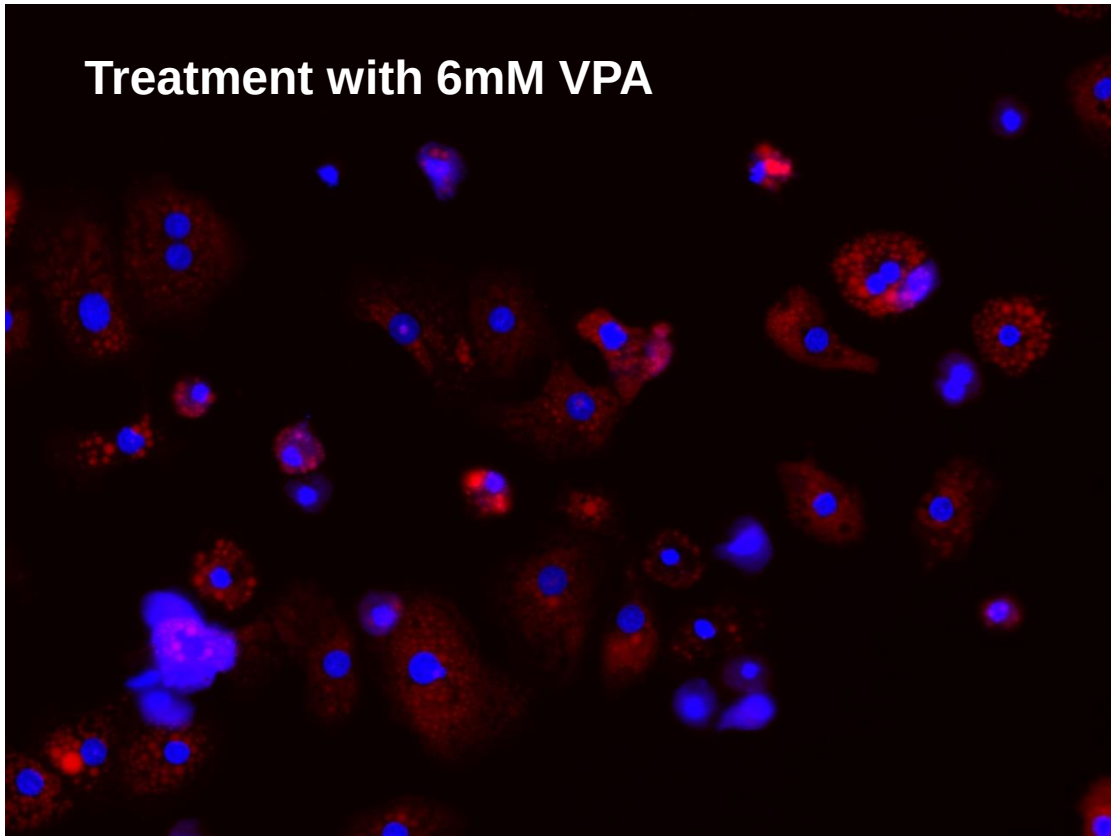
NAFLD induction with Tetracycline



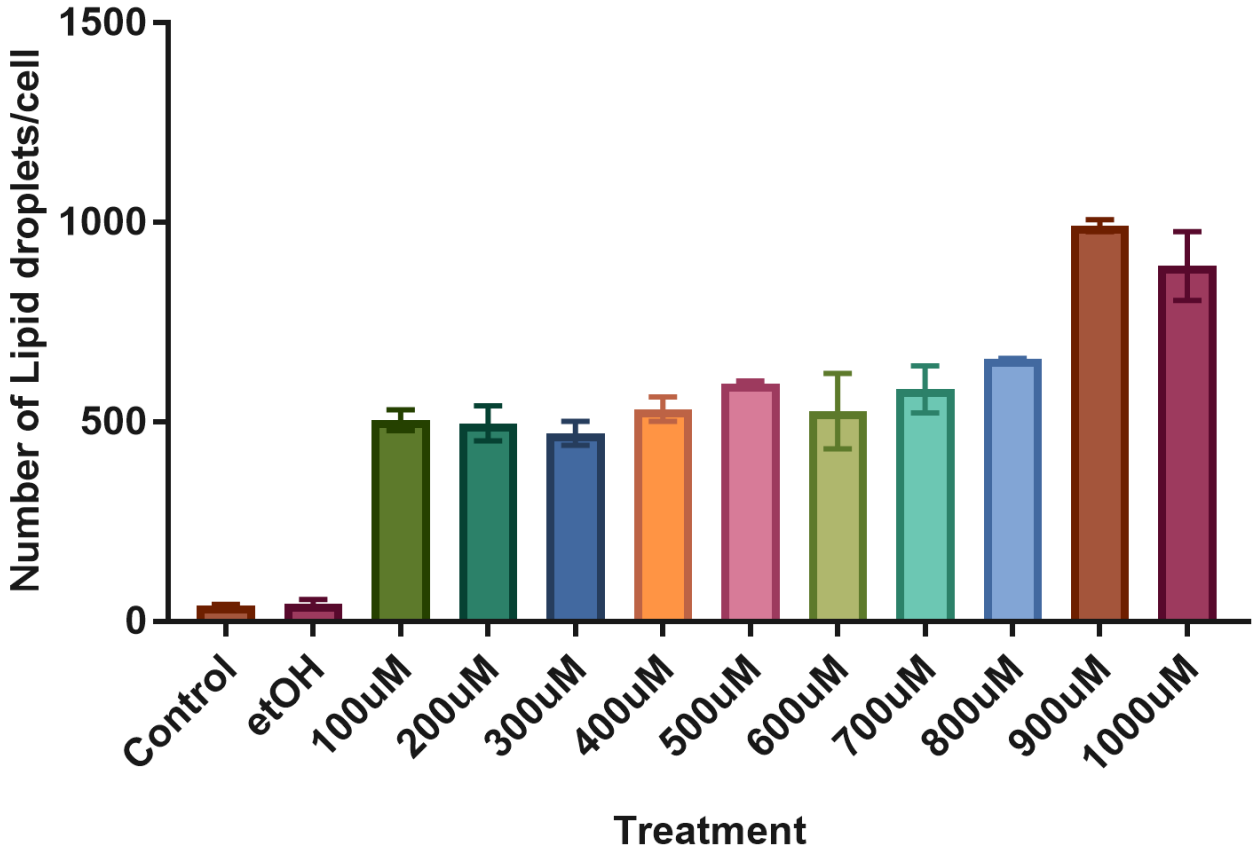


NAFLD induction with Valproic Acid





Quantification of intracellular fat accumulation



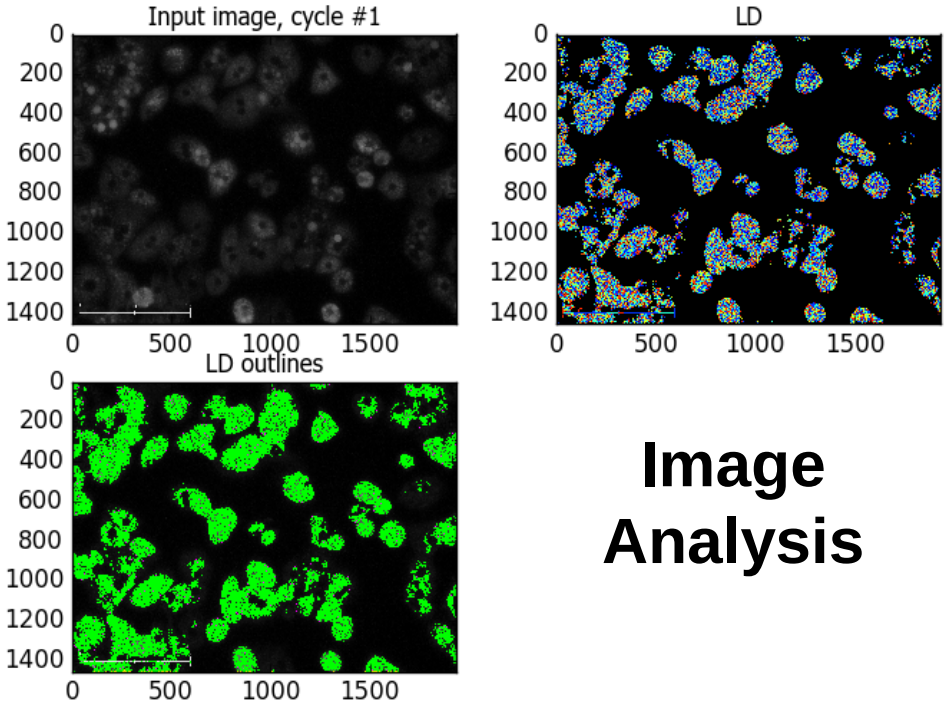
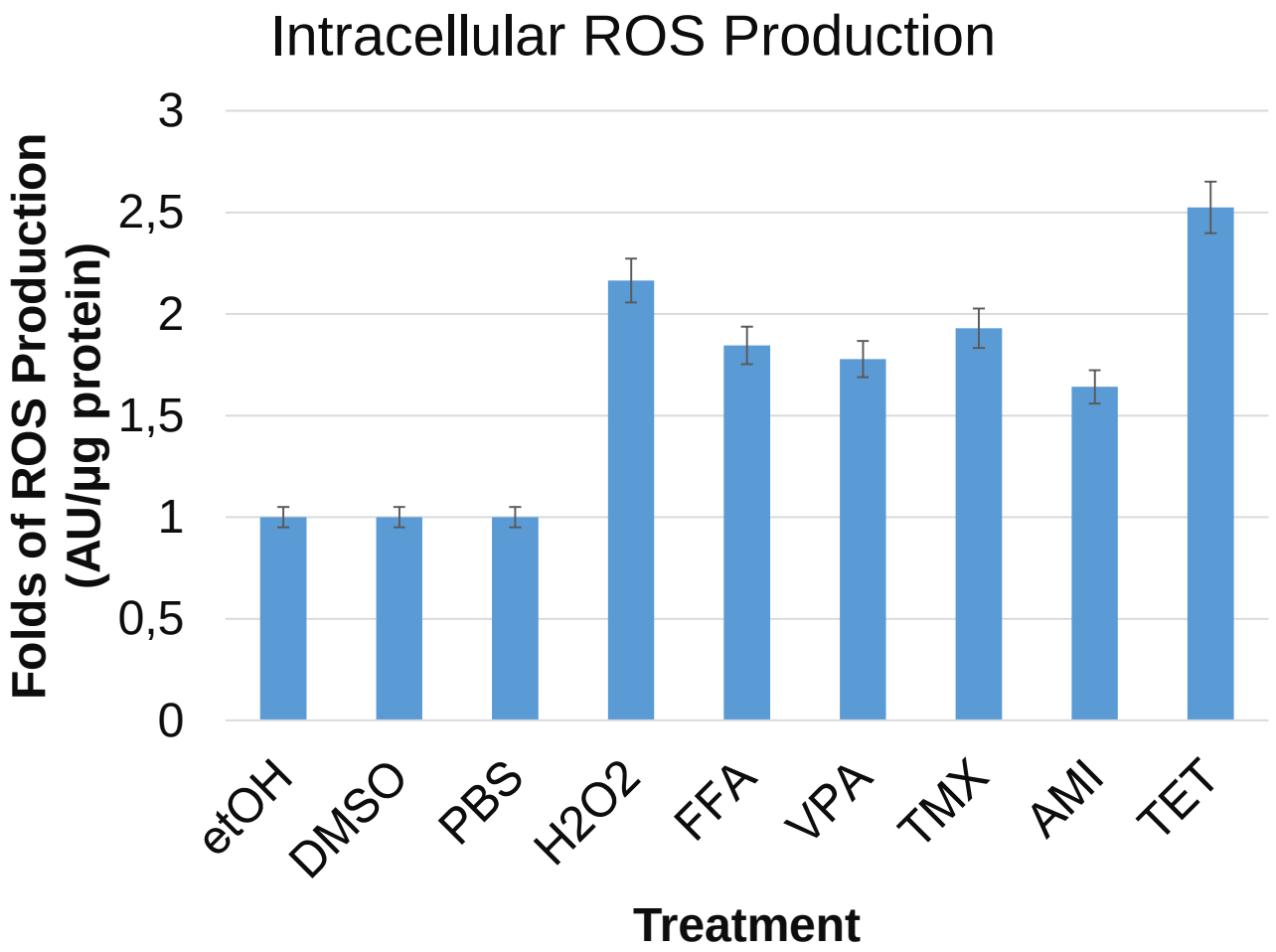
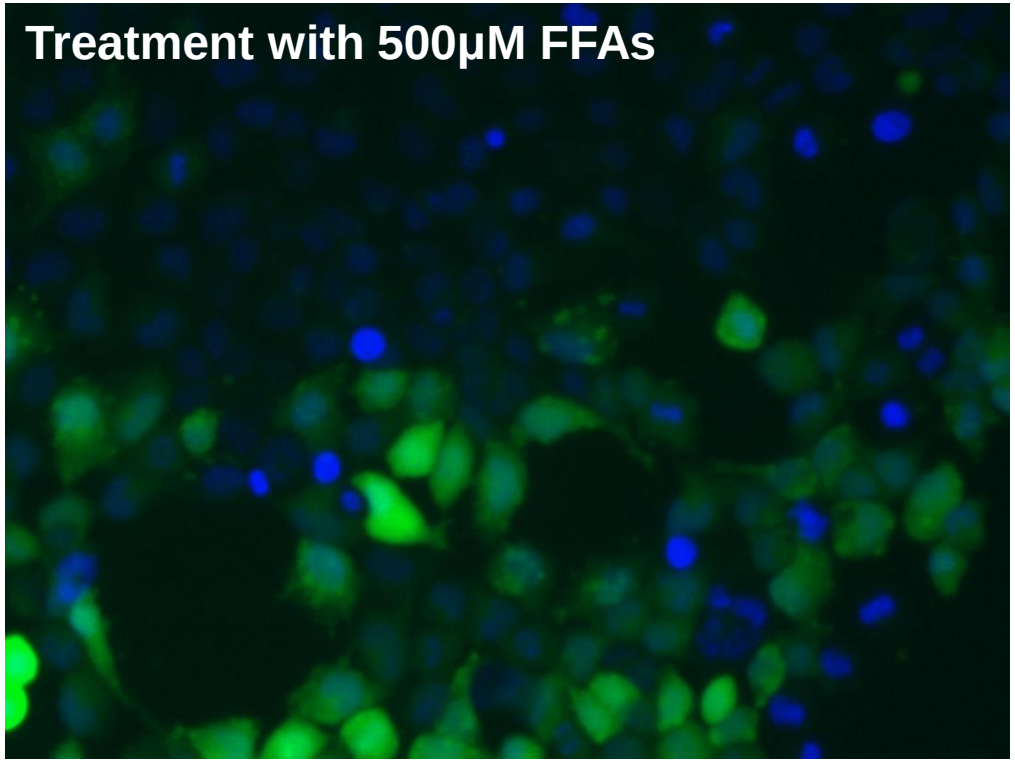


Image Analysis

Quantification of ROS Production

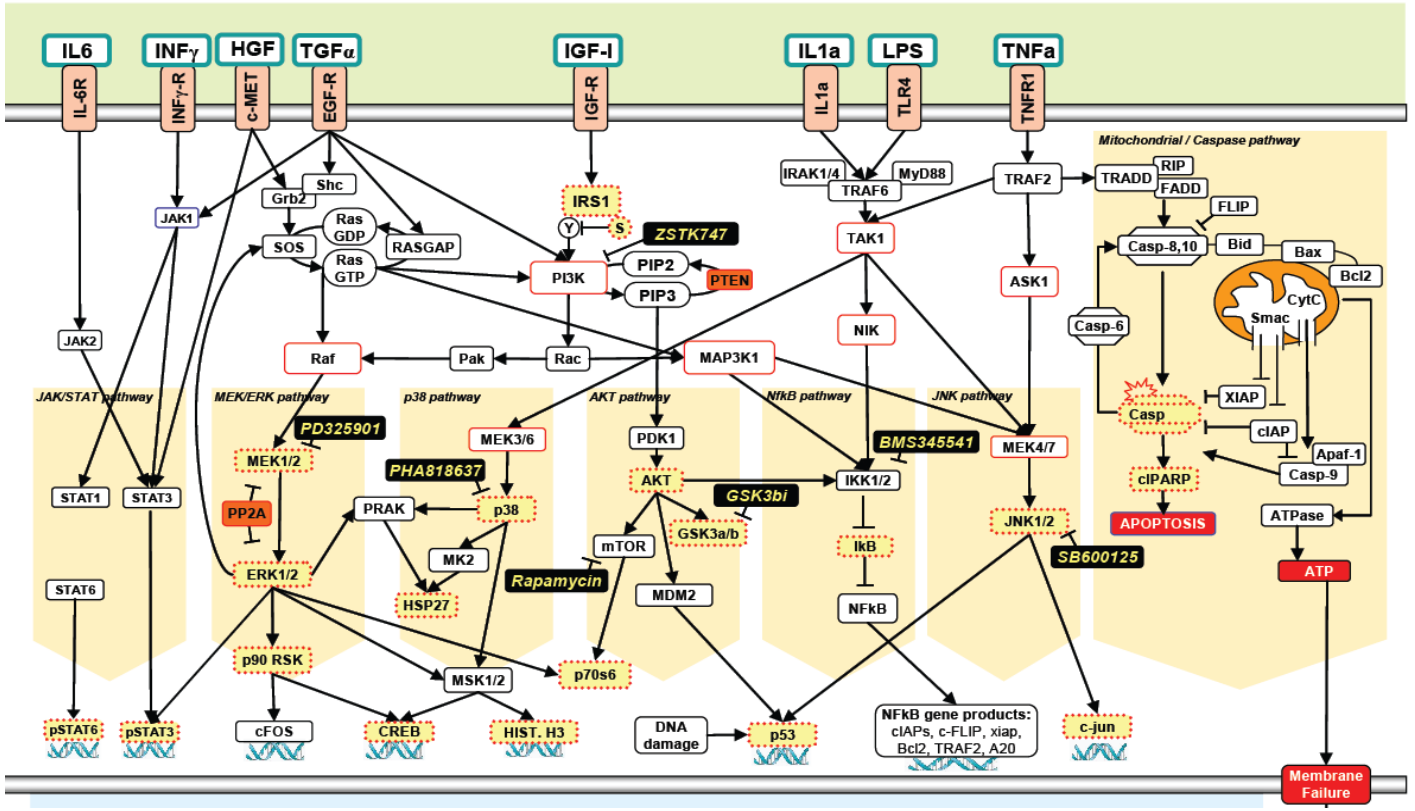




Phosphoproteomic and cytokine release profiling

NAFLD PHENOTYPE (Fat accumulation, ROS)

Measured phopshoprotein and cytokine signals



NAFLD MECHANISMS

Phosphoproteomic and cytokine release profiling

Secreted Proteins

FFA Treatment (µM)	CCL5	CXL10	IFNG	IL1A	IL20	IL6	IL8	TNF10	TNFA
100	0.87	0.84	0.70	0.93	0.91	0.71	1.09	**0.71**	0.88
200	0.97	1.01	0.87	0.96	0.98	1.11	1.16	**0.75**	0.83
300	0.89	0.76	**0.75**	1.00	1.00	0.99	1.18	0.78	0.83
400	0.90	0.66	**0.67**	0.77	0.76	0.46	0.95	**0.65**	0.96
500	0.89	0.76	**0.72**	0.95	0.98	0.73	1.36	0.78	0.87
600	0.91	0.80	**0.69**	0.96	0.96	0.70	1.34	**0.68**	0.82
700	0.85	0.78	**0.71**	0.94	0.92	0.79	1.43	**0.71**	**0.77**
800	0.85	0.71	0.61	0.91	0.93	0.72	1.44	**0.65**	**0.77**
900	0.84	0.68	**0.65**	0.97	0.90	0.68	1.41	**0.66**	**0.71**
1000	1.03	0.77	**0.73**	0.99	0.99	0.78	1.47	**0.75**	0.77

LEGEND	
value	CV < 20%
value	< 0.75

Phosphoproteins

FFA Treatment (µM)	AKT1	FAK1	HSBP1	IKBA	JUN	STAT6	WNK1
100	**0.67**	0.88	0.66	0.60	**0.73**	**0.75**	0.66
200	**0.65**	0.83	0.65	0.69	0.76	**0.75**	0.64
300	0.72	0.86	0.71	0.80	0.80	0.81	0.66
400	**0.64**	0.81	0.67	0.67	**0.73**	**0.67**	0.54
500	0.66	0.81	0.64	0.66	0.73	**0.68**	0.57
600	0.67	0.86	0.67	0.62	**0.75**	**0.66**	0.59
700	0.69	0.85	0.70	**0.68**	0.78	**0.73**	**0.66**
800	0.67	0.85	0.76	0.73	0.85	**0.71**	**0.61**
900	0.67	0.87	0.72	**0.70**	0.79	**0.73**	**0.60**
1000	0.62	0.91	0.75	**0.70**	**0.74**	**0.74**	**0.60**

Fold change of the proteomic measurements of three biological replicates at increasing concentrations of FFAs.

Differences between groups were compared by using Students *t* test.

Conclusion

1. We build *in vitro* model for NAFLD/NASH induced by different DRUS & FFAs

2. We quantified NAFLD by ROS and fat accumulation

3. We measured signaling effects on the hepatocyte network

4. We identifies decreased AKT (known) and Irregular phosphorylation patterns in IKBA, JUN, STAT6 and WNK1 as well as in the secretion of TNFA, TNF10 ad TNFG

MORE ANALYSIS UNDER DEVELOPMENT

Acknowledgments

Antoniou A Efstathios team & Konstadoulakis M Manoussos team

Protatonce Ltd.

Pliaka Vicky, Minia Angeliki, Rožanc Jan

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