Supplemental Information

Development of an In Vitro Human Liver System for Interrogating Non-Alcoholic Steatohepatitis

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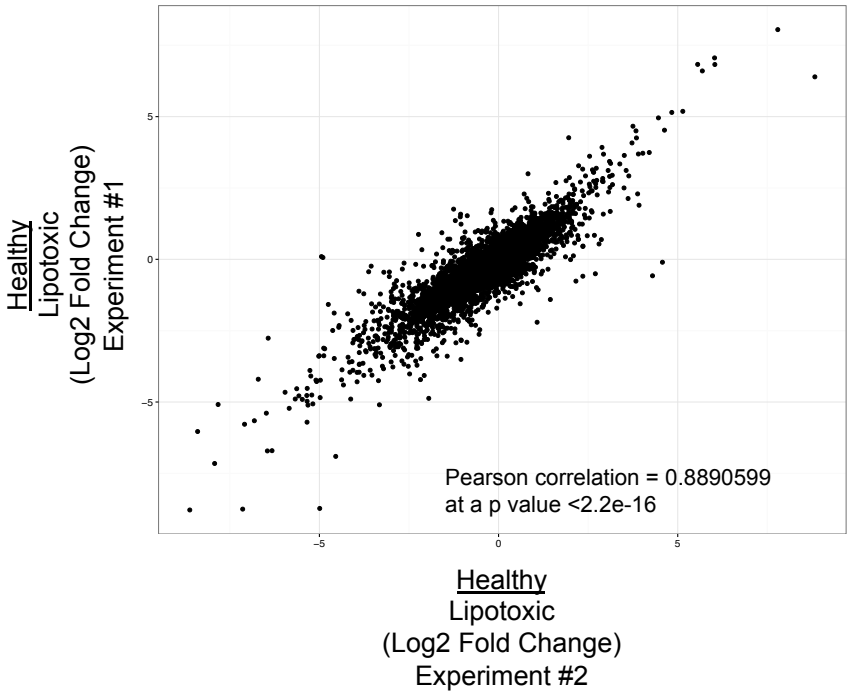
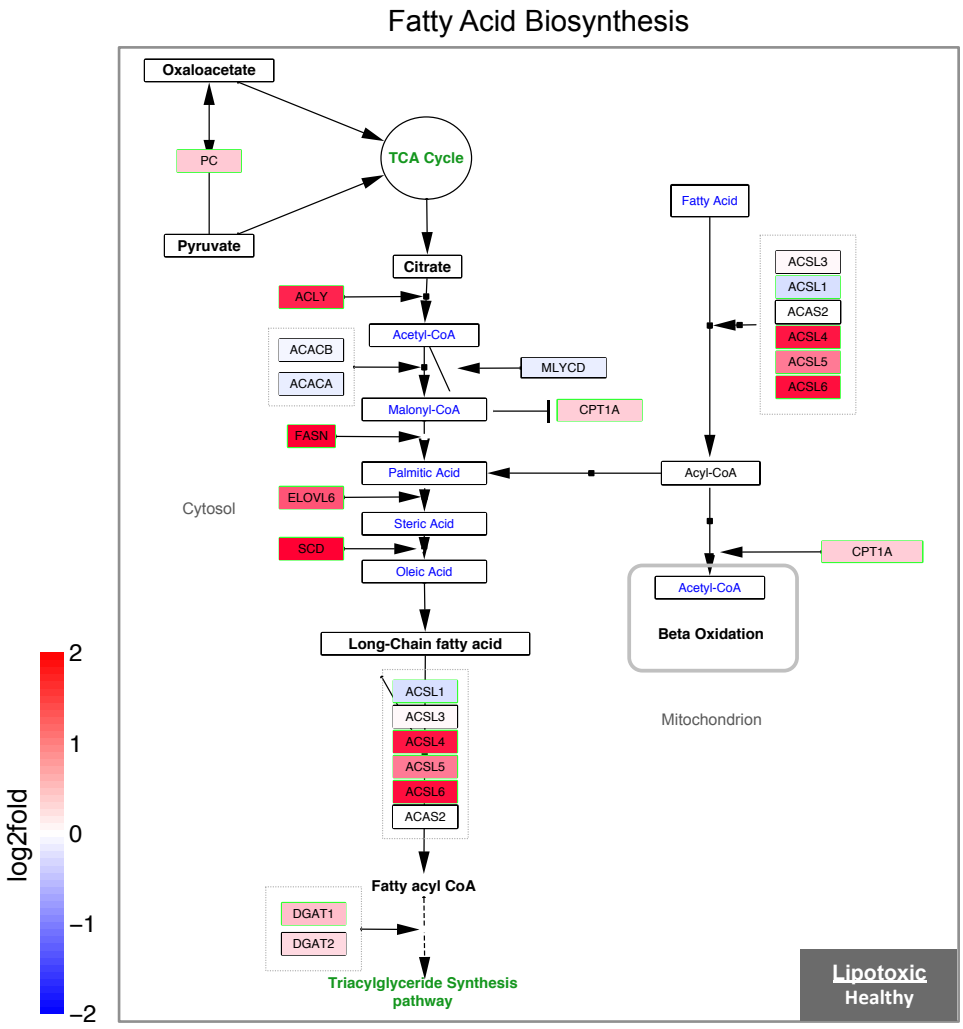
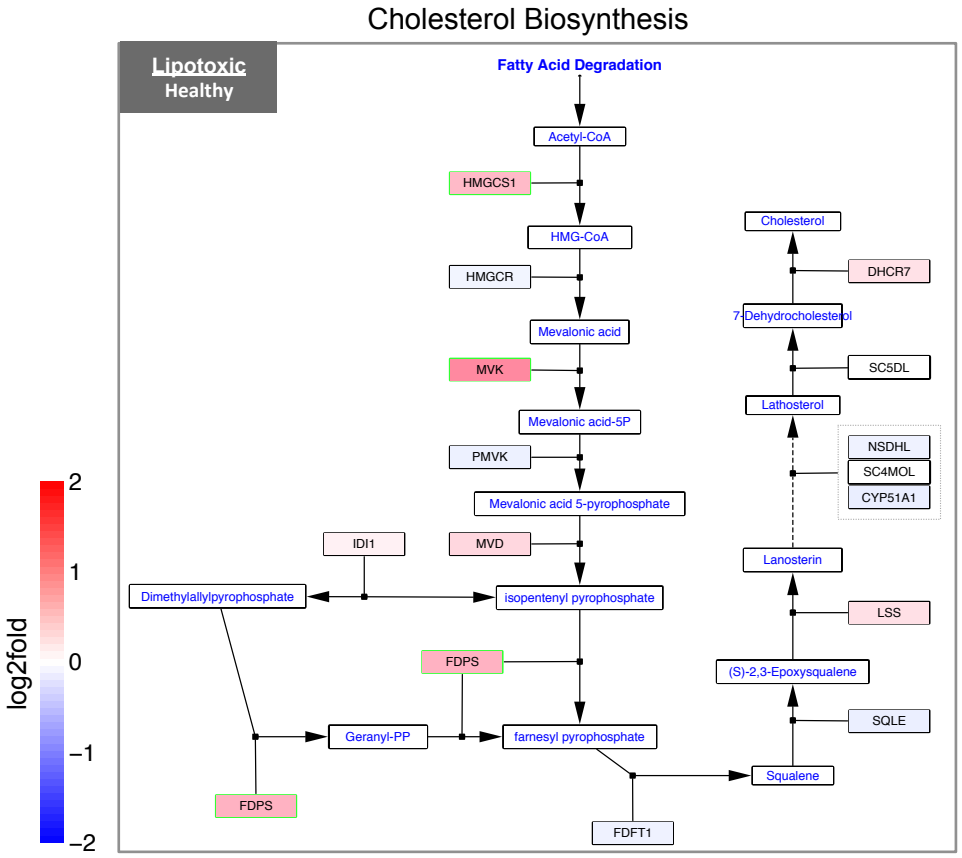
Supplemental Figures and Tables:



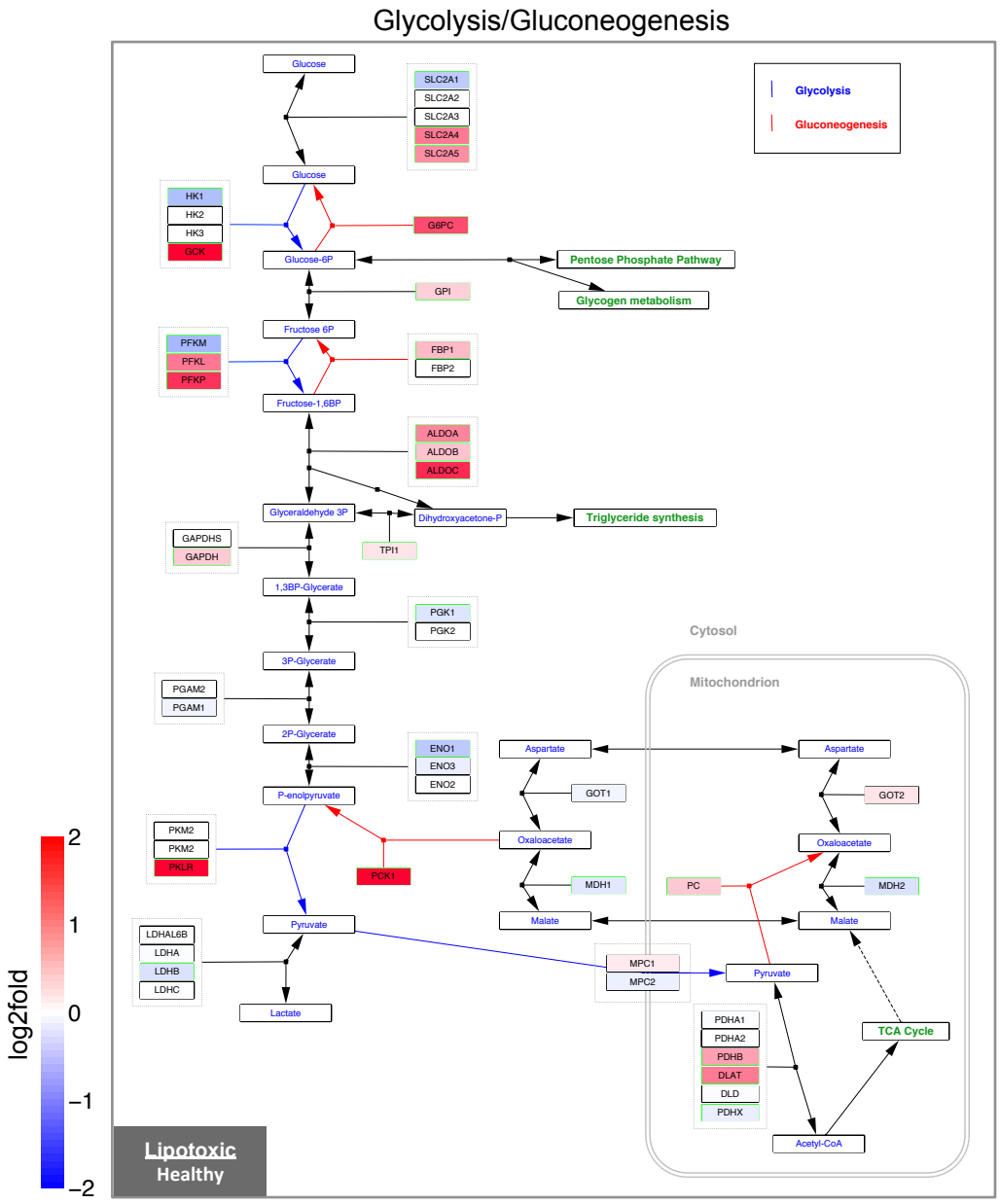
Supplemental Figure 1. Correlation plot demonstrating the reproducibility of data from different experiments in the lipotoxic system. Hepatocyte transcriptomic data of all genes (each gene is an individual dot) from two different experiments (#1 and #2) performed 2 months apart with *n*=3 and *n*=4 donors, respectively, is represented in this correlation plot. Comparison is healthy vs. lipotoxic + 0.1ng/ml TNFα milieu for each experiment. The scatterplot shows that genes from both experiments exhibit a strong positive correlation in gene response, and thus high reproducibility.



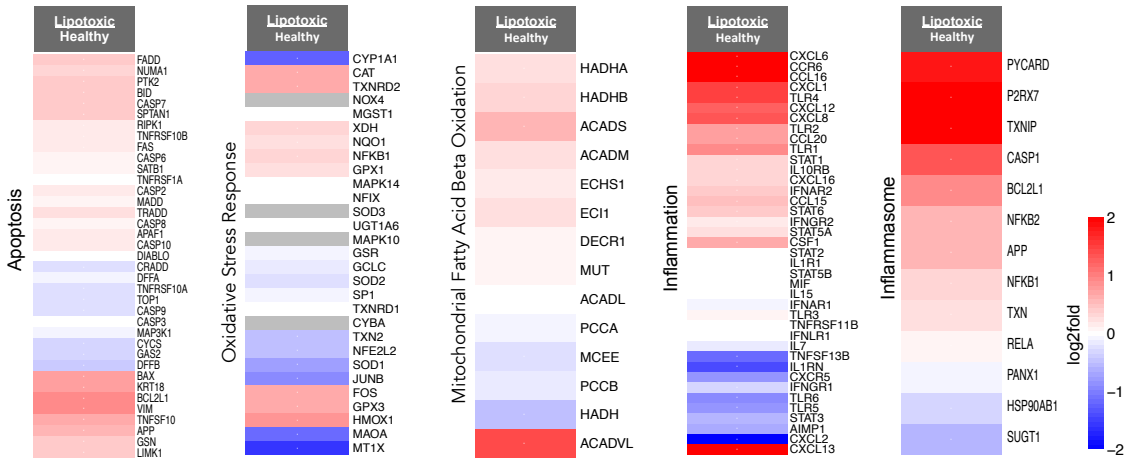
Supplemental Figure 2. Transcriptomic data from hepatocytes exposed to the lipotoxic compared to the healthy milieu is overlaid onto a diagram representing the fatty acid biosynthesis pathway. A red-filled box indicates the gene is upregulated while a blue-filled box indicates the gene is downregulated; the intensity of the color corresponds to greater log2fold-changes. Boxes with a green perimeter indicate the gene expression log2fold-change is significant (FDR<10%).



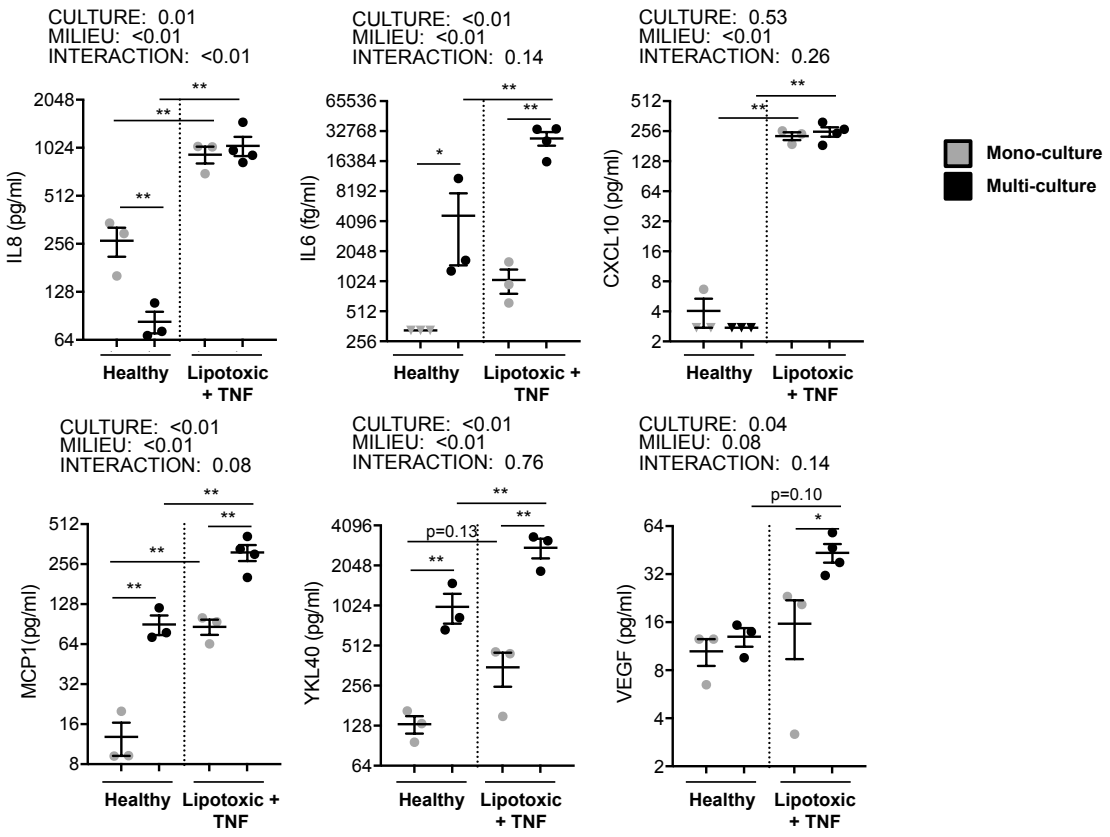
Supplemental Figure 3. Transcriptomic data from hepatocytes exposed to the lipotoxic compared to the healthy milieu is overlaid onto a diagram representing the cholesterol biosynthesis pathway. A red-filled box indicates the gene is upregulated while a blue-filled box indicates the gene is downregulated; the intensity of the color corresponds to greater log2fold-changes. Boxes with a green perimeter indicate the gene expression log2fold-change is significant (FDR<10%).



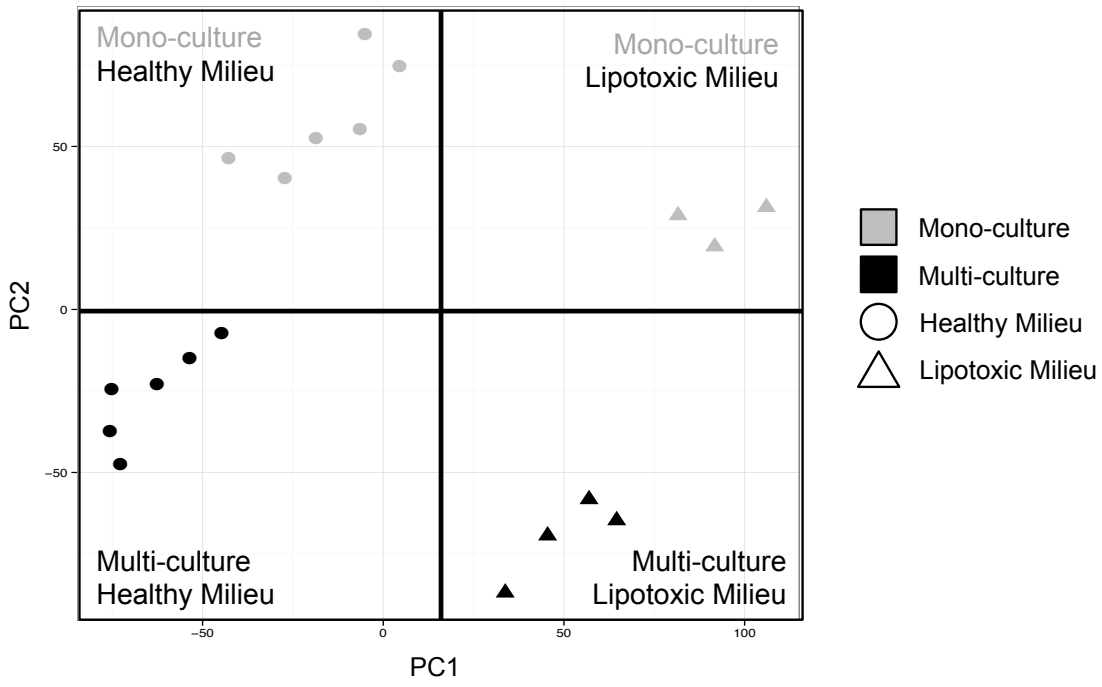
Supplemental Figure 4. Transcriptomic data from hepatocytes exposed to the lipotoxic compared to the healthy milieu is overlaid onto a diagram representing the glycolysis/gluconeogenesis pathway. A red-filled box indicates the gene is upregulated while a blue-filled box indicates the gene is downregulated; the intensity of the color corresponds to greater log2fold-changes. Boxes with a green perimeter indicate the gene expression log2fold-change is significant (FDR<10%).



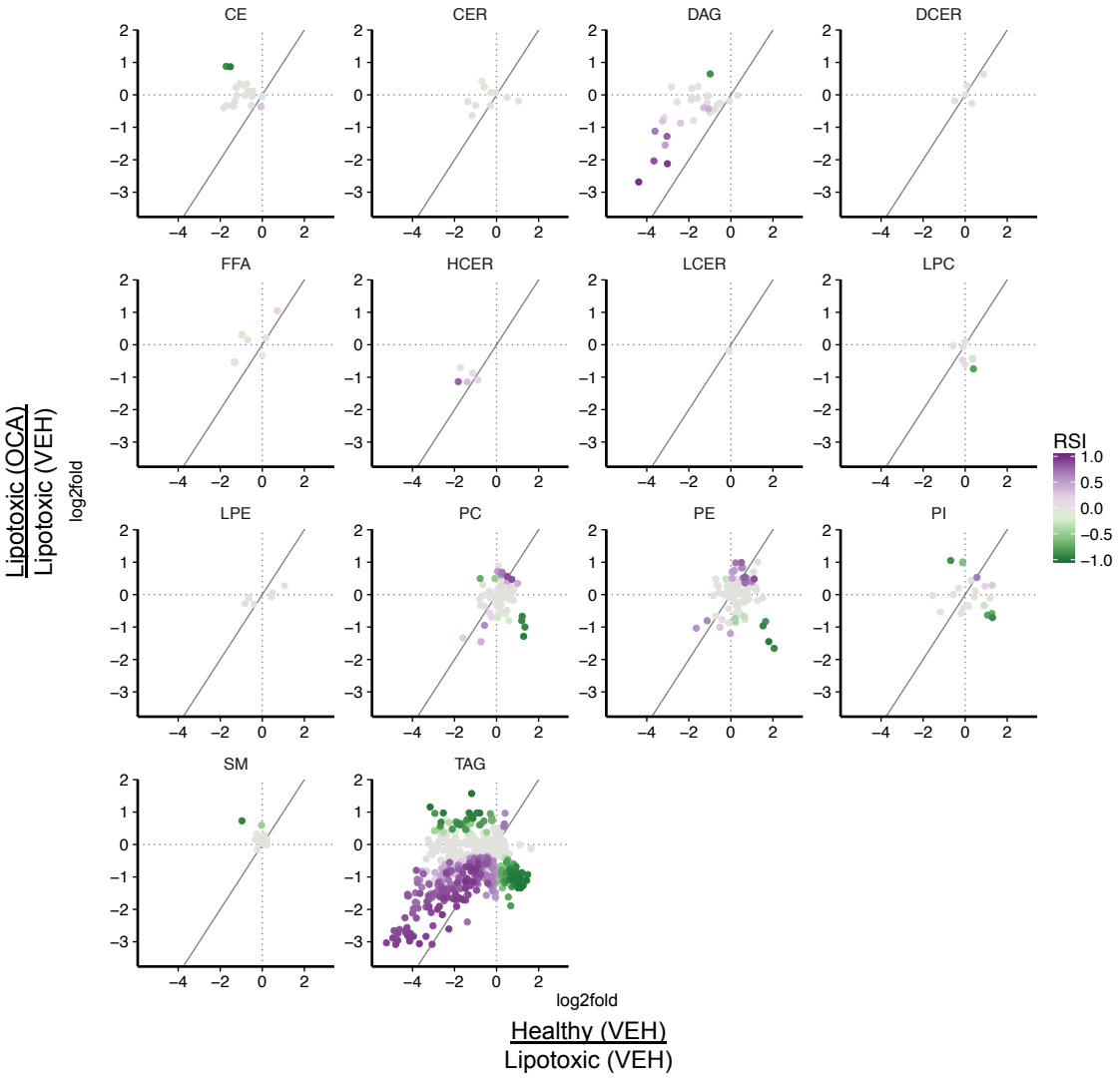
Supplemental Figure 5. Transcriptomic data from hepatocytes exposed to the lipotoxic compared to the healthy milieu is presented for various biological pathways associated with cellular stress and inflammation. Expression of each gene is represented as log2fold-change of lipotoxic vs. healthy milieu (red=upregulation, blue=downregulation). *n*=6 experiments, 3 donors.



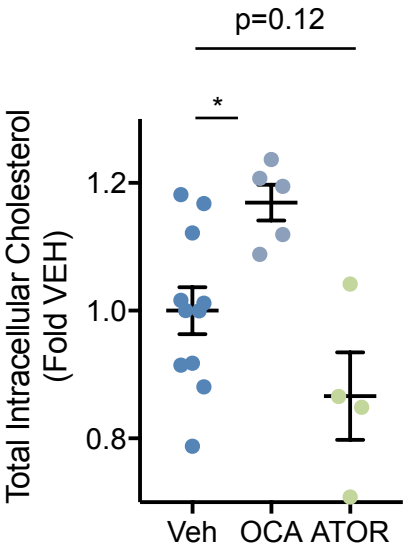
Supplemental Figure 6. The presence of non-parenchymal cells (NPCs) in the in vitro human liver system significantly impacts its inflammatory profile. Secreted analytes were measured in the media effluent from devices at day 10. The lipotoxic milieu for these experiments is composed of 65μM oleic acid and 0.1ng/ml TNFα. *n*=4 experiments, 1 donor. Triangles indicate samples were below lower limit of quantification. Tables above charts provide overall significance from 2-way ANOVA for cell culture and milieu treatment conditions and the interaction p-value. Multiple comparison were performed as indicated with *p<0.05, **p<0.01, 2-way ANOVA, Tukey.



Supplemental Figure 7. The presence of non-parenchymal cells (NPCs) in the in vitro human liver system significantly impacts the transcriptomic profile of hepatocytes. Transcriptomic data was acquired from hepatocytes exposed for 10 days to a lipotoxic milieu composed of 65μM oleic acid and 0.1ng/ml TNFα in the presence or absence of NPCs. *n*=3-6 experiments, 1 donor. Gene expression profile reveals that the hepatocytes from healthy and lipotoxic treatments separate along principle component 1, and these further separate along principle component 2 if co-cultured with NPCs.



Supplemental Figure 8. OCA treatment in the lipotoxic milieu promotes a change in lipid profile towards the healthy controls. Lipids from hepatocytes from device exposed to the healthy or lipotoxic milieu with 0.5μM OCA or vehicle control were measured by metabolomics. Scatterplot representation of differentially expressed lipids in these hepatocytes are shown as log2fold-change and colored by RSI. *n*=4 experiments, 2 donors. Each lipid species is represented in an individual plot: CE (cholesterol ester), CER (ceramide), DAG (diacylglycerol), DCER (dihydroceramide), FFA (free fatty acid), HCER (hexosylceramide), LCER (lactosylceramide), LPC (lysophosphatidylcholine), LPE (lysophosphatidylethanolamine), PC (phosphatidylcholine), PE (phosphatidylethanolamine), PI (phosphatidylinositol), SM (sphingomyelin), TAG (triacylglycerol).



Supplemental Figure 9. Obeticholic acid (OCA) increases total intracellular cholesterol levels. Total intracellular cholesterol was measured from hepatocytes from the device exposed to vehicle control, 0.5μM OCA, or 300nM atorvastatin (ator) in the lipotoxic milieu for 10 days and represented as fold change relative to vehicle controls. Atorvastatin serves as a positive control as it inhibits HMG-CoA reductase, the rate-limiting enzyme of the cholesterol-producing mevalonate pathway. *n*=4 experiments, 4 donors. *p<0.05, student's 2-tailed t-test.

	In Vitro Model	Figure/Table	References / Reviews
Steatosis / Lipogenesis	**Yes**	Fig 2	1-3
Hypertriglycermia	**Yes**	Table 2	2-5
Increased Cholesterol	**Yes**	Table 2	2-5
Increased Glucose Output	**Yes**	Fig 3A	2,3 6
Insulin Resistance	**Yes**	Fig 3	1-3,6,7
Cell Stress / Elevated ALT	**Yes**	Fig 4	1-3,7,8
Inflammatory Signaling	**Yes**	Fig 4	2,3,7,8
Increased Apoptosis	**Yes**	Fig 4	2,3,7-9
Histological Features (e.g. ballooning)		NA	1-3
Early Fibrosis	**Yes**	Fig 5	2,3,7,10
Advanced Fibrosis		Fig 5	2,3,10
Cirrhosis		NA	2,3,10
Oncogenesis		NA	2,3

Supplemental Table 1. Pathological features of NAFLD in the *in vitro* human liver lipotoxic system.

Donor	Donor ID	Age	Gender	BMI	Vendor
1	HC5-30	21	Male	25	XenoTech
2	QHuf14024	55	Female	29	QPS
3	QHuf15028	58	Female	21	QPS
4	HC3-31	42	Female	31	XenoTech
5	QHu13035	39	Male	30	QPS

Supplemental Table 2. Hepatic donors used in this study with their sourcing details and demographic information.

Supplemental References:

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