Hepatotoxic Response in 2D and 3D Co-culture Models Differs From Hepatocyte-alone Models

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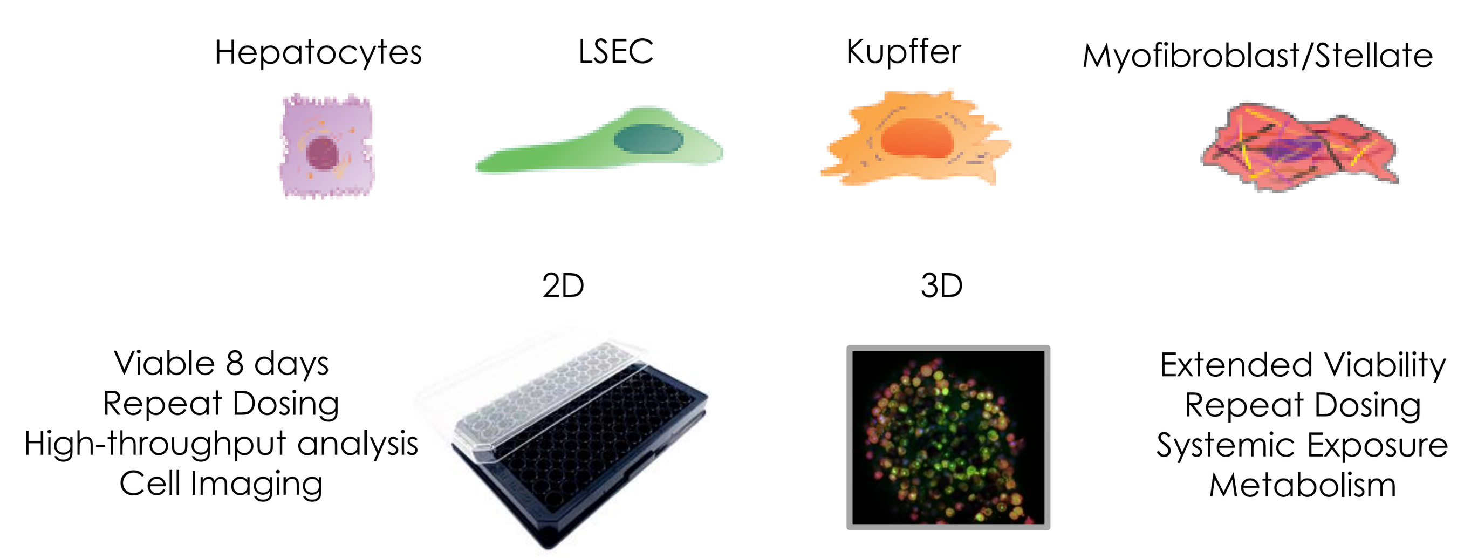
Abstract

Safety assessment strategies that rely on testing species-relevant cells in vitro are advancing, yet there are currently no in vitro alternatives for hepatotoxicity testing accepted by regulators. An organotypic rat model, capable of predicting rat in vivo responses to hepatotoxicants, will provide an affordable and ethical alternative to in vivo rat models currently utilized. An ideal in vitro liver model for hepatotoxicity testing would include hepatocytes and the non-parenchymal cells (NPCs) (i.e., hepatic stellate cells, Kupffer cells, and liver sinusoidal endothelial cells), and would support hepatocyte viability, phenotypic maintenance, and metabolic competence for an extended time in culture to allow for repeated exposures and long-term dosing. The current studies have focused on developing organotypic two-dimensional 96-well plate-based and three-dimensional alginate bead-based culture systems that include primary rat hepatocytes and NPCs and can support hepatocyte viability in vitro out to eight days in 2D and beyond 21 days in 3D. Using several canonical hepatotoxicants, we have compared 2D and 3D hepatocyte-alone (mono-) and hepatocyte and NPC (co-) culture systems to determine the robustness of these models. After treatment with acetaminophen (APAP; 1 mM, 5 mM, 10 mM, 15 mM 20 mM) for 3 consecutive days, APAP-induced cytotoxicity was significantly increased in the mono-culture model, compared to the co-culture model. Transcriptomic analysis of 2D mono- and co-culture models revealed that co-culture response to phenobarbital (10 μM) is more representative (32% increase in similarity of the ontology enrichment) of in vivo responses (using OPEN TG_GATEs in vivo data, 300 mg/kg, 24 h). These studies have demonstrated that the organotypic model can recapitulate rodent in vivo liver phenotypes observed in response to canonical hepatotoxicants and suggest that the co-culture model could be useful for testing the effects of compounds in vitro as an early stage alternative to in-life studies.

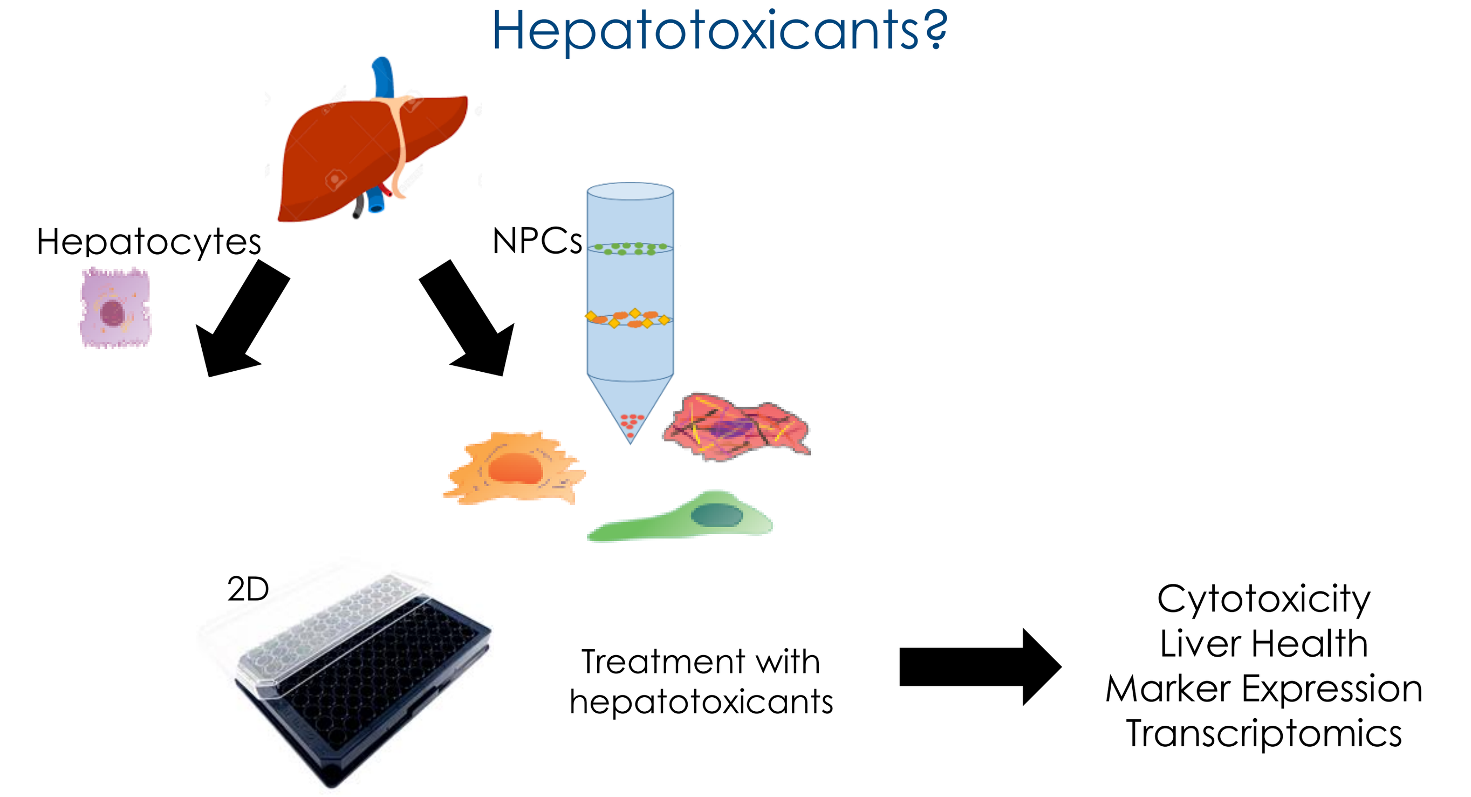
Background and Approach

In Vitro Rat Hepatotoxicity Assay Development

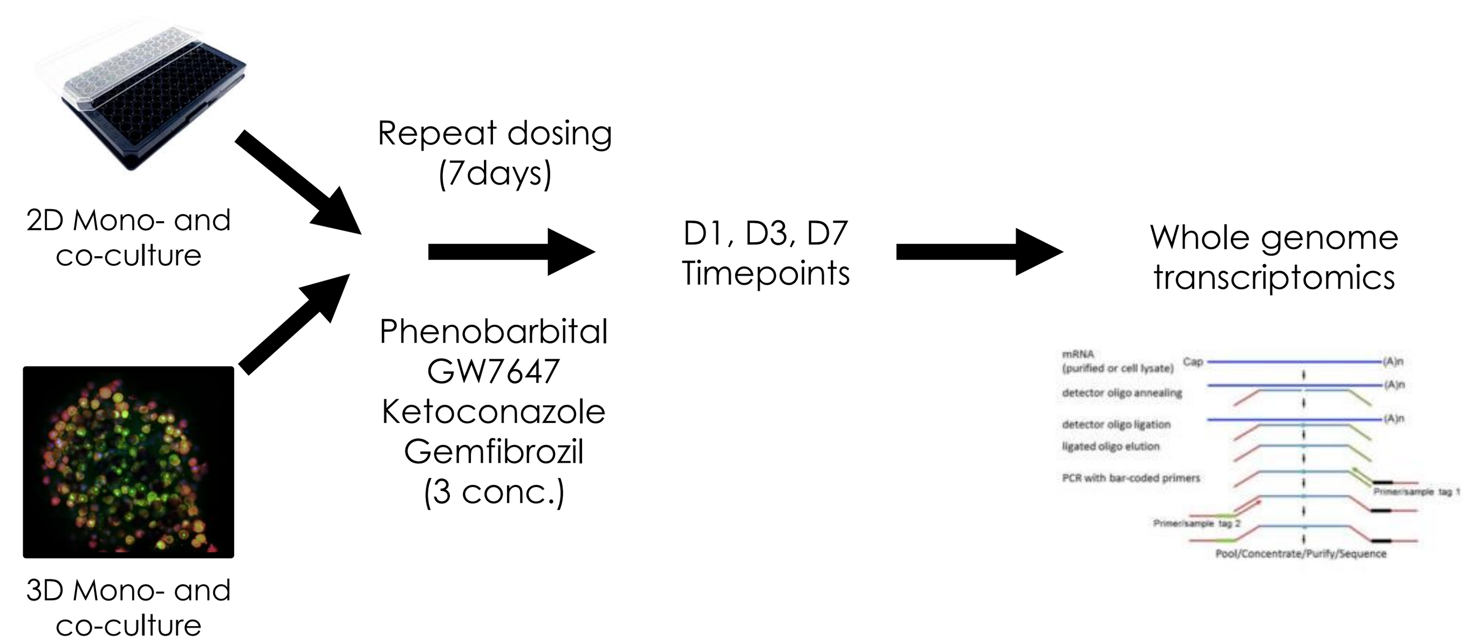
Goal: To include non-parenchymal cells into 2D or 3D hepatocyte cultures and more accurately model species-specific in vivo responses to chemical insult



Aim 1: Does Co-Culture Improve Response to Known Hepatotoxicants?

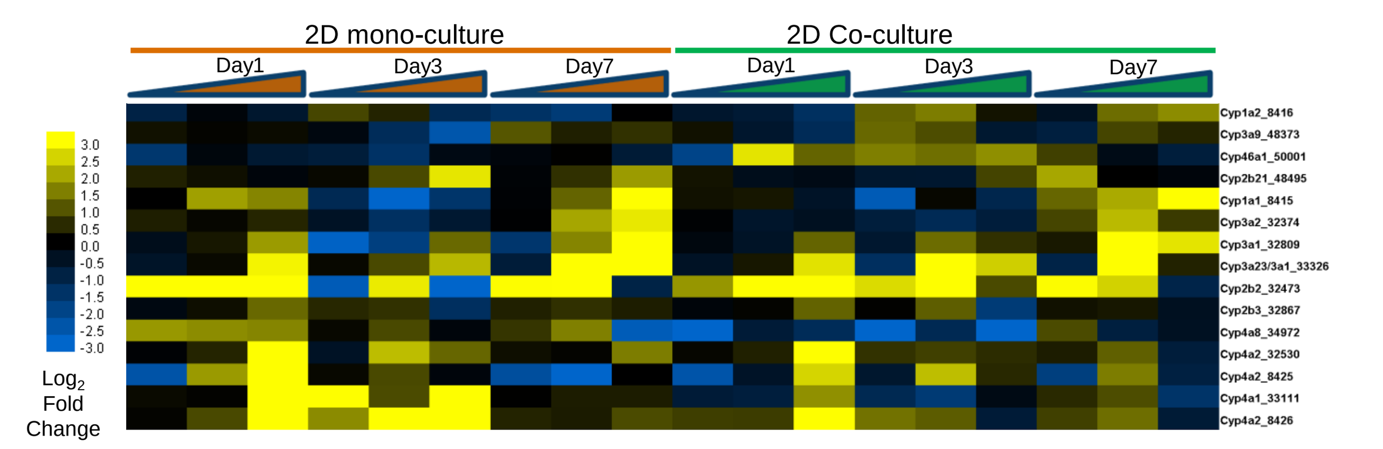


Aim 2: Does Increased Complexity Alter Transcriptomic Response?



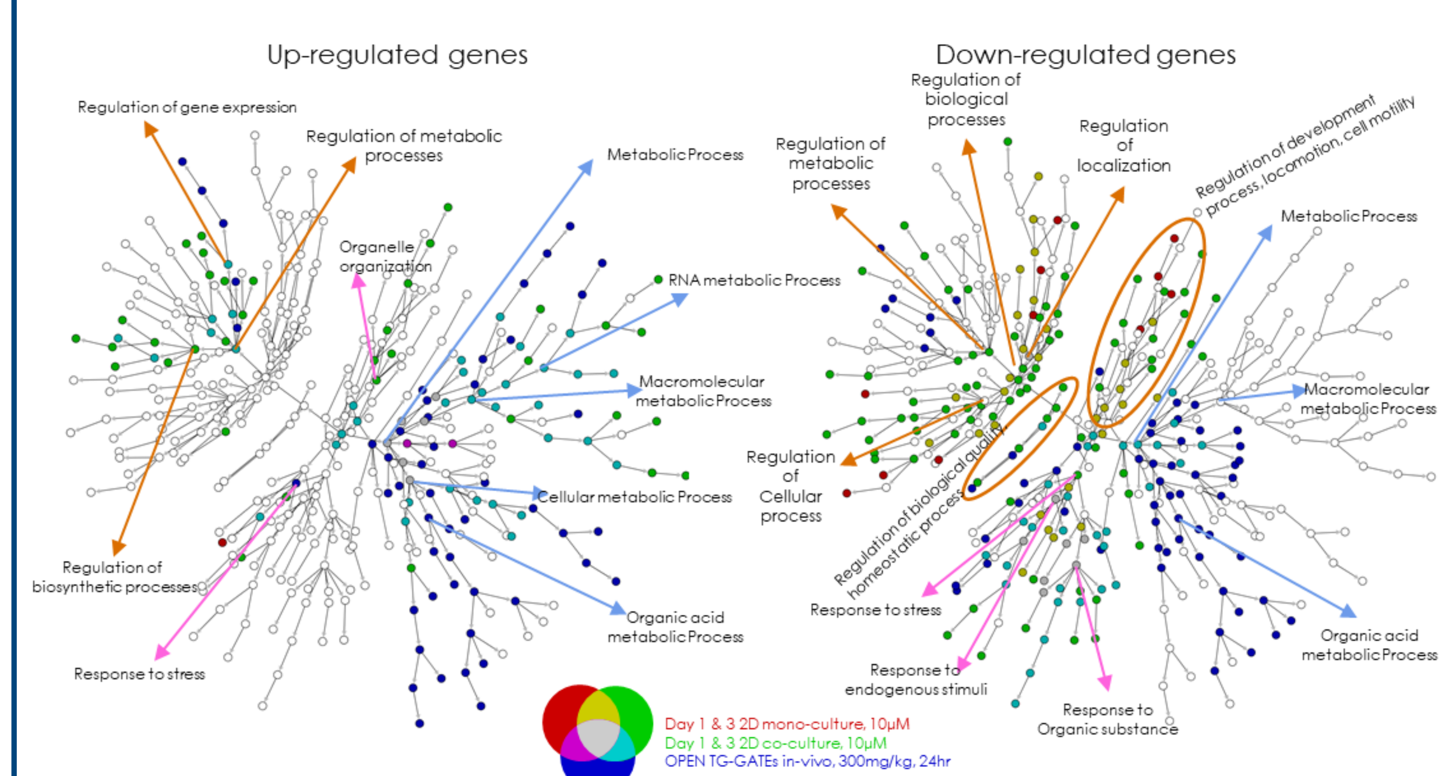
Results

Gene Expression Induction in CYP450s upon exposure to Phenobarbital



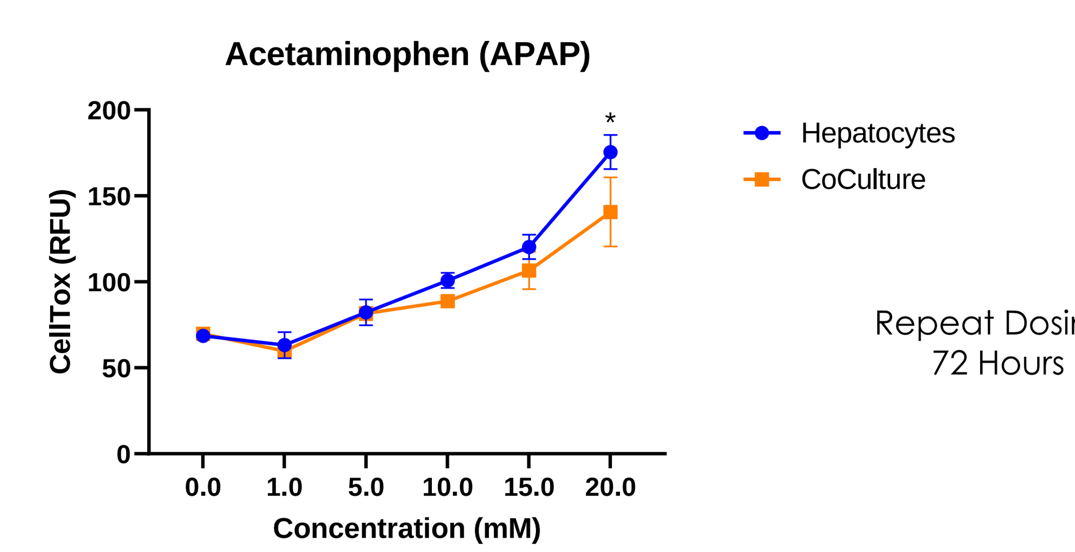
Single Dose of Phenobarbital (0.1mM, 1mM, and 10mM)

Co-Culture Response to Phenobarbital is More Representative of In Vivo Responses



Overlap in ontology enrichment with 2D mono- and co-culture systems and OPEN TG-GATEs in vivo single dose exposure for phenobarbital. Red indicates enrichment using the union of up- or downregulated genes from days 1 and 3 from the 2D mono-culture system, while green indicates enrichment using the analogous lists from the 2D co-culture system. Blue indicates enrichment using genes from OPEN TG-GATEs microarray experiments from in vivo rat liver samples after exposure to a single dose (gavage) at 300 mg/kg and 24 hours.

APAP-induced Toxicity is Increased in 2D Mono-culture compared to Co-culture after Repeat Dosing

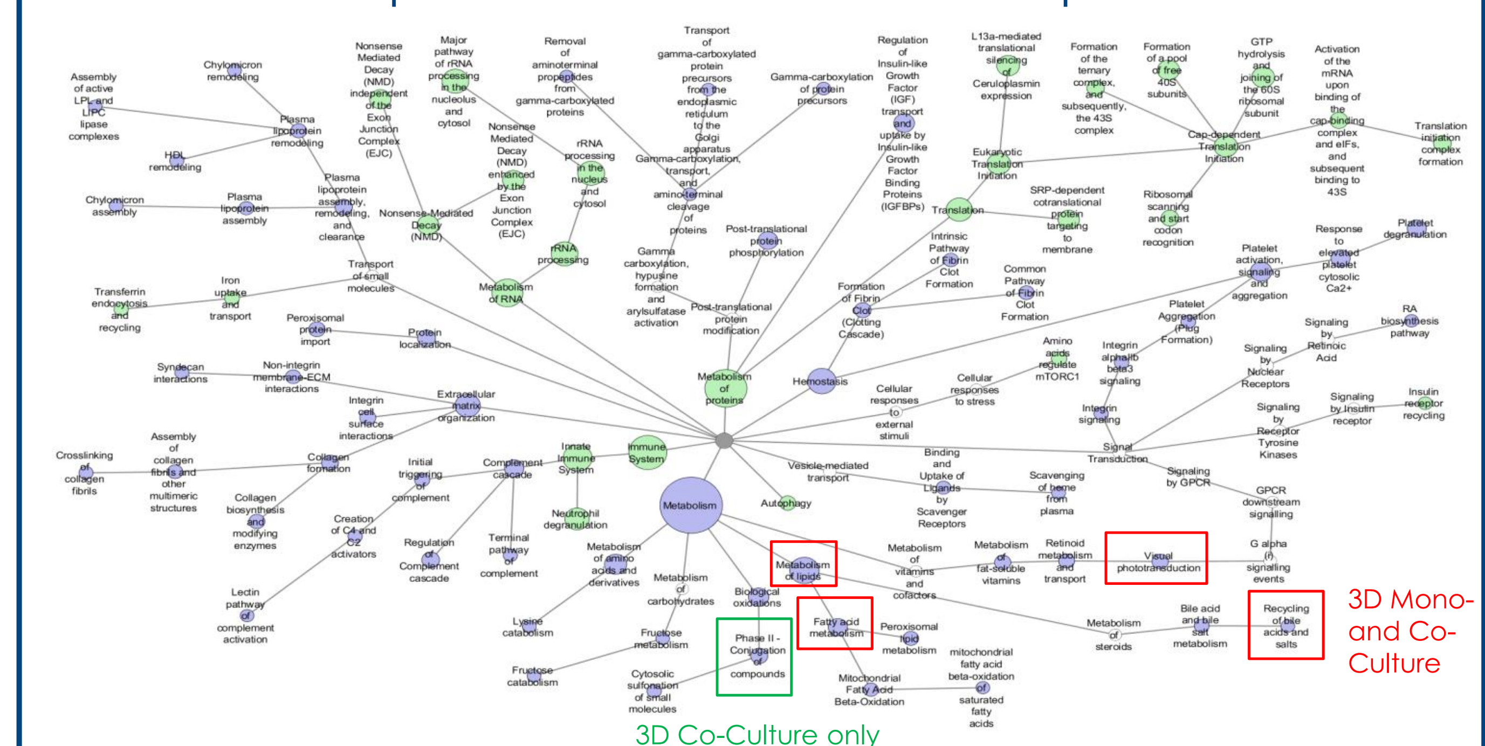


Repeat Dosing
72 Hours

All 4 Models Show CYP Inhibition in Response to Repeat Dosing with Ketoconazole

Gene Symbol	2D mono-culture 24 hours 1µM	2D mono-culture 24 hours 10µM	2D mono-culture 24 hours 30µM	2D mono-culture 72 hours 1µM	2D mono-culture 72 hours 10µM	2D mono-culture 72 hours 30µM	2D mono-culture 168 hours 1µM	2D mono-culture 168 hours 10µM	2D co-culture 24 hours 1µM	2D co-culture 24 hours 10µM	2D co-culture 24 hours 30µM	2D co-culture 72 hours 1µM	2D co-culture 72 hours 10µM	2D co-culture 72 hours 30µM	2D co-culture 168 hours 1µM	2D co-culture 168 hours 10µM	3D mono-culture 24 hours 1µM	3D mono-culture 24 hours 10µM	3D mono-culture 24 hours 30µM	3D mono-culture 72 hours 1µM	3D mono-culture 72 hours 10µM	3D mono-culture 72 hours 30µM	3D mono-culture 168 hours 1µM	3D mono-culture 168 hours 10µM	3D co-culture 24 hours 1µM	3D co-culture 24 hours 10µM	3D co-culture 24 hours 30µM	3D co-culture 72 hours 1µM	3D co-culture 72 hours 10µM	3D co-culture 72 hours 30µM	3D co-culture 168 hours 1µM	3D co-culture 168 hours 10µM
Cyp2c12	-0.87	-2.37	-1.76	-4.31	-3.20	-3.01	-0.38	-1.20	-0.47	-1.43	-2.64	0.42	-3.04	-2.86	-2.36	-3.31	-0.20	-1.01	-3.42	0.14	-1.73	-7.42	-2.43	-5.01	-0.16	-1.28	-3.81	-0.05	-3.25	-7.18	-1.57	-5.41
Cyp4a1	0.20	0.06	0.77	-1.02	-4.80	0.54	0.65	0.84	0.72	1.06	1.43	-1.15	-2.54	0.21	2.24	1.66	0.10	-0.69	-2.78	0.23	-1.99	-6.85	-0.90	-1.83	-0.09	-0.67	-3.21	-0.88	-2.29	-6.53	-0.40	-0.78
Cyp2d2	0.25	0.94	1.40	0.58	-1.15	0.61	-0.70	-3.43	-0.42	1.37	1.49	-1.80	-4.09	1.19	3.28	0.00	-0.12	-0.86	-1.90	-0.63	-1.97	-6.55	-0.42	-1.70	0.12	-0.28	-3.69	-0.40	-1.01	-6.15	-0.04	-0.40
Cyp4f4	0.51	0.31	-1.07	0.76	-1.26	-4.95	0.40	-1.16	0.19	-1.06	-1.20	0.13	-1.20	-4.63	0.70	-1.37	0.17	-0.44	-2.34	-0.68	-2.23	-6.41	-0.88	-2.42	-0.84	-1.03	-4.45	-0.28	-1.86	-5.49	-0.12	-1.82
Cyp2b3	0.16	-0.62	-0.66	-0.61	-3.50	-3.50	-0.45	-1.29	0.06	-0.41	-0.80	-1.93	-3.52	-4.95	0.31	-0.53	-0.29	-1.52	-2.98	-0.47	-2.08	-5.84	-0.55	-1.80	-0.50	-1.54	-3.59	-0.49	-2.01	-6.91	-0.13	-1.57
Cyp4a2	-0.12	-0.79	0.32	0.22	-0.85	0.63	0.66	-0.27	1.19	2.06	1.04	-0.05	2.42	2.04	2.55	1.82	-1.42	-3.25	-4.96	-0.40	-2.41	-5.45	-1.51	-3.88	-0.28	0.16	-1.64	-2.56	-1.43	-3.63	-0.66	-1.20
Cyp2c13	0.39	0.25	0.62	-0.41	-0.76	1.28	-0.89	-1.01	0.28	0.44	0.93	-1.57	-1.14	2.01	-0.68	-1.87	-0.34	-2.56	-2.96	-2.71	-4.75	-5.41	-0.11	-0.93	-0.62	-2.44	-2.45	-1.43	-4.90	-5.55	0.41	1.20
Cyp2d1	0.54	0.38	0.57	0.62	-0.36	4.09	0.99	-0.77	0.39	0.57	1.17	-0.48	0.31	2.47	0.12	-1.23	-0.19	-0.64	-1.42	0.12	-1.29	-4.95	-0.50	-2.48	0.17	-0.29	-1.58	-1.43	-1.97	-5.35	-0.60	-1.80
Cyp11a1	0.15	0.01	-2.28	-1.26	0.40	2.07	-1.23	-2.67	-0.89	-0.89	-0.79	-0.39	0.23	-2.70	-0.84	-1.55	-0.89	1.43	-0.92	-2.31	-1.43	-3.78	0.93	-0.90	-0.59	-0.64	-1.62	-0.32	-0.08	-2.53	-1.31	-0.57
Cyp2c6v1	0.46	0.34	0.49	-0.96	-2.22	0.00	0.26	-0.83	0.45	0.65	0.76	-1.82	-2.13	-0.25	0.81	-0.25	-0.31	-1.11	-1.36	-0.35	-1.15	-2.66	-0.29	-0.30	-0.45	-0.89	-1.66	-1.03	-1.37	-4.00	-0.18	-0.18
Cyp2f4	0.21	-0.28	-1.49	-0.63	-0.70	-2.99	-0.05	-1.24	0.65	-0.02	-1.02	0.25	-0.29	-1.74	-0.32	0.37	-0.13	-0.54	-0.92	-0.22	-0.39	-2.47	-0.09	-0.18	-0.15	-0.12	-1.13	-0.06	-0.76	-1.97	-0.10	-0.32
Cyp46a1	-0.40	1.71	0.02	1.51	1.81	-2.33	-0.26	-2.29	-0.70	0.89	-2.33	0.18	0.48	-4.08	-0.73	-1.64	-0.03	0.45	-1.78	-0.79	0.95	-2.41	0.69	1.45	-1.09	-1.07	0.72	-2.01	-0.91	0.54	-0.92	-0.32
Cyp3a9	-0.07	-0.17	-1.06	-0.13	-0.08	-0.79	0.61	-0.23	0.15	-0.11	-0.98	0.24	0.03	-0.32	1.37	0.32	-0.03	-0.07	-1.04	-0.06	-0.92	-2.37	-0.04	-1.85	0.16	-0.12	-1.30	-0.44	-0.71	-2.64	0.35	-0.93
Cyp2b2	1.42	-1.04	-0.11	-0.46	-1.99	-3.77	0.91	-3.65	0.27	-0.77	-1.18	-3.92	-4.75	-4.57	1.39	-1.49	-0.14	-2.24	-1.82	-0.45	-0.53	-1.93	0.24	-2.10	0.55	-1.49	-1.13	-1.09	-2.57	-4.57	-0.85	-2.23
Cyp2c22	-0.25	-0.76	-1.86	-1.76	-2.47	-4.17	-0.37	0.32	-0.02	-0.12	-1.43	-2.11	-2.82	-4.36	0.43	1.20	-0.13	-1.15	-1.56	-1.35	-1.60	-1.26	-0.78	-0.20	-0.38	-1.46	-2.30	-1.36	-1.43	-1.37	-0.66	0.07
Cyp27a1	-0.31	0.01	-0.12	-0.20	-0.32	-0.47	0.07	-0.20	0.13	-0.04	-0.33	-0.13	-0.35	-0.11	0.35	0.24	-0.03	0.08	0.09	-0.53	-0.88	-1.18	-0.19	-0.21	-0.32	0.21	-0.43	-0.22	-0.18	-0.72	-0.04	0.15
Cyp2c7	0.47	0.17	0.07	-0.16	-0.21	1.34	-0.04	-2.46	0.71	1.00	0.92	-0.70	-0.20	1.04	0.02	-1.37	0.25	-0.04	-0.28	0.46	-0.74	-1.16	-0.52	-0.42	0.34	-0.16	-0.48	-0.79	-1.83	-2.19	-0.46	-0.86
Cyp2e1	0.47	0.27	1.69	0.25	-2.04	2.96	1.40	0.73	0.32	0.80	1.91	-2.53	-0.03	3.80	-1.33	-3.21	-1.02	0.06	-0.41	-1.27	-1.35	-1.15	0.36	-0.04	-0.54	-0.55	-1.42	-0.02	-1.34	-1.74	-0.25	-0.28
Cyp2u1	0.49	-0.02	0.24	0.15	0.24	-1.21	1.01	0.73	-1.53	-2.46	-1.17	-0.65	-0.99	-1.16	0.38	-0.11	-0.01	0.29	-0.41	-1.10	-1.41	-1.07	-0.21	0.41	-0.94	-0.66	-0.40	0.02	-0.24	0.32	-0.31	-0.01
Cyp2d4	0.32	0.26	-0.95	0.64	0.02	-2.10	0.48	0.20	-0.44	-0.55	-1.27	0.16	-0.02	-1.40	0.00	-0.07	0.16	0.12	-0.50	-0.39	-0.45	-1.06	0.43	0.20	-0.24	-0.14	-0.79	-0.27	-0.66	-1.64	-0.03	0.09
Cyp2c24	-1.11	0.81	-3.96	-3.07	-1.89	-3.68	0.51	1.73	0.54	1.24	-3.13	0.35	-1.36	-1.18	0.08	0.59	0.16	0.76	0.42	0.65	3.51	-0.68	0.57	2.50	0.73	1.67	0.13	-0.14	3.26	-1.94	0.85	3.11
Cyp4v3	0.32	0.45	-0.61	0.15	0.14	-0.06	0.17	0.42	0.29	-0.78	-0.61	-0.09	-0.10	-0.28	0.06	0.34	0.01	-0.04	-0.46	-0.03	-0.42	-0.66	-0.14	-0.20	-0.06	-0.27	-0.68	-0.10	-0.36	-0.61	-0.21	-0.02
Cyp2r1	-0.12	0.24	0.32	0.05	0.84	-0.79	0.10	-0.42	-0.12	0.12	0.44	-0.10	0.41	0.75	-1.42	-0.67	-0.13	-0.08	0.21	-0.56	-0.06	-0.62	-0.29	-0.31	-0.02	0.38	-0.23	0.34	0.57	0.18	-0.21	-0.15
Cyp4f6	0.07	0.09	0.13	-0.28	-0.12	-0.87	-0.39	0.10	-0.04	0.12	-1.26	-0.39	-0.05	-0.16	-0.41	-0.35	0.20	-0.02	-0.12	-0.64	-0.62	-0.51	0.39	-0.03	-0.32	-0.21	-0.22	-0.29	-0.12	0.02	0.05	-0.04
Cyp2t1	0.14	0.38	-0.58	0.38	0.38	-1.64	0.43	0.68	0.21	0.35	-0.73	0.20	0.28	-1.56	0.22	0.47	0.02	0.05	-0.12	0.10	0.27	-0.38	0.32	0.41	0.09	0.34	-0.06	0.09	0.40	-0.58	0.21	0.24
Cyp2c11	0.38	0.05	0.56	0.05	1.09	2.34	0.05	-0.09	0.28	0.62	0.90	0.01	0.10	1.58	-1.96	-0.06	-0.40	-0.19	-0.49	0.18	0.00	-0.33	1.19	0.26	-0.18	0.25	-0.61	-0.02	0.55	-0.92	0.06	-0.09
Cyp2j4	-0.11	0.20	-0.66	0.18	0.18	-0.05	0.15	0.13	-0.27	-0.18	-0.26	0.03	0.11	0.12	0.46	0.63	0.00	0.06	-0.42	-0.21	-0.34	-0.32	-0.27	-0.24	0.17	-0.10	-0.26	-0.18	-0.32	-0.24	0.15	0.22
Cyp2j10	-0.16	-0.38	-0.27	0.12	0.74	0.57	0.15	-0.47	0.13	-0.33	0.29	-0.50	-0.47	0.20	-0.13	0.60	-0.32	-0.66	-1.22	0.06	-0.01	-0.28	-0.21	-0.51	-0.24	-0.58	-0.78	-0.61	-0.22	-1.21	-0.05	-0.06
Cyp4f17	-0.07	-0.04	-0.07	0.20	0.27	0.85	0.09	-0.32	0.53	0.39	-0.22	0.03	0.27	0.27	0.28	0.30	-0.43	-0.28	-0.22	-0.60	-0.37	0.08	0.02	0.35	-0.16	0.17	-0.14	-0.02	-0.31	0.23	0.08	-0.03
Cyp20a1	-0.04	0.26	0.81	-0.08	0.17	0.73	-0.02	0.70	-0.05	0.30	0.83	0.13	0.17	0.86	-0.16	0.18	-0.05	-0.16	0.47	-0.24	-0.09	0.52	0.02	0.07	-0.28	-0.01	0.41	0.03	0.36	0.79	0.03	0.25
Cyp1a2	0.67	0.57	0.24	-0.13	-0.22	1.78	0.55	1.55	0.45	0.83	0.31	0.02	-0.92	1.64	0.24	0.73	-0.39	1.76	1.24	0.26	2.95	0.57	-0.17	-0.18	0.96	3.34	1.40	-0.16	2.60	0.15	0.22	1.26
Cyp51	0.14	2.07	2.01	0.33	1.82	1.60	0.08	0.80	0.02	1.79	1.55	0.28	1.60	1.63	-0.20	0.91	0.24	0.76	1.62	0.83	0.96	1.17	0.27	0.90	0.42	1.11	1.52	0.15	1.03	1.13	0.67	1.06
Cyp1b1	-0.45	0.07	0.84	0.04	0.06	0.68	0.10	0.08	0.50	0.54	1.81	-0.14	-0.04	0.70	0.51	0.73	-0.46	0.61	1.97	-0.09	0.14	1.49	0.42	-0.08	0.92	1.55	3.69	0.19	-0.47	1.89	0.49	-0.08
Cyp27b1	0.34	1.48	-0.15	0.62	-0.56	-0.37	0.34	0.68	0.83	-0.89	1.23	0.97	-2.32	0.45	-4.05	-2.51	1.06	0.55	0.57	1.33	0.90	1.53	1.46	1.55	2.92	0.00	1.68	-2.12	-2.94	0.25	-2.98	-3.19
Cyp7b1	-1.15	-1.13	-0.11	-3.05	-0.88	-0.07	-0.40	-1.55	2.72	3.23	3.86	-2.09	0.90	0.23	-0.36	-1.94	2.37	0.92	1.00	-1.12	1.44	1.56	-3.01	-2.98	2.79	3.58	0.83	-0.82	-0.79	-2.58	1.13	0.00
Cyp1a1	0.34	1.09	-4.18	0.29	-0.71	-3.18	5.86	7.38	1.86	2.73	-2.37	1.17	-0.13	-3.85	5.65	6.27	1.00	4.78	6.72	1.75	2.49	3.22	0.27	1.96	-2.23	2.90	2.88	1.16	4.08	4.19	0.00	2.47

3D Models Capture Complex Metabolic Processes in Response to Ketoconazole Exposure



Ketoconazole Reactome enrichment for genes that were significantly up-regulated (FDR<0.05 & FC> +1.5-fold, Green nodes) and significantly down-regulated (FDR<0.05 & FC< -1.5-fold – blue nodes) at 30μM and 72 hours exposure in 3D co-culture.

3D Models Capture Inhibition of Major Metabolic Pathways in a more Consistent Manner

Down-regulated genes (lower triangle); Up-regulated genes (upper triangle)

A.	2D monoculture	3D monoculture	2D co-culture	3D co-culture
2D monoculture		0.550	0.719	0.706
3D monoculture	0.155		0.517	0.718
2D co-culture	0.423	0.134		0.515
3D co-culture	0.145	0.738	0.135	

Combined

B.	2D monoculture	3D monoculture	2D co-culture	3D co-culture
2D monoculture		0.266	0.522	0.280
3D monoculture	---		0.255	0.731
2D co-culture	---	---		0.247
3D co-culture	---	---	---	

MJI score between 3D mono-culture and 3D co-culture showing a 28.6% increase over the next best score of 0.522 between 2D co-culture and 2D mono-culture.

The Modified Jaccard Index (MJI)scores between pair-wise comparisons of the enrichment with ketoconazole at 30μM and 72 hours

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