



These projects have received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 963845 (ONTOX), No. 965406 (PrecisionTox), No. 964537 (RISK-HUNT3R)

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Open **2-3 July 2026** Symposium

Maastricht
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The TXG MAPr toolbox: qualitative and quantitative interpretation of toxicogenomics data

Giulia Callegaro

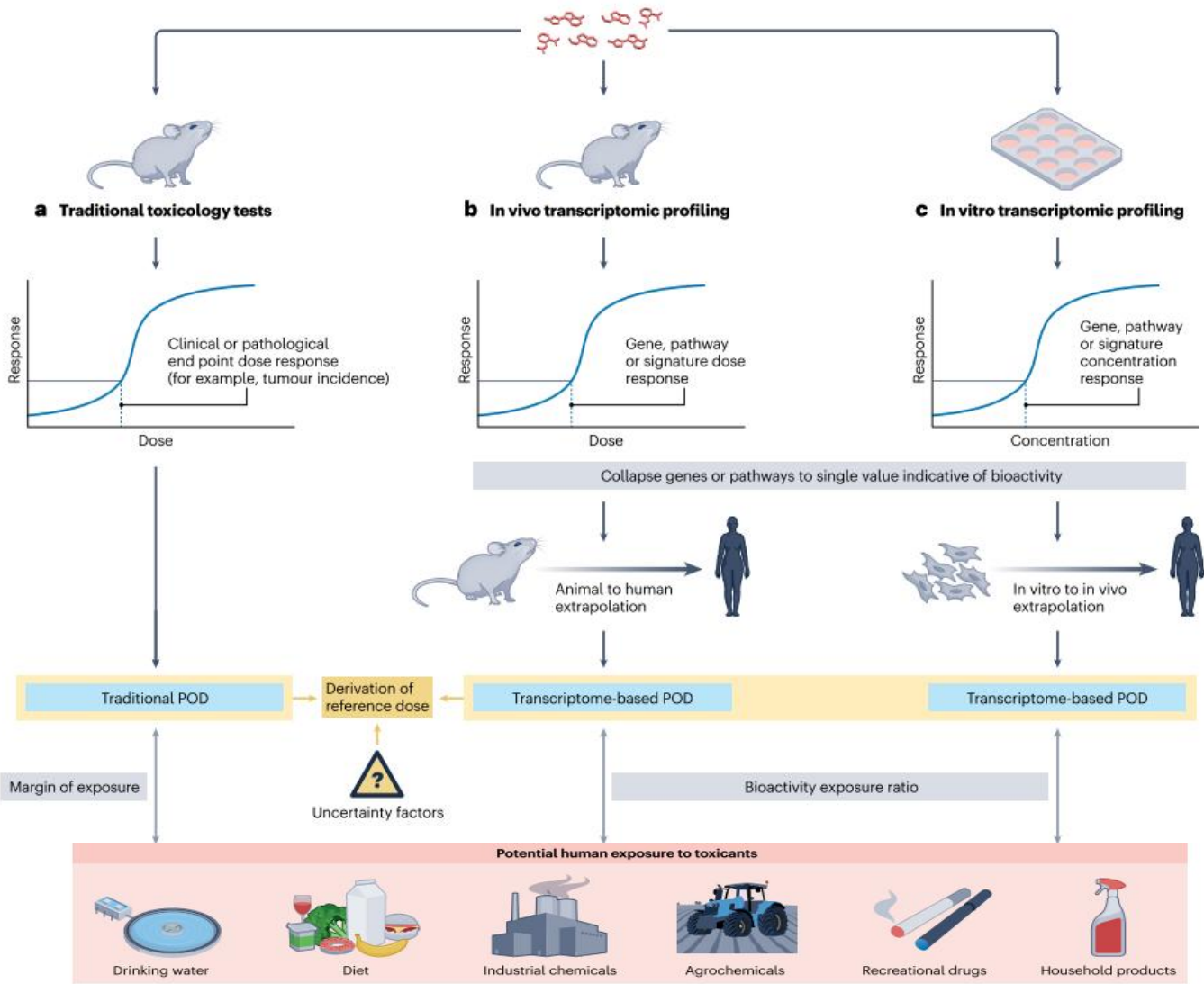
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RISK-HUNT3R project

aspis-cluster.eu

Toxicogenomics is well-positioned to support mechanistic safety assessment

- Mechanistic read-out
- Holistic view
- Well accepted





Toxicogenomics is well-positioned to support mechanistic safety assessment

Mechanistic read-out

Holistic view

Well accepted

However still suffers from limitations

Unclear interpretation framework

Unclear translation to human pathophysiology

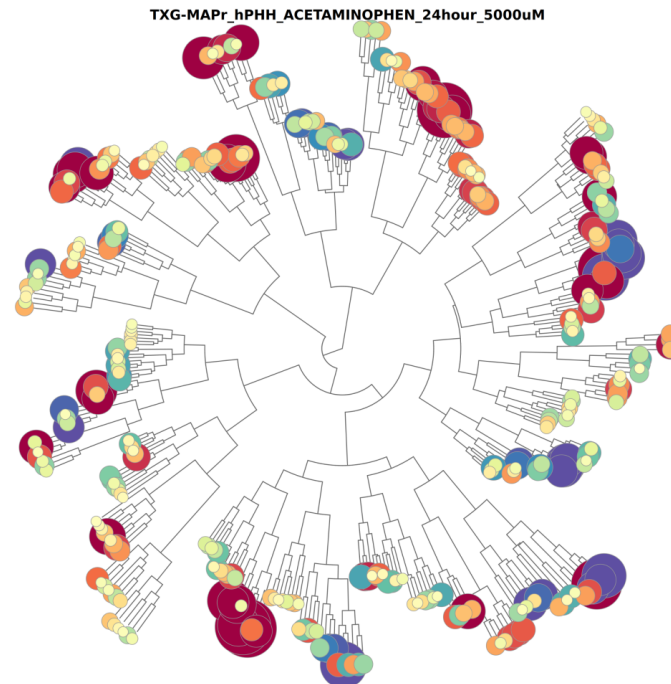
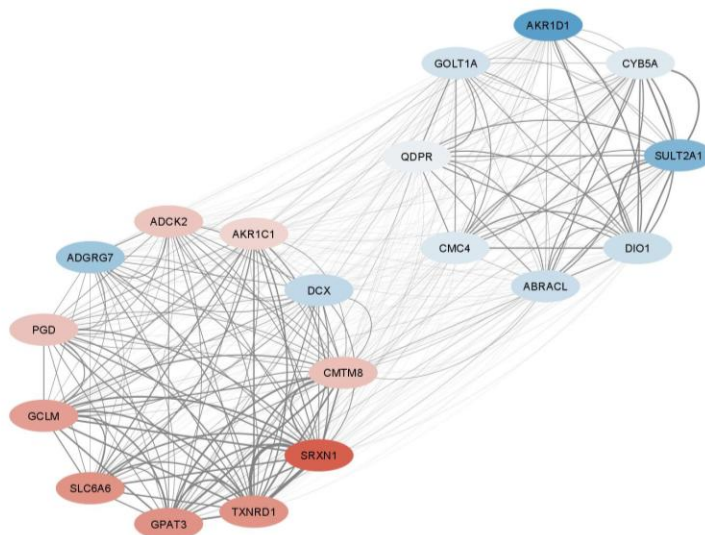
Unmet context-specific requirements





Why network-based methods?

- Organize highly dimensional / complex omics data
- Biological function is retained
- Network dynamics can be quantified
- Network visualization improves data interpretation
- Conservation of networks used for interspecies translation



However still suffers from limitations

Unclear interpretation framework

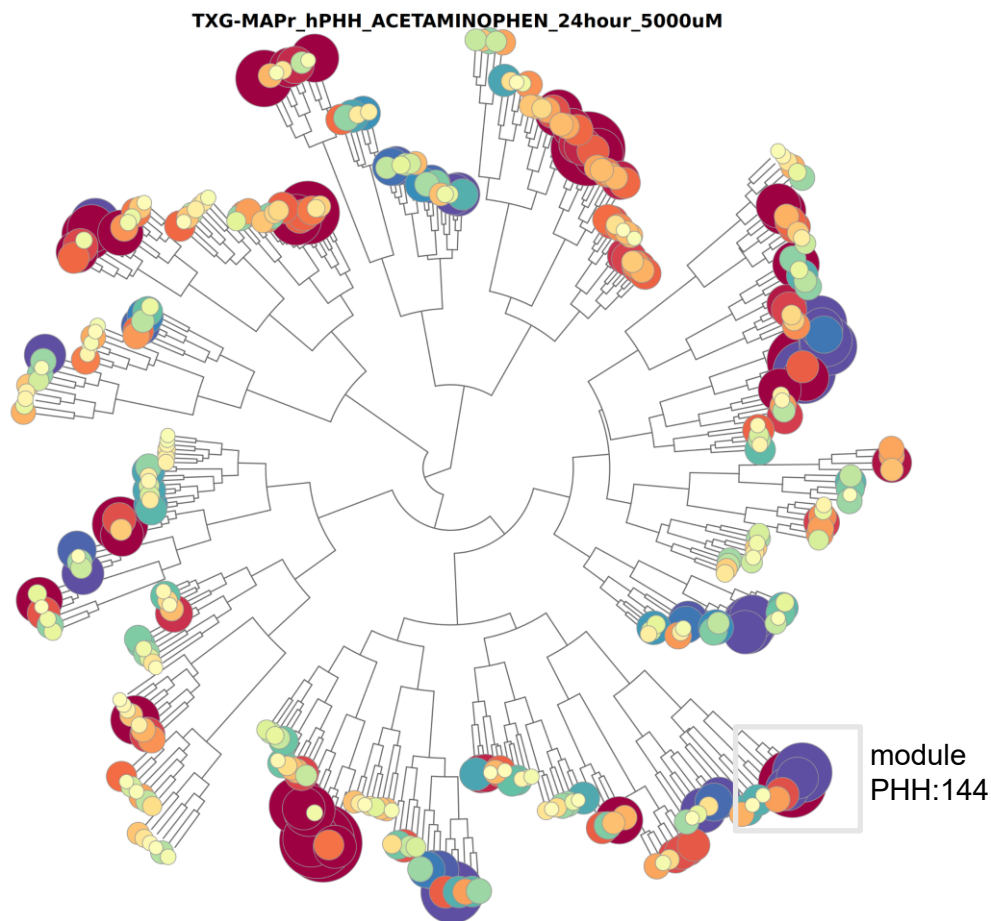
Unclear translation to human pathophysiology

Unmet context-specific requirements

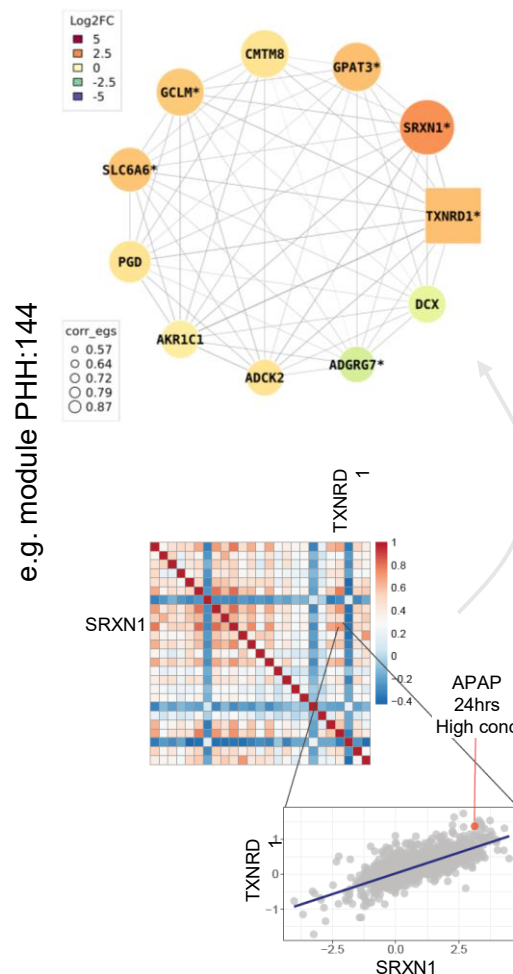


The TXG-MAPr tools

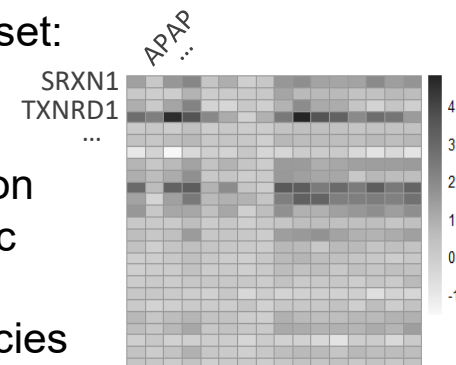
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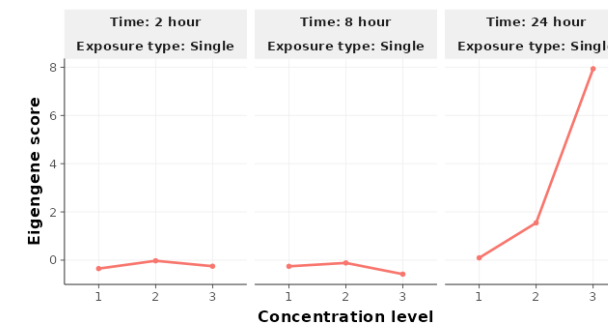
An ensemble of subnetworks, each representing a group of genes co-expressed in the



The input dataset:
a large perturbational gene expression dataset specific for each cell line/tissue/species



Each subnetwork (module): one score of activity & interpretation



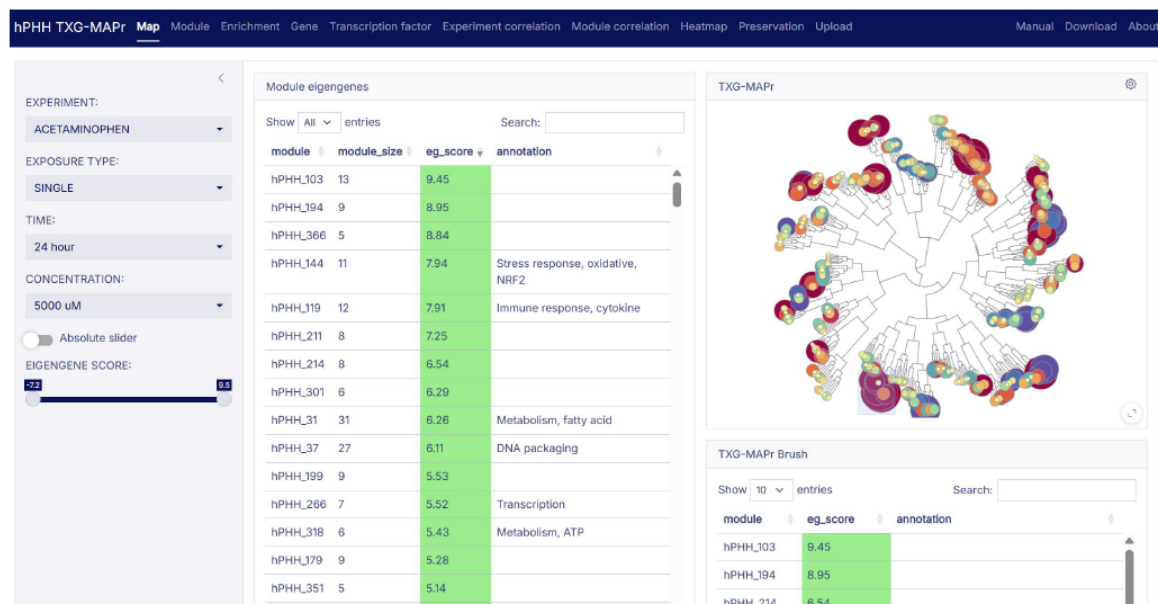
Stress response, oxidative, NRF2

Term	P adj
NRF2 pathway (WikiPathway)	0.00000564
Glutathione metabolism - Homo sapiens (human, KEGG)	0.000706
Oxidoreductase (UniProt)	0.00570



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TXG-MAPr toxicogenomics interpretation toolbox



Interactive tabs for mechanistic insights

hPHH TXG-MAPr Map Module Enrichment Gene Transcription factor Experiment correlation Module correlation Heatmap Preservation Upload

Gene network qualification and quantification

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Gene network ontology enrichment search

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Gene level interpretation and network association

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Transcription factor activity

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Experimental correlation based on gene network activity.

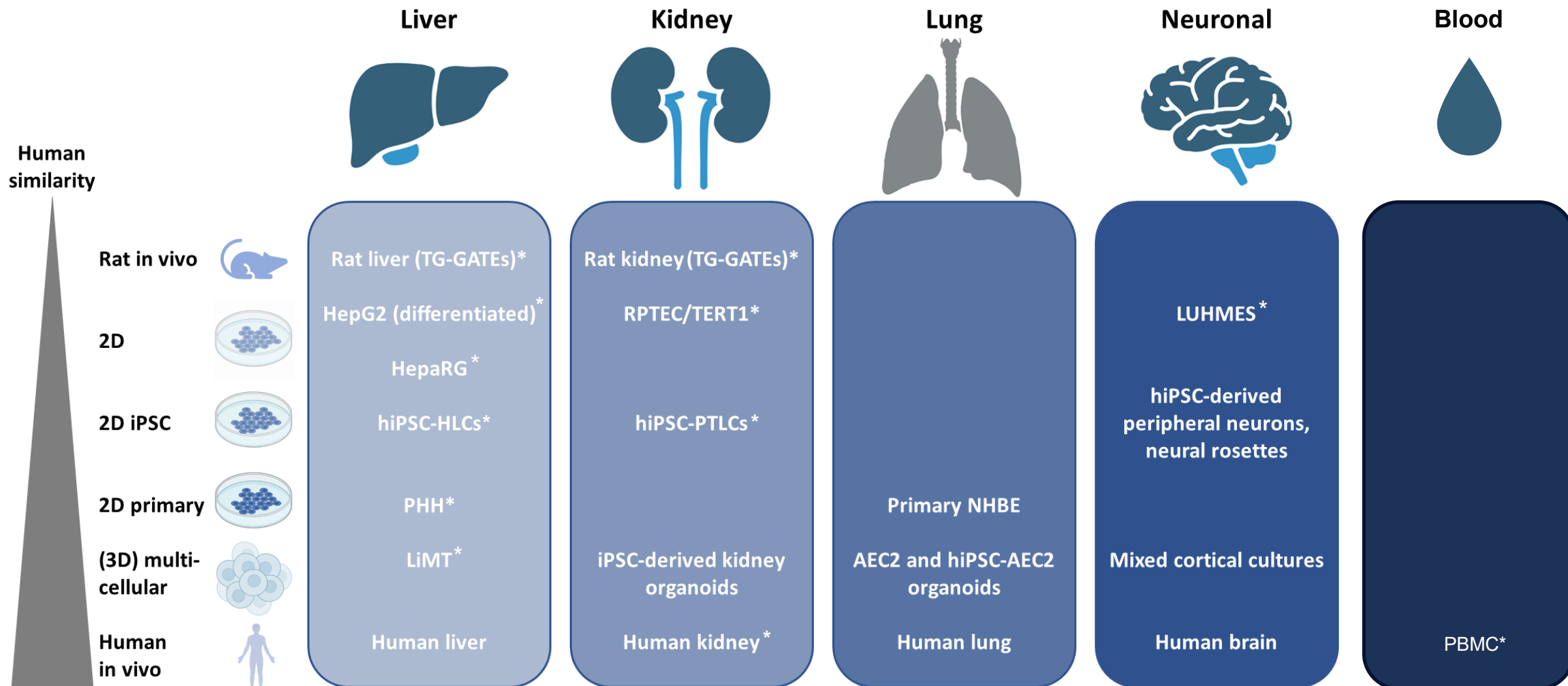
hPHH TXG-MAPr Map Module Enrichment Gene Transcription factor Experiment correlation Module correlation Heatmap Preservation Upload

Uploading of own datasets for toxicogenomics interpretation.



The TXG-MAPr tools development

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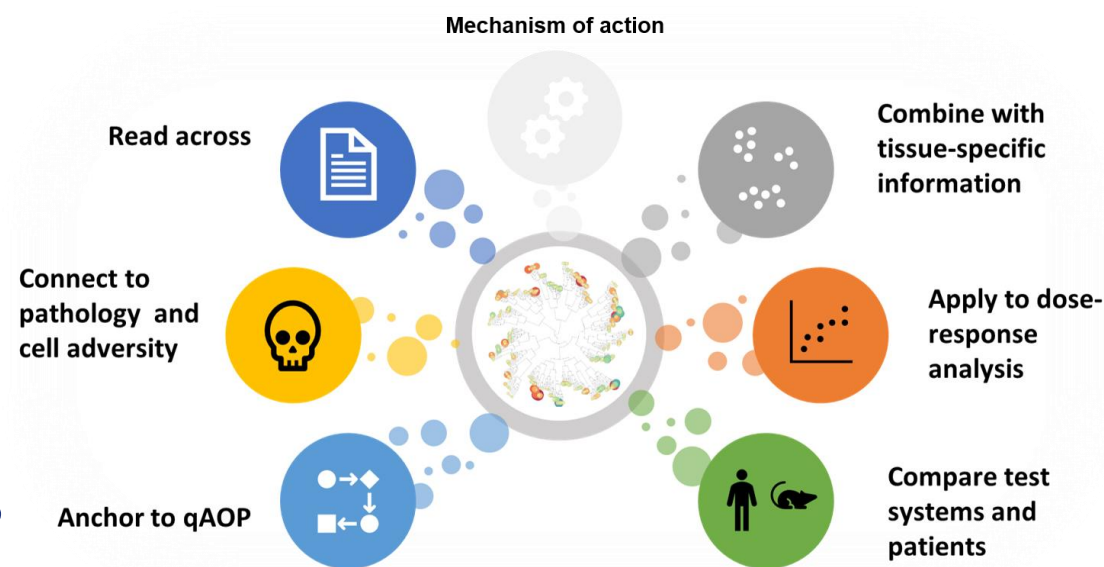


* TXG-MAPr tools already established



We have applied the TXG-MAPr paradigm transversely in RH3R in multiple case studies:

- Connecting in vitro test systems to human pathophysiology: the kidney example
- Transcriptomics for compound prioritization
- RAX with transcriptomics data: conazoles and beyond
- Time dependency of MoA assessment: non-genotoxic carcinogens as a case study
- Systematic comparison of liver test systems for the modulation of KE1 of the liver fibrosis AOP 38
- Comparison of transcriptomics and metabolomics for assessment of bioactivity: a liver test system comparison
- ...

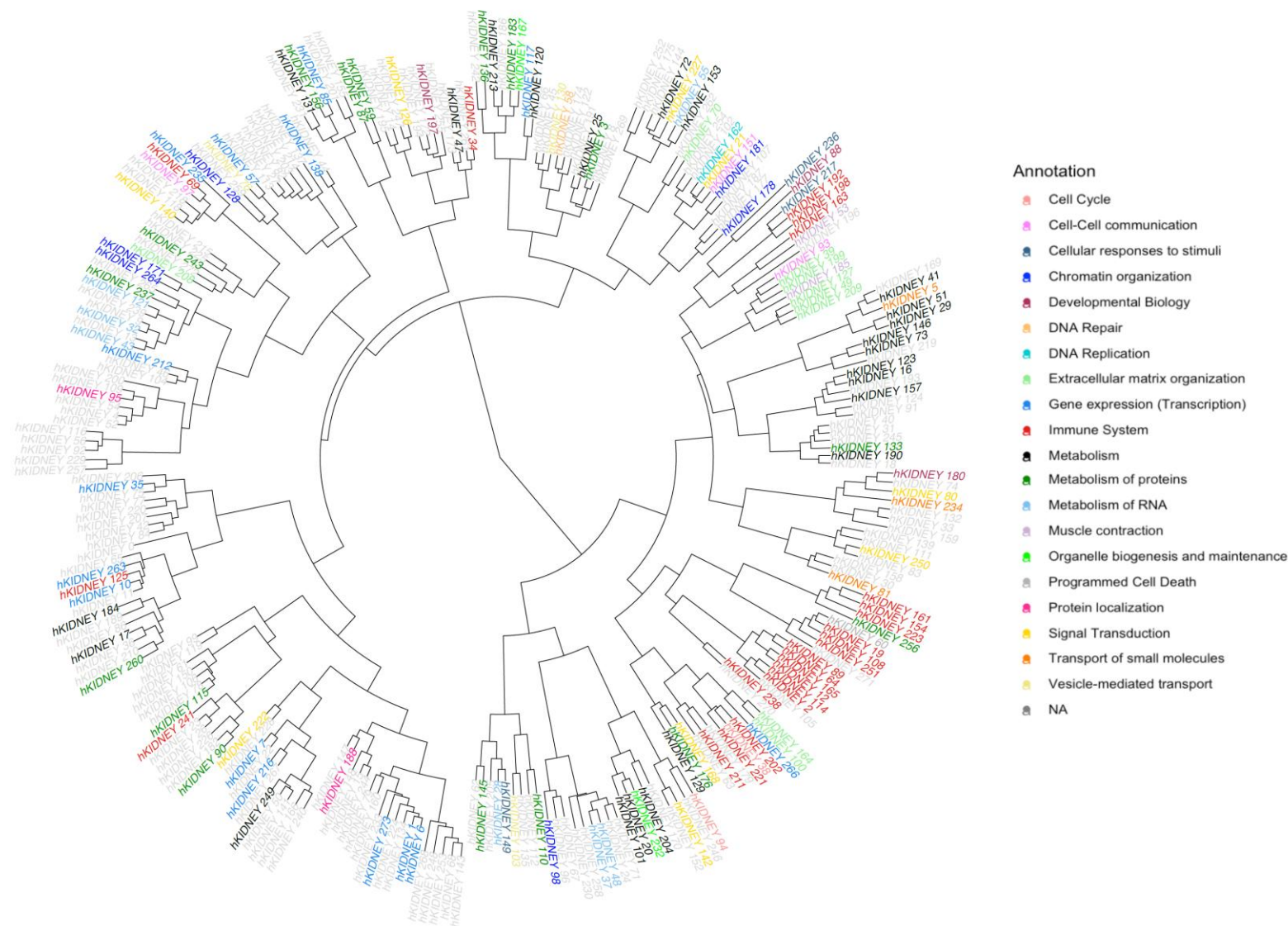




Connecting in vitro test systems to human pathophysiology: the kidney example

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- >1000 kidney biopsy samples
- Diversity of renal pathologies
- Pathology scoring
- TempO-Seq of all biopsies

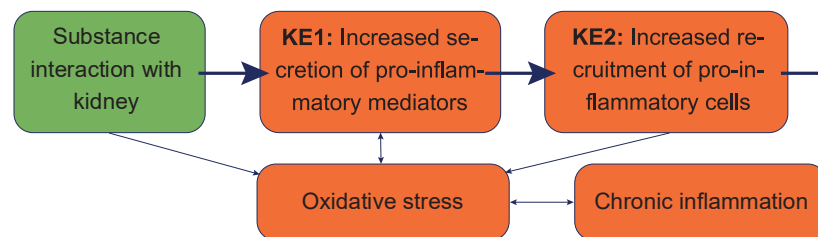




Connecting in vitro test systems to human pathophysiology: the kidney example

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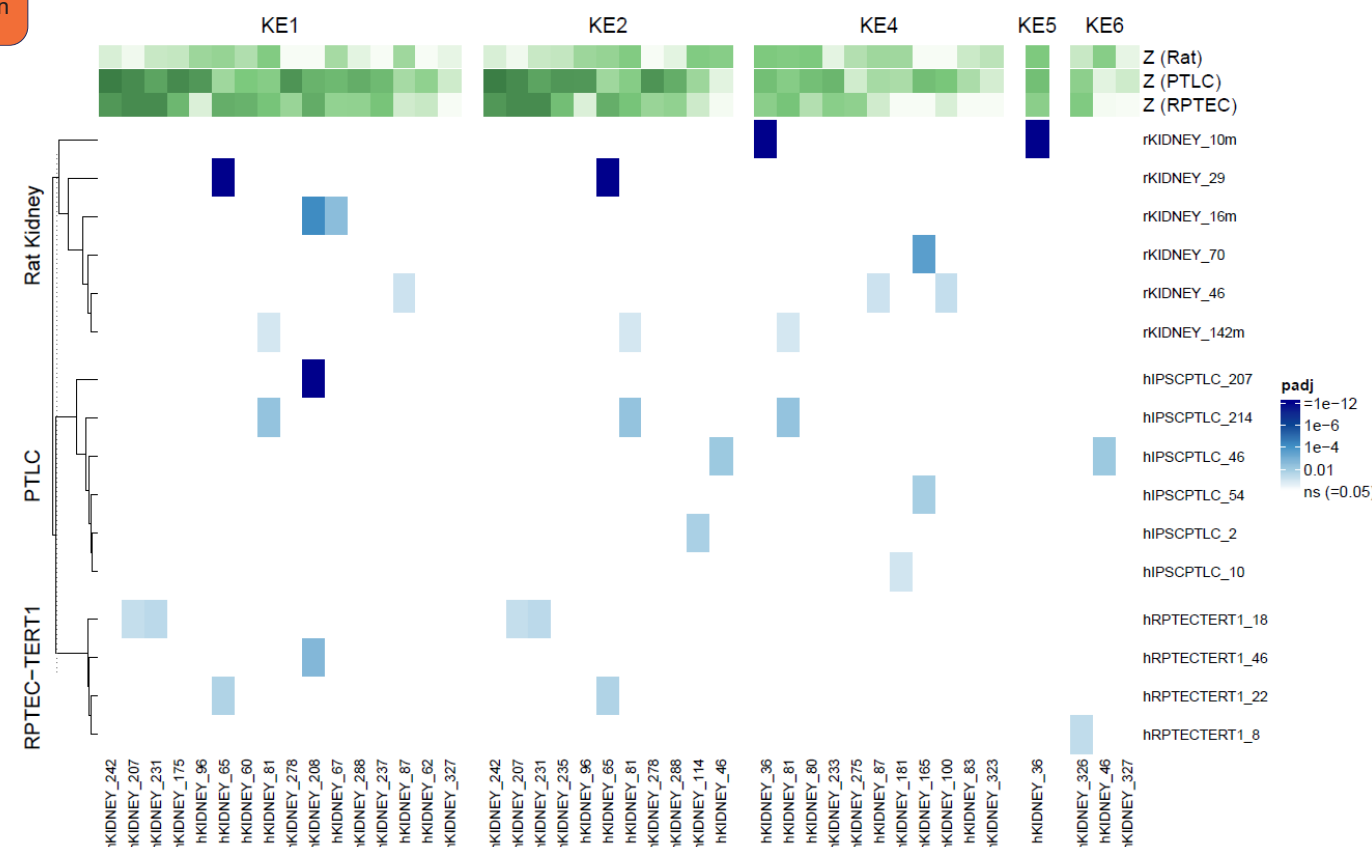
Molecular Initiating Event



Key Events

Adverse Outcome

- Different human modules can be associated with KEs
- (in vitro) renal test systems have differential capacities to capture different KEs



- Subchronic oral rat/mouse studies reviewed, adversity discussed
- Three sources used: RepDose / ECHA CHEM/ToxRef DB/ GHS C&L

→ 64 compounds selected

29 low toxic - no adverse effect up to 1000 mg/kg bw/d

35 toxic - adverse effect below 10 mg/kg bw/d

Experiment:

- PHH 10 donor-pool (BioIVT) (50/50 male/female)
- 9 concentrations (based on PBPK modelling in context of LOAEL)
- 3 replicates
- whole transcriptome TempO-Seq (BioSpyder)
- Bioinformatics using TXG-MAPr transcriptomics toolbox

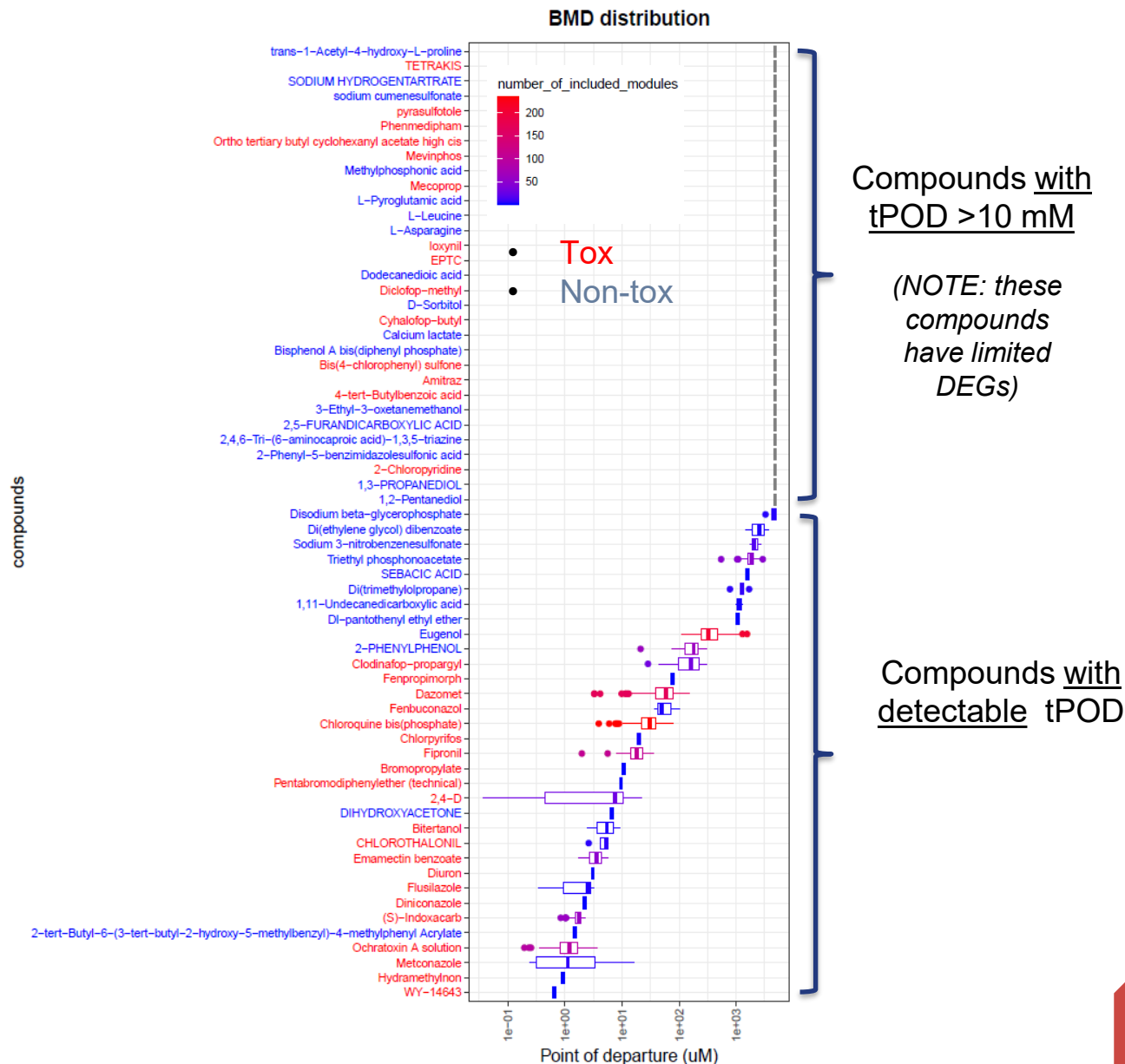
Target organ at LOAEL	Number of compounds
CNS	2
General	2
Heart	1
Hematology	5
Kidney	10
Liver	20
Spleen	1
Testes	1
Thyroid	1
Urinary Bladder	1



Toxicogenomics can prioritize chemicals with liability to be highly toxic

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- Chemicals with lowest BMCs are high toxic cmpds
- Various high toxic chemicals are not picked up by PHH transcriptomics
- 21/35 high toxic chemicals with tPOD < 100 μM
- 2/29 low toxic chemicals with tPOD < 100 μM
- Application:
 - Prioritization for ECHA
 - SSbD

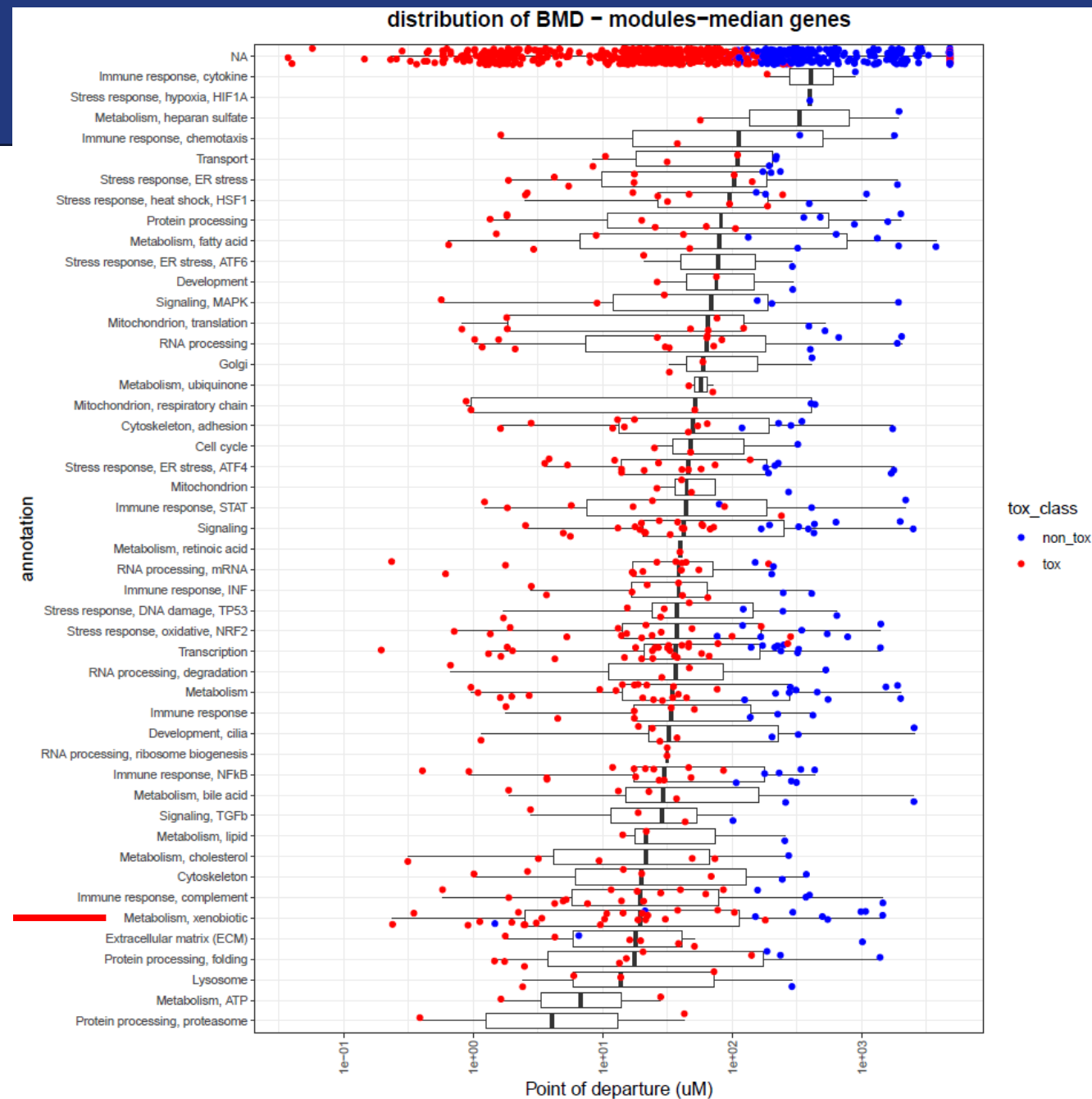
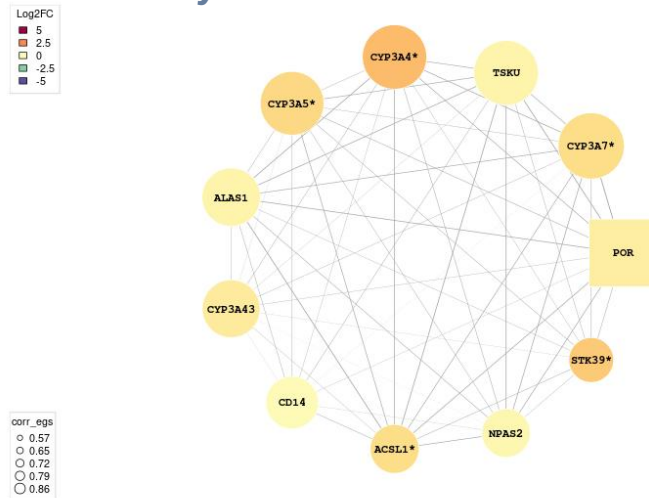




Mechanistic insight of distribution of transcriptional BMC

- High toxic compounds affect various biological processes
- Low toxic compounds have similar distribution of biological perturbations
- High toxic compounds show lower tPODs

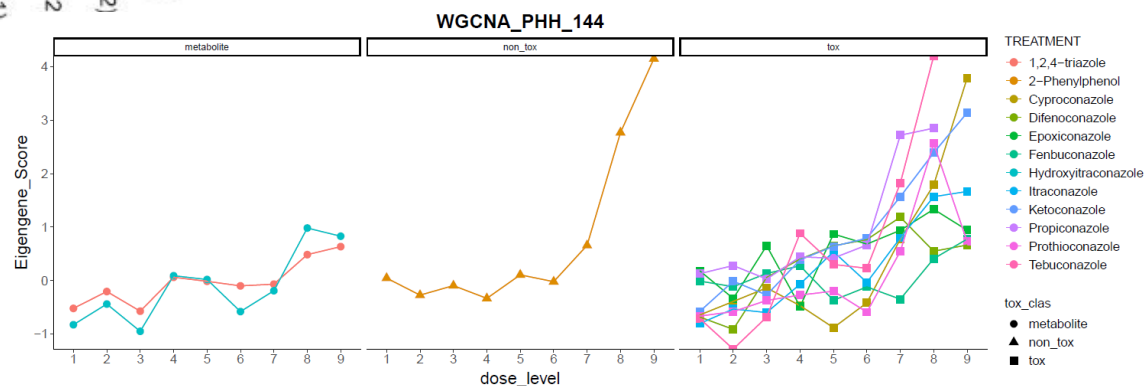
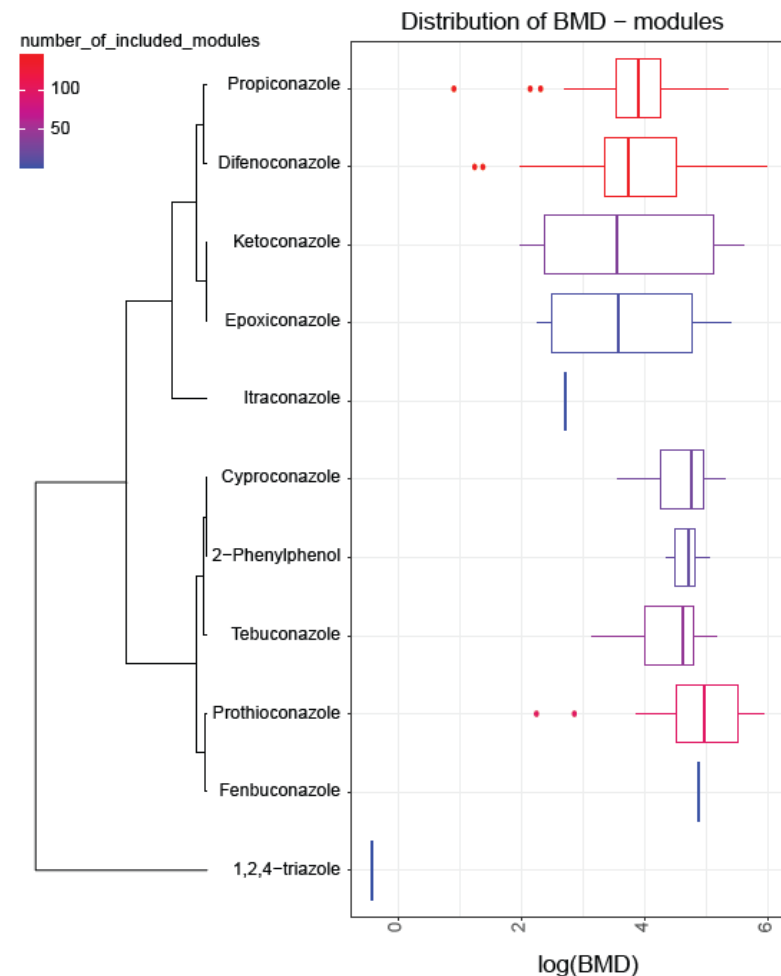
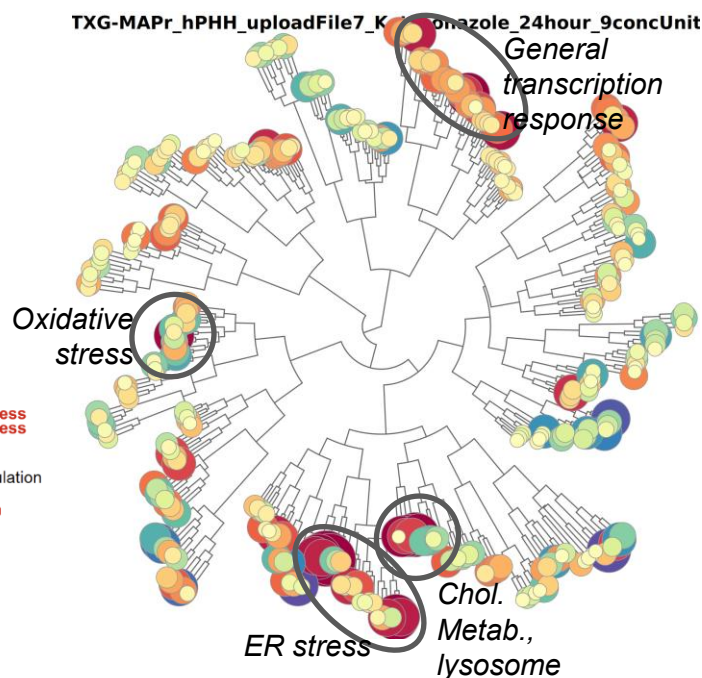
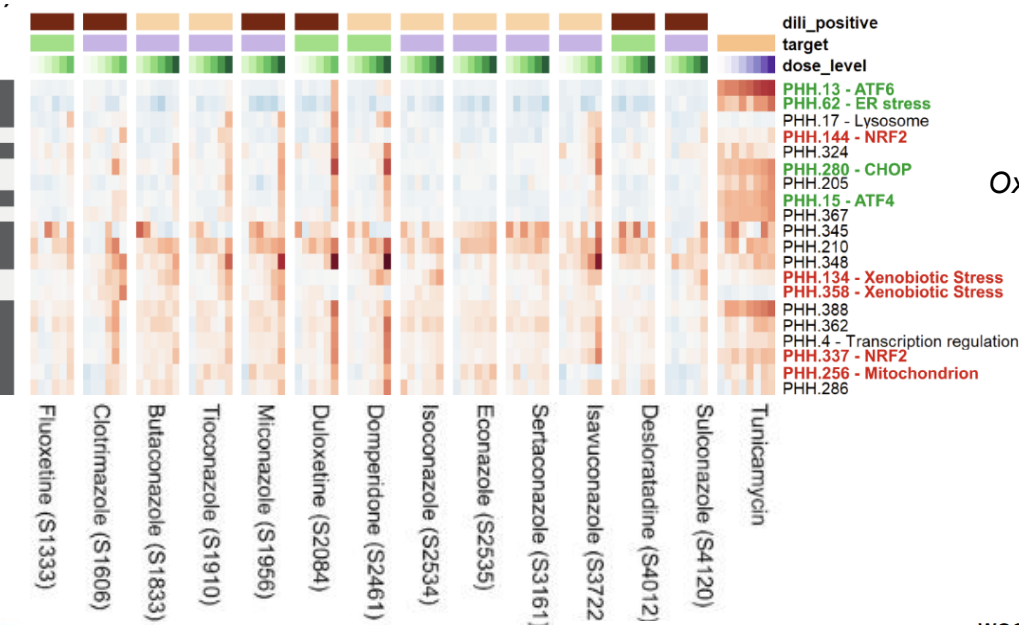
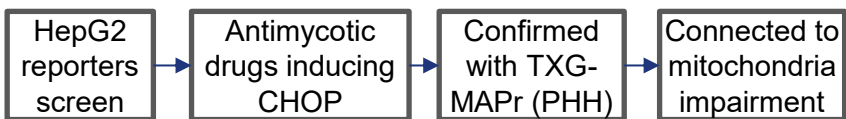
Module 134 composition: Cytochrome P450 metabolism





TXG-MAPr: from safety alerts *in vitro* to Rax Conazole case study

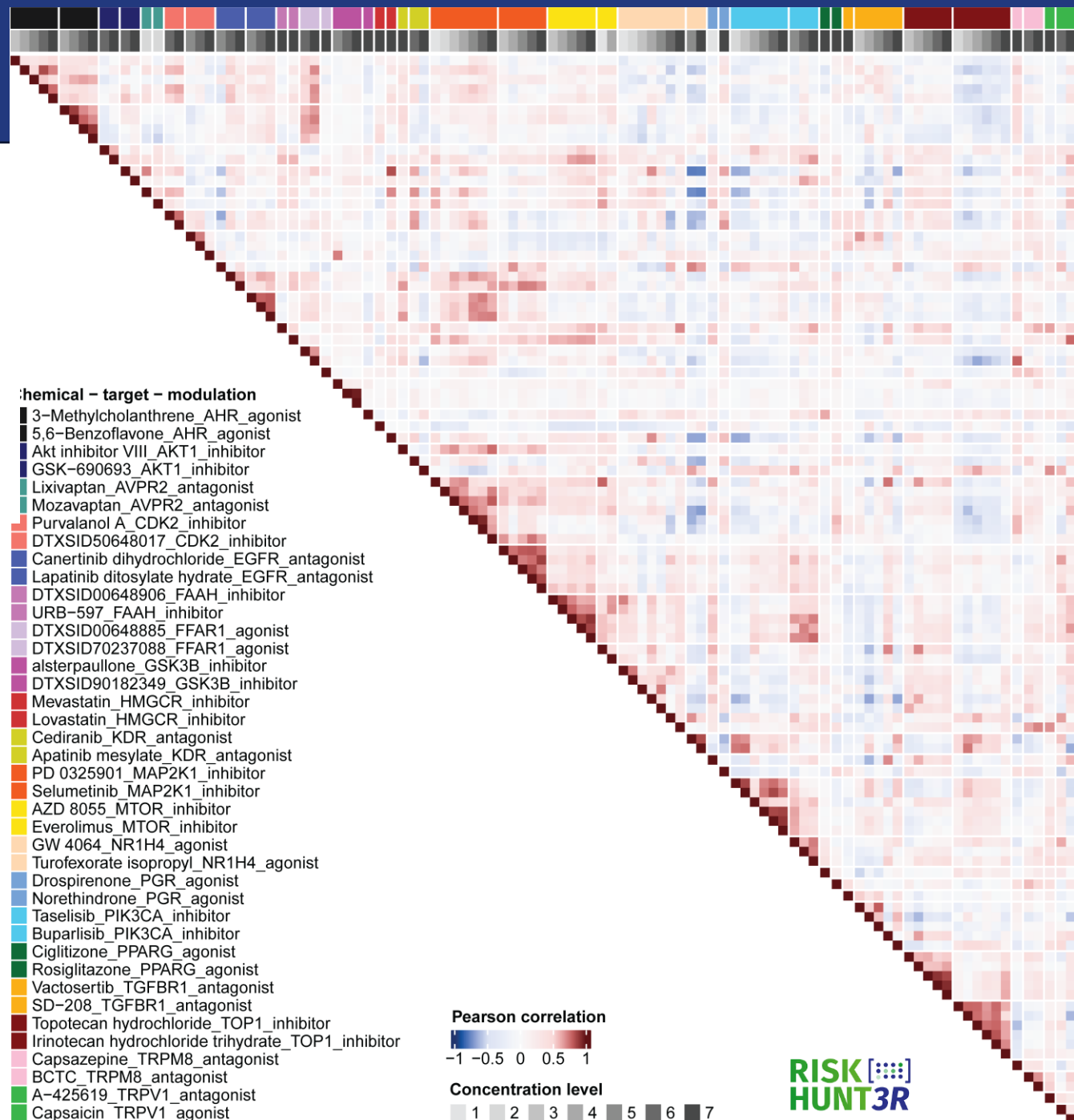
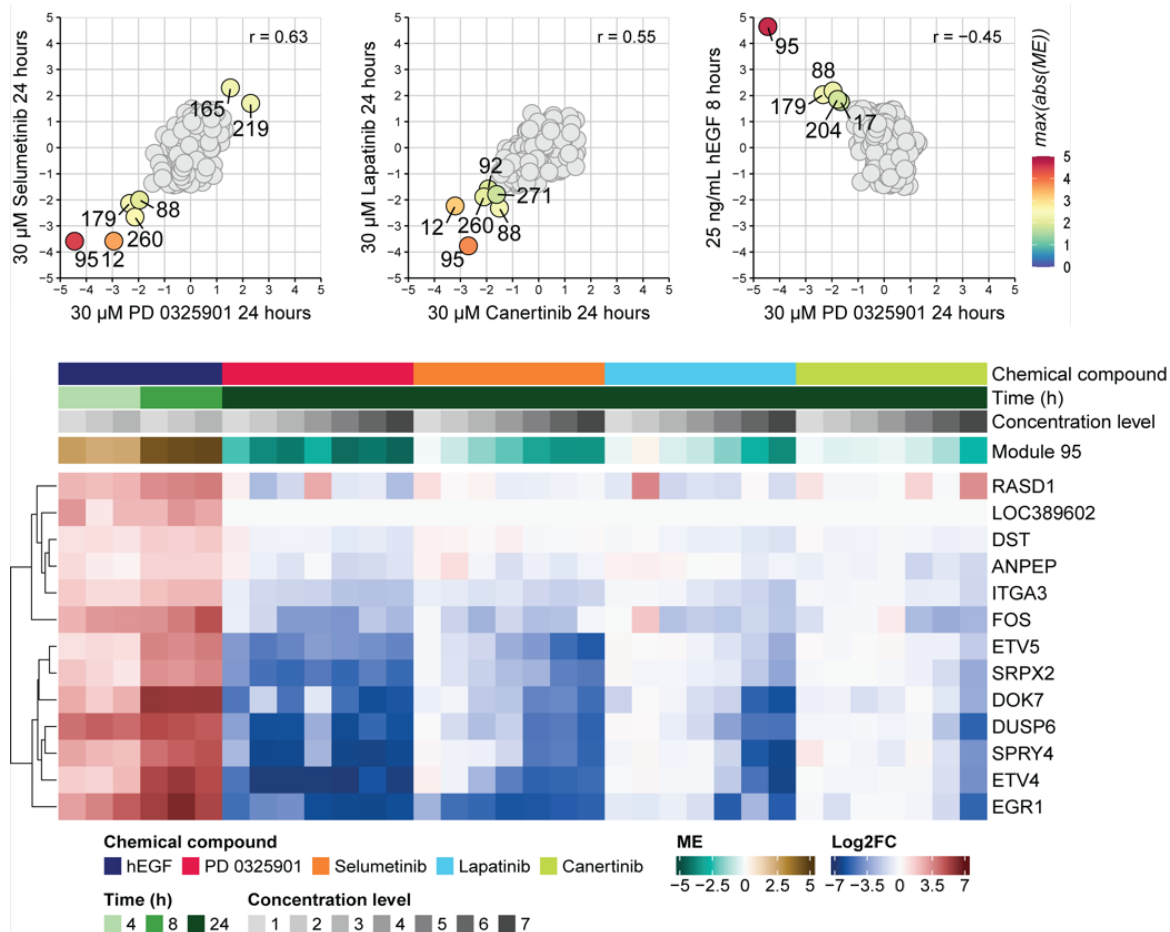
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Vlasveld M, Callegaro G, ..., van de Water B. Liver Int. 2024 Mar;44(3):760-775. doi: 10.1111/liv.15822.



RAX: biological similarity





What is still ongoing and next developments

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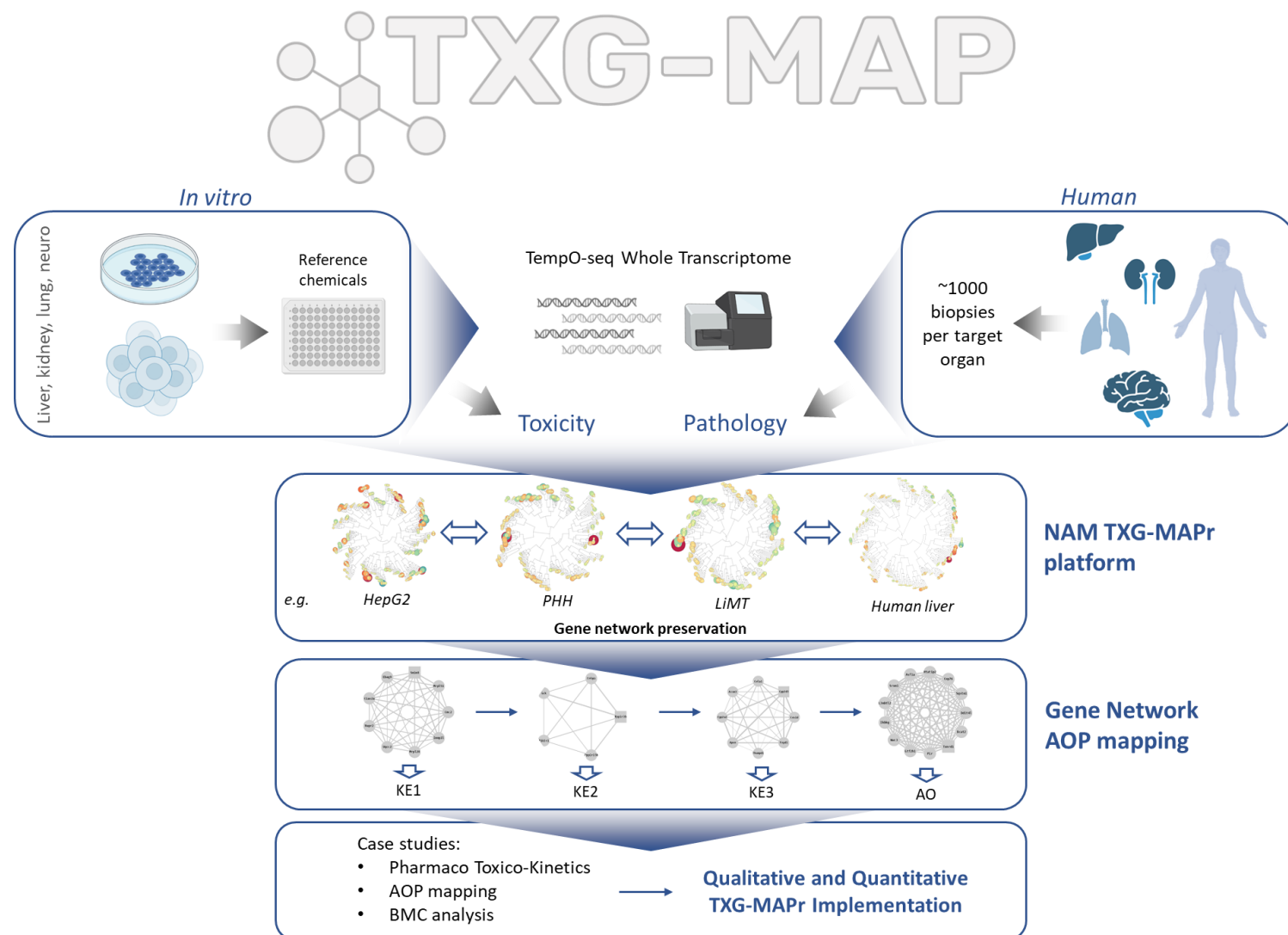
Expand the biological space

Agree on mechanistic terminology, anchoring to AOP

Gain confidence on reproducibility

Improve data management of toxicogenomics data

Keep on building (regulatory relevant) case studies



Expand the biological space

Agree on mechanistic terminology, anchoring to AOP

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P5.3.1.b_grOMICS: Fostering the use of omics for grouping and read-across in risk assessment

Omics data, including high-throughput transcriptomics (HTTr) and high-throughput phenotypic profiling (HTPP) can support rapid and mechanistic-based hazard assessment. Grouping and RAx are a clear area of imminent application

Objectives

Develop a database and interface for the OECD Omics Regulatory Reporting Framework

Develop standardized, interoperable and toxicologically-oriented pathways and gene set tools and ontologies

Showcase three case studies the integration of chemical structural and physiochemical similarity measurements with mode of action features

Impacts

Streamline data reporting, increase the confidence in data processing

Reduce the complexity and redundancy of omics data interpretation. Lower the barrier to understand the output of these analyses to a general toxicologically oriented audience

Provide indications on how to use mode of action data for RAx and grouping



Acknowledgements

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TXG-MAPr team @ UL

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Mick van Schaick

Gerhard Burger

Kirsten Veltman

Tamara Danilyuk

LACDR Lukas Wijaya, Sylvia Le
Devedec, Tessa Hagens

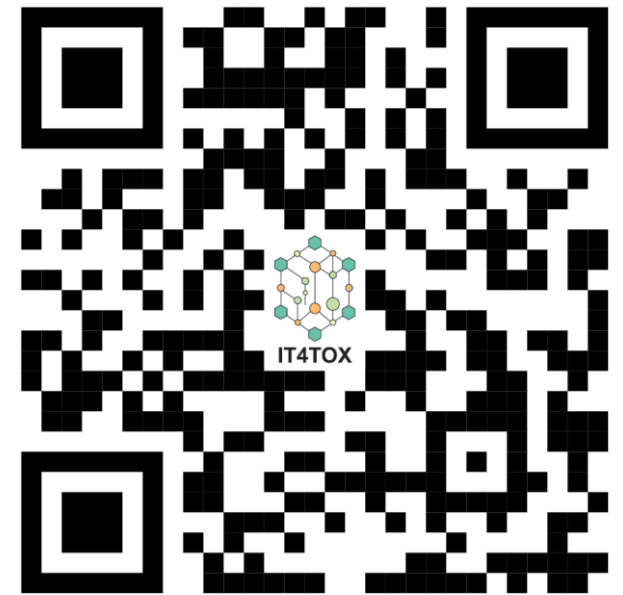
AMC Jesper Kers

US EPA Josh Harrill

RH3R Sylvia Escher, Matthias Wehr,
Barira Islam

And the whole RH3R project!

More information,
including publications
with more case
studies, on the UL
spin-off website
IT4TOX



Universiteit
Leiden



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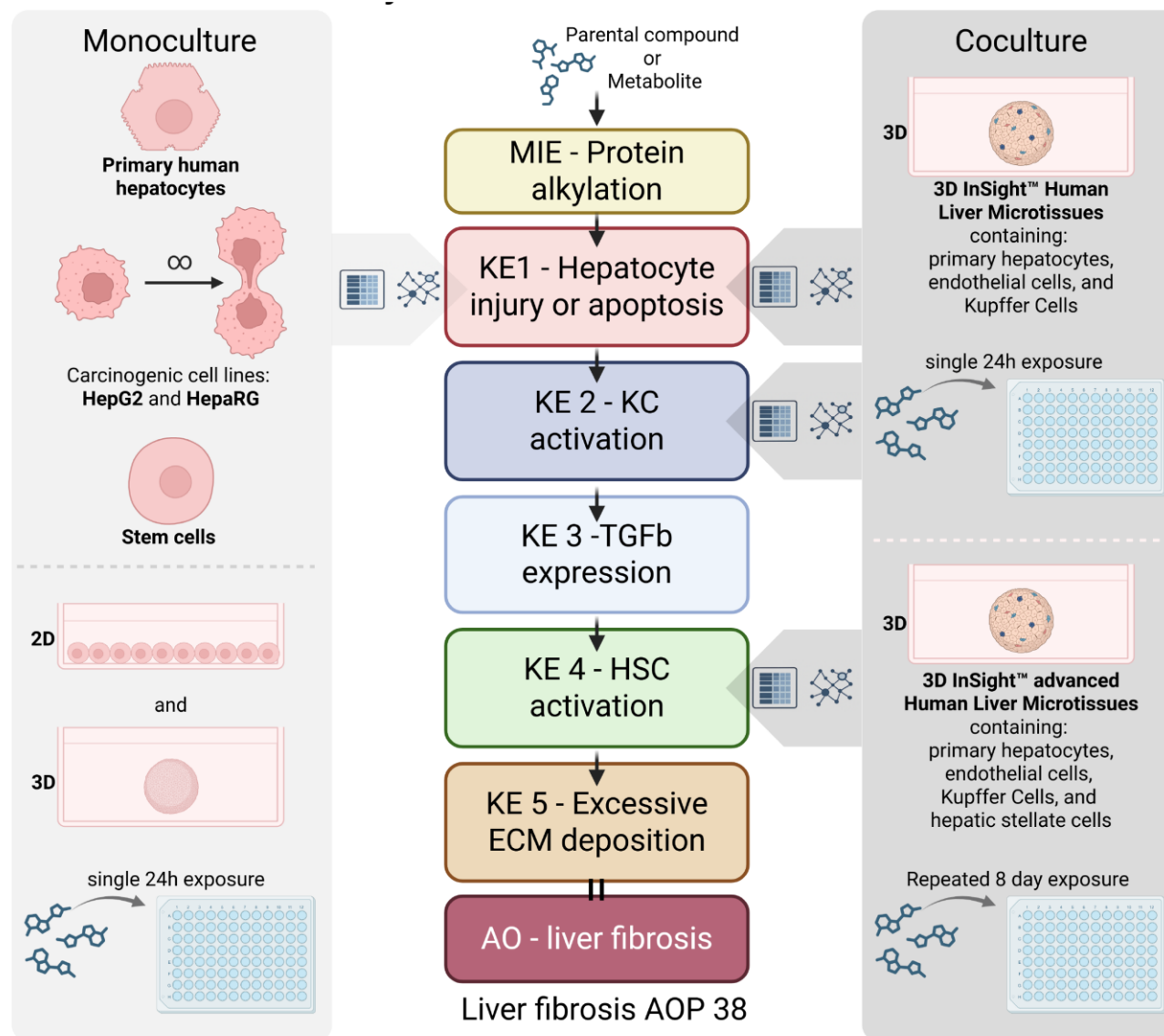
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Extras



Systematic comparison of liver test systems for the modulation of KE1 of the fibrosis AOP 38

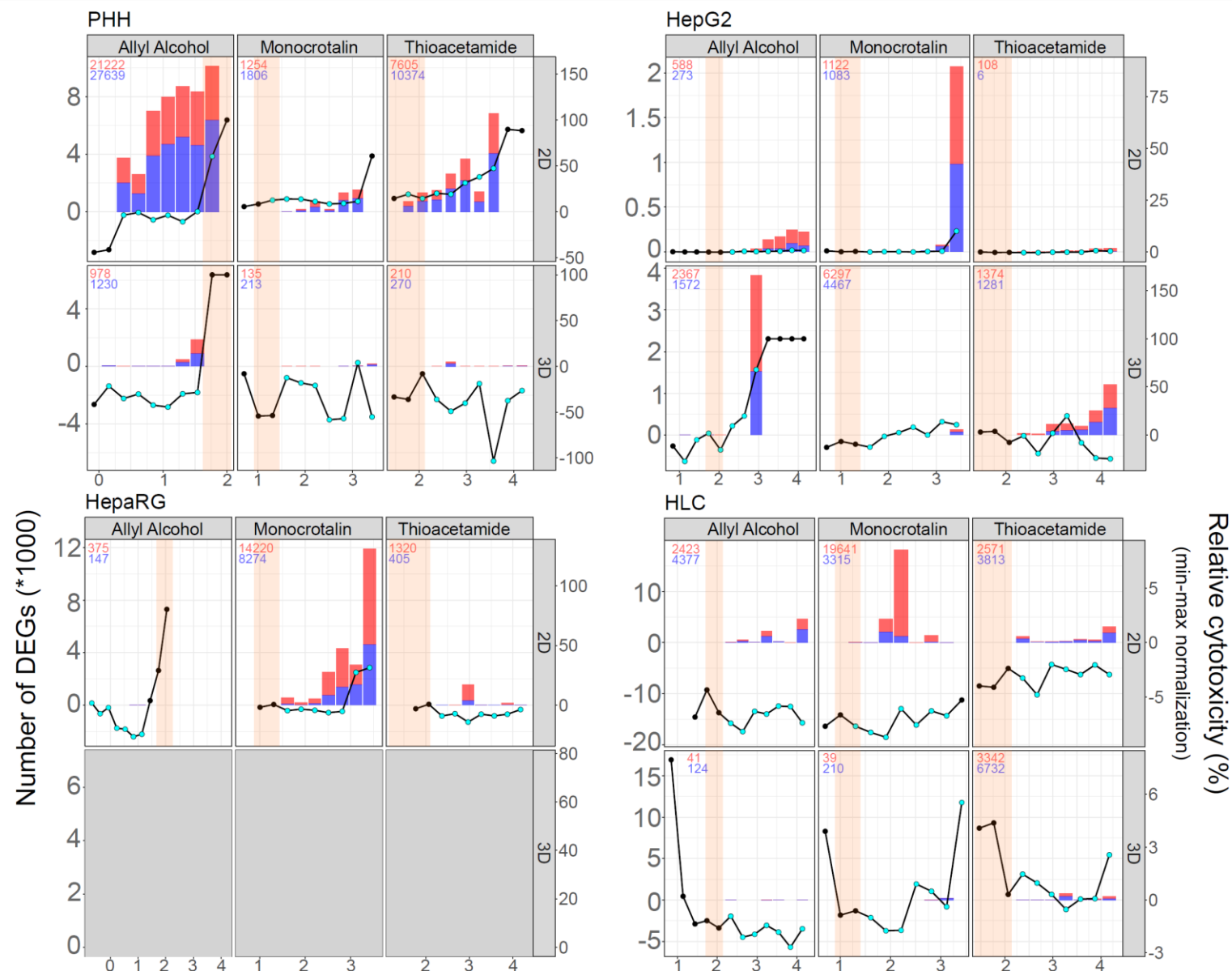
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Cytotoxicity and DEGs comparison between different liver test systems after Allyl-alcohol, Monocrotaline and Thioacetamide treatment

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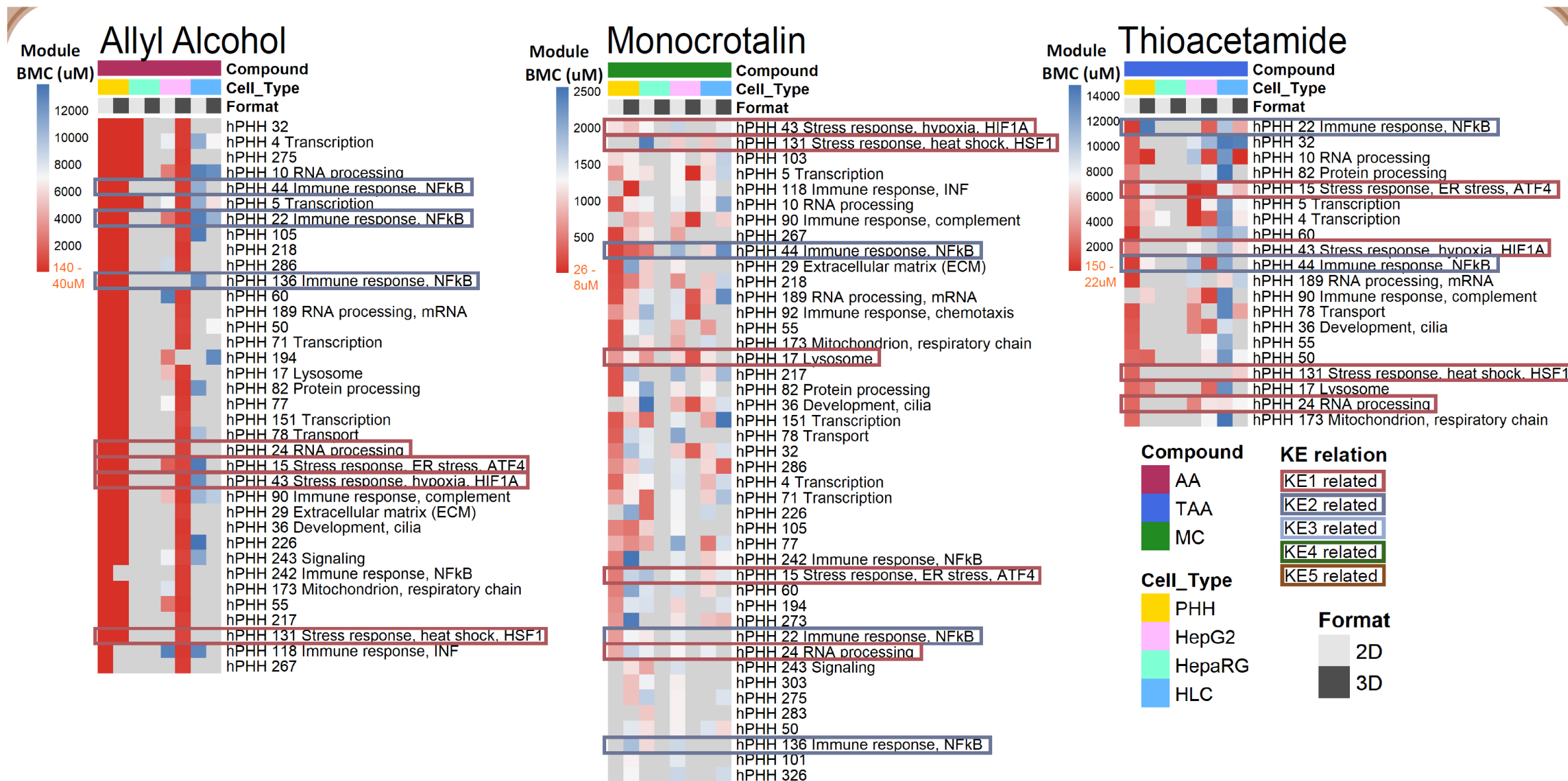


Tessa Hagens et al., manuscript in preparation



Transcriptional BMC analysis shows PHH as most sensitive for activation of cellular stress response that are related to KE1 of AOP 38

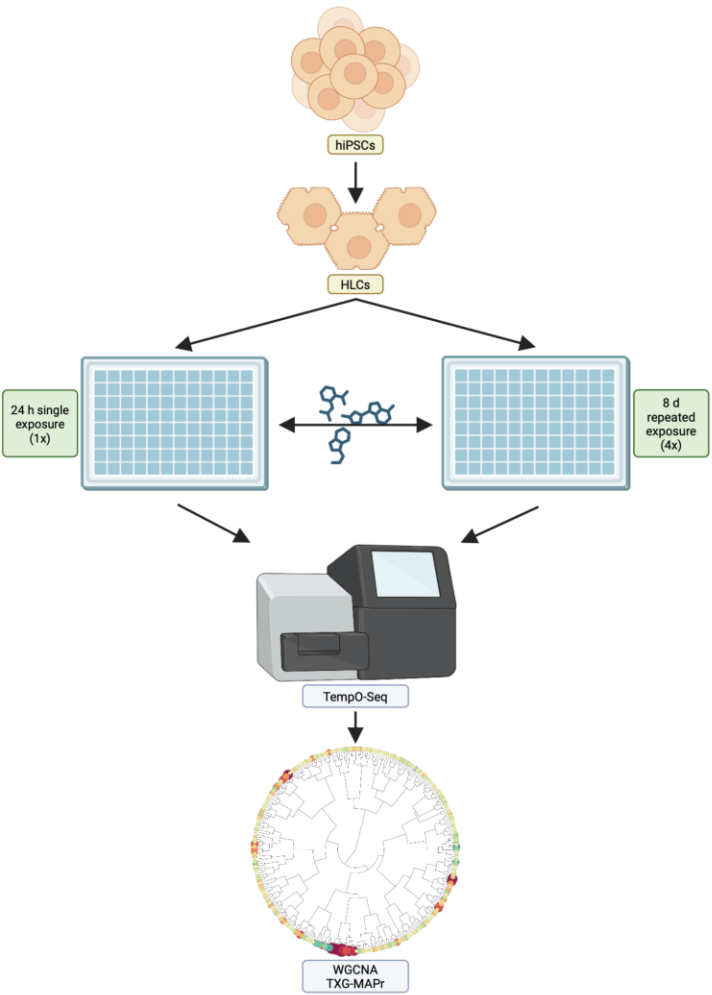
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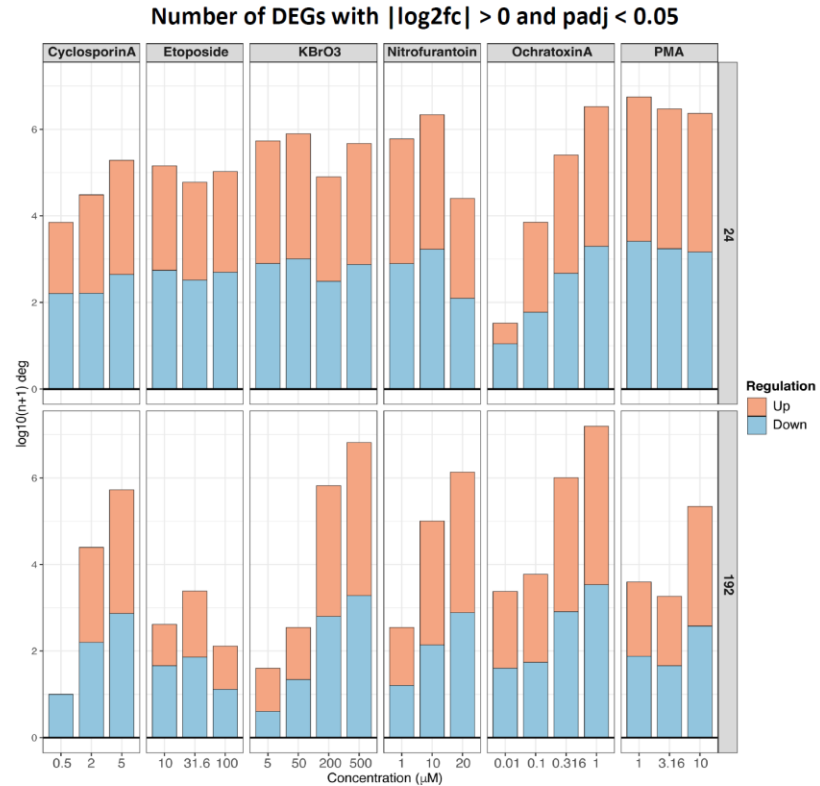


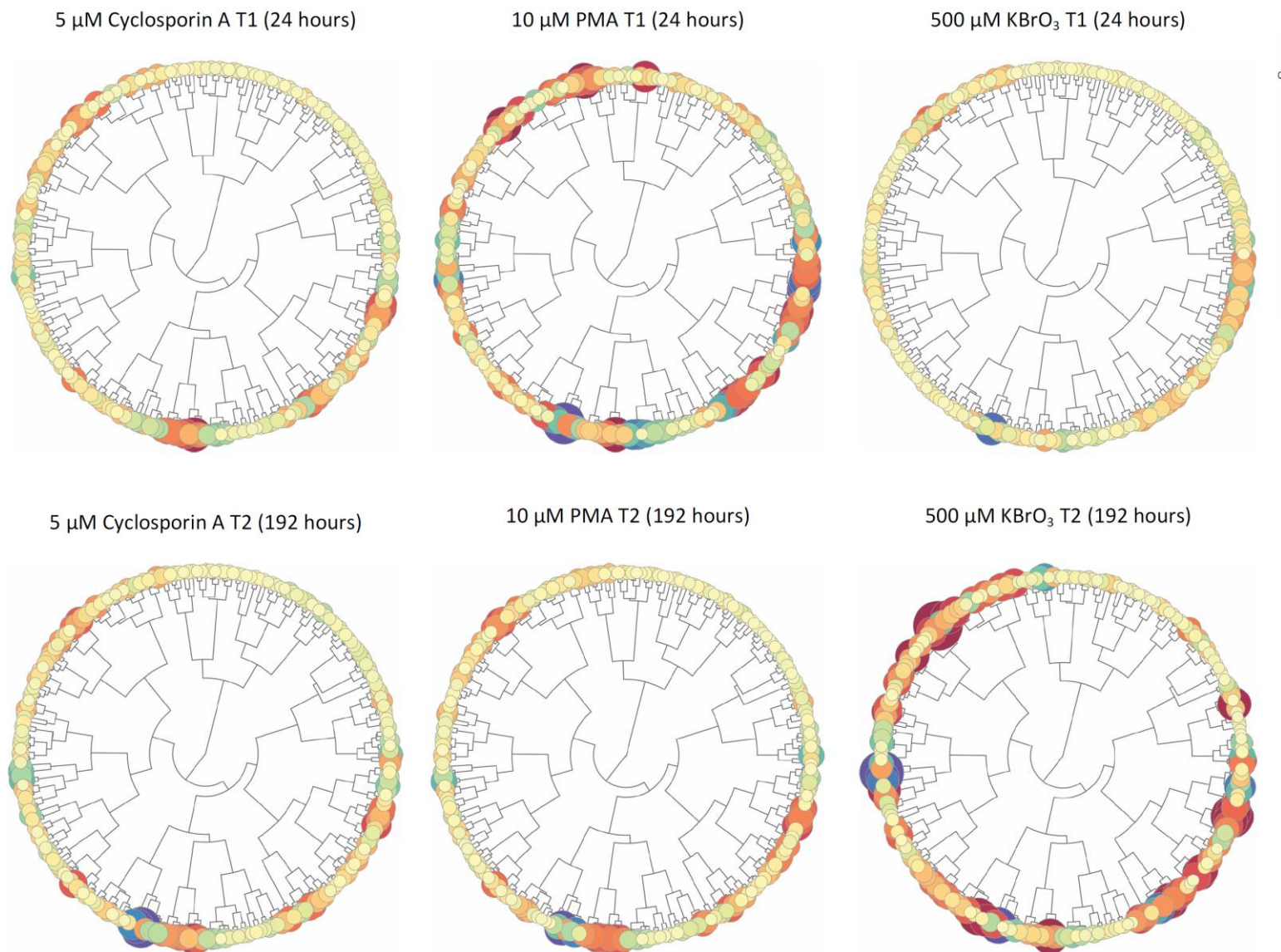
Time dependency of MoA assessment: non-genotoxic carcinogens as a case study.

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Compound	MOA	Carcinogenic	Concentrations (μM)			
			C1	C2	C3	C4
Ochratoxin A	Oxidative DNA Damage	Yes, IARC 2B	0,01	0,1	0,316	1
Potassium Bromate (KBrO ₃)	Oxidative stress	Yes, IARC 2B	5	50	200	500
Nitrofurantoin	Oxidative stress	No	1	10	20	
Phorbol 12-myristate 13-acetate (PMA)	Mitogenic, inflammation	Yes	1	3,16	10	
Cyclosporin A	Immune suppression	Yes, IARC 1	0,5	2	5	
Etoposide	DNA damage	Yes, IARC 1	10	31,6	100	

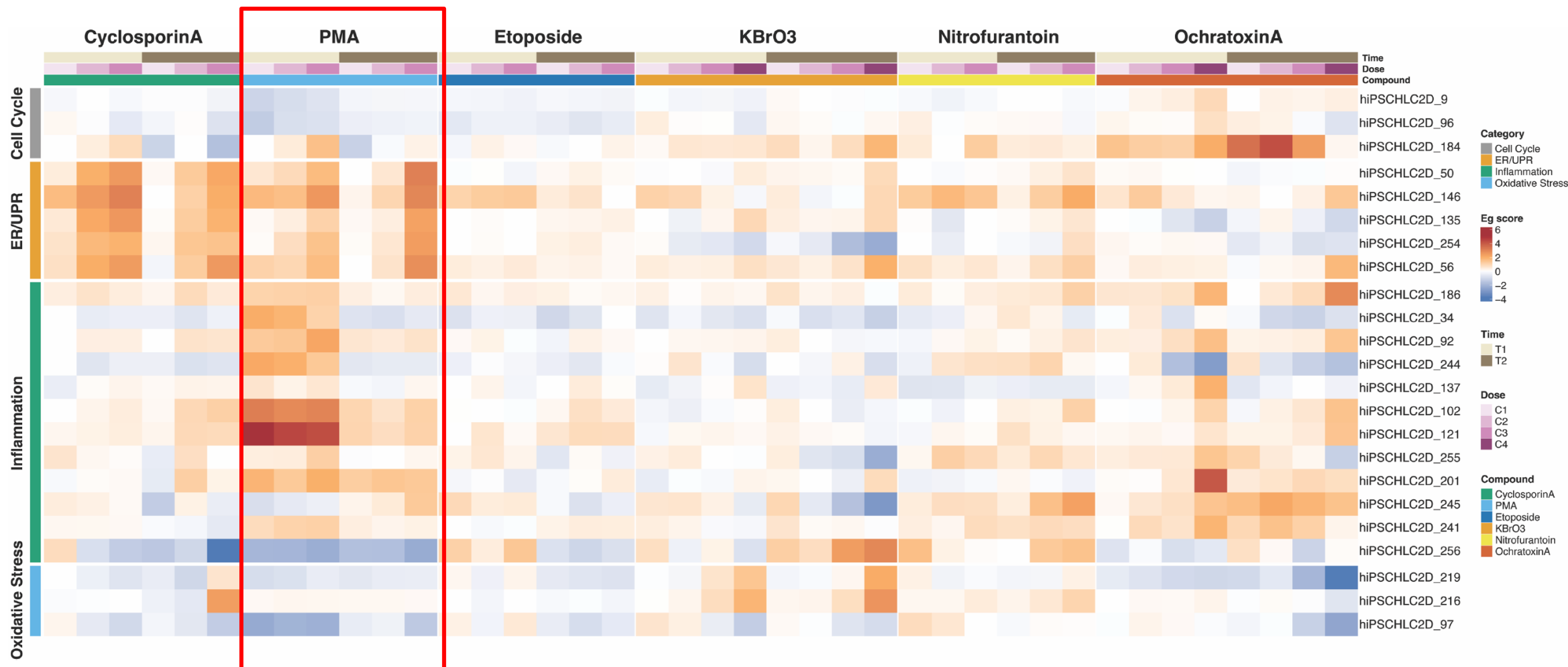






Differential modulation of gene networks in iPSC-HLC after treatment with CsA, PMA and KBrO3.

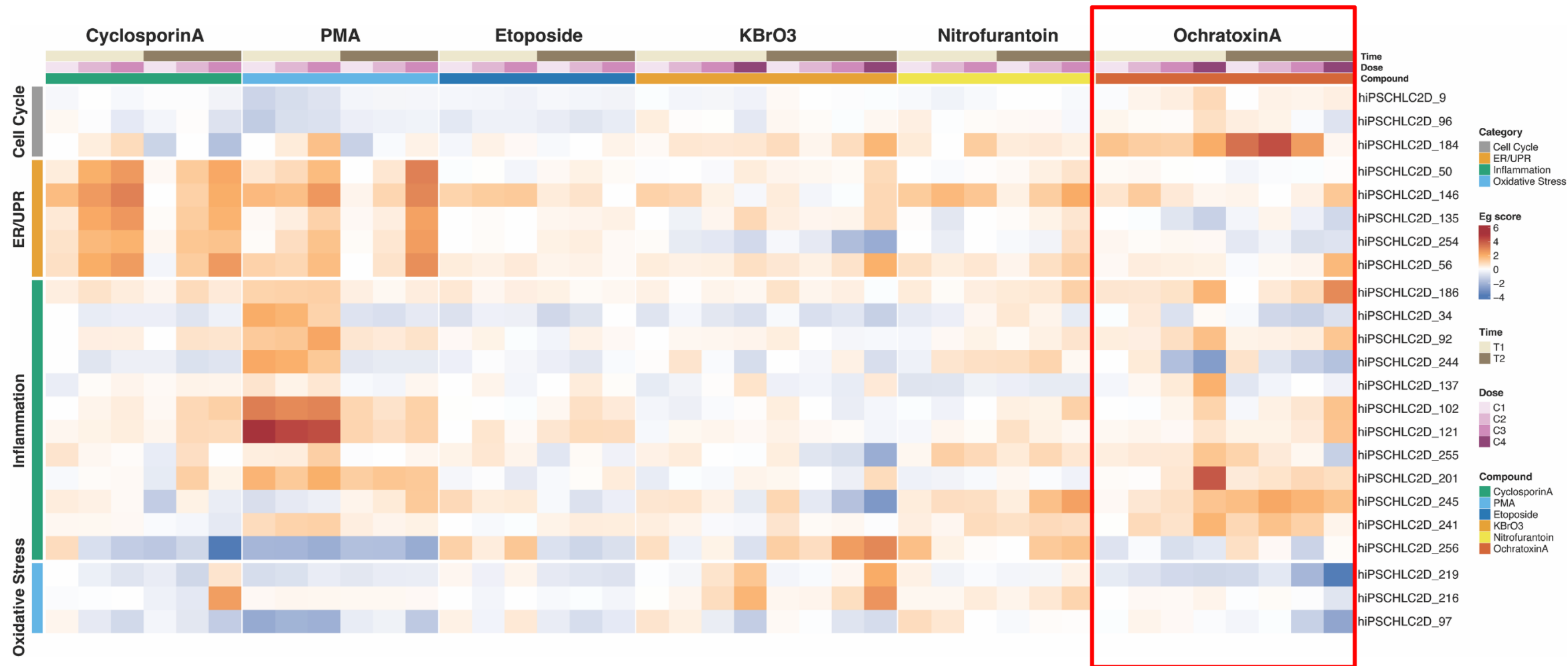
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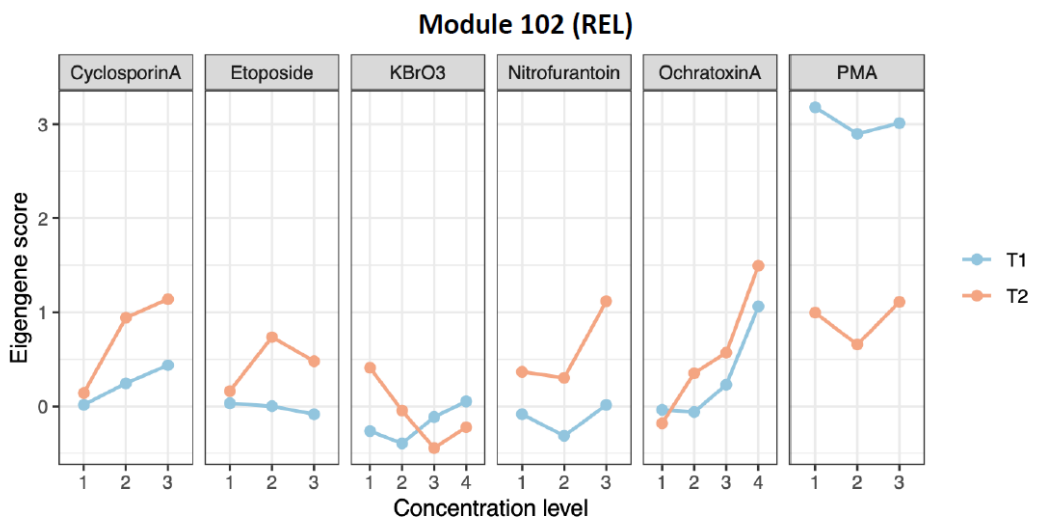
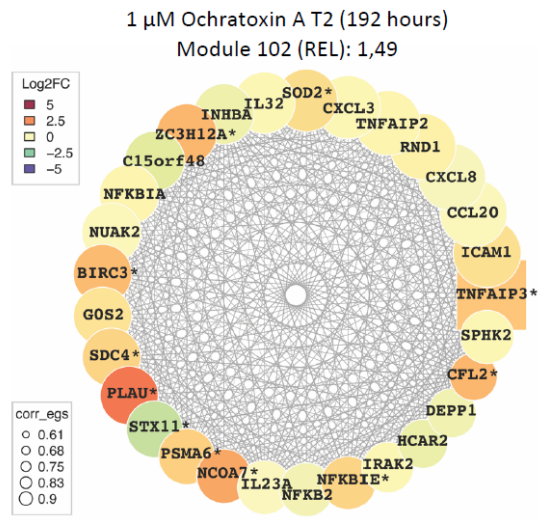
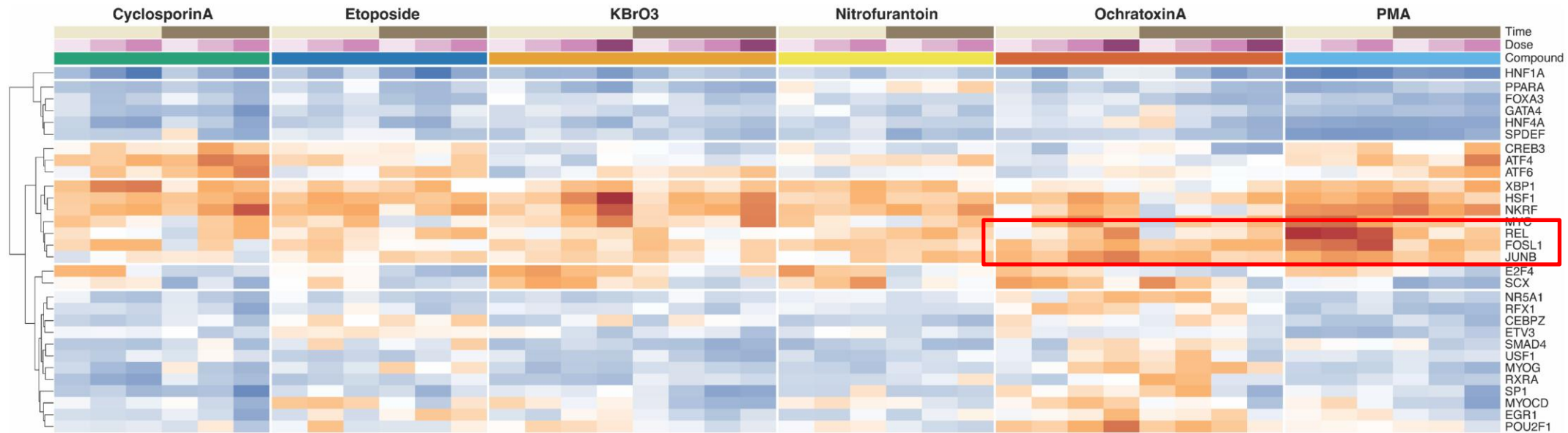
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Sustained transcription factor activation by OchratoxinA and PMA is related to NFkB and AP-1.

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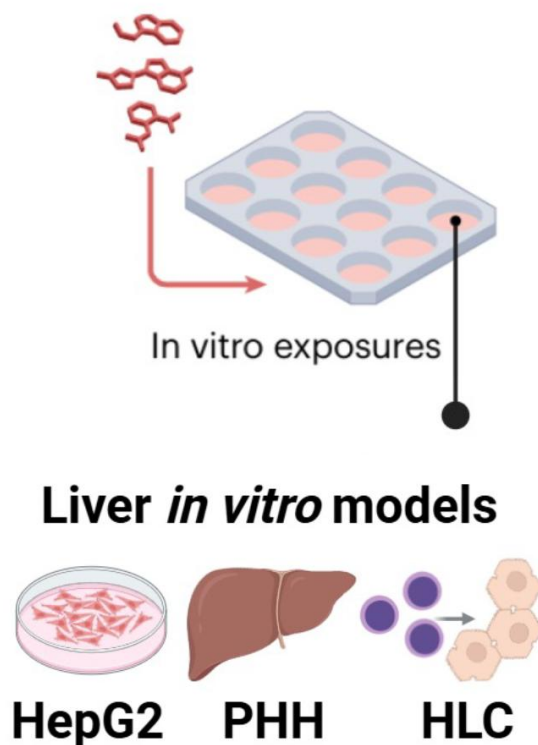




Comparison of transcriptomics and metabolomics for assessment of bioactivity: a liver test system comparison.

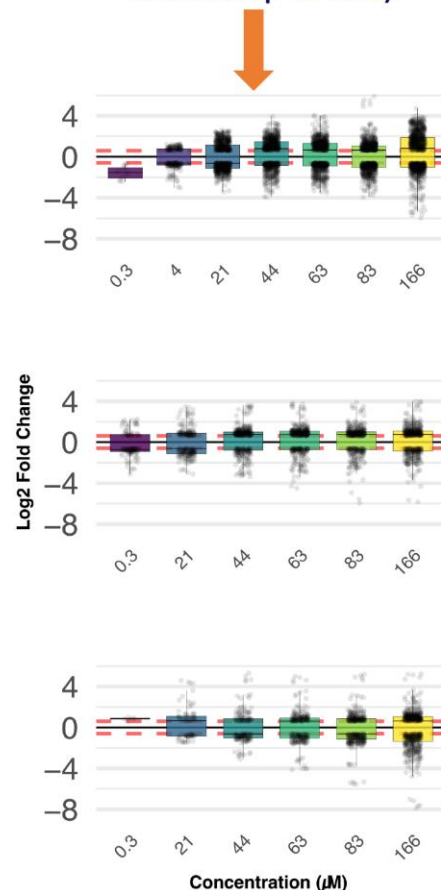
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Compound	DILI Class	Associated DILI pathology
Nitrofurantoin	DILI Class A	idiosyncratic acute liver injury, acute hepatitis
Diclofenac	DILI Class A	acute lobular hepatitis
Ketoconazole	DILI Class A	idiosyncratic acute liver injury, acute hepatitis

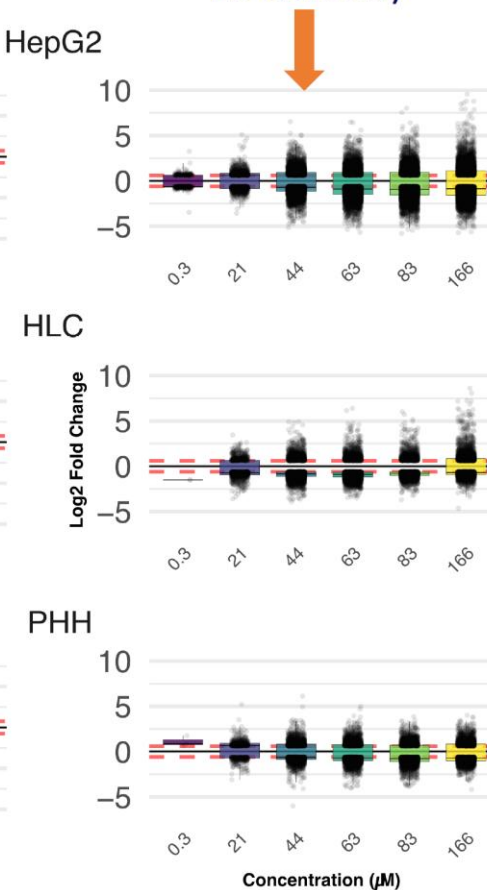


Experimental Feature	HepG2	PHH	HLC
Dose response (Both Omics @ 24h, 6 dose levels)	✓	✓	✓
Time Course (Metabolomics: 3, 6, 24, 48h)	✓	--	--

Targeted transcriptomics
(TempoSeq, whole transcriptome)



Targeted metabolomics
(~255 Metabolites, 13 classes)

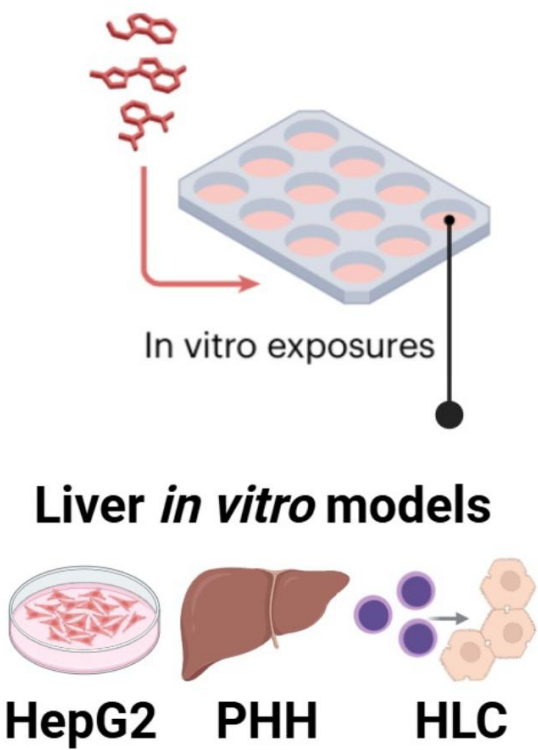


Ex: Ketoconazole 24 hours ($|\log_2FC| \geq 0.6$, $p_{adj} \leq 0.05$)

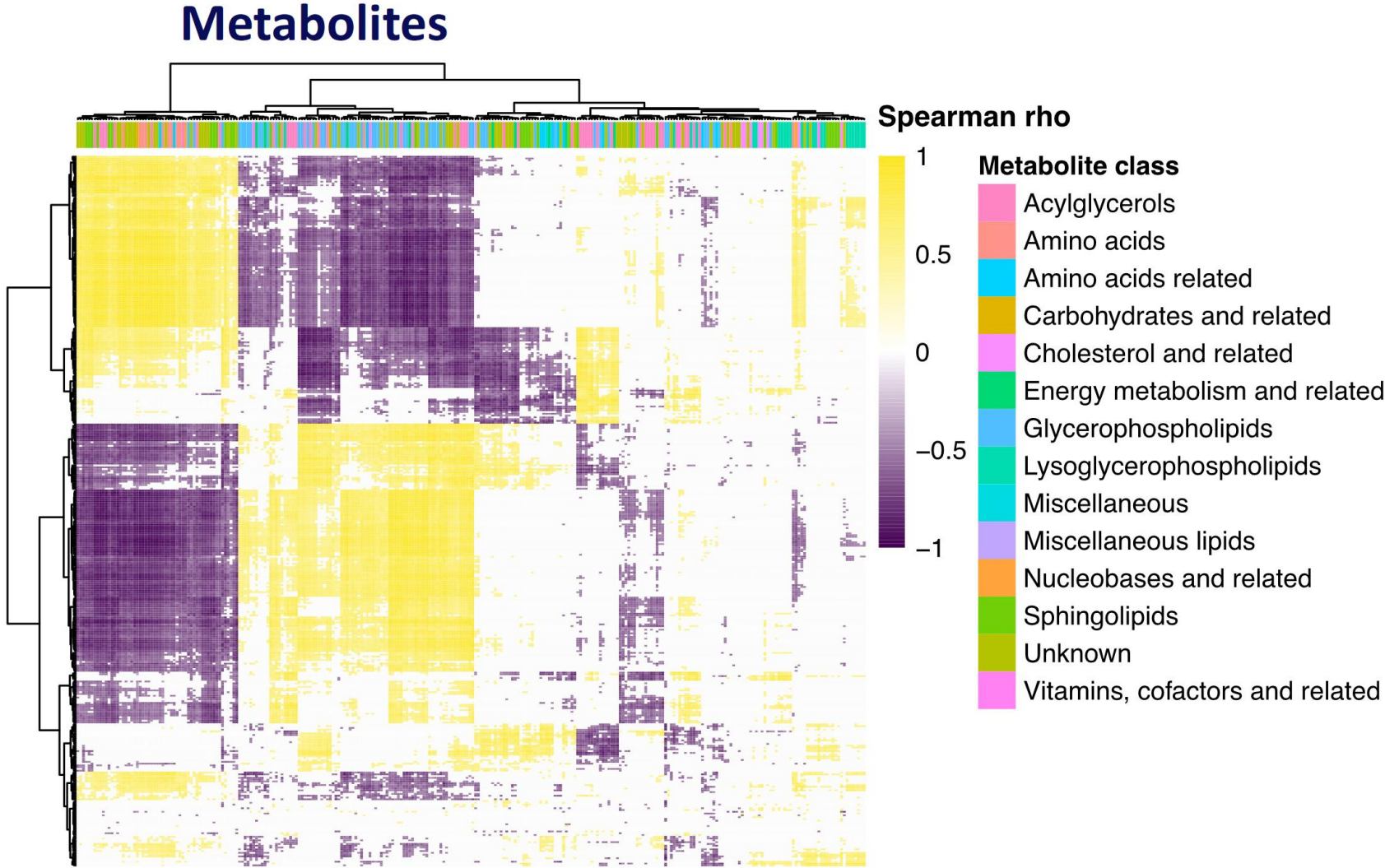


Positive and negative correlation between transcriptomics and metabolic responses.

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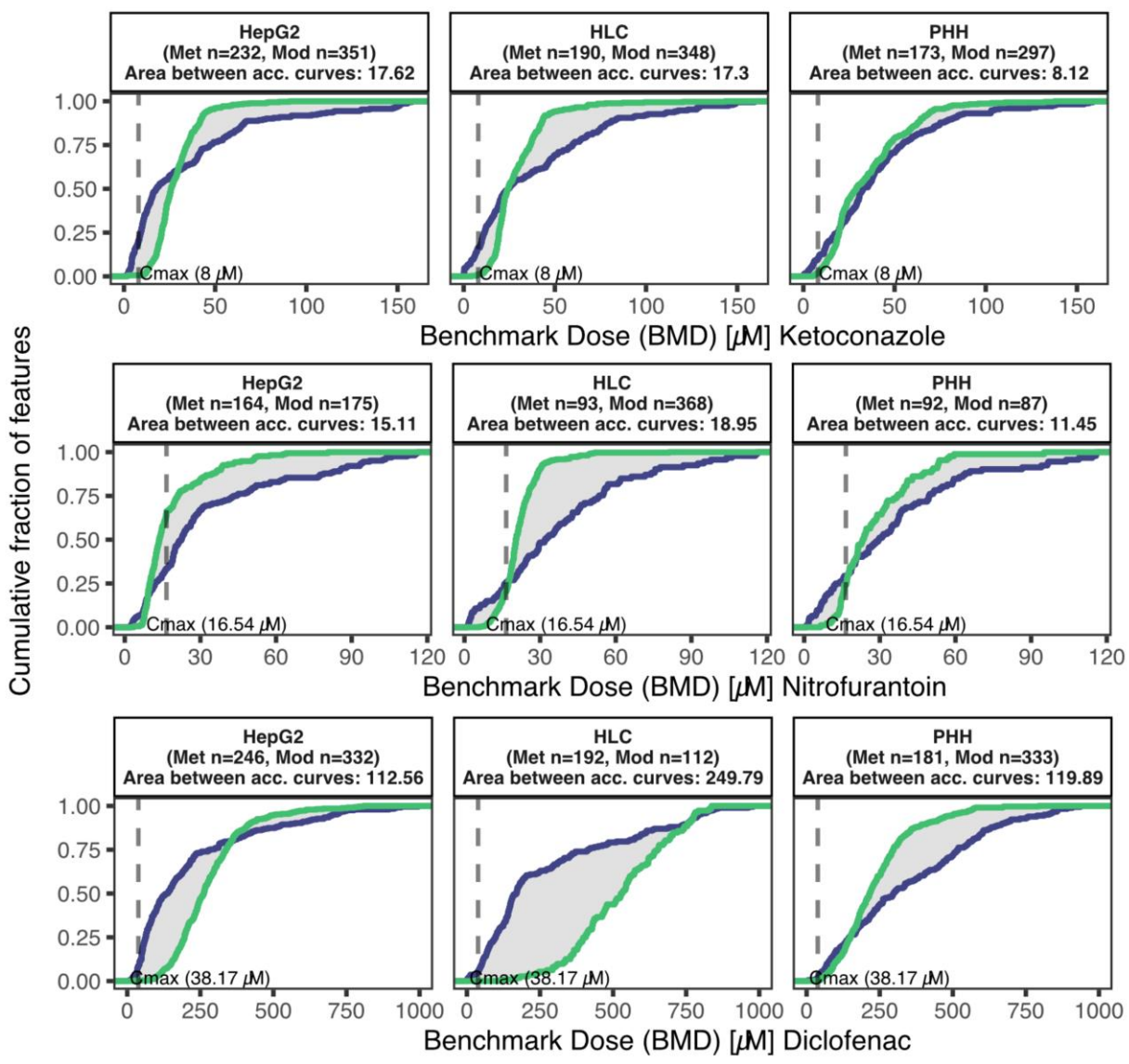
PHH TxGMAPr gene modules



Ex: HepG2 Ketoconazole 24 hours dose response (fdr <= 0.01)

Compound	DILI Class	Associated DILI pathology
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Omics Layer

Metabolomics

Transcriptomic gene networks