ASPIS

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RISK HUNT3R

Open Symposium

2-3 July 2026

Maastricht
The Netherlands

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

Giulia Callegaro

Leiden University

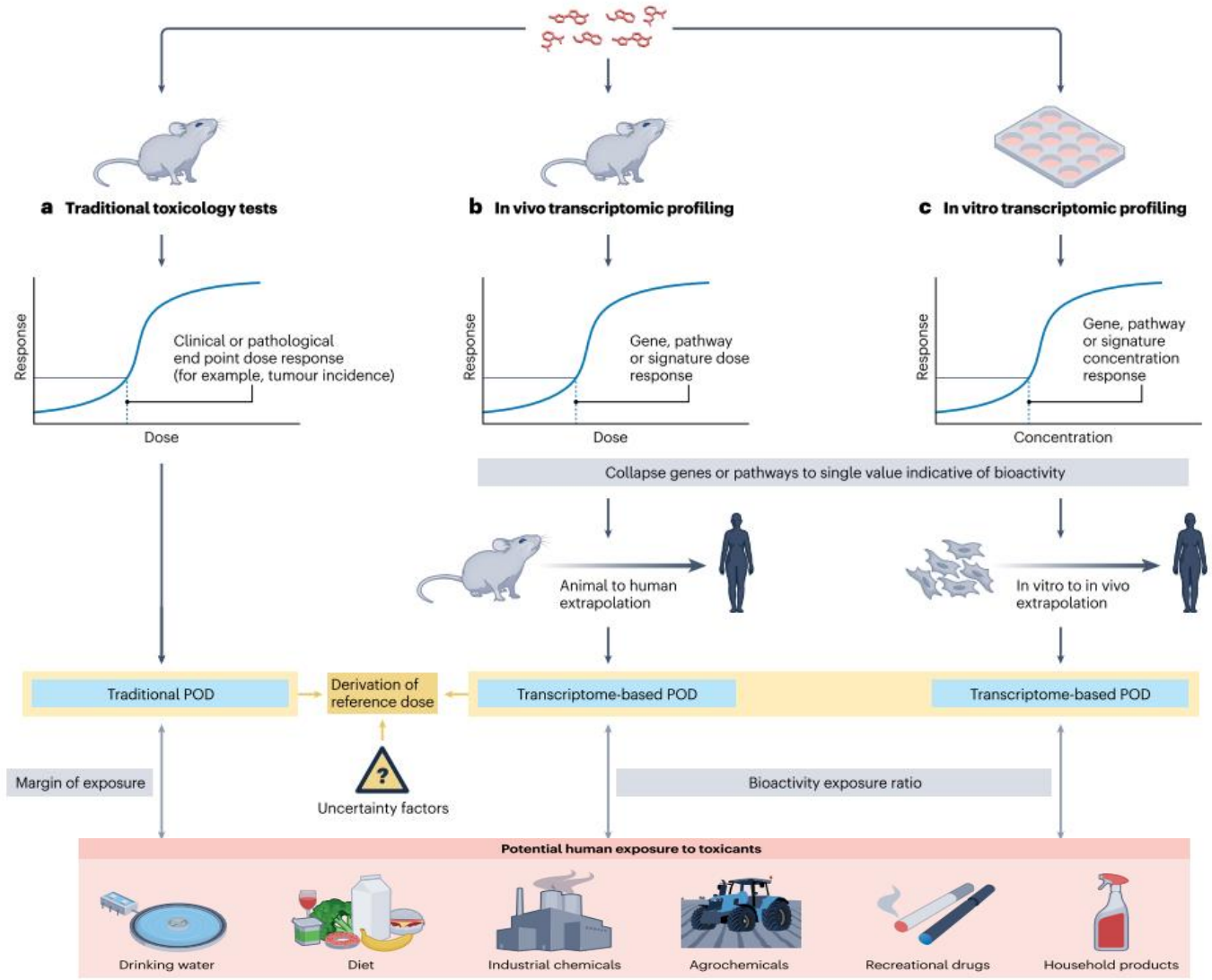
RISK-HUNT3R project

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)

Push 3Rs, develop NAMs, keep up with thousands of chemicals to be evaluated: how?

Toxicogenomics is well-positioned to support mechanistic safety assessment

- Mechanistic read-out
- Holistic view
- Well accepted



Adapted from Meier et al. (2024)

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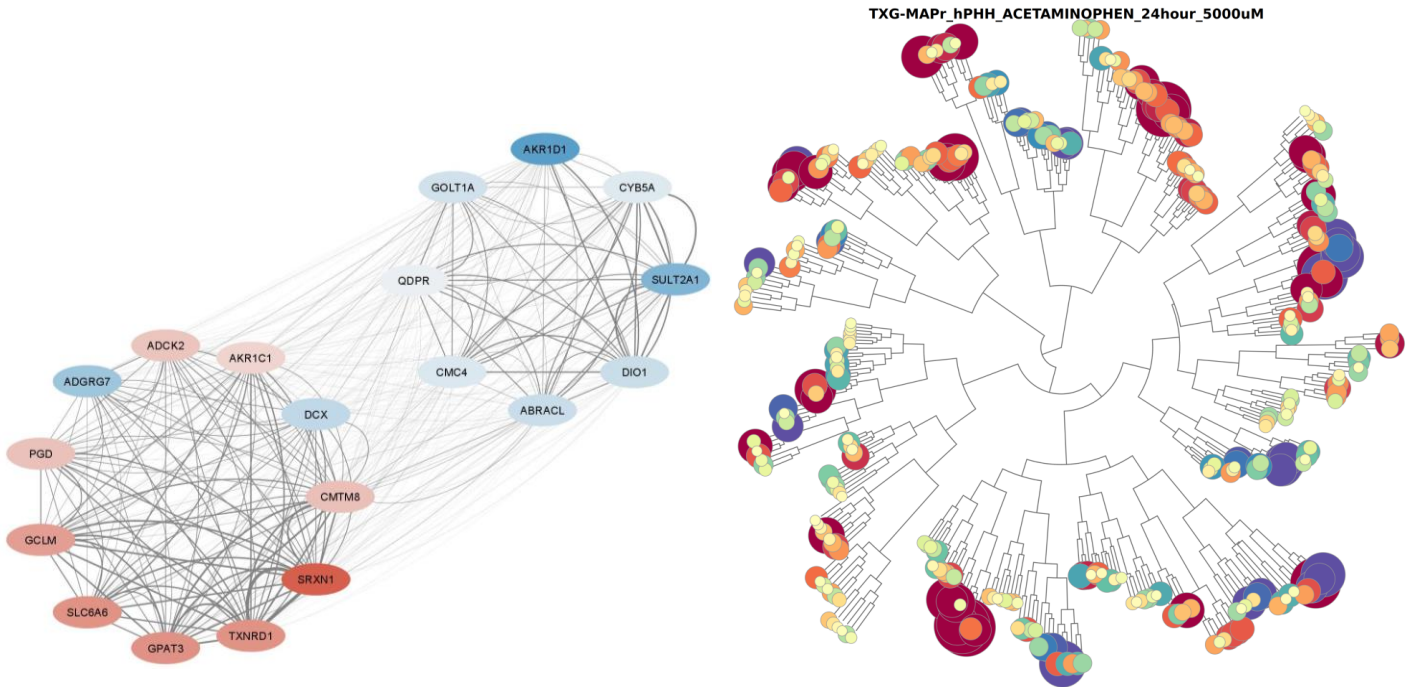
However still suffers from limitations

- Unclear interpretation framework
- Unclear translation to human pathophysiology
- Unmet context-specific requirements

Toxicogenomics: networks are our solution

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



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The TXG-MAPr tools

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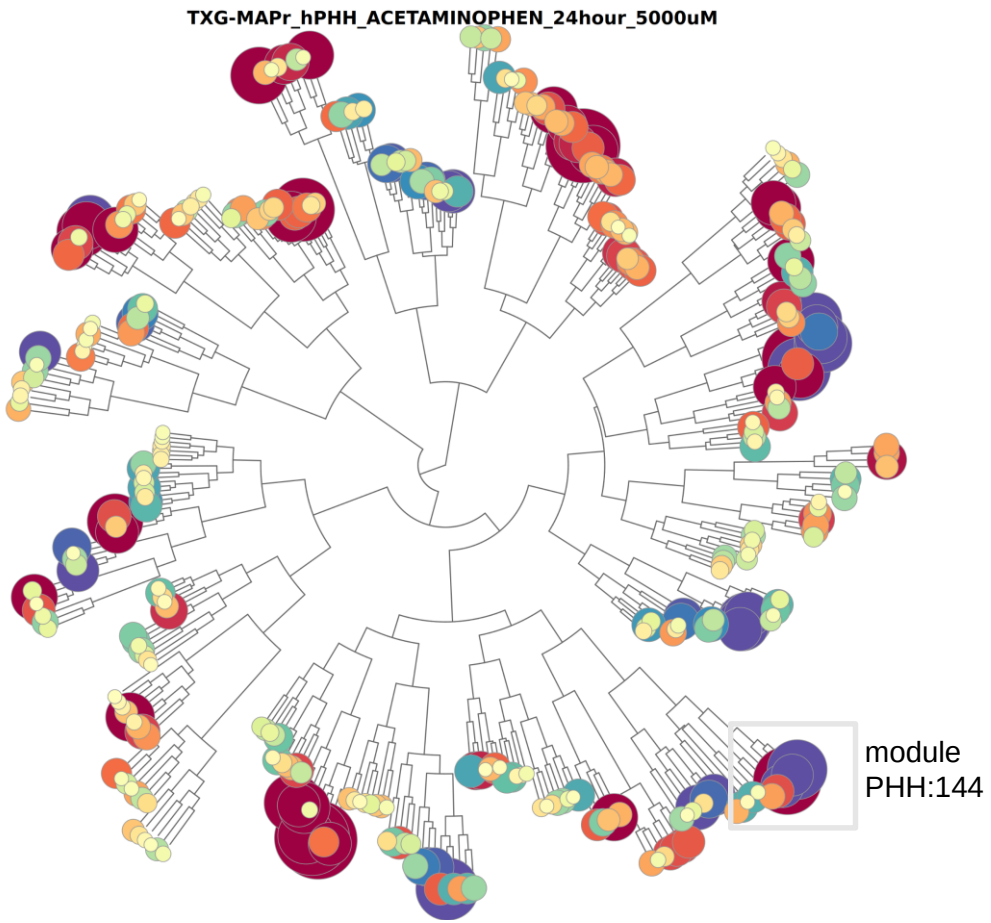

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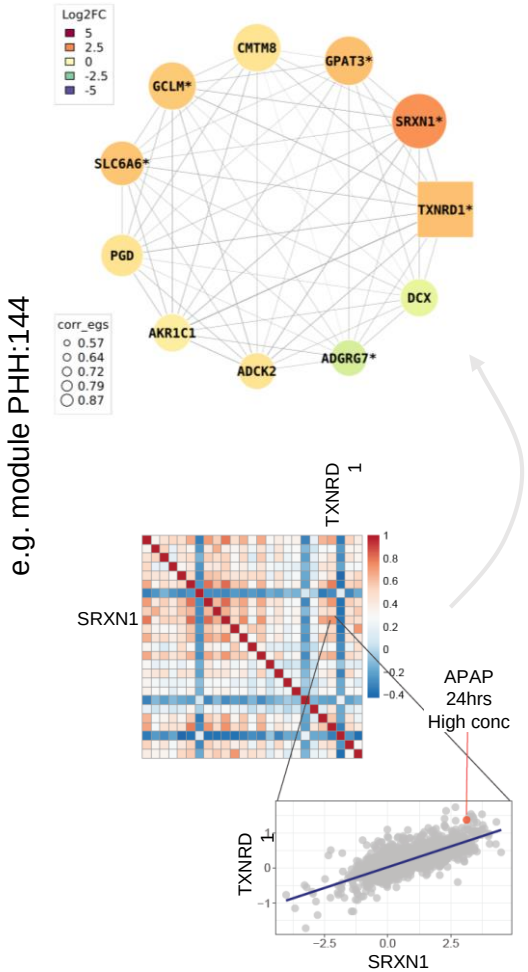
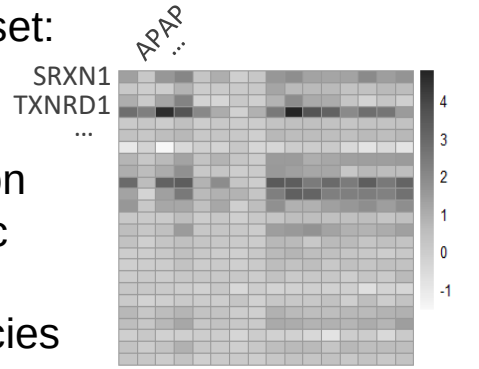
An ensemble of subnetworks, each representing a group of genes co-expressed in the

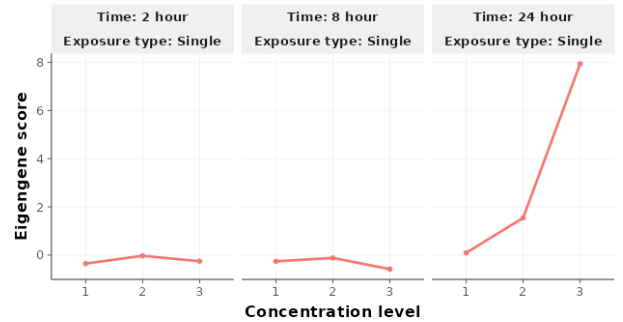
e.g. module PHH:144



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



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

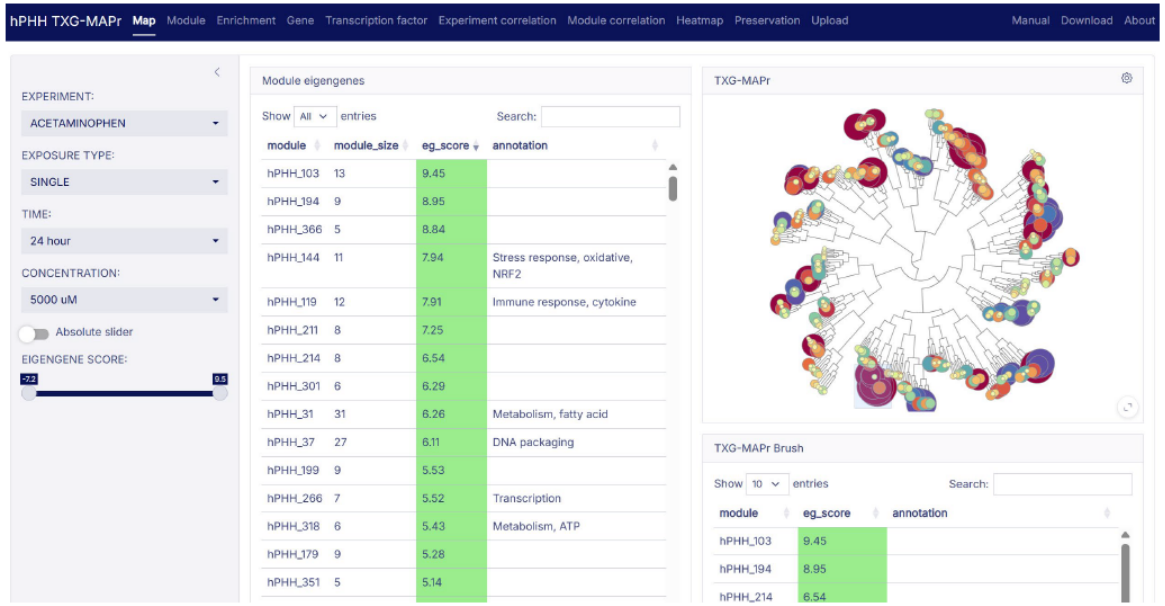


Stress response, oxidative, NRF2

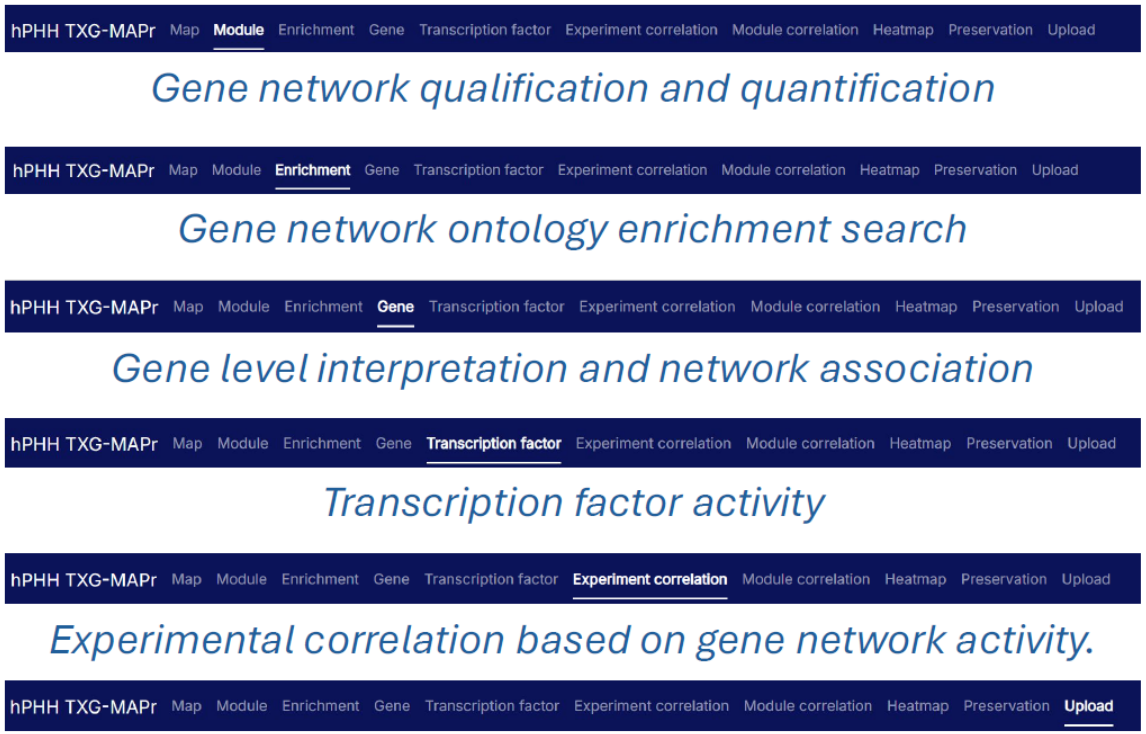
Term	P adj
NRF2 pathway (WikiPathway)	0.00000564
Glutathione metabolism - Homo sapiens (human, KEGG)	0.000706
Oxidoreductase (UniProt)	0.00570

The TXG-MAPr tools: interactive platform

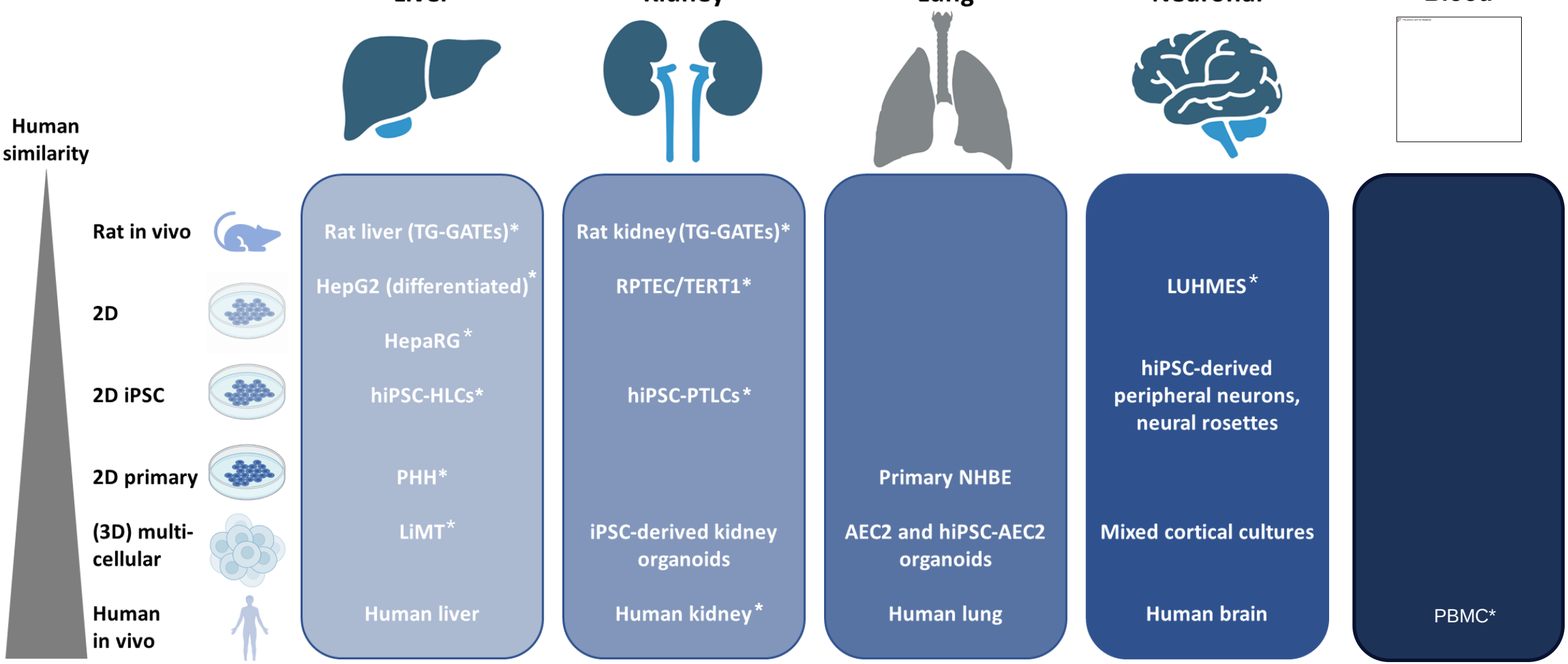
TXG-MAPr toxicogenomics interpretation toolbox



Interactive tabs for mechanistic insights



The TXG-MAPr tools development

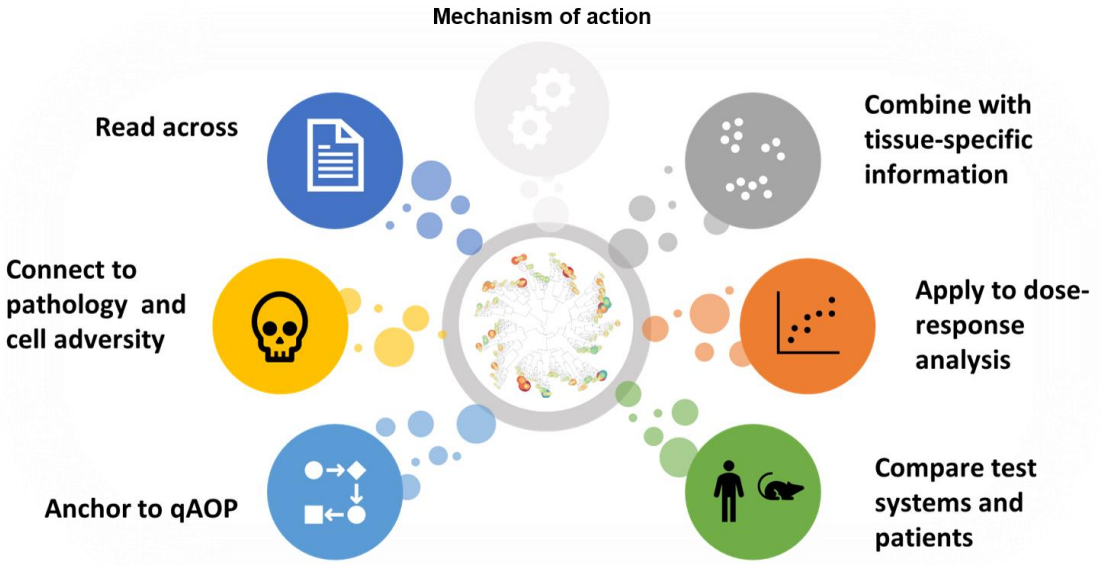


*** TXG-MAPr tools already established**

The TXG-MAPr tools case studies

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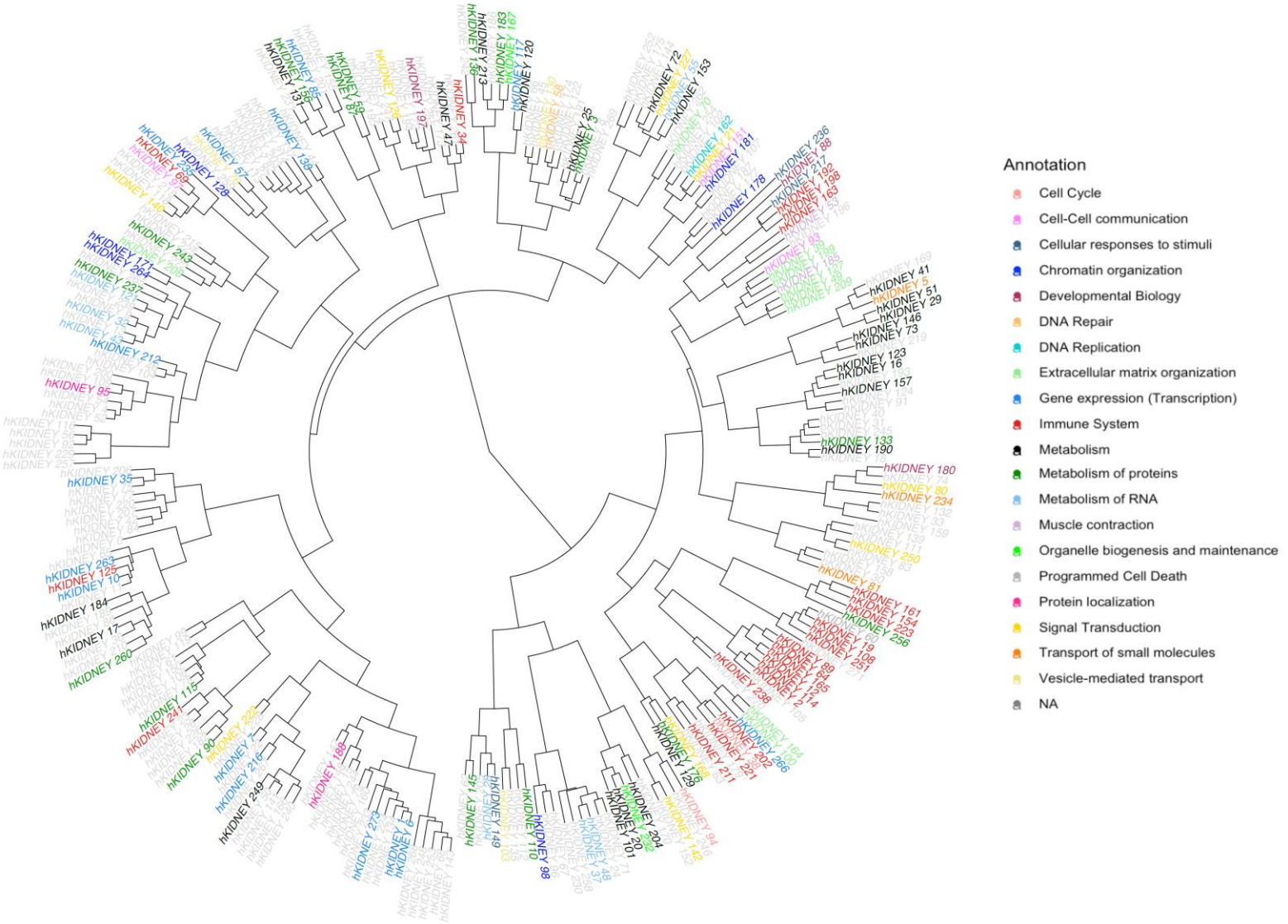
- >1000 kidney biopsy samples
- Diversity of renal pathologies
- Pathology scoring
- TempO-Seq of all biopsies



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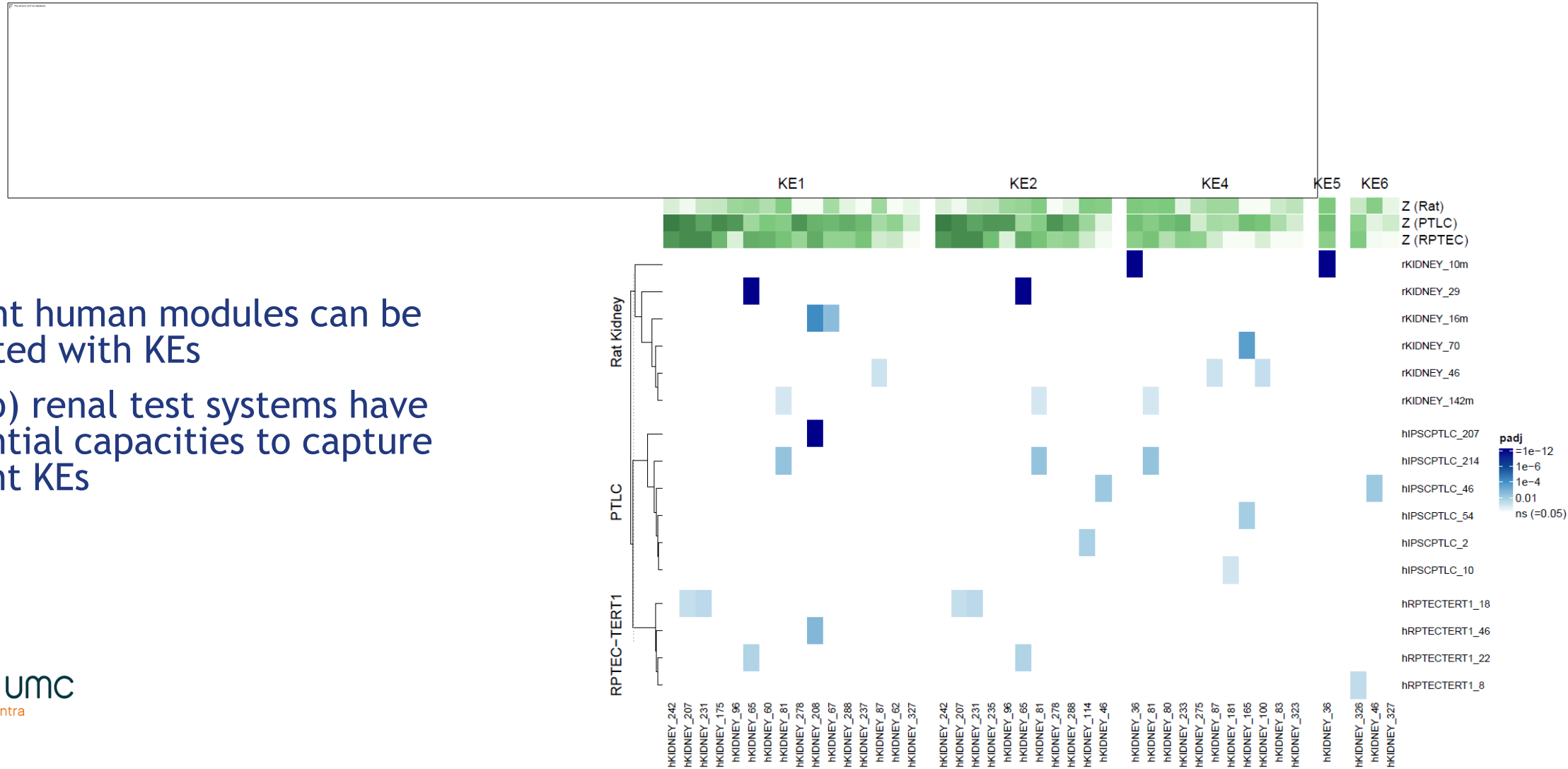
Imke Bruns et al., manuscript in preparation

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

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



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efsa

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Imke Bruns et al., manuscript in preparation

RISK-HUNT3R case study: can we use 24 hr PHH transcriptomics predict 90-day rat high toxicity chemicals?

- 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 advese effect up to 1000 mg/kg bw/d

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

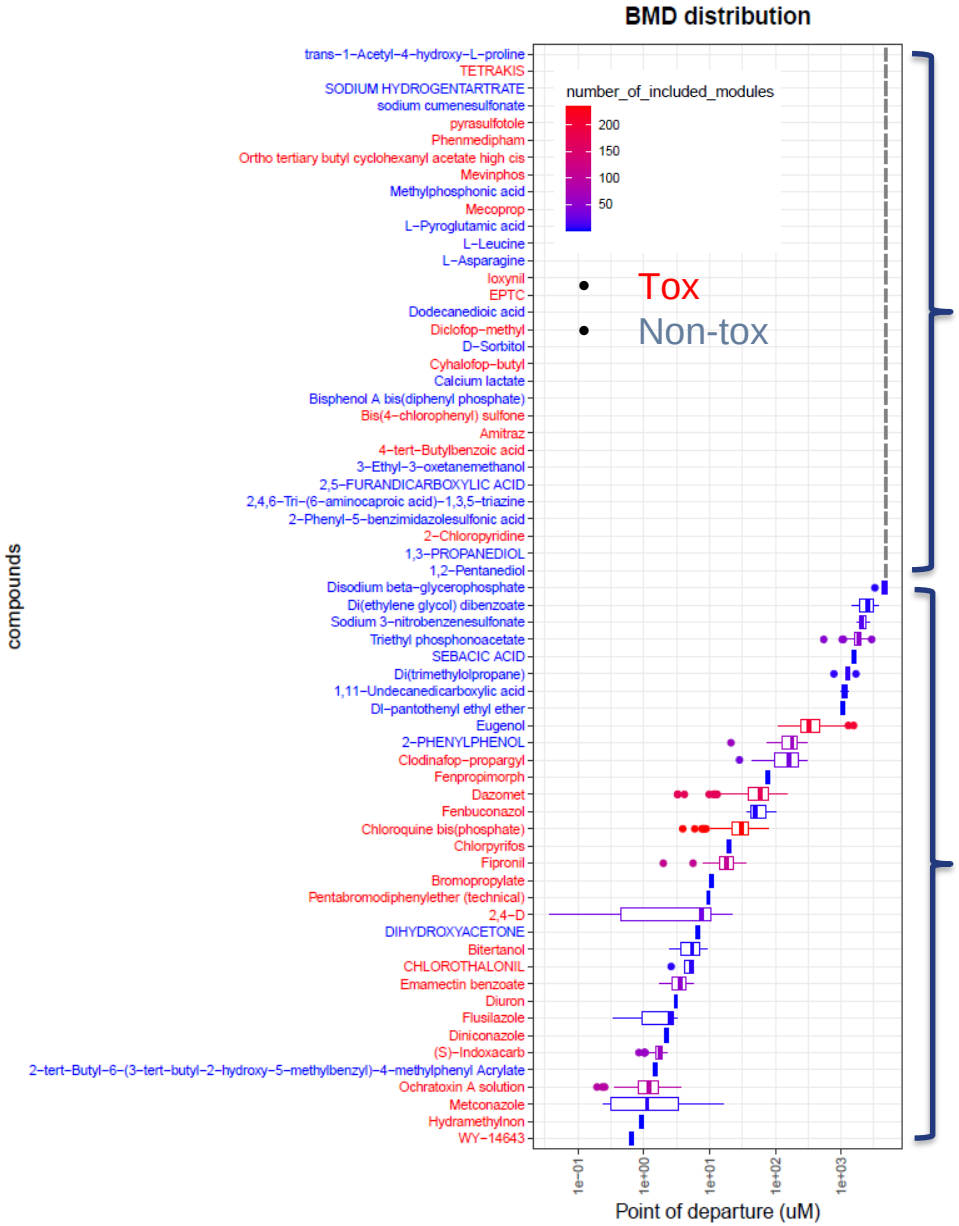
Target organ at LOAEL	Number of compounds
CNS	2
General	2
Heart	1
Hematology	5
Kidney	10
Liver	20
Spleen	1
Testes	1
Thyroid	1
Urinary Bladder	1

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

Toxicogenomics can prioritize chemicals with liability to be highly toxic

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



Compounds with tPOD >10 mM

(NOTE: these compounds have limited DEGs)

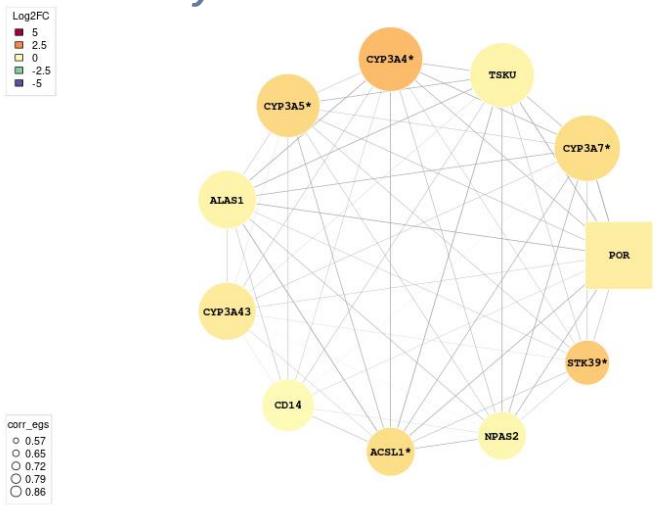
Compounds with detectable tPOD

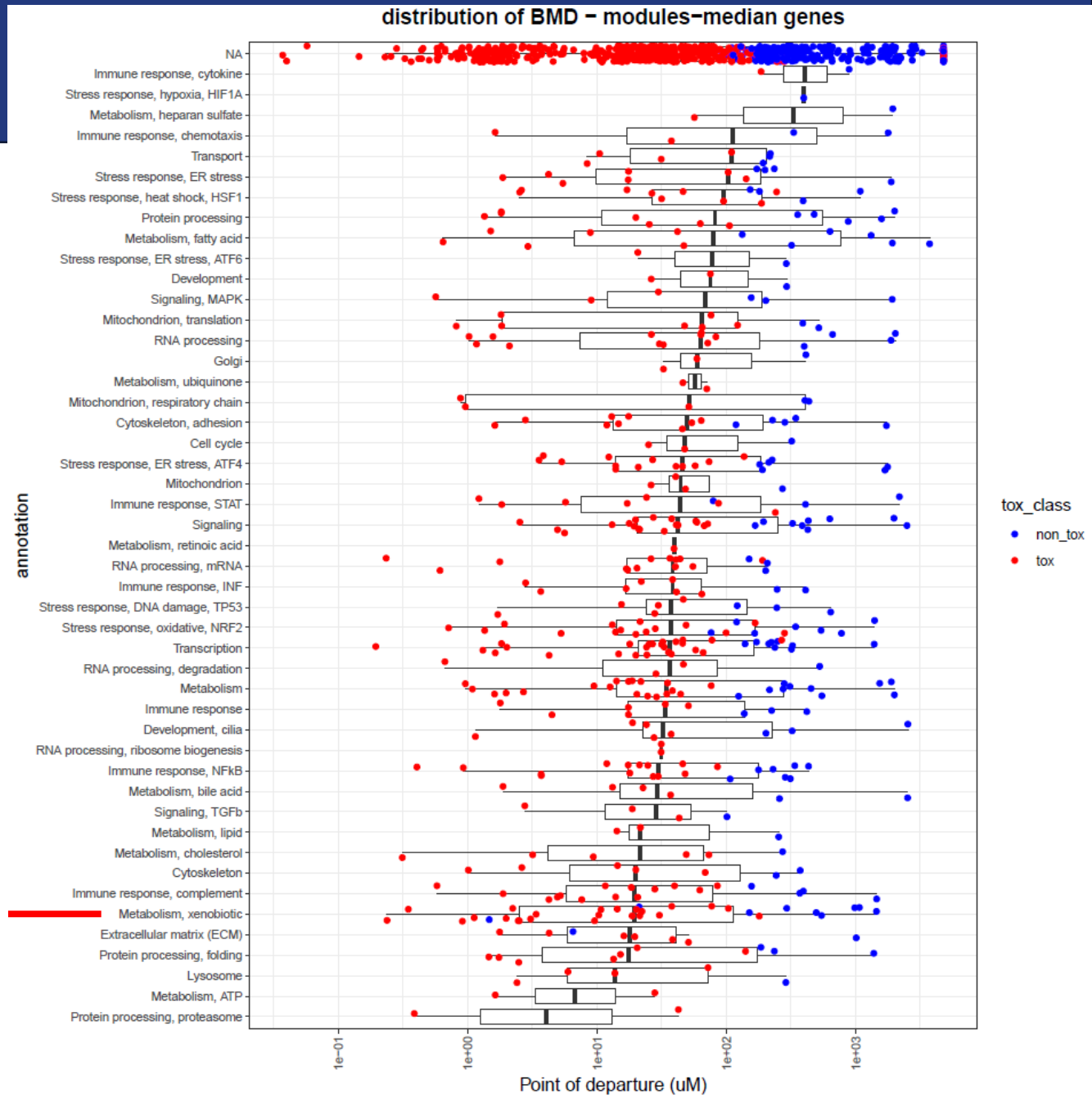
Wijaya et al. in preparation

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

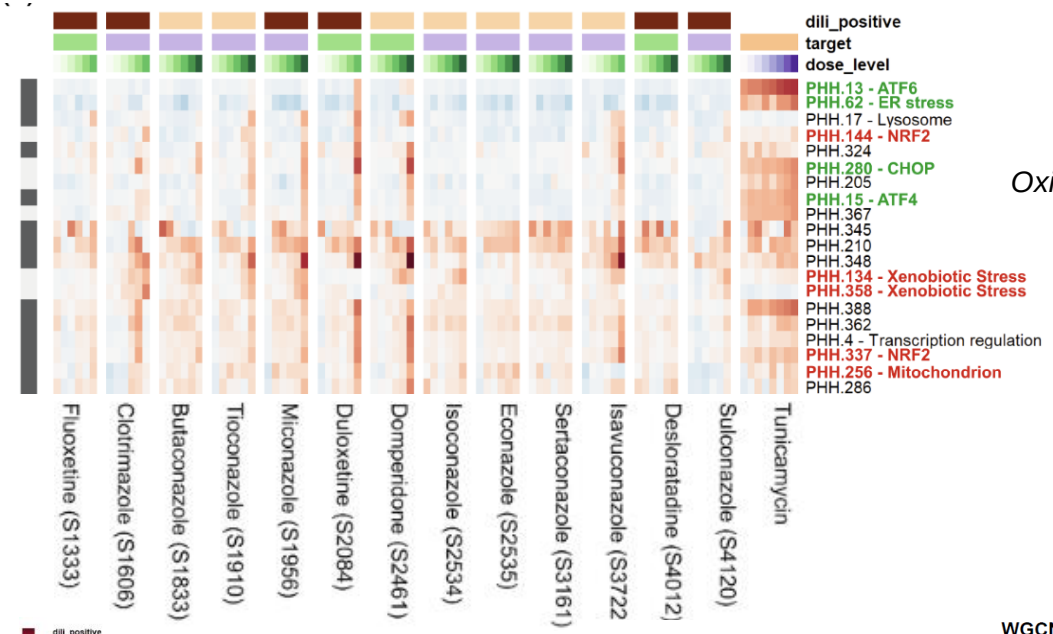


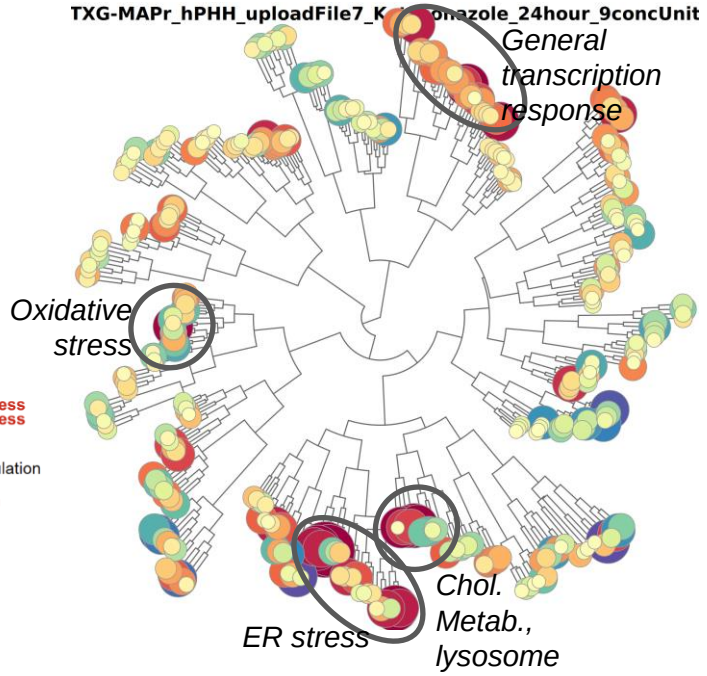


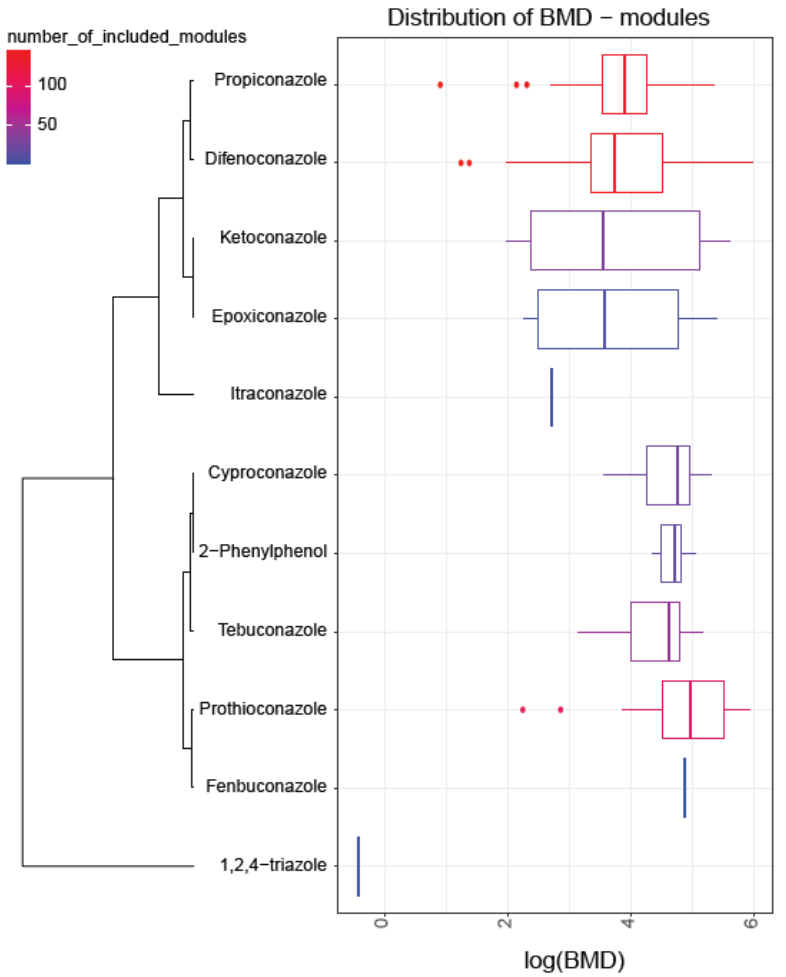
Wijaya et al. in preparation

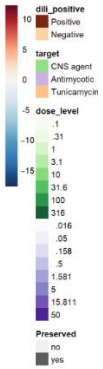
TXG-MAPr: from safety alerts *in vitro* to Rax Conazole case study

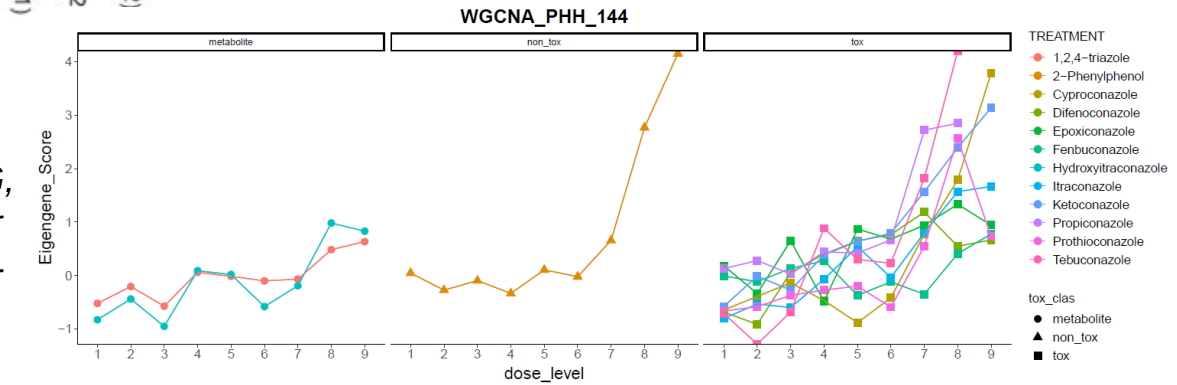
HepG2 reporters screen → Antimycotic drugs inducing CHOP → Confirmed with TXG-MAPr (PHH) → Connected to mitochondria impairment





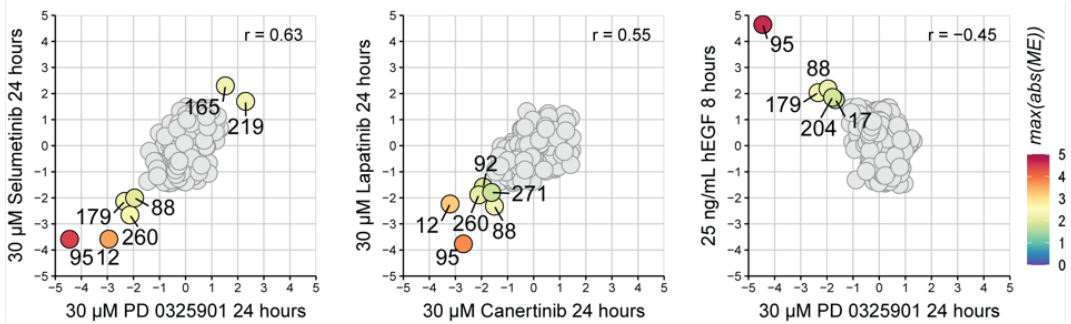


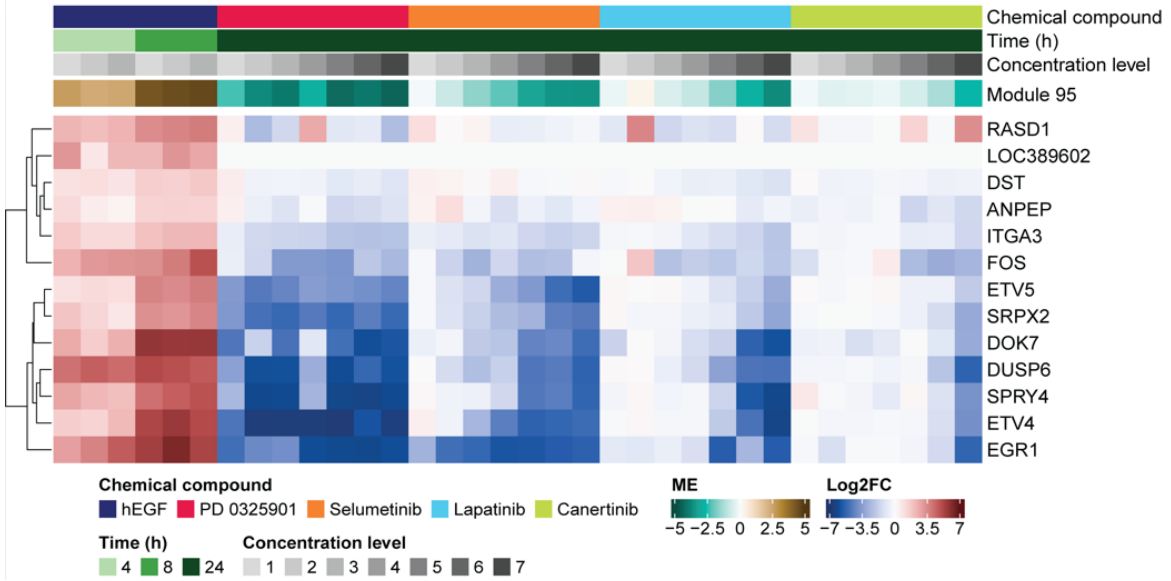


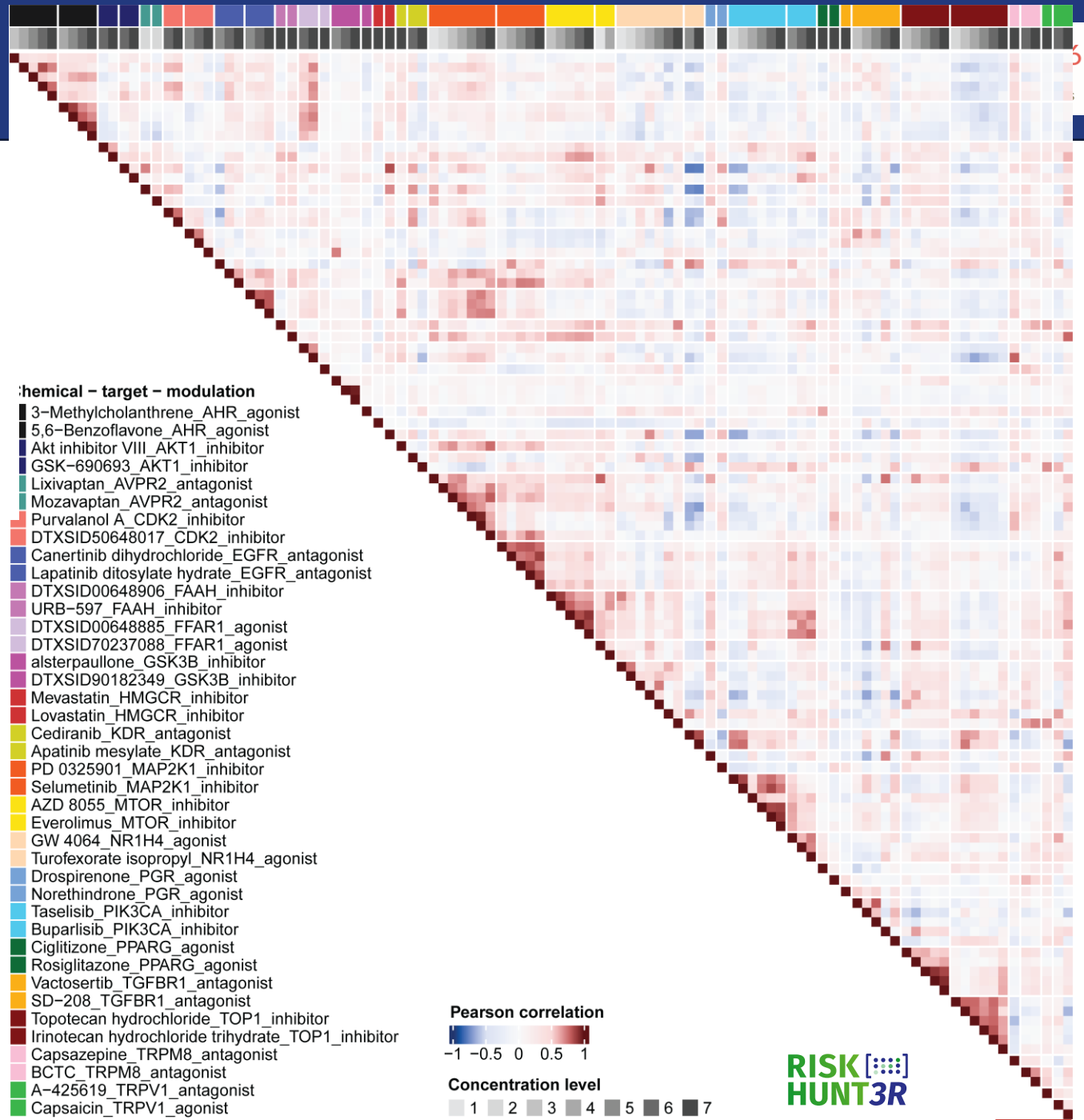


Vlasveld M, Callegaro G, .., van de Water B. Liver Int. 2024 Mar;44(3):760-775. doi: 10.1111/liv.15822.

RAx: biological similarity







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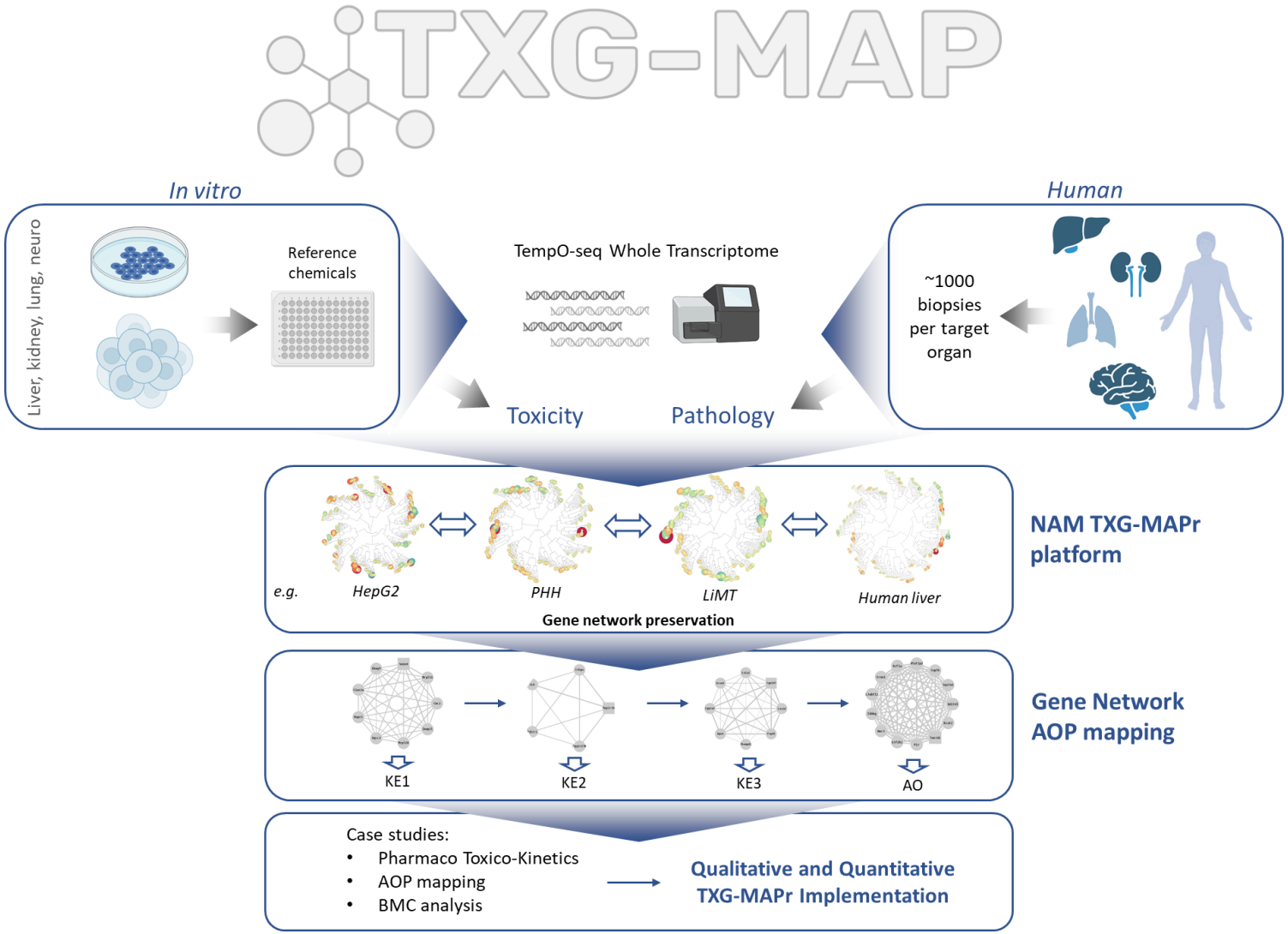
Hugo van Kessel et al., manuscript ready for submission

What is still ongoing and next developments

- 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



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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	Impacts
Develop a database and interface for the OECD Omics Regulatory Reporting Framework	Streamline data reporting, increase the confidence in data processing
Develop standardized, interoperable and toxicologically-oriented pathways and gene set tools and ontologies	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
Showcase three case studies the integration of chemical structural and physiochemical similarity measurements with mode of action features	Provide indications on how to use mode of action data for RAx and grouping

Acknowledgements

TXG-MAPr team @ UL

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Lucy Sinke

Mick van Schaick

Gerhard Burger

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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

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This output reflects only the author's view and the Directorate-General for Research and Innovation (DG RTD) cannot be held responsible for any use that may be made of the information contained therein.

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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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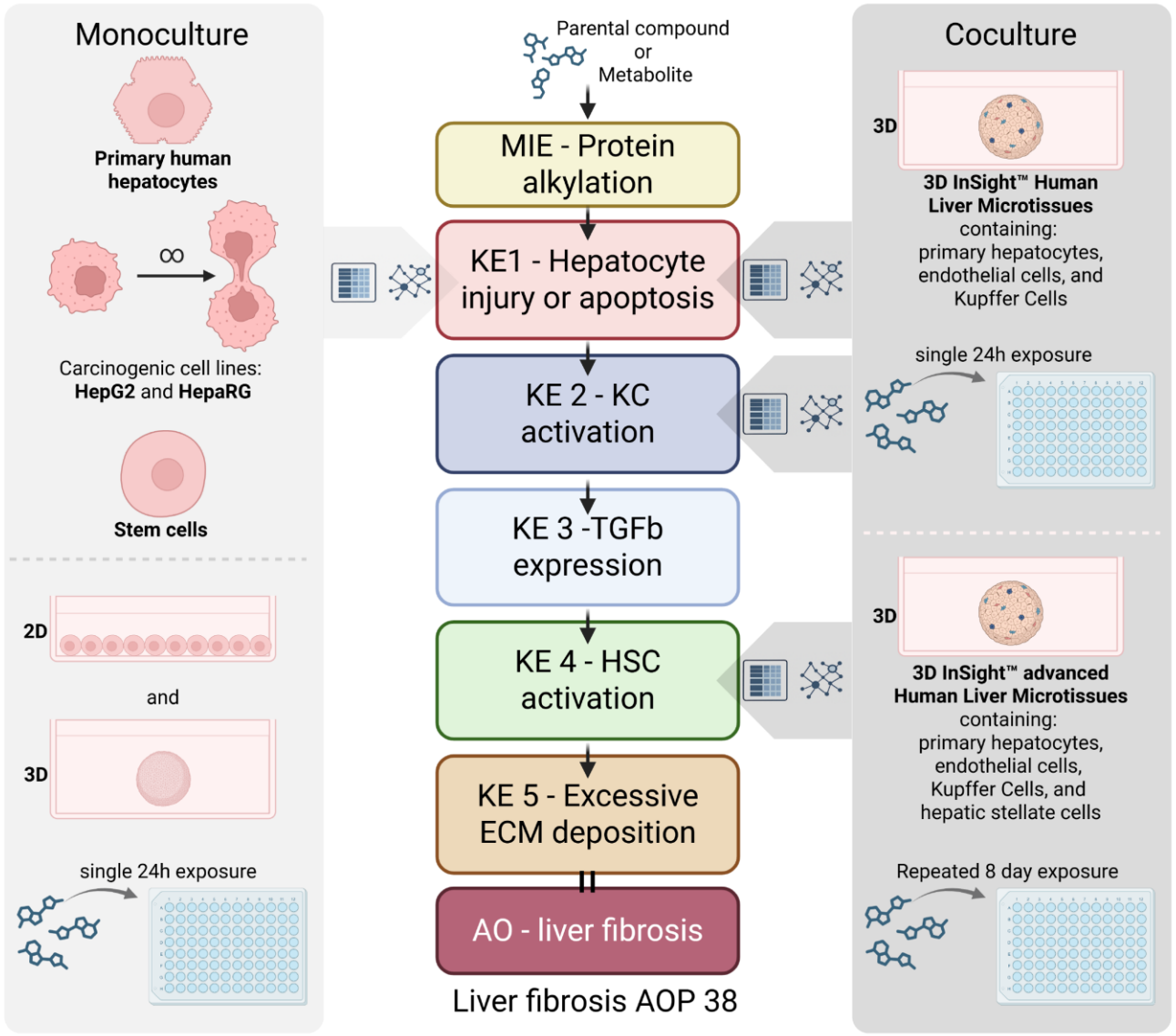

Tessa Hagens et al., manuscript in preparation

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

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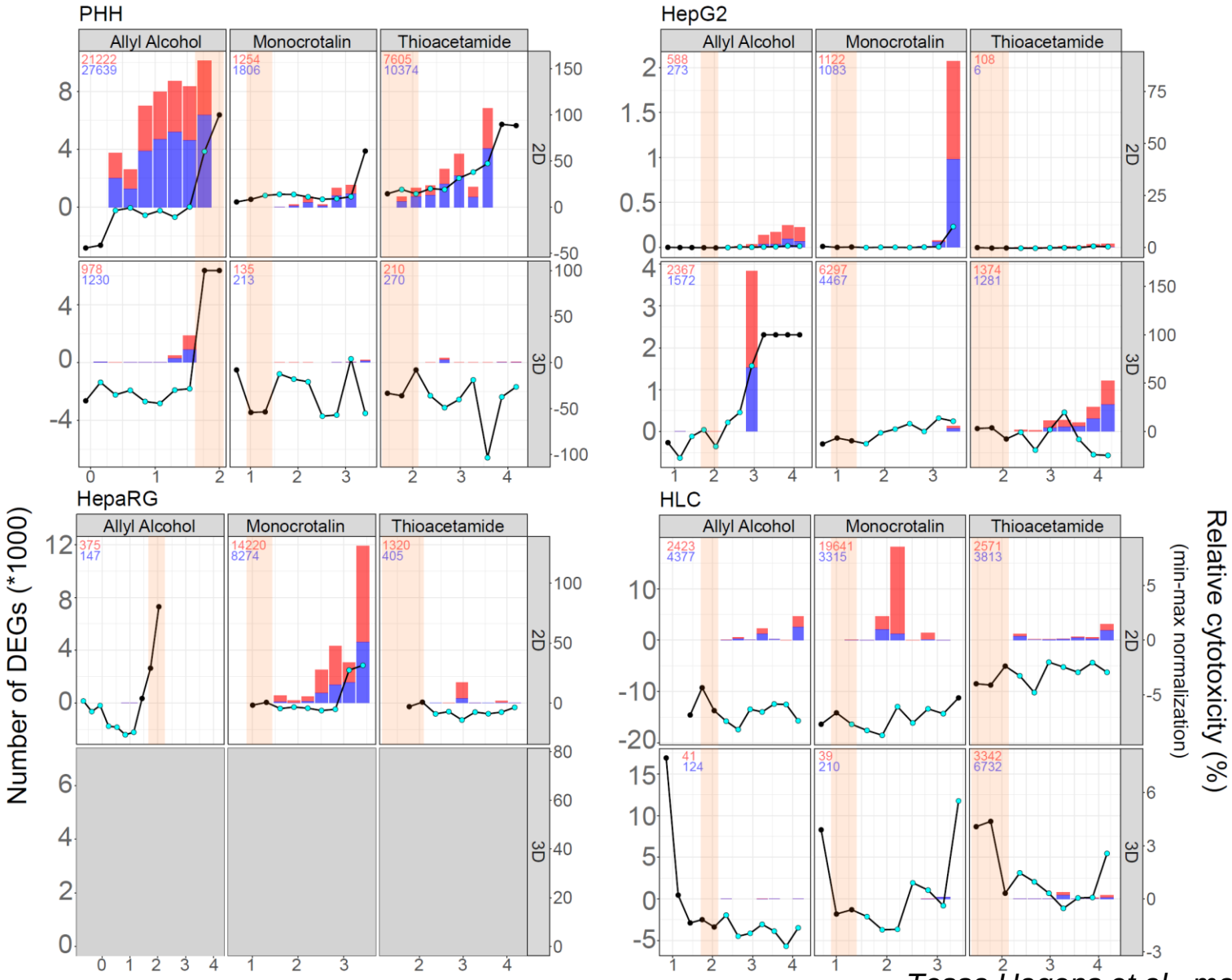

Tessa Hagens et al., manuscript in preparation

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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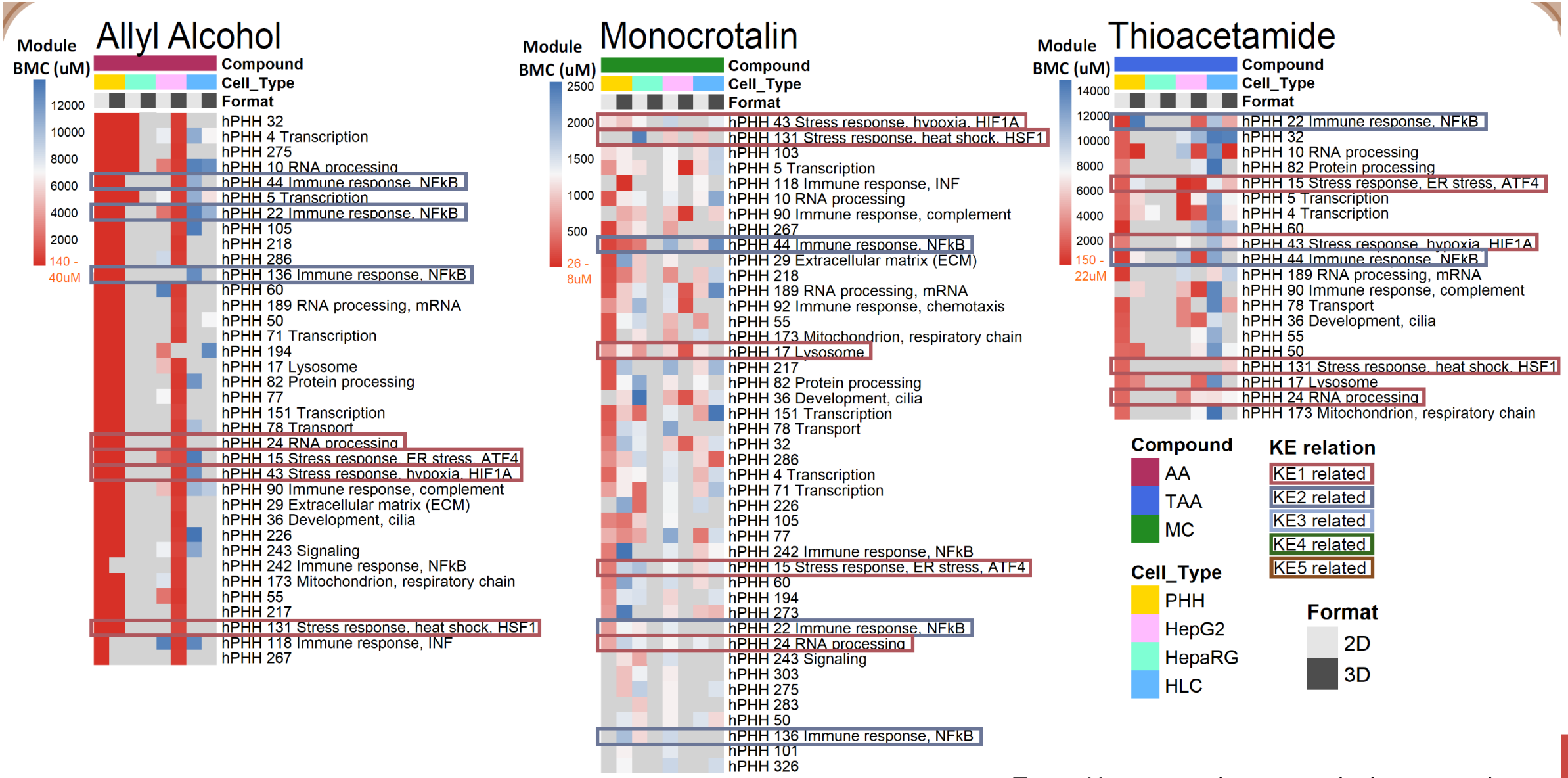

Tessa Hagens et al., manuscript in preparation

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

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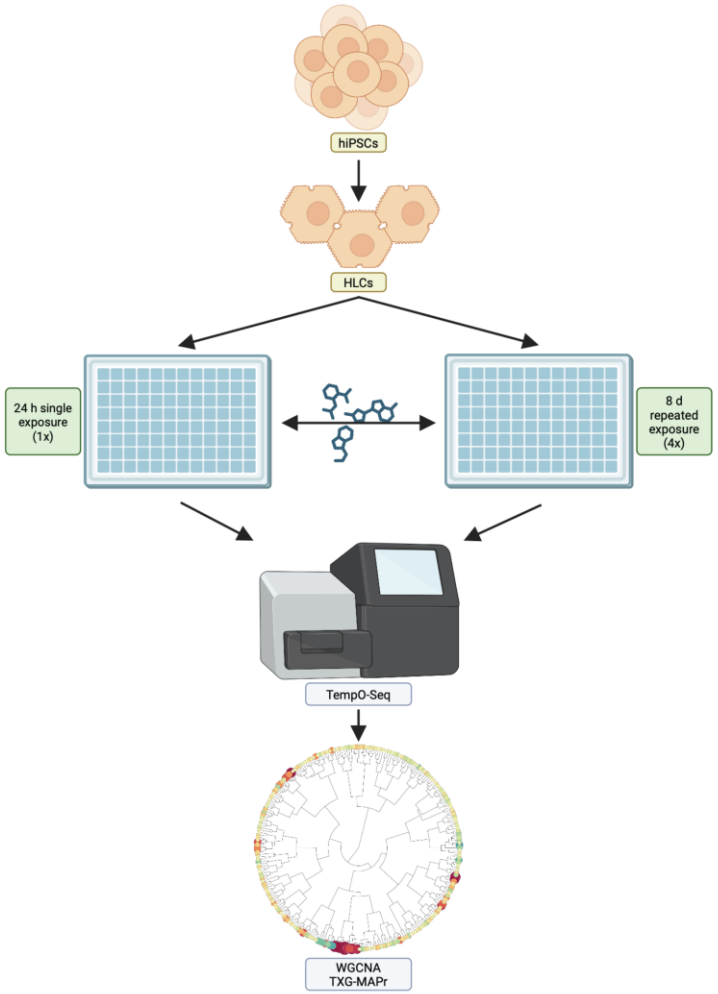
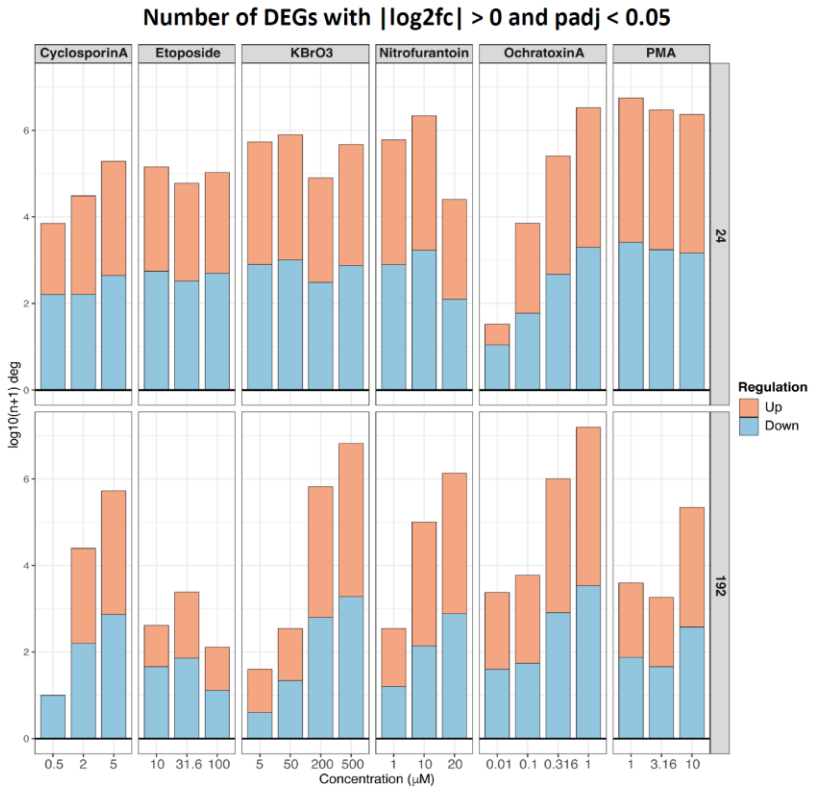
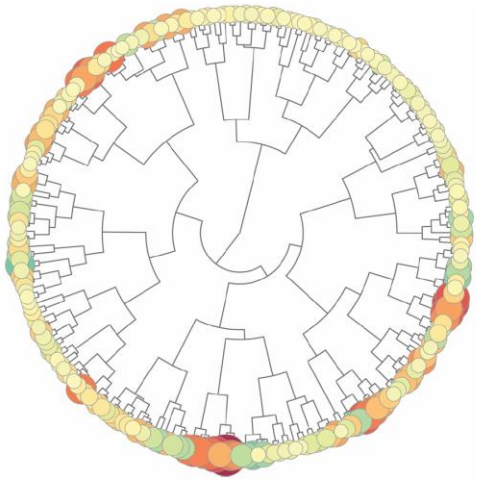


Compound	MOA	Carcinogenic	Concentrations (µM) C1	C2	C3	C4
Ochratoxin A	Oxidative DNA Damage	Yes, IARC 2B	0,01	0,1	0,316	1
Potassium Bromate ($KBrO_3$)	Oxidative stress	Yes, IARC 2B	5	50	200	500
Nitrofurantoin	Oxidative stress	No	1	10	20	
Phorbol 12-myristate 13-acetate (PMA)	Mitogenic, inflammation	Yes	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	

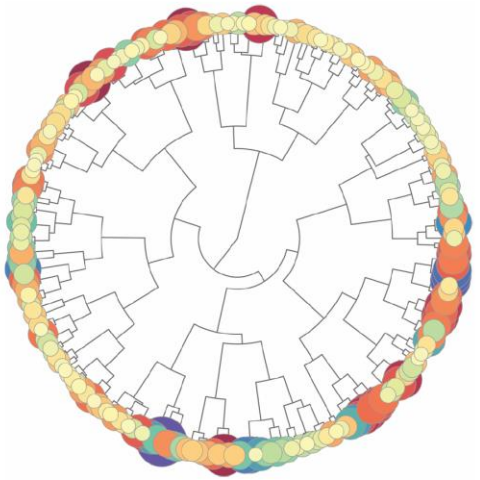


Differential modulation of gene networks in iPSC-HLC after treatment with cyclosporine A, phorbol ester (PMA) and KBrO3.

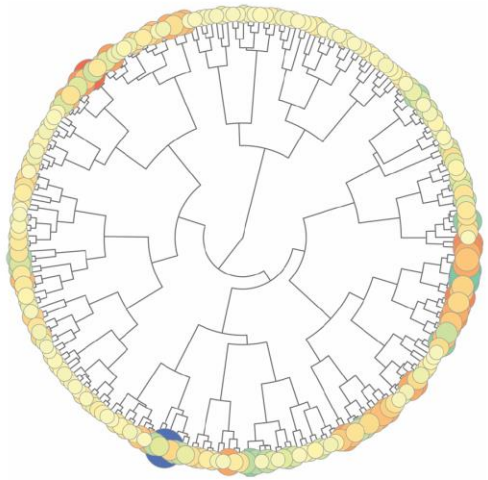
5 µM Cyclosporin A T1 (24 hours)



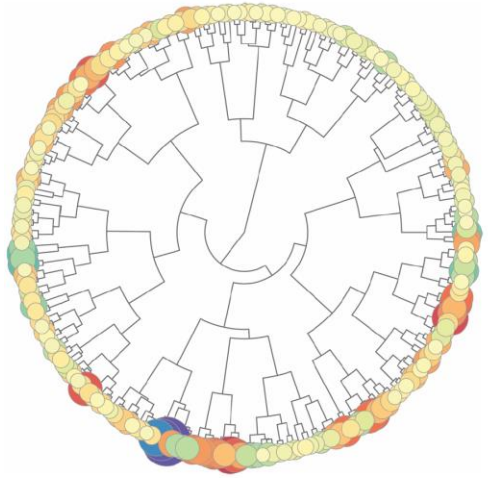
10 µM PMA T1 (24 hours)



500 µM $KBrO_3$ T1 (24 hours)



5 µM Cyclosporin A T2 (192 hours)



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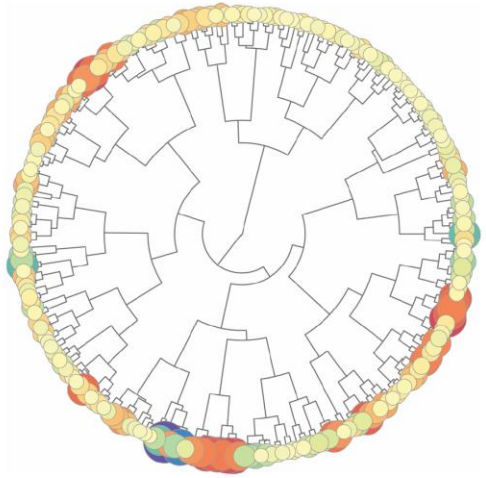

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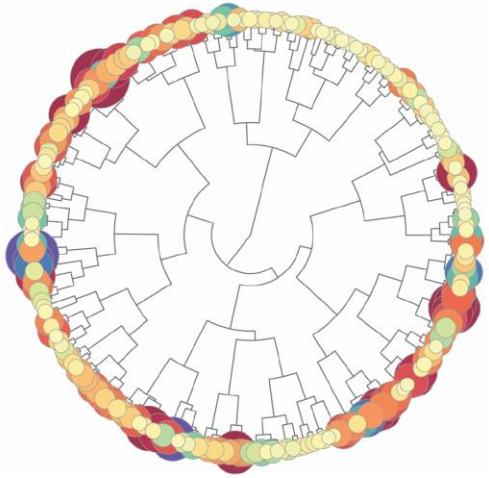
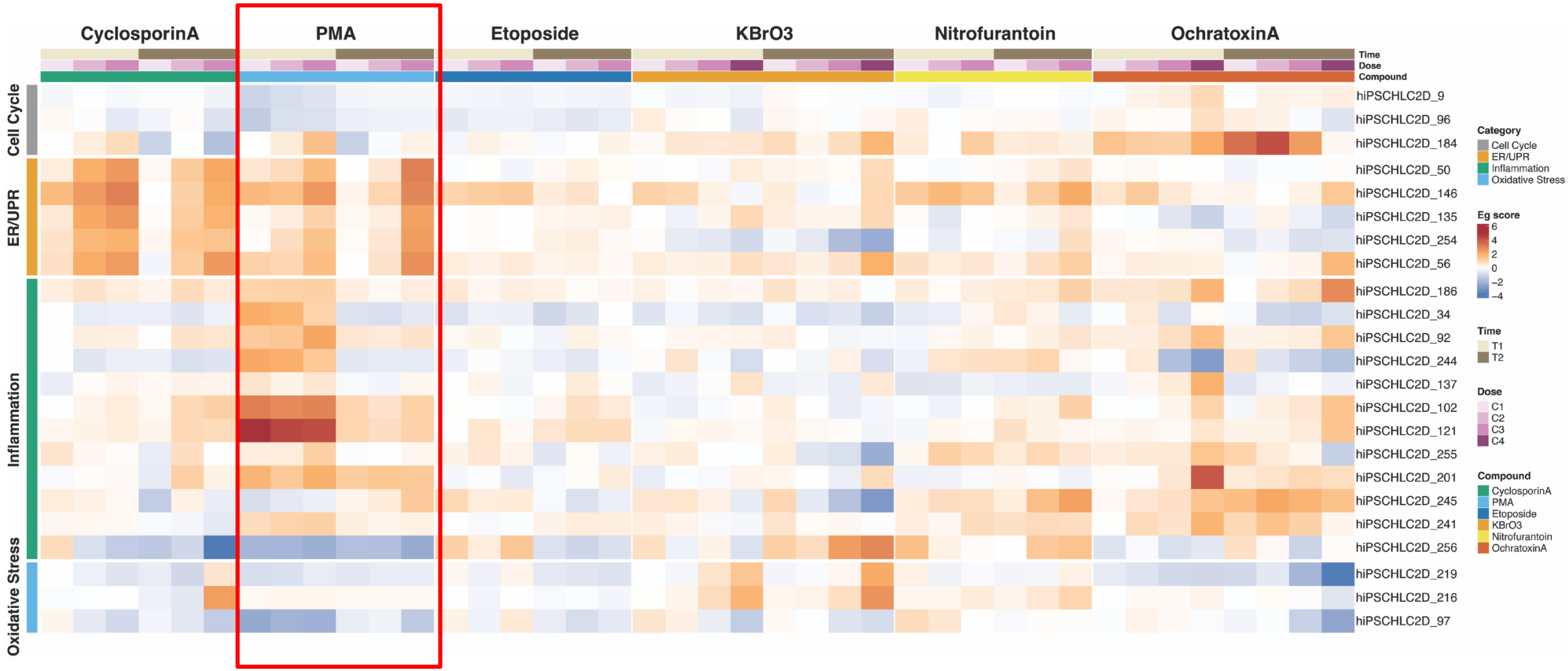


Differential modulation of gene networks in iPSC-HLC after treatment with CsA, PMA and KBrO3.



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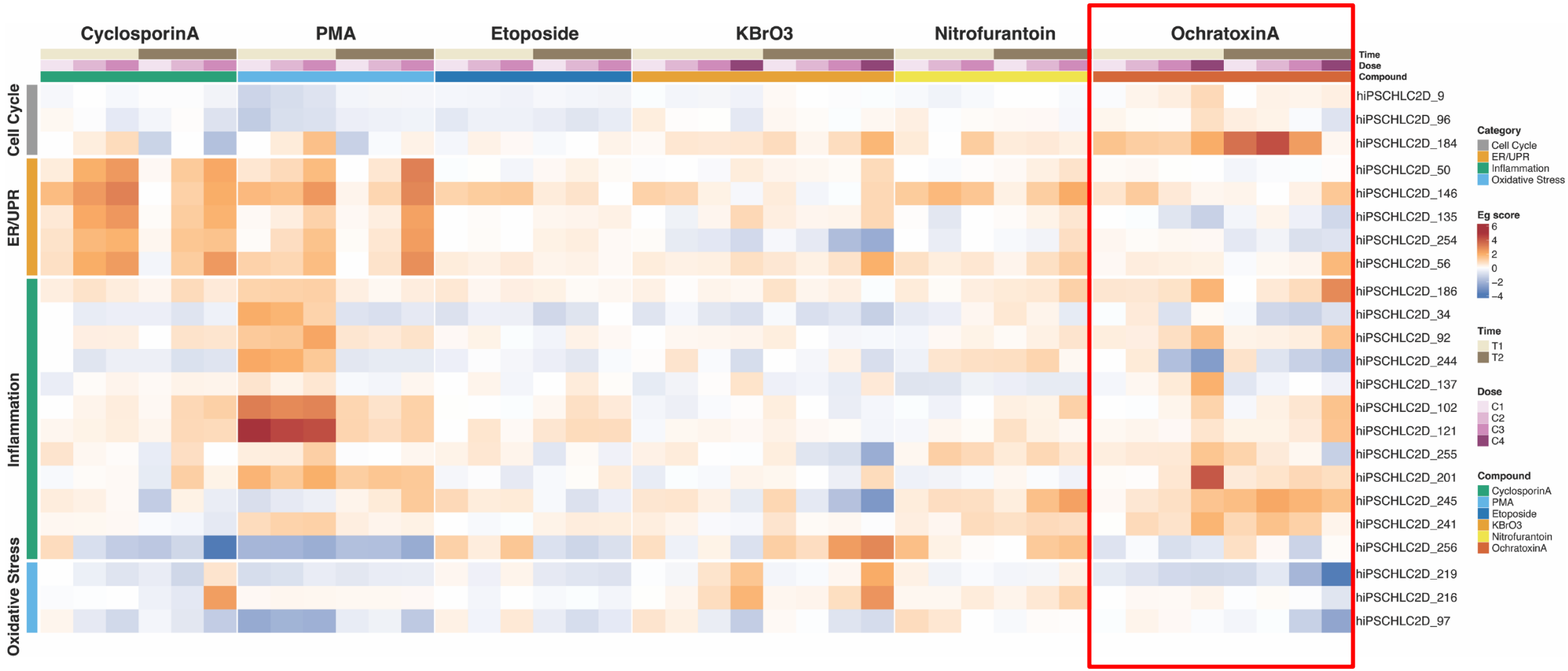

Sustained transcription factor activation by OchratoxinA and PMA is related to NFkB and AP-1.

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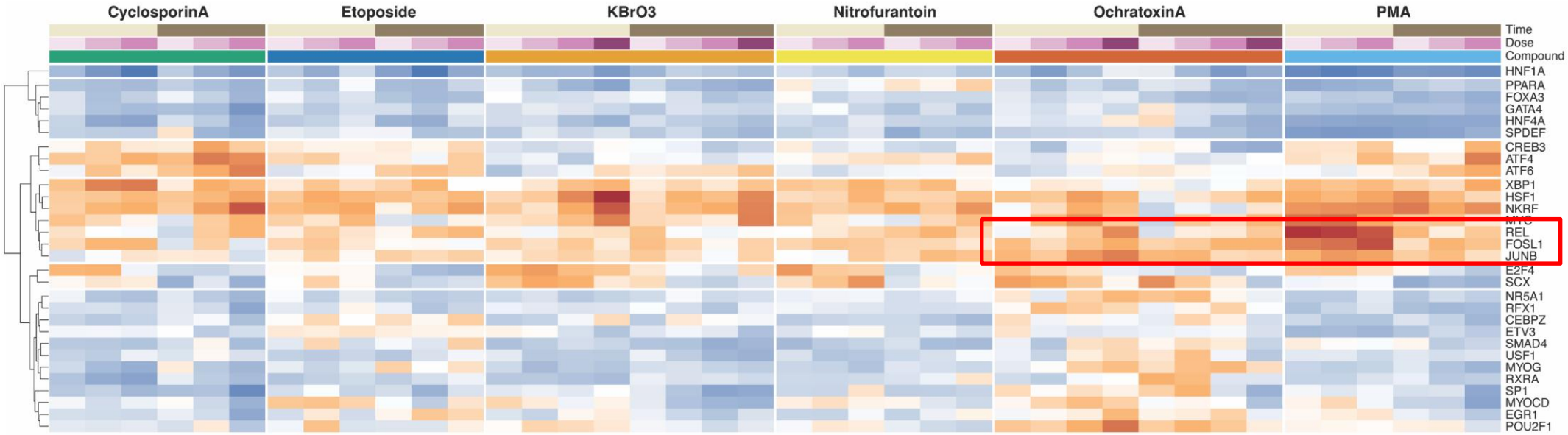
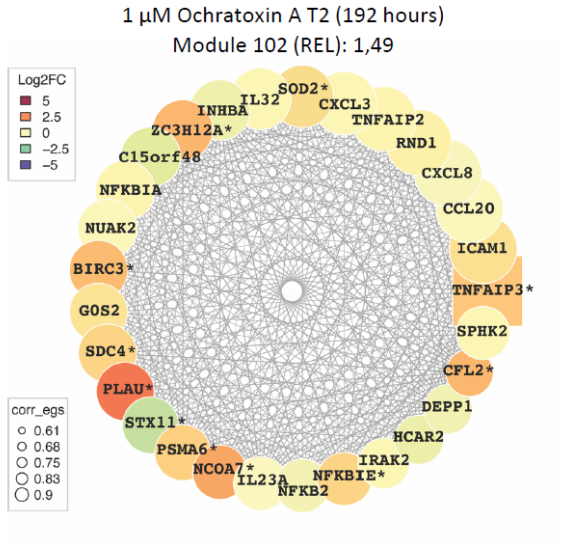
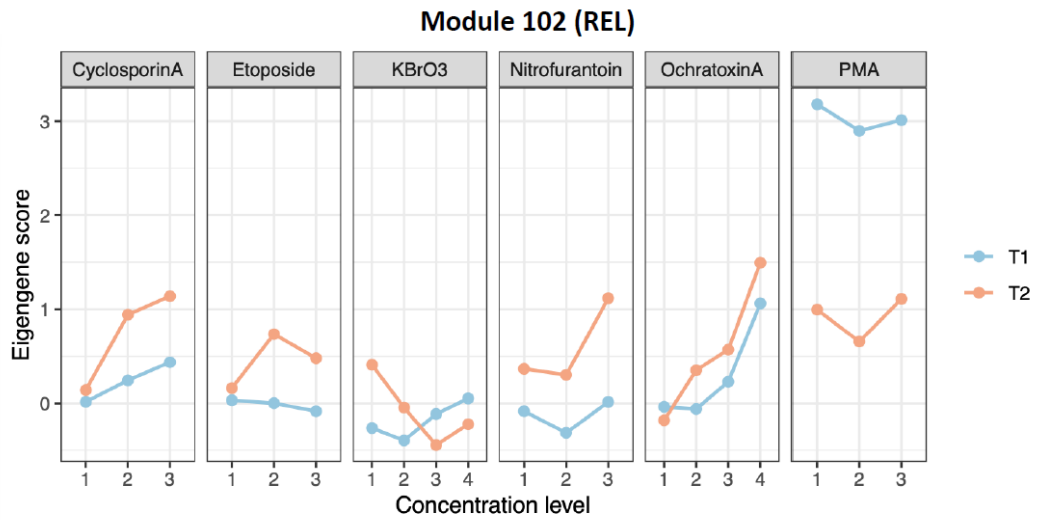
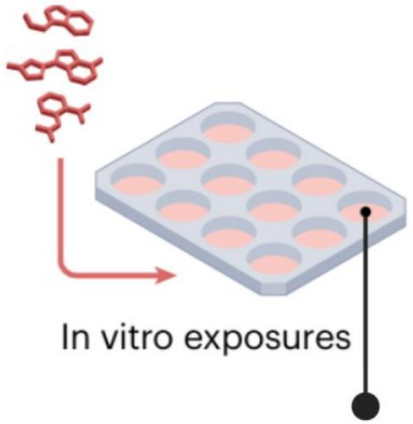




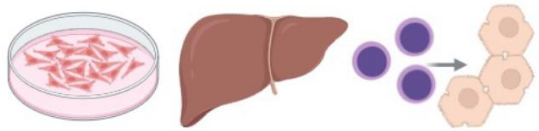


Comparison of transcriptomics and metabolomics for assessment of bioactivity: a liver test system comparsion.

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



Liver *in vitro* models

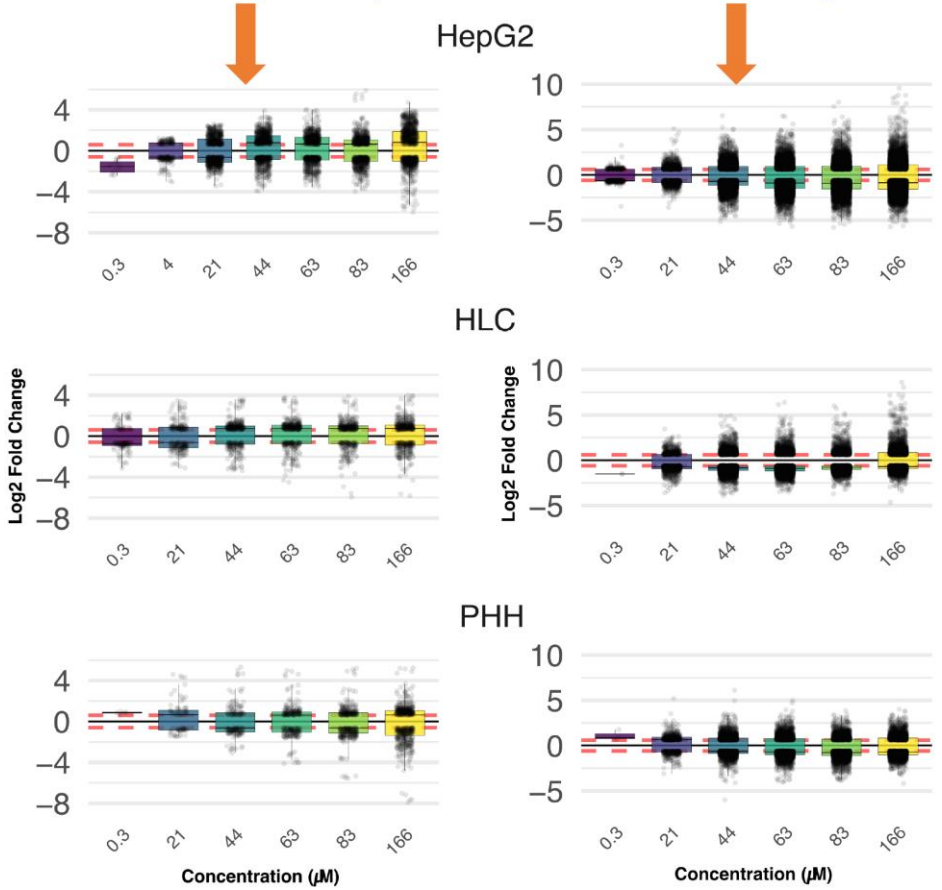


HepG2 **PHH** **HLC**

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

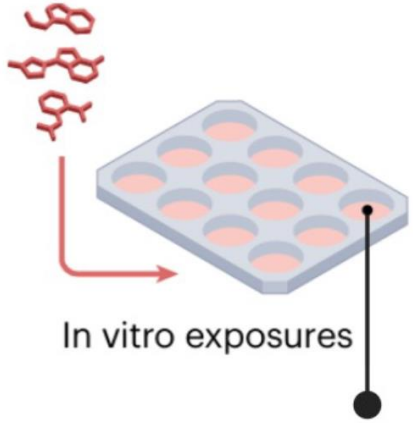
Targeted transcriptomics (TempoSeq, whole transcriptome)

Targeted metabolomics (~255 Metabolites, 13 classes)

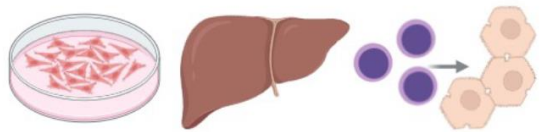


Ex: Ketoconazole 24 hours (|log2FC|>=0.6, p_adj <=0.05)

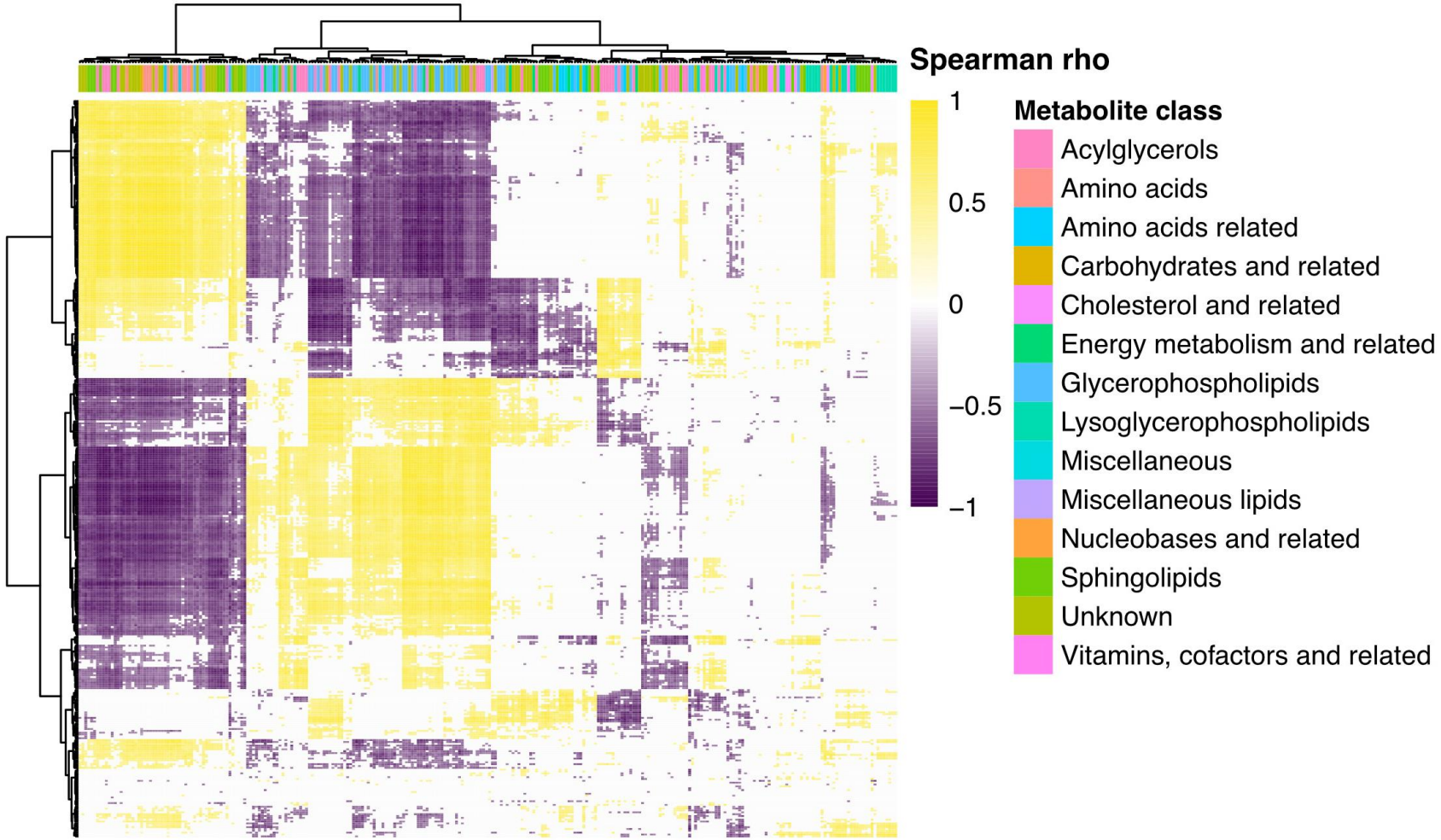
Positive and negative correlation between transcriptomics and metabolic responses.



Liver *in vitro* models



HepG2 PHH HLC



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

BMC analysis indicates similar sensitivity for tPOD and mPOD across test systems.

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)	✓	--	--