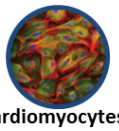
House et al.:

Grouping of UVCB Substances with New Approach Methodologies (NAMs) Data

Supplementary Figures

Fig. S1: iPSC-derived cell types (including FujiFilm-CDI [Madison, WI] catalogue numbers), positive and vehicle controls that were used for each cell type
See Supplemental File 1 for details on each cell type and chemical listed herein.

Neurons
Cat #: R1013



Cardiomyocytes
Cat #: R1007

Endothelium
Cat #: R1022

Hepatocytes
Cat #: R1027

Macrophages
Cat #: R1099

Positive Assay Specific Controls		Vehicle Controls
Mitomycin C (0.1, 1, 10, 100 µM)	Cytotoxic Agent, DNA Crosslinker	Media (n=14)
Rotenone (0.1 ,1, 10, 100 µM)	Mitotoxicant, Complex I Inhibitor	DMSO (n=13)
CCCP (0.13, 1.3, 13, 130 µM)	Mitotoxicant , gradient uncoupler	Method Blank (n=28)
Retinoic Acid (0.5, 5, 50, 500 µM)	Altered Neuronal Signaling	
Brefeldin A (0.1, 1, 10, 100 µM)	Inhibitor of Neurite Outgrowth	
Tetraoctylammonium Bromide (50 µM, n=14)	Cytotoxic Agent	
Isoproterenol (0.1, 0.5, 1, 10, 50 µM)	Positive Inotropic Agent	Media (n=14)
Propranolol (0.1, 0.5, 1, 10, 50 µM)	Negative Inotropic Agent	DMSO (n=13)
Cisapride (0.1, 0.5, 1, 10, 50 µM)	Induces Qt Prolongation	Method Blank (n=28)
Sotalol (0.1, 0.5, 1, 10, 50 µM)	Induces Qt Prolongation	
Tetraoctylammonium Bromide (50 µM, n=10)	Cytotoxic Agent	
Nocodazole (0.001, 0.01, 0.1, 1 µM)	Inhibits Microtubule Formation	Media (n=14)
Suramin (0.5, 5, 15, 50 µM)	Disrupts Angiogenesis Assay	DMSO (n=13)
Histamine (0.1, 1, 10, 100 µM)	Endothelial Cell Gene Alteration	Method Blank (n=28)
Colchicine (0.3, 3, 30, 100 µM)	Alters Microtubule Formation	
Chloroquine (1, 10, 100, 500 µM)	Induces Apoptosis in Endo Cells	
SU5402 (0.1, 1, 10, 50 µM)	VEGFR inhibitor	
Tetraoctylammonium Bromide (50 µM, n=12)	Cytotoxic Agent	
Doxorubicin (0.01, 0.1, 1, 10 µM)	Cytotoxic Agent / ROS Inducer	Media (n=14)
Amiodarone (0.1, 1, 10, 100 µM)	Steatosis Inducer	DMSO (n=13)
Rotenone (0.1, 1, 10, 100 µM)	Mitotoxicant, Complex I Inhibitor	Method Blank (n=28)
CCCP (0.1, 1, 10, 50µM)	Mitotoxicant , gradient uncoupler	
Acetaminophen (0.1, 1, 10 mM)	Hepatotoxic Agent, ROS Inducer	
Tetraoctylammonium Bromide (50 µM, n=10)	Cytotoxic Agent	
Cytochalasin D (0.01, 0.1, 1, 10, 100 µM)	Inhibitor of Actin Polymerization	Media (n=14)
SB203580 (0.1, 1, 10, 100, 1000 µM)	MAPK Inhibitor	DMSO (n=13)
Phobol 12-myristate 13-acetate (0.01, 0.1, 1, 10, 100 nM)	Stimulates Macrophage Function	Method Blank (n=28)
LPS (1, 10, 100, 500 ng/ml)	Stimulates Macrophage Function	
Tetraoctylammonium Bromide (50 µM, n=10)	Cytotoxic Agent	

doi:10.14573/altex.2006262s1

Fig. S2: Schematic diagram for preparation of serial dilutions for petroleum substance extracts from a master plate for subsequent use in bioactivity profiling experiments

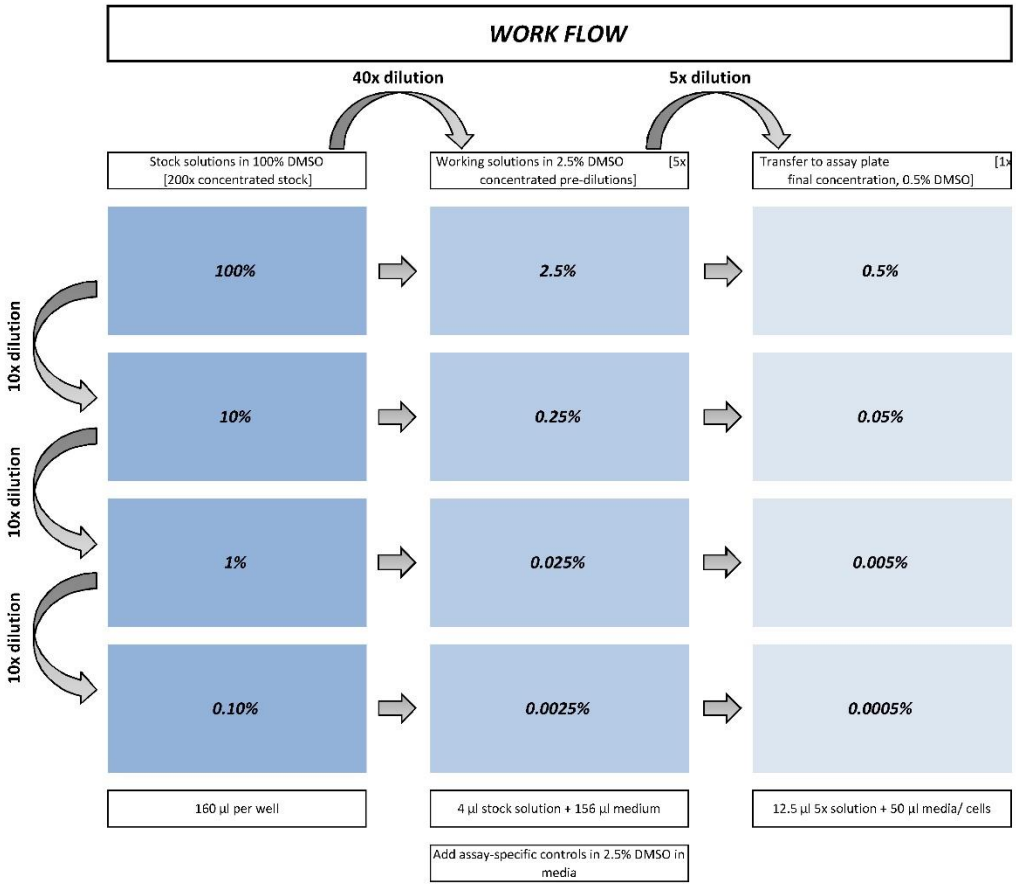


Fig. S3: Decision-tree for derivation of the point of departure (POD) values from *in vitro* data
See Supplemental File 3 for the R scripts used in these calculations.

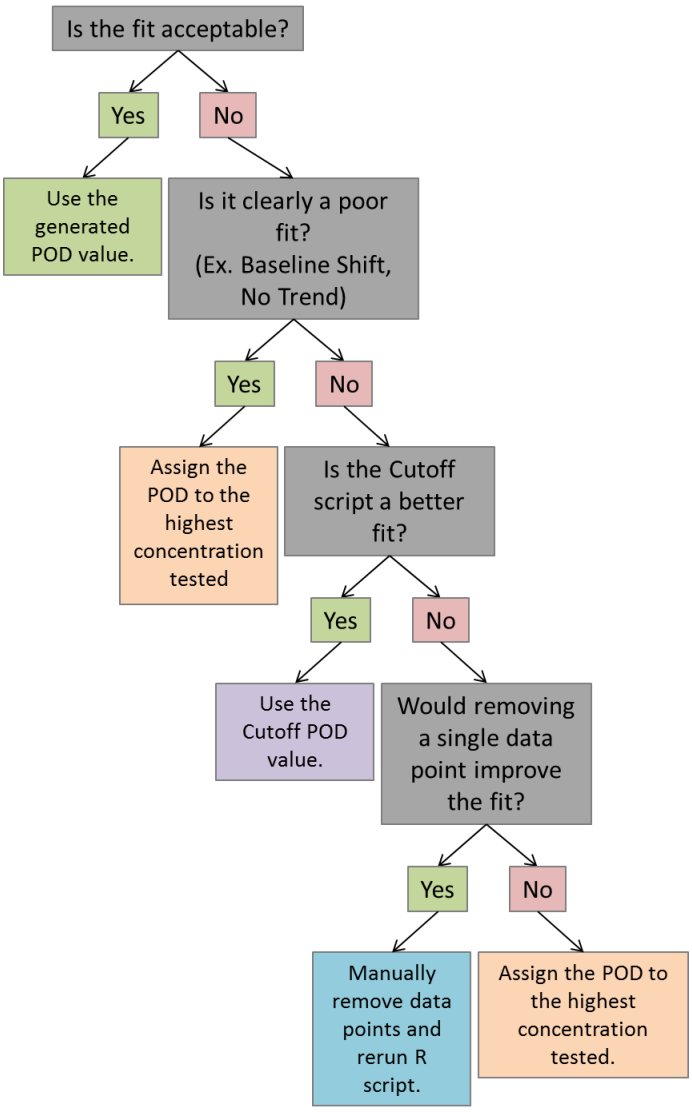
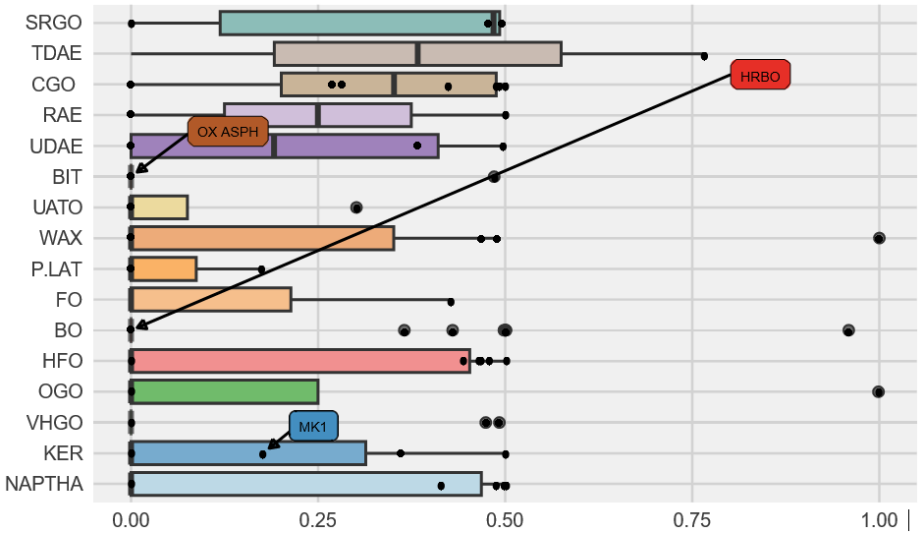
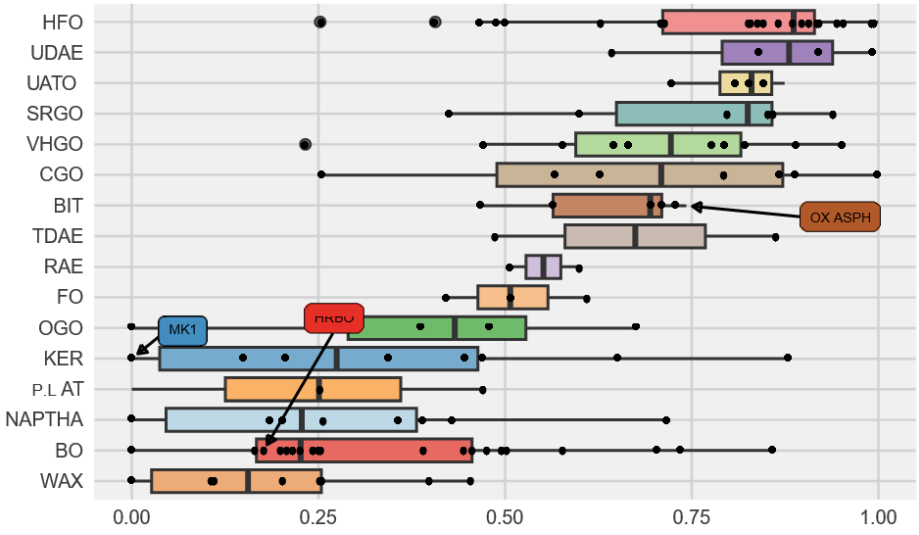


Fig. S4: Manufacturing stream-based grouping of the bioactivity for individual petroleum-derived UVCBs using data on the individual cell types not shown in Figure 6
Cell type abbreviations are defined in Table 2. Each dot represents a UVCB sample total ToxPi score derived from all phenotypes (top) or cell-specific phenotypes. Box is the inter-quartile range, vertical line is the median, and whiskers are min-max range of values. X-axis in all plots is the ToxPi score for each cell type.

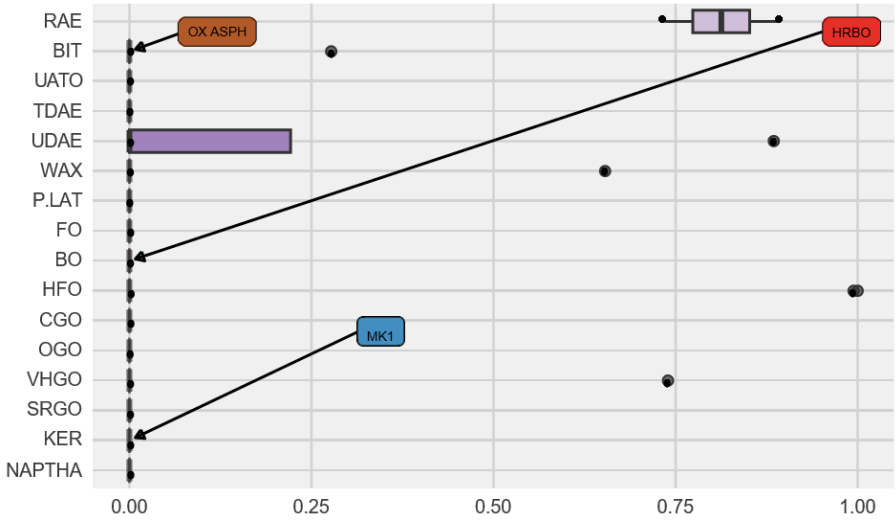
A. A375 cells.



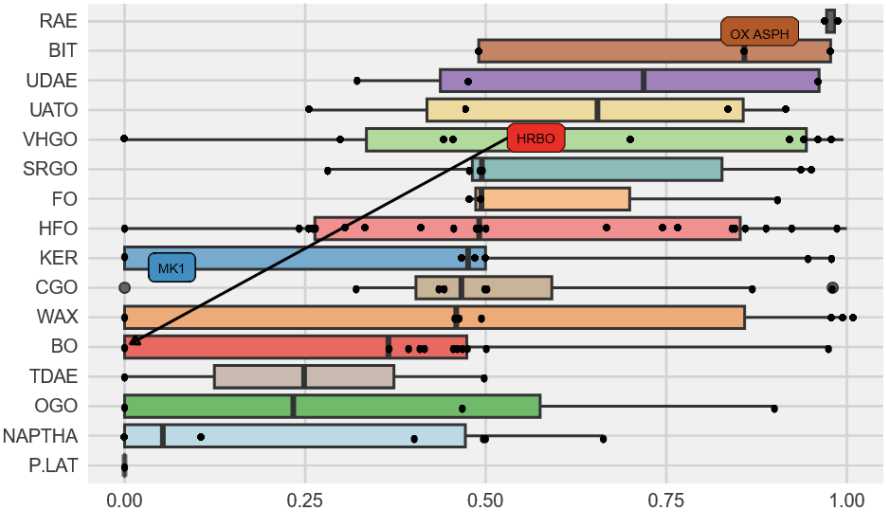
B. ENDO cells.



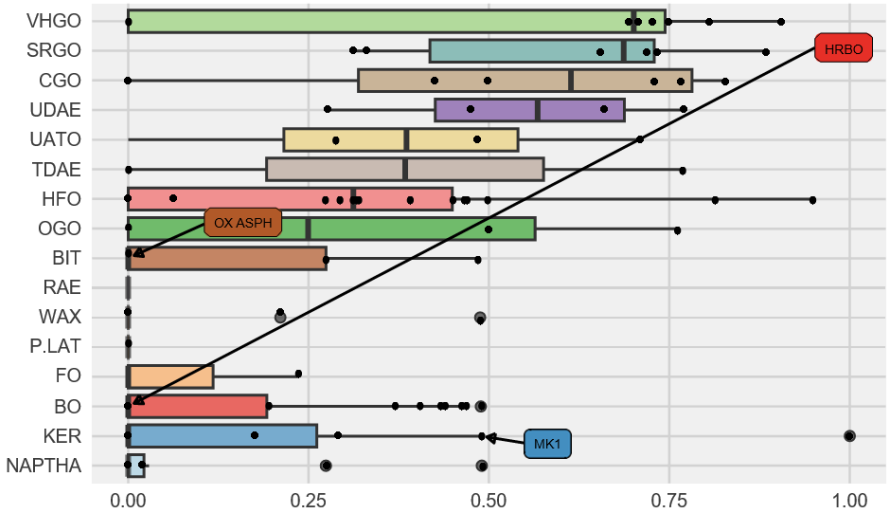
C. HEPARG cells.



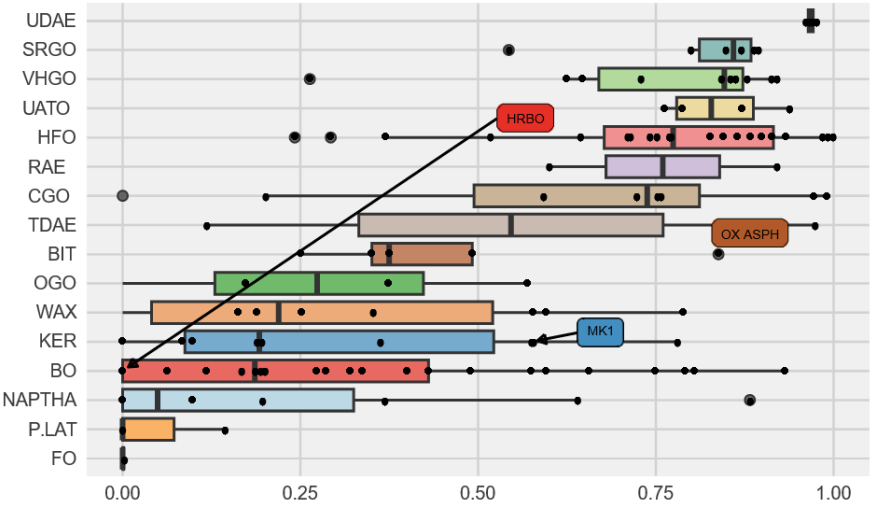
D. HepG2 cells.



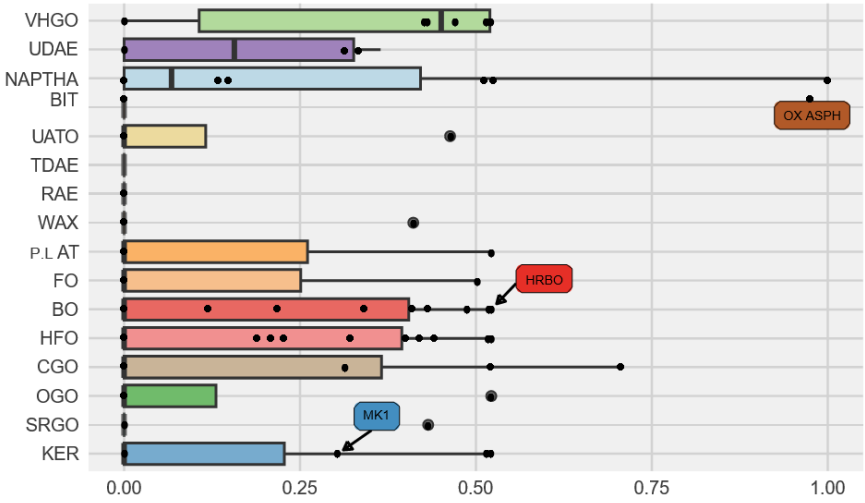
E. HLMVEC cells.



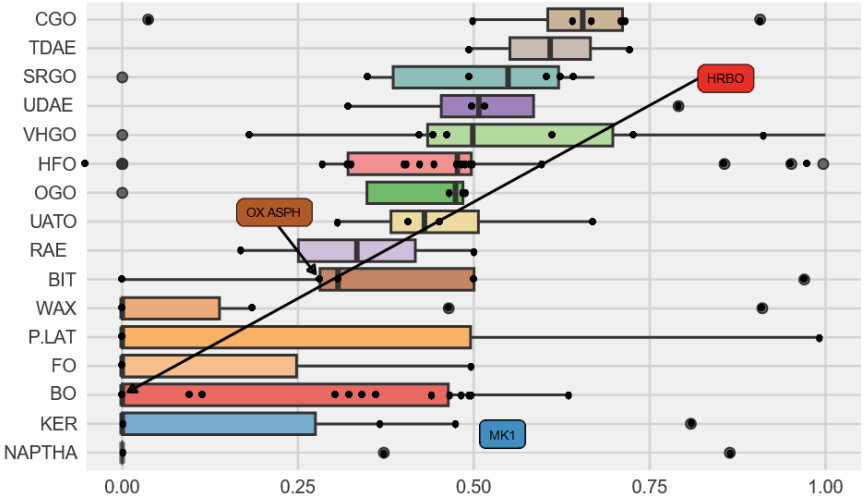
F. HUVEC cells.



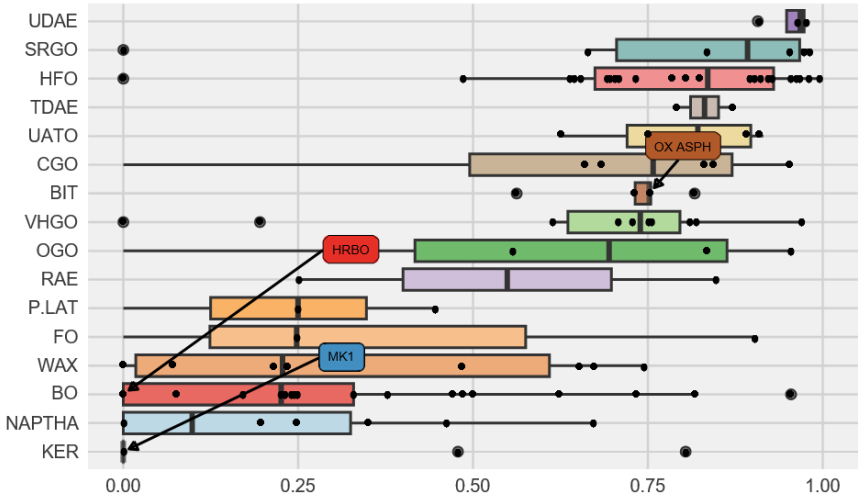
G. LN229 cells.



H. MCF7 cells.



I. NEUR cells.



J. SH-SY5Y cells.

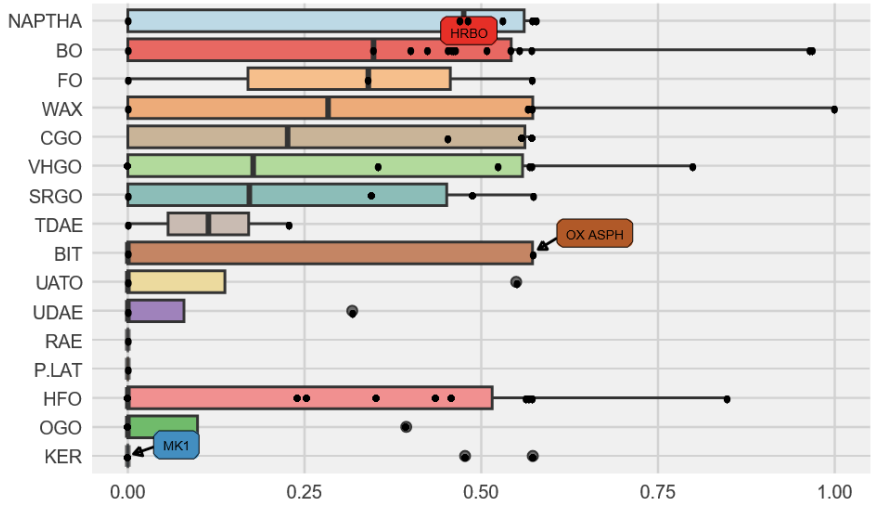
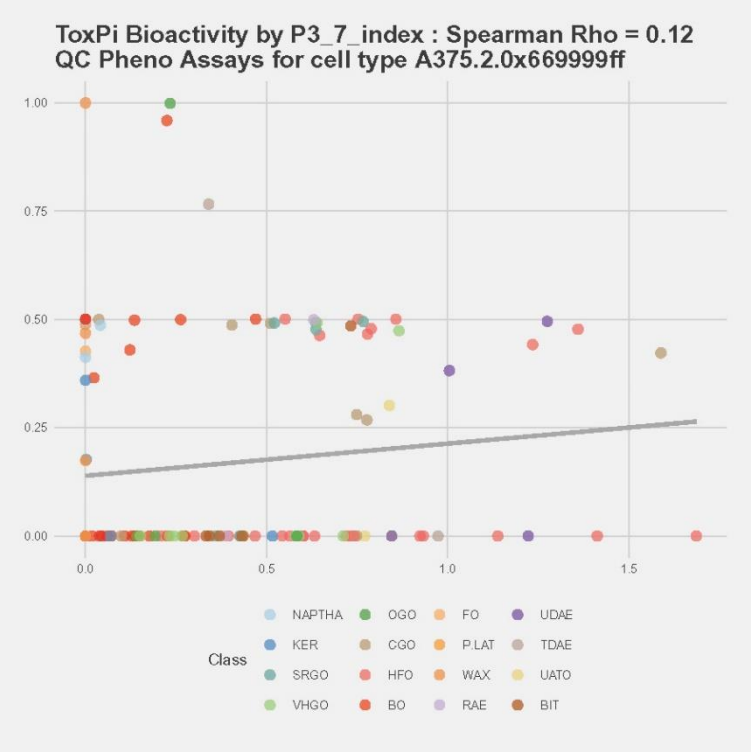
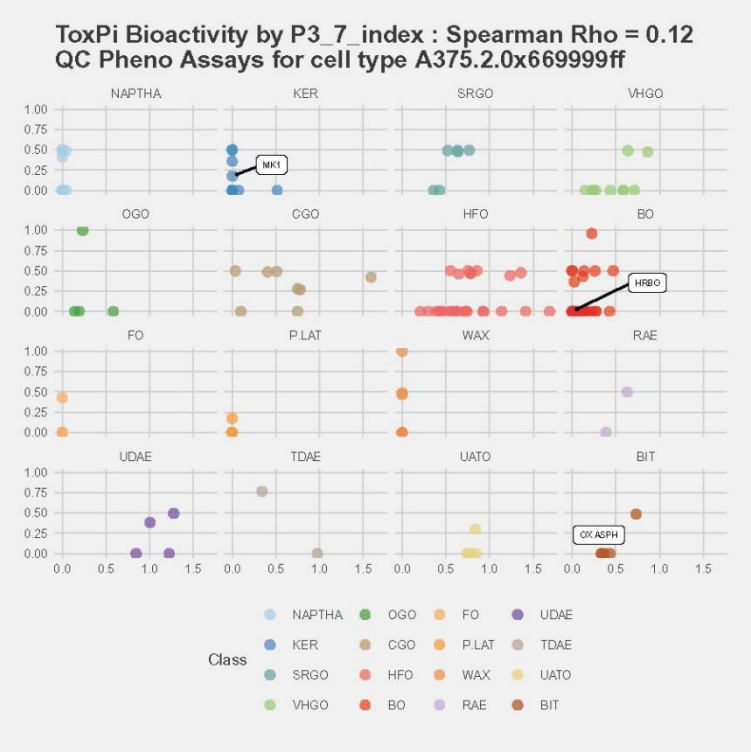


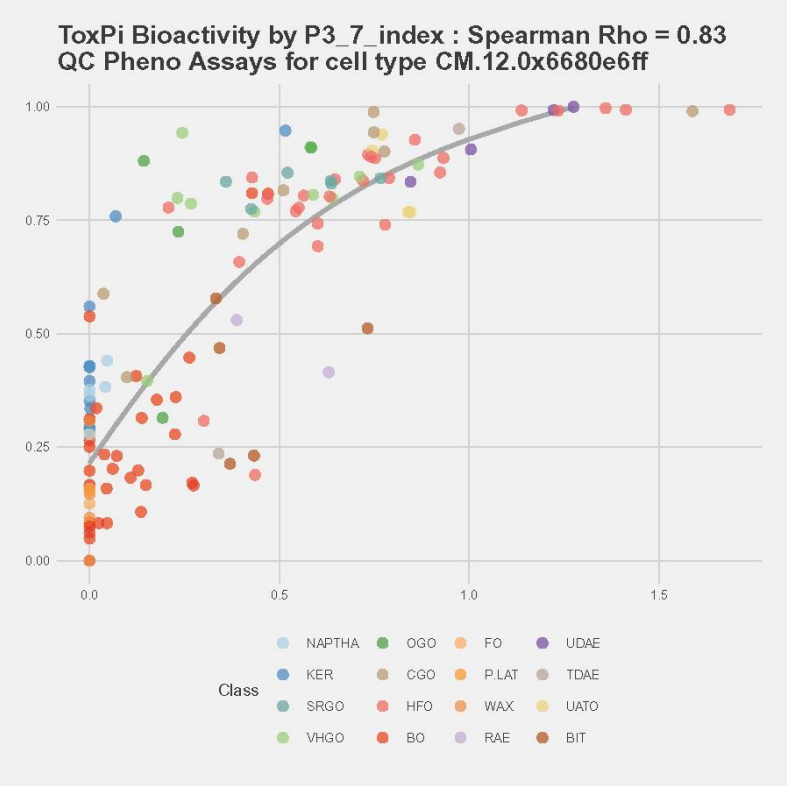
Fig. S5: Correlation of polycyclic aromatic compound (PAC) score for 3-7 ring compounds in each UVCB sample with cell-specific ToxPi bioactivity scores
Spearman Rho correlation is shown on each plot.

A. A375 cells.





B. CM cells.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0.00
0.0
0.5
1.0
1.5
Class
NAPTHA
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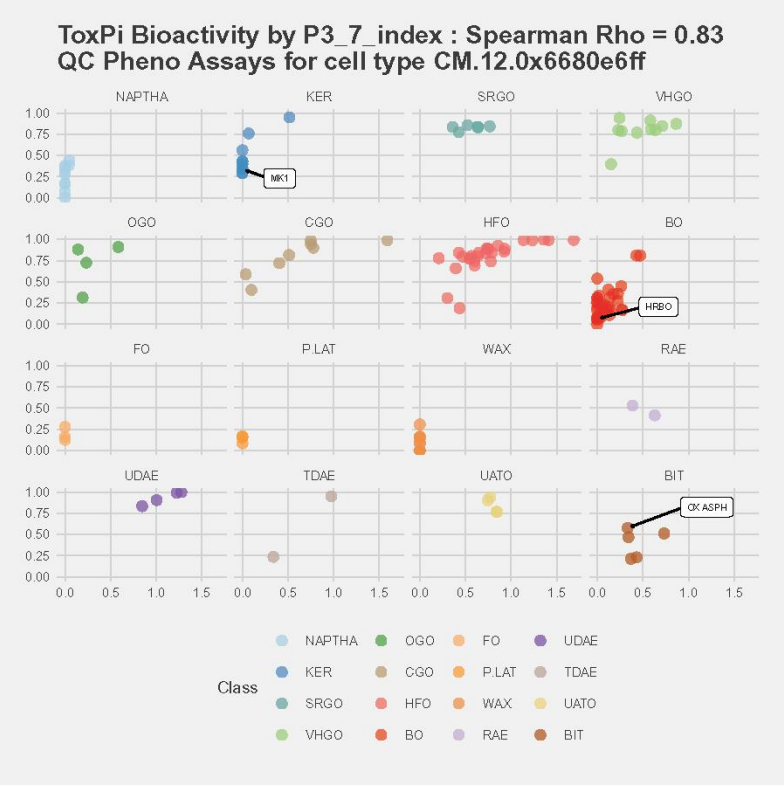
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0.75
0.50
0.25
0.00
0.0
0.5
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SRGO
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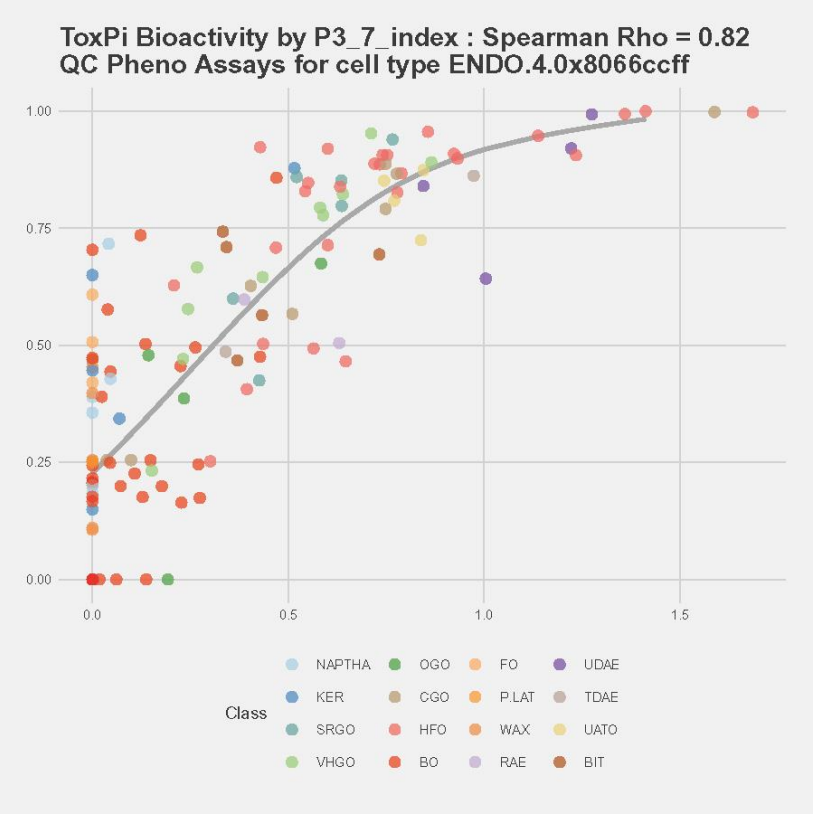
C. ENDO cells.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0.0
0.5
1.0
1.5
Class
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WAX
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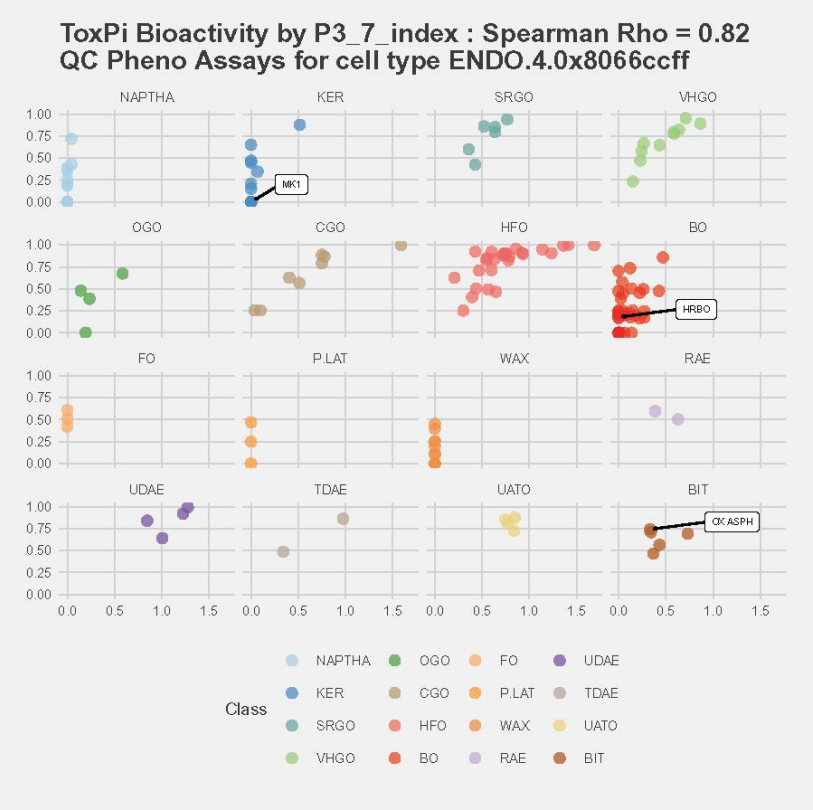
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OX ASPH
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0.50
0.25
0.00
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0.5
1.0
1.5
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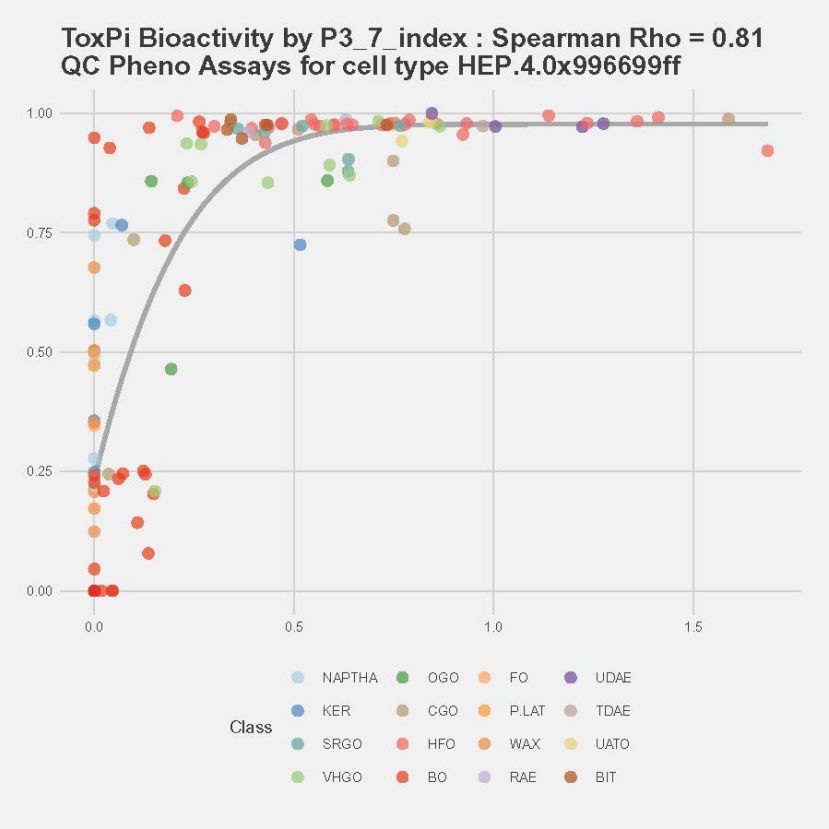
UDAE
TDAE
UATO
BIT

D. HEP cells.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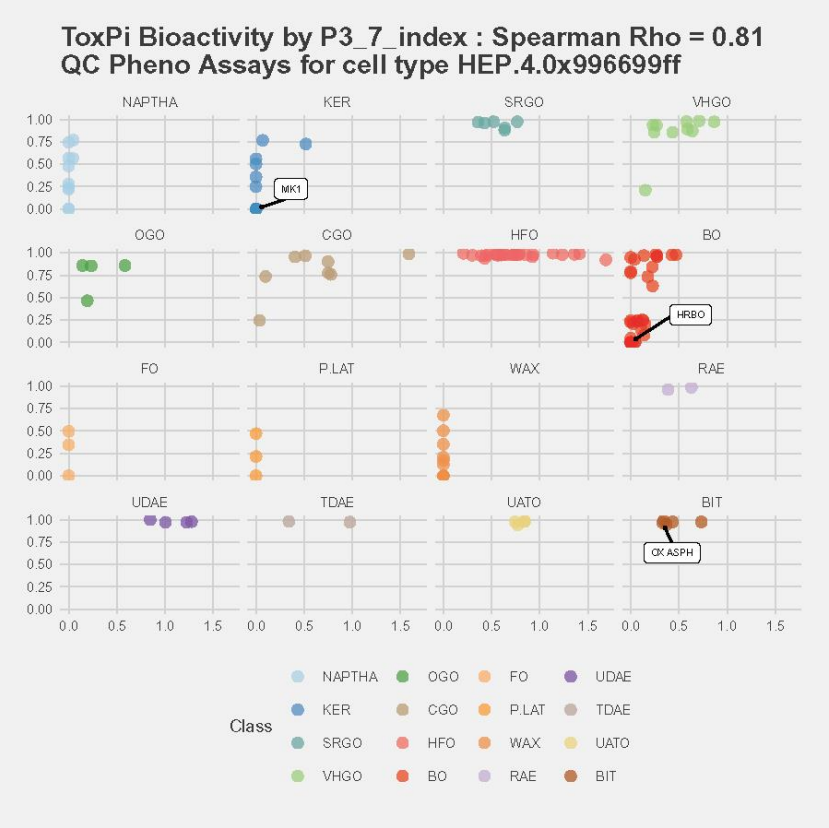
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UDAE
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VHGO
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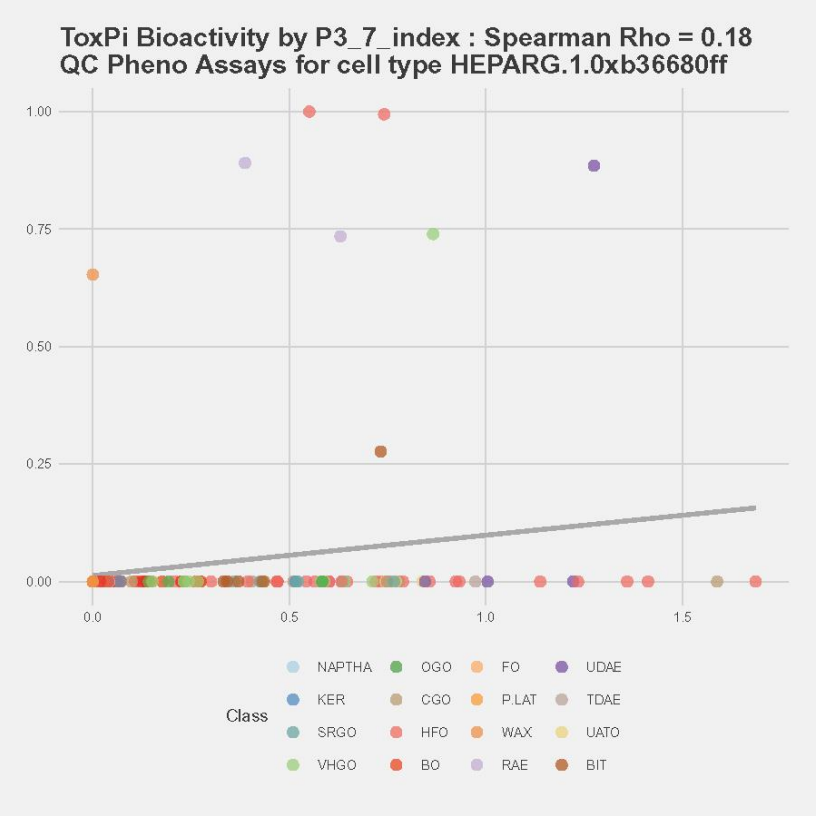
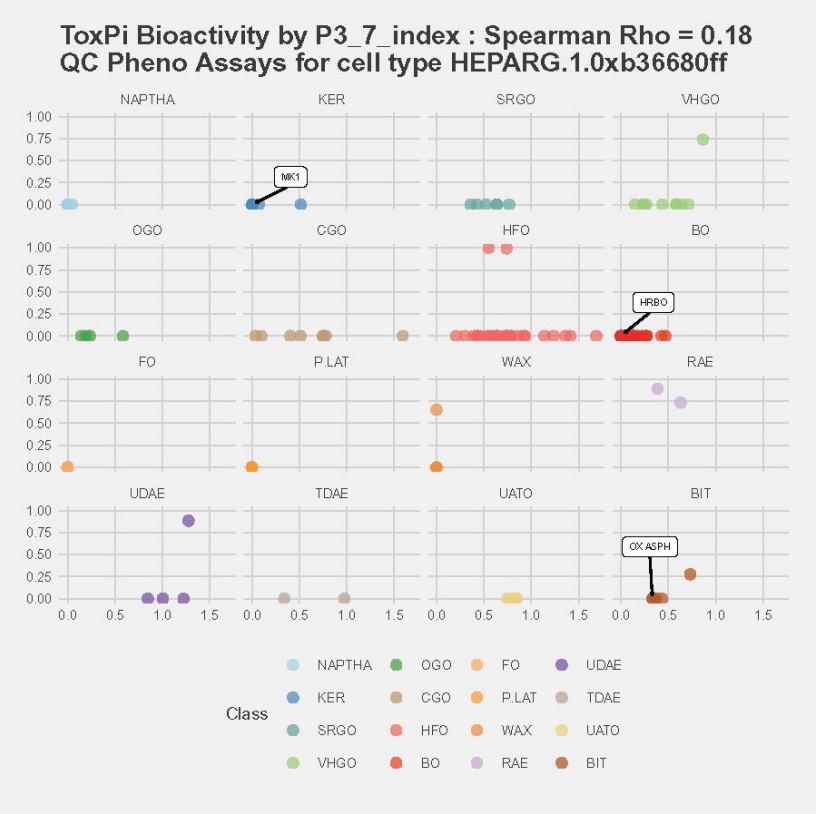
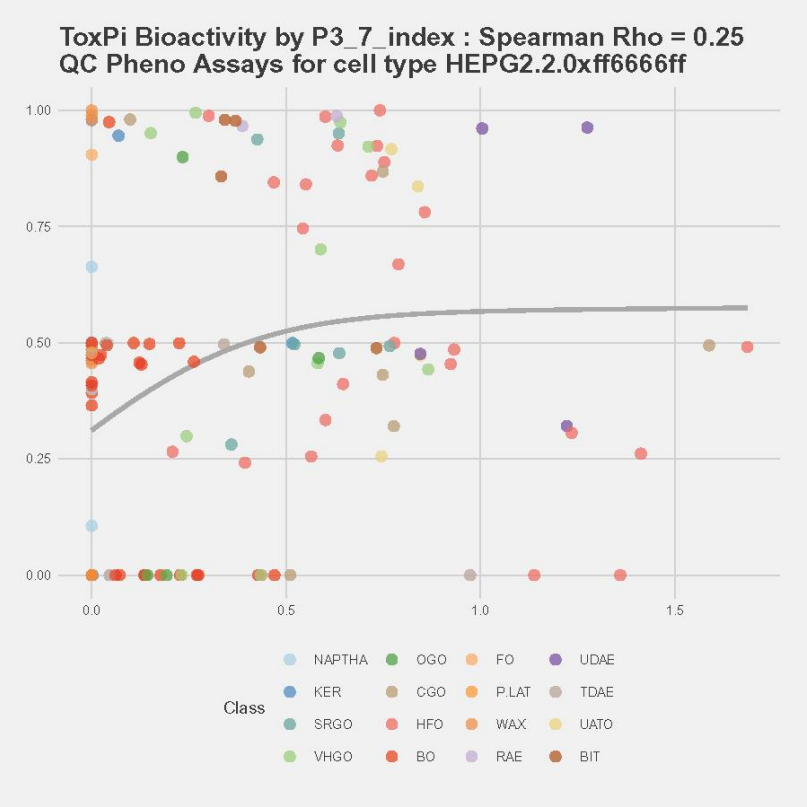
RAE
BIT

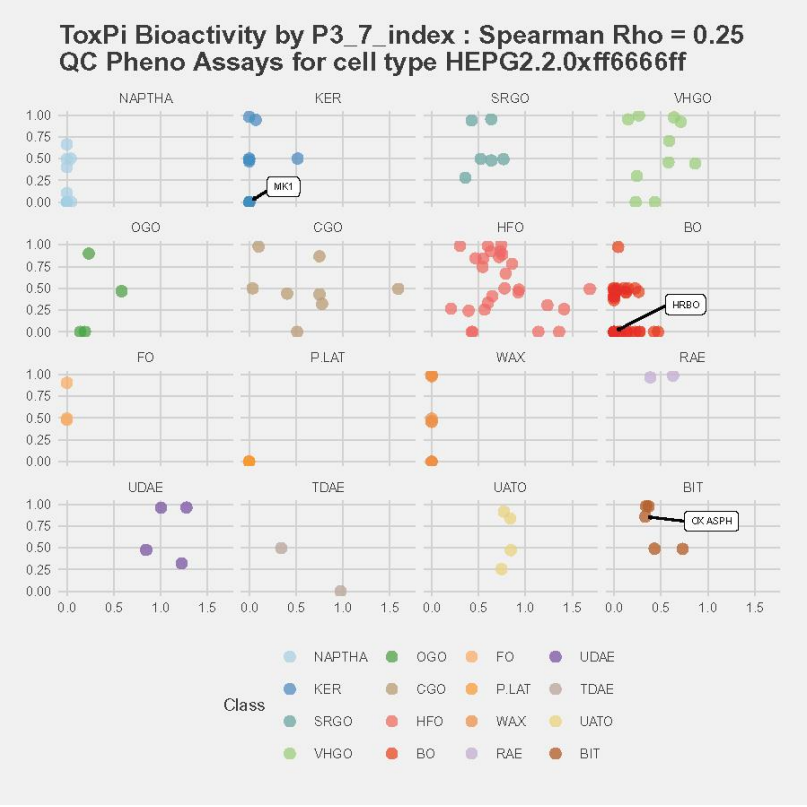
E. HEPARG cells.



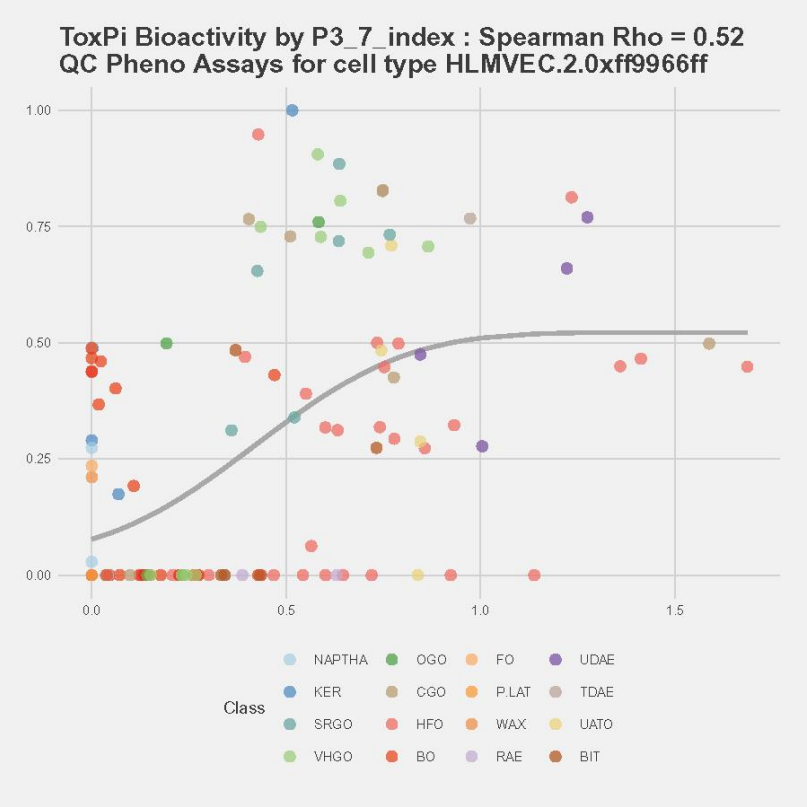


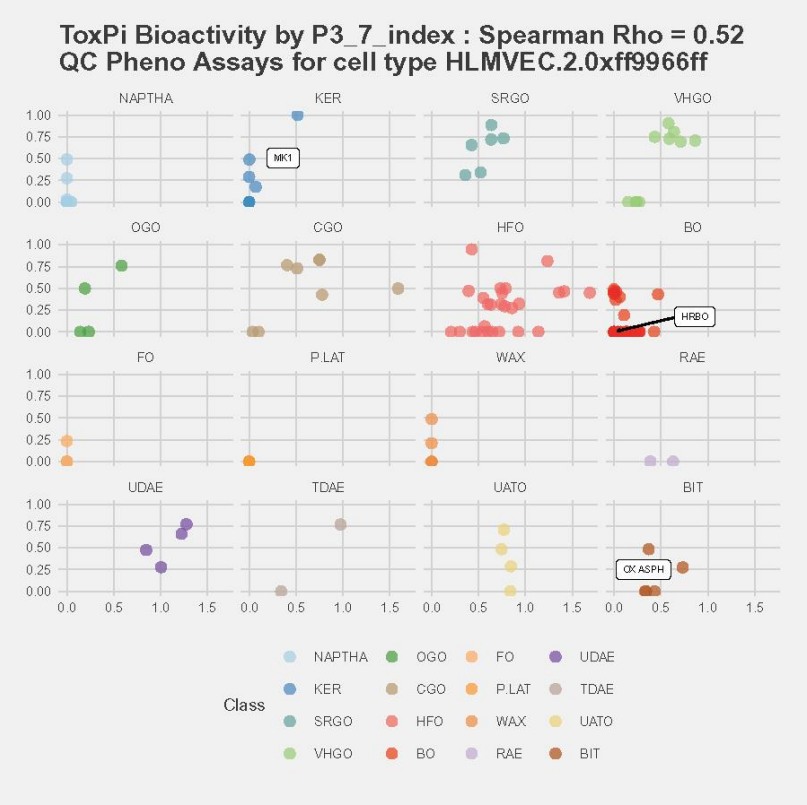
F. HepG2 cells.





G. HLMVEC cells.





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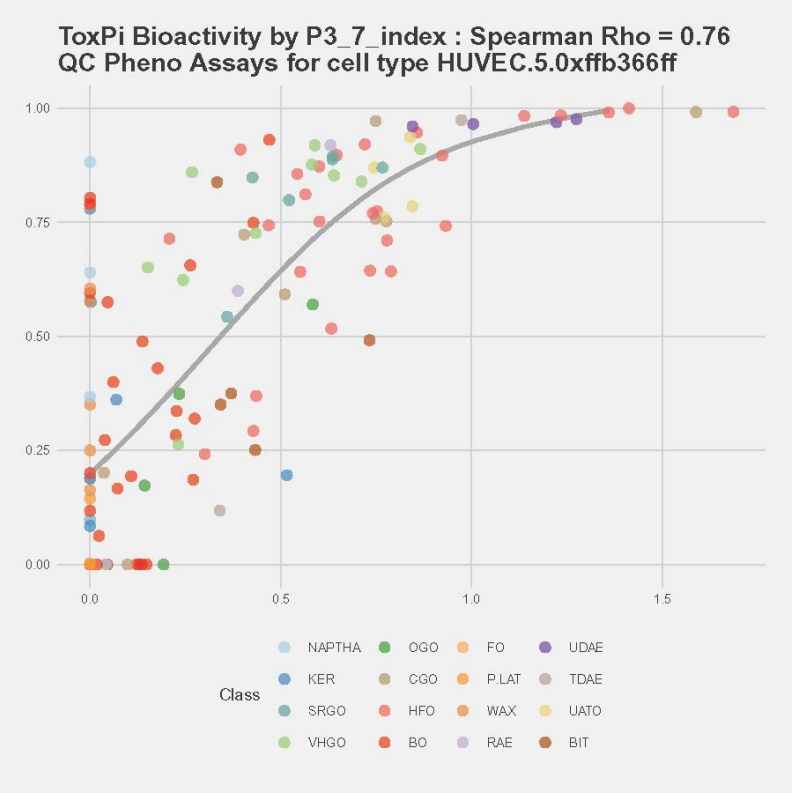
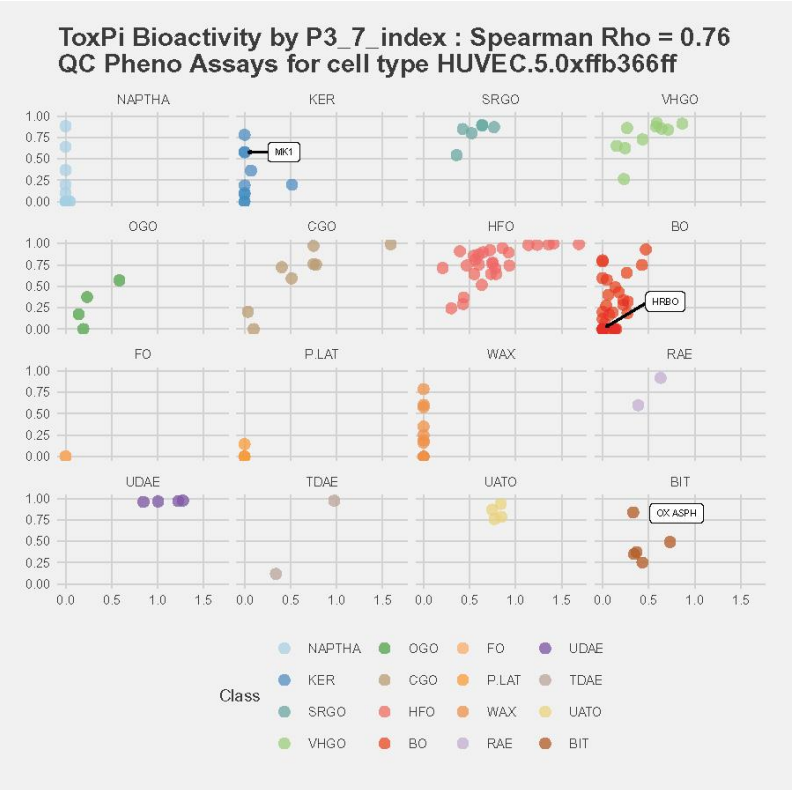
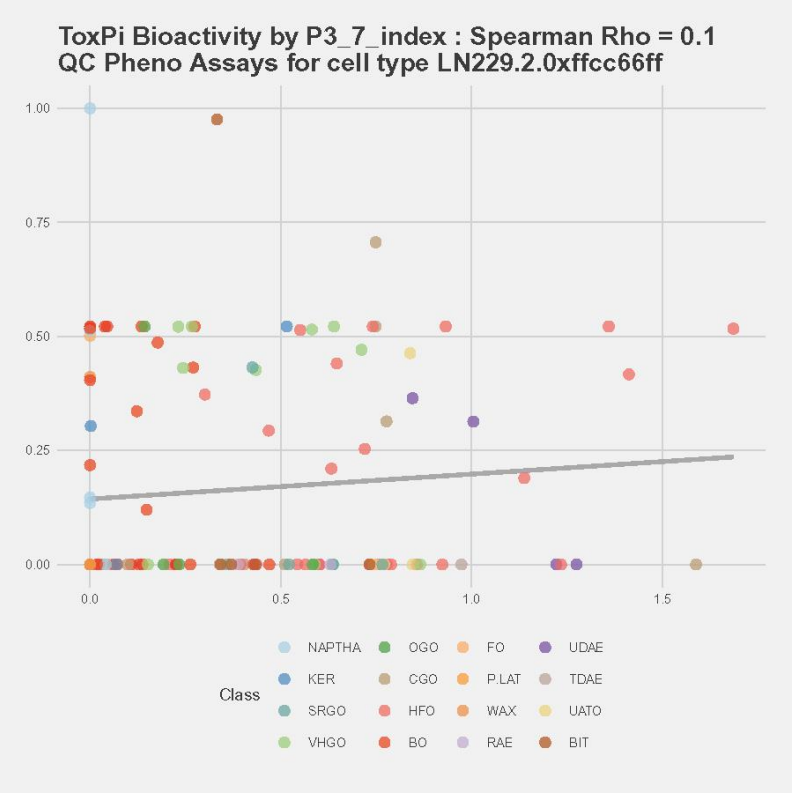
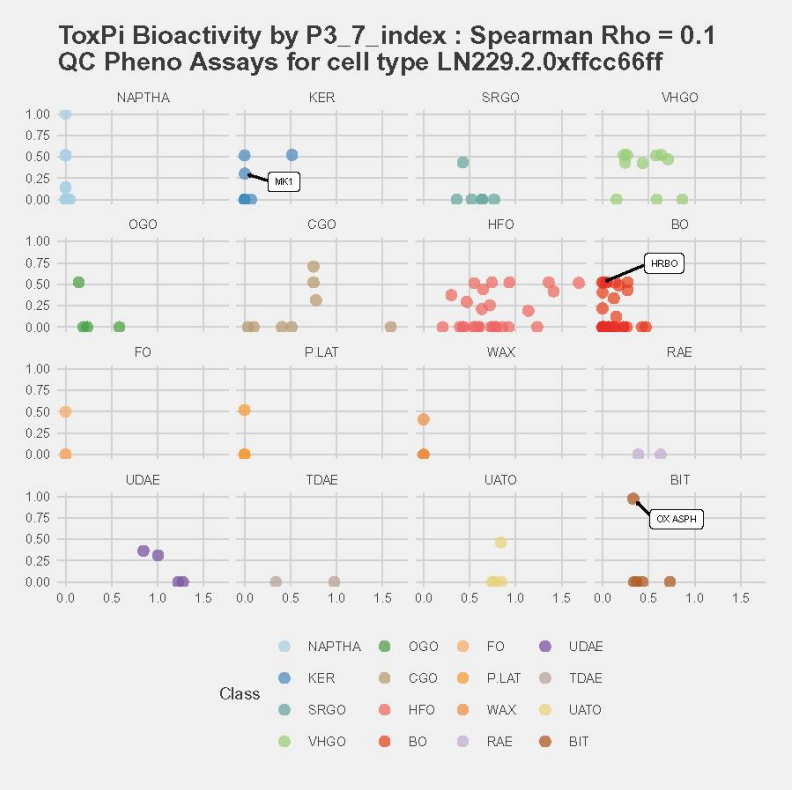


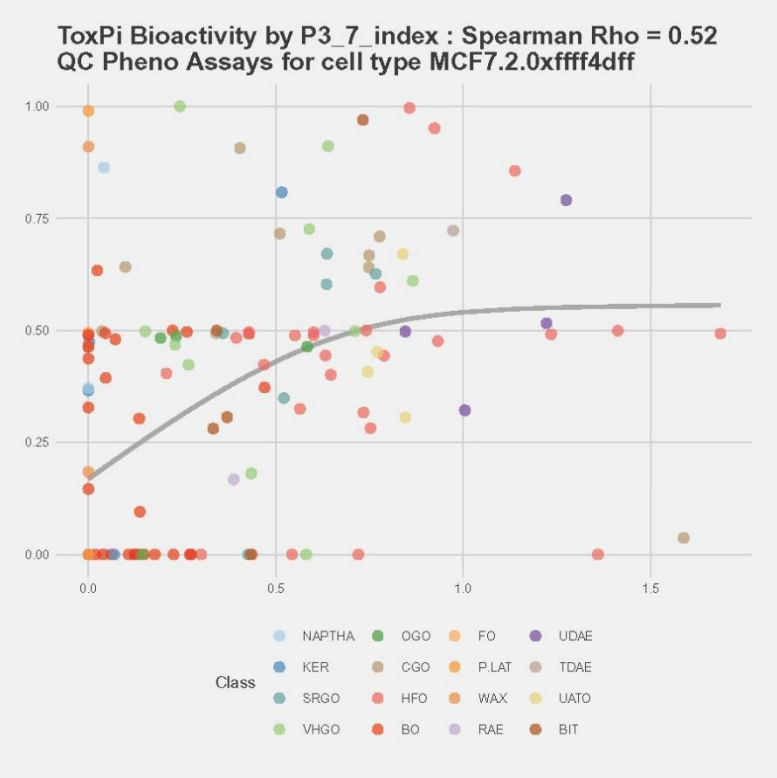


I. LN229 cells.





J. MCF7 cells.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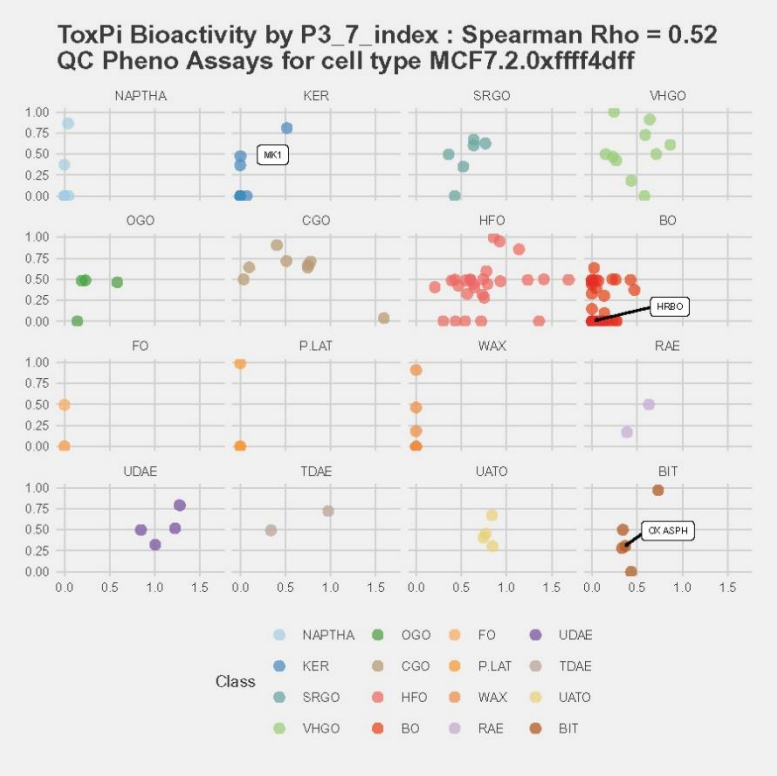
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UDAE
TDAE
UATO
BIT
CK ASPH
1.00
0.75
0.50
0.25
0.00
0.0
0.5
1.0
1.5
Class
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KER
SRGO
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OGO
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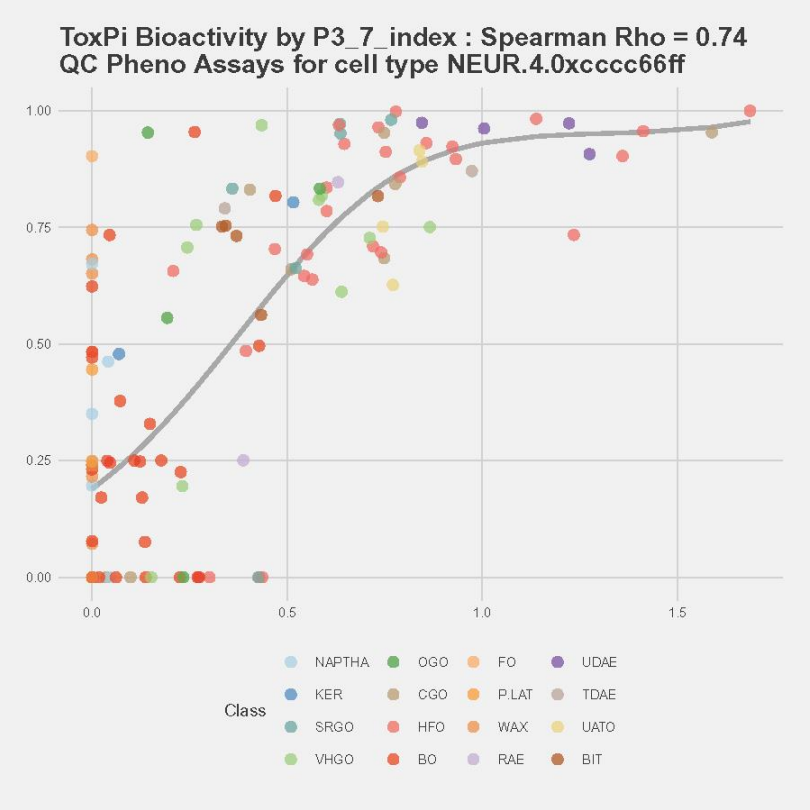
BIT

K. NEUR cells.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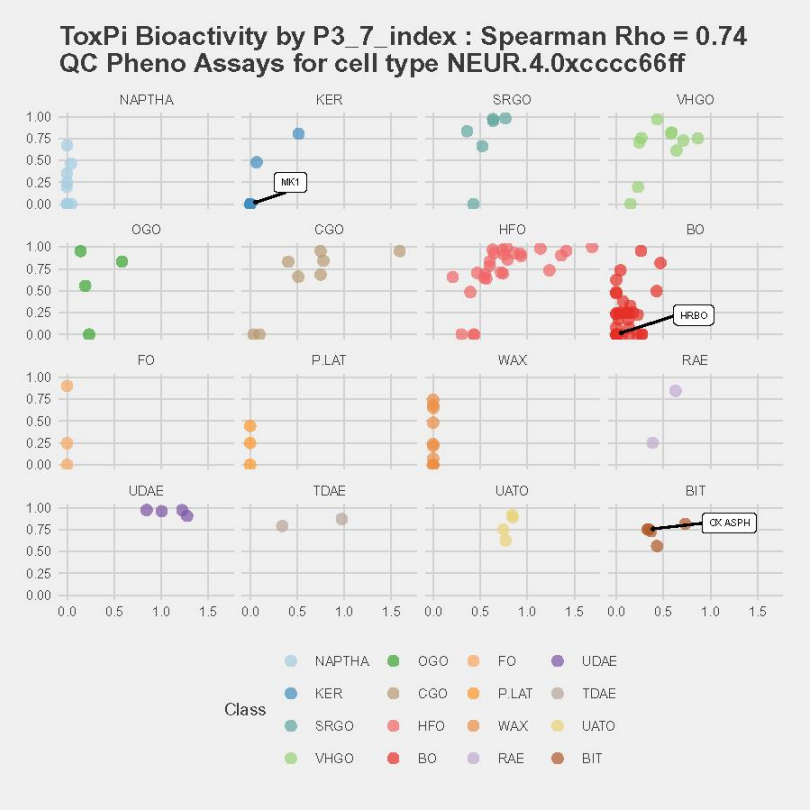
BO
RAE
BIT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UDAE
TDAE
UATO
BIT
CK ASPH
Class
NAPTHA
OGO
FO
UDAE
KER
CGO
P.LAT
TDAE
SRGO
HFO
WAX
UATO
VHGO
BO
RAE
BIT

L. SH-SY5Y cells.