Chen et al.:

A High-Throughput and Highly Automated Genotoxicity Screening Assay

Supplementary Data

Cell viability assay

The cytotoxicity of each compound was measured using the MTT assay. TK6 cells were treated with a range of concentrations from 2 µM to 1 mM using 10 different concentrations with two-fold dilution for 4 h in triplicate. For agents requiring metabolic activation, cells were treated with agents in the absence or presence of S9 rat liver extract as described previously (Buick et al., 2015). At the end of 4 h treatment, the medium was removed, and cells were washed twice with PBS. Fresh medium was added to the cells, and cells recovered at 37°C in 5% CO_2 for 20 h. Cell viability was measured at the end of recovery period using the MTT Assay Kit (Cayman Chemical) following the manufacturer's instruction.

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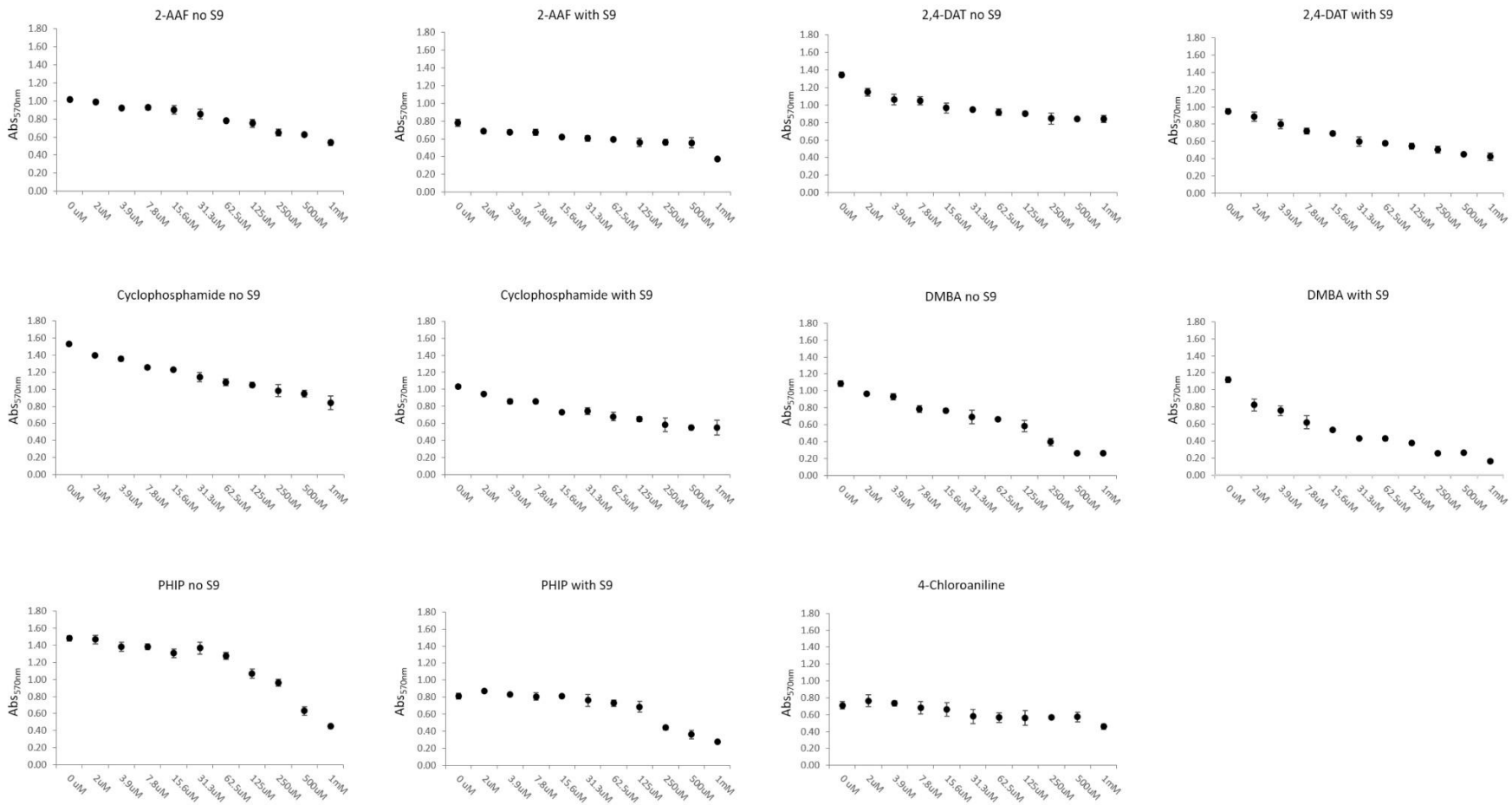
A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2-AAF with S9
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DMBA with S9
PHIP no S9
PHIP with S9
4-Chloroaniline
Abs570nm
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0.20
0.40
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1.00
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1.40
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1mM

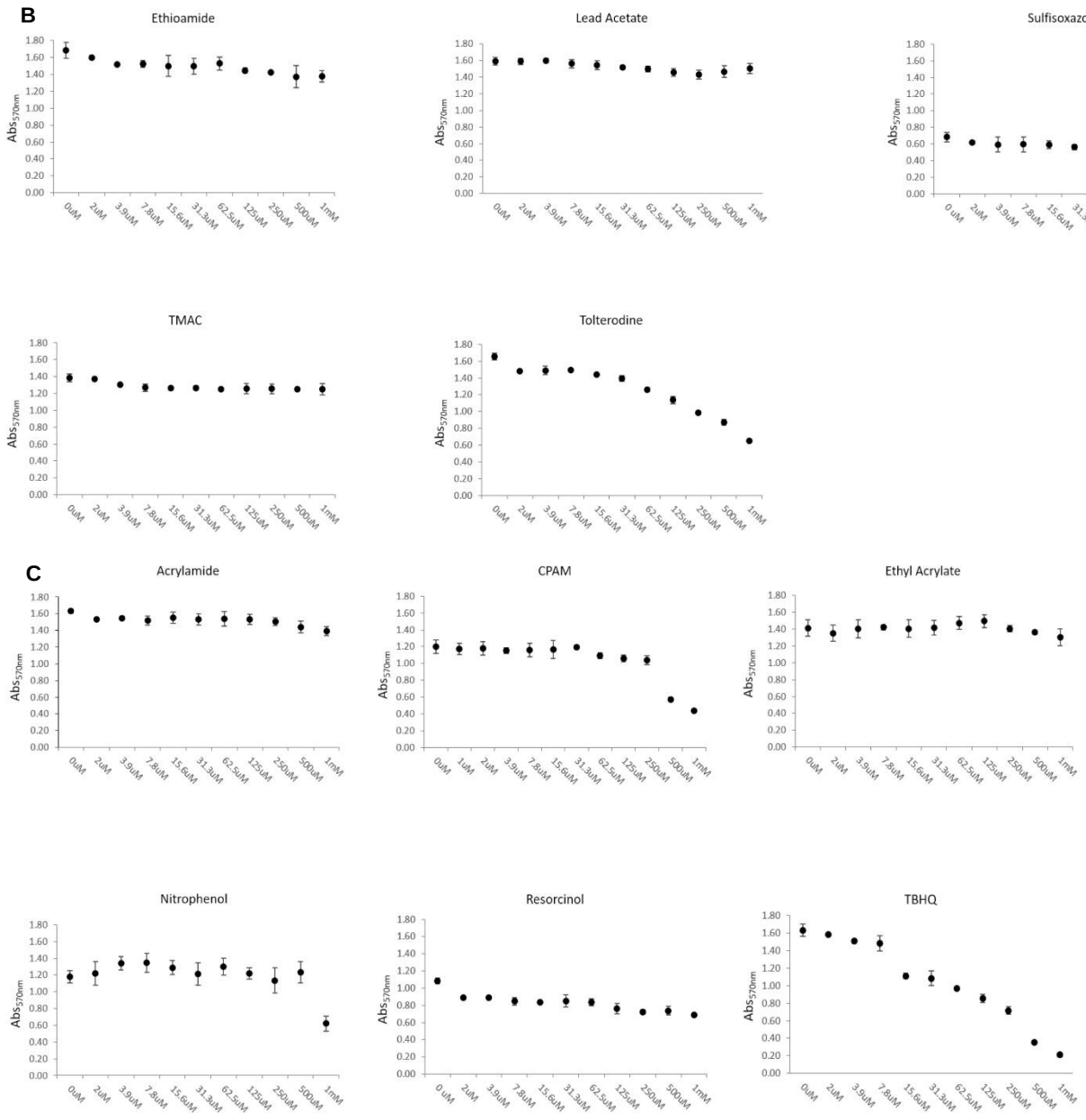
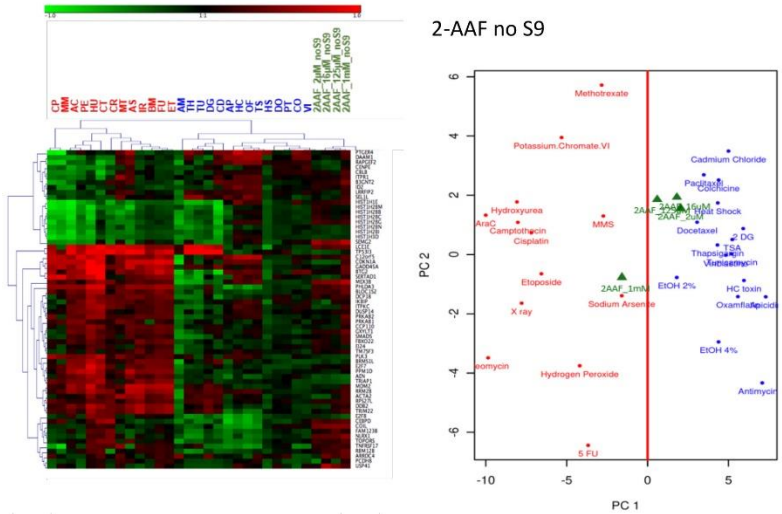


Fig S1: Cell viability for each agent was measured using the MTT assay After the 4 h treatment, cells were incubated in fresh medium for 20 h. Cell viability was measured at the end of the 20-h recovery period using the MTT assay. A) Cell viability of group 1 agents. For agents requiring metabolic activation, cell viability was measured both in the absence and presence of S9 rat liver extract. B and C) Cell viability of group 2 and 3 agents, respectively.

A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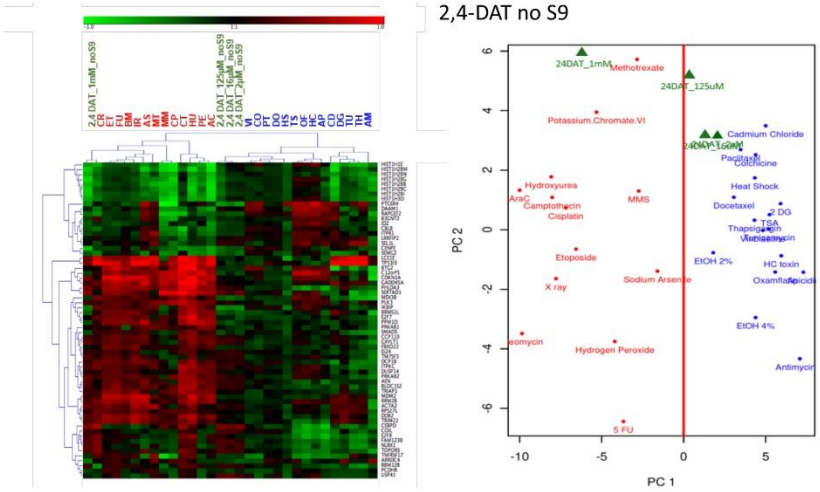
Cisplatin
MMS
Heat Shock
Docetaxel
2 DG
TSA
Thapsigargin
Vinblastine
Etoposide
2AAF_1mM
EtOH 2%
HC toxin
Sodium Arsenite
Oxamflatin
Apicidin
X ray
EtOH 4%
Hydrogen Peroxide
Antimycin
5 FU
PC 1
PC 2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Colchicine
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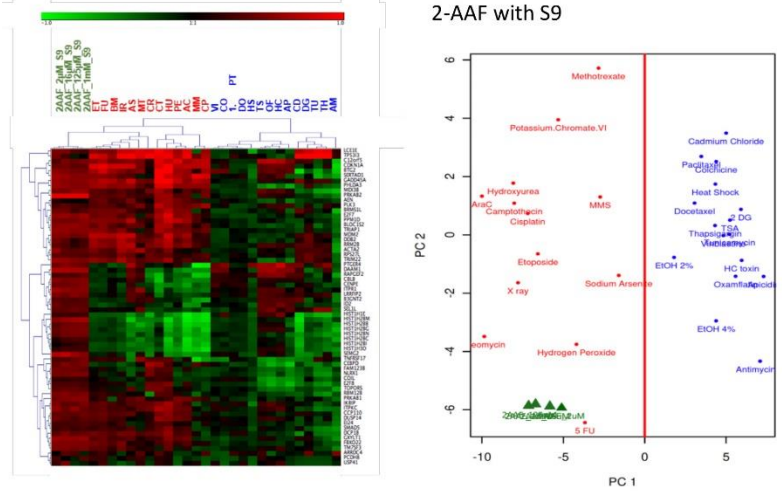
AraC
Camptothecin
Cisplatin
MMS
Heat Shock
Docetaxel
2 DG
TSA
Thapsigargin
Vinblastine
Etoposide
EtOH 2%
HC toxin
X ray
Sodium Arsenite
Oxamflatin
Apicidin
EtOH 4%
Hydrogen Peroxide
Antimycin
5 FU
PC 1
PC 2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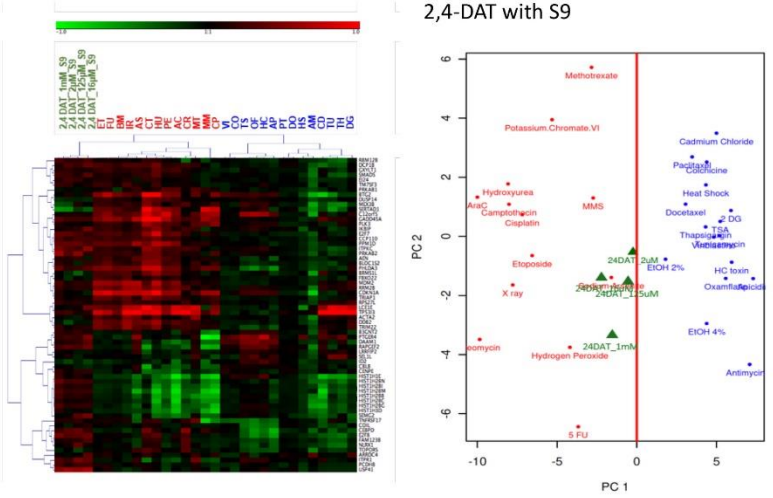
Cisplatin
MMS
Heat Shock
Docetaxel
2 DG
TSA
Thapsigargin
Vinblastine
Etoposide
EtOH 2%
HC toxin
X ray
Sodium Arsenite
Oxamflatin
Apicidin
EtOH 4%
Hydrogen Peroxide
Antimycin
5 FU
PC 1
PC 2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Camptothecin
Cisplatin
MMS
Heat Shock
Docetaxel
2 DG
TSA
Thapsigargin
Vinblastine
Etoposide
24DAT_2uM
EtOH 2%
HC toxin
X ray
24DAT_125uM
Oxamflatin
Apicidin
EtOH 4%
24DAT_1mM
Hydrogen Peroxide
Antimycin
5 FU
PC 1
PC 2

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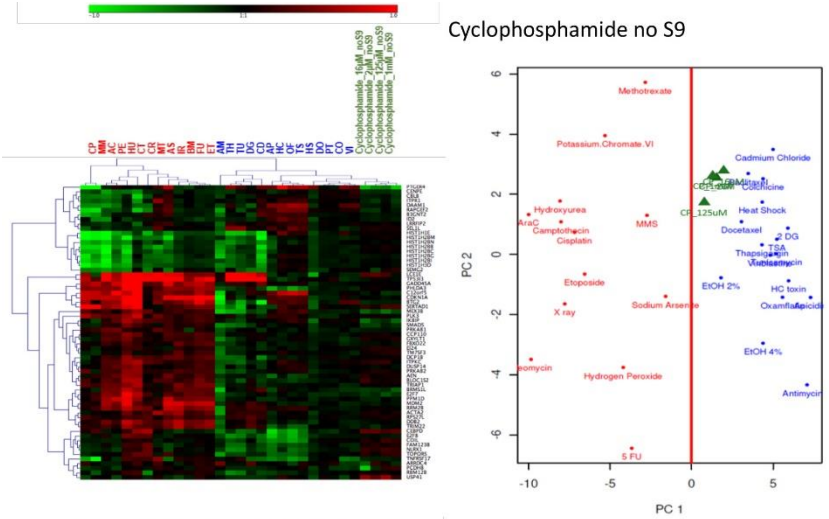

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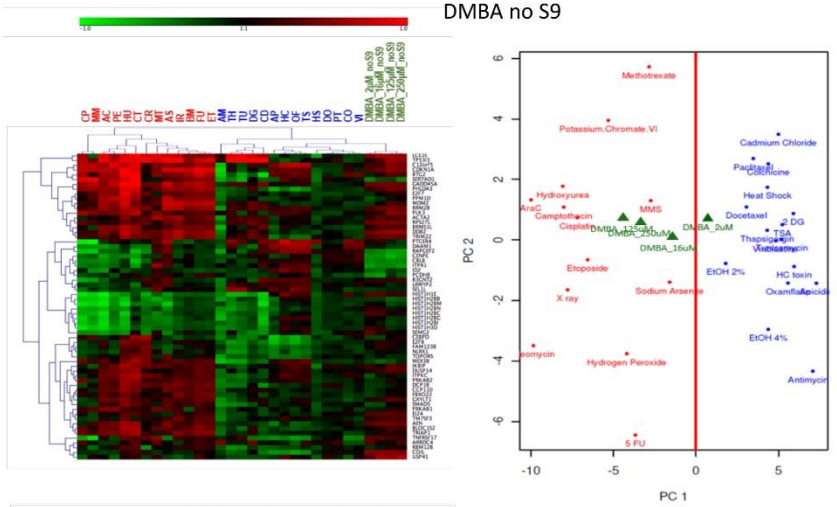

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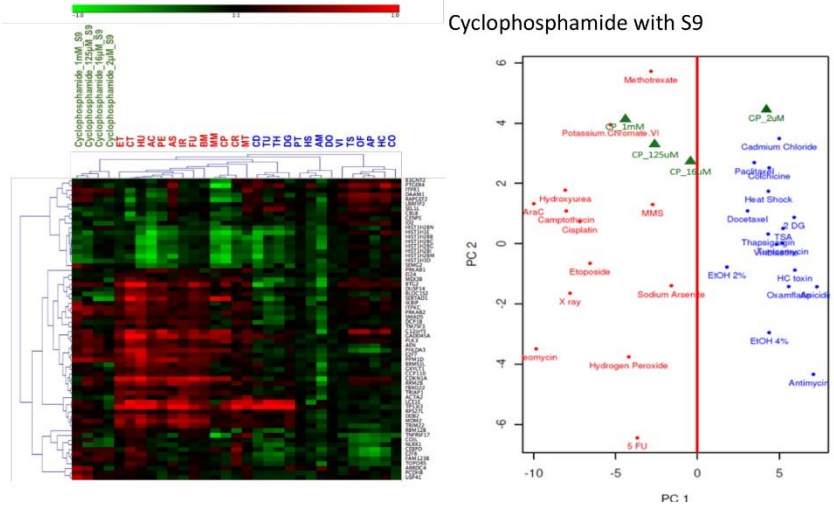

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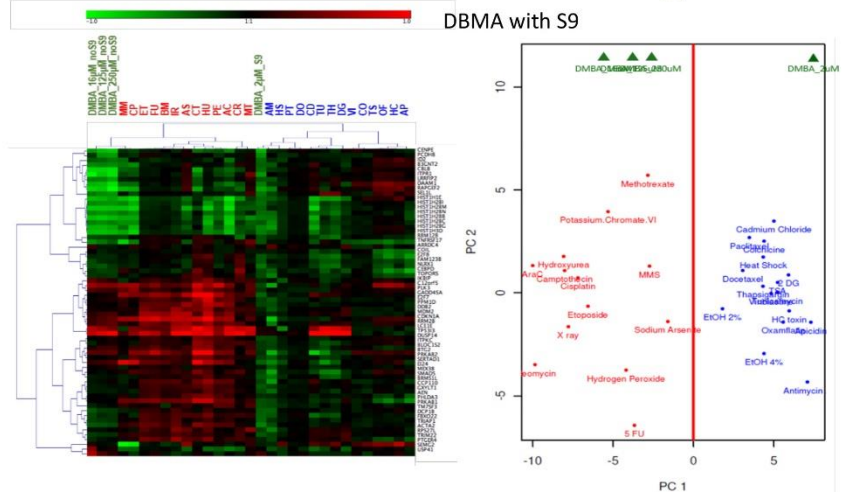

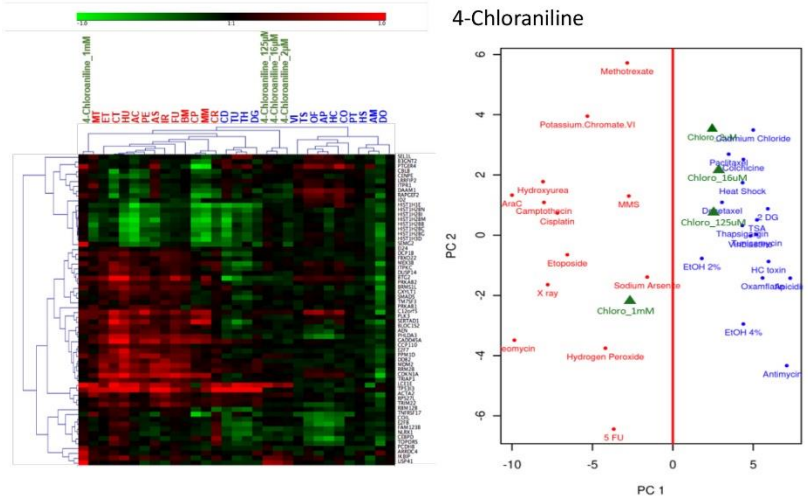
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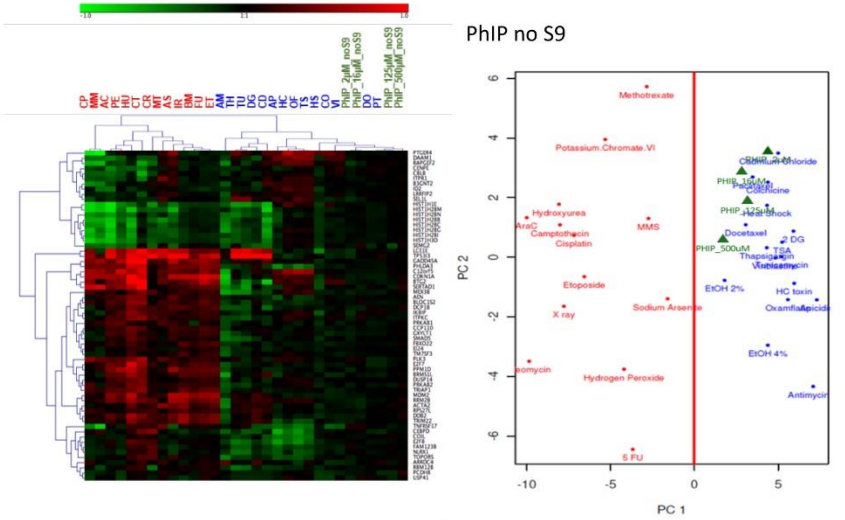
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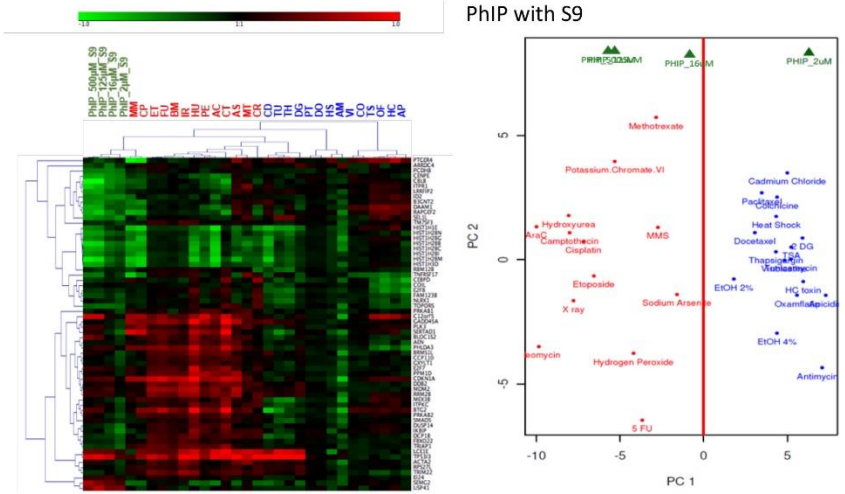
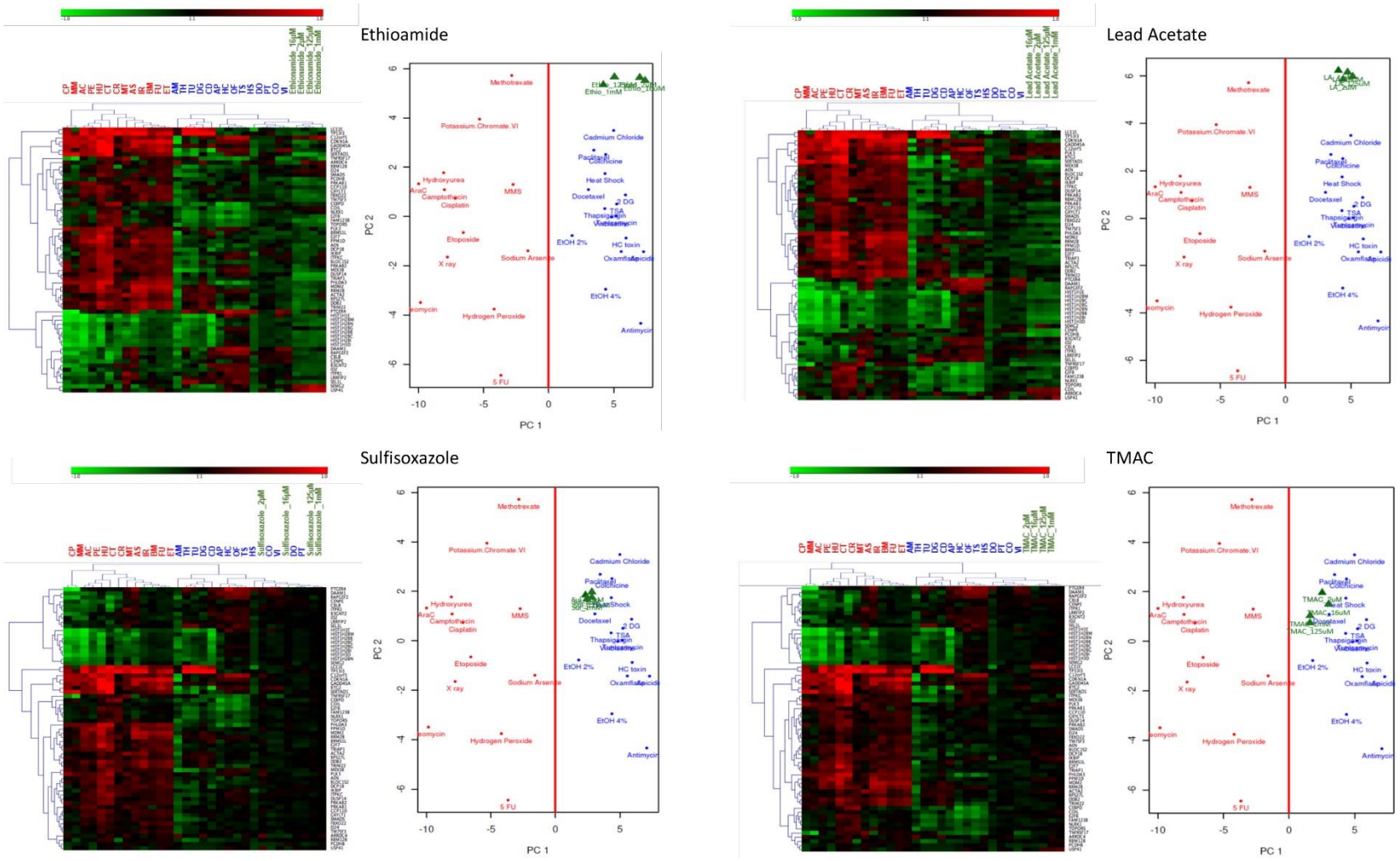

PhIP with S9
PC 1
PC 2

B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TMAC
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PC 2
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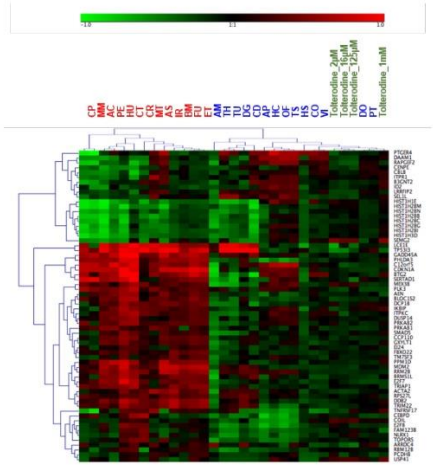
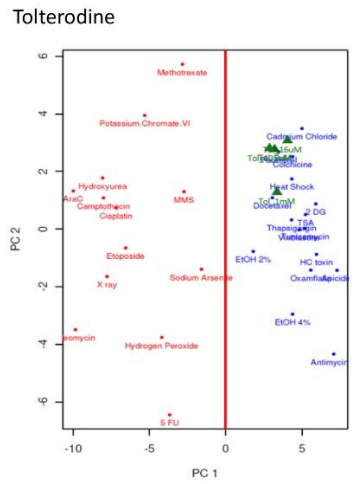
Potassium.Chromate.VI
Cadmium Chloride
Paclitaxel
Colchicine
Heat Shock
Docetaxel
2 DG
TSA
Thapsigargin
Vinblastine
HC toxin
Oxamflatin
Apicidin
EtOH 4%
Antimycin
5 FU
Hydrogen Peroxide
Sodium Arsenite
EtOH 2%
X ray
Etoposide
MMS
Cisplatin
Camptothecin
AraC
Hydroxyurea

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AraC
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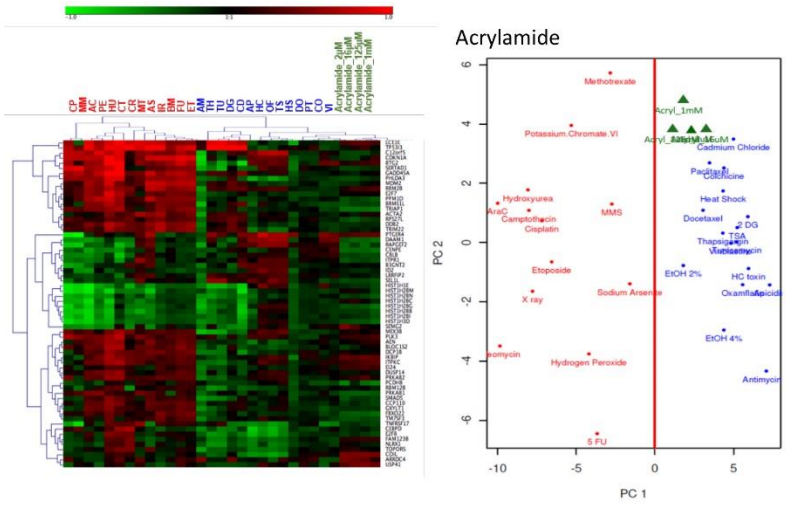
Heat Shock
Docetaxel
2 DG
TSA
Etoposide
EtOH 2%
HC toxin
Sodium Arsenite
X ray
EtOH 4%
Hydrogen Peroxide
Antimycin
5 FU
PC 1
PC 2

C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Heat Shock
Docetaxel
2 DG
TSA
Vinblastin
Thapsigargin
Hydroxyurea
AraC
Camptothecin
Cisplatin
MMS
Etoposide
EtOH 2%
HC toxin
X ray
Sodium Arsenite
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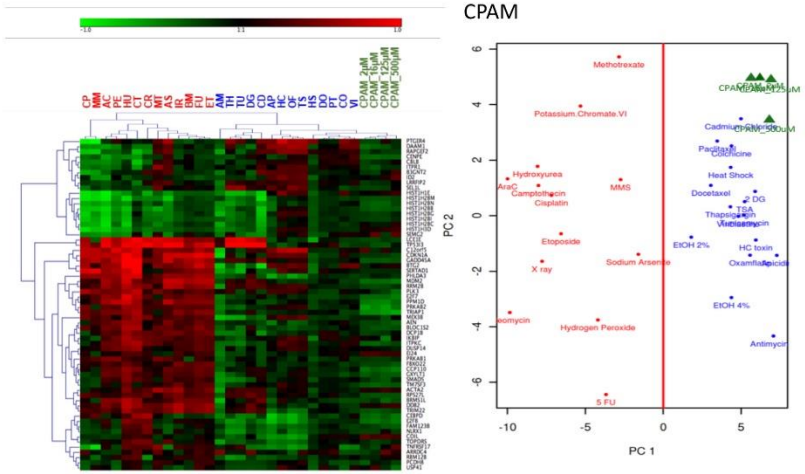
Apicidin
EtOH 4%
Hydrogen Peroxide
Antimycin
5 FU
PC 1
PC 2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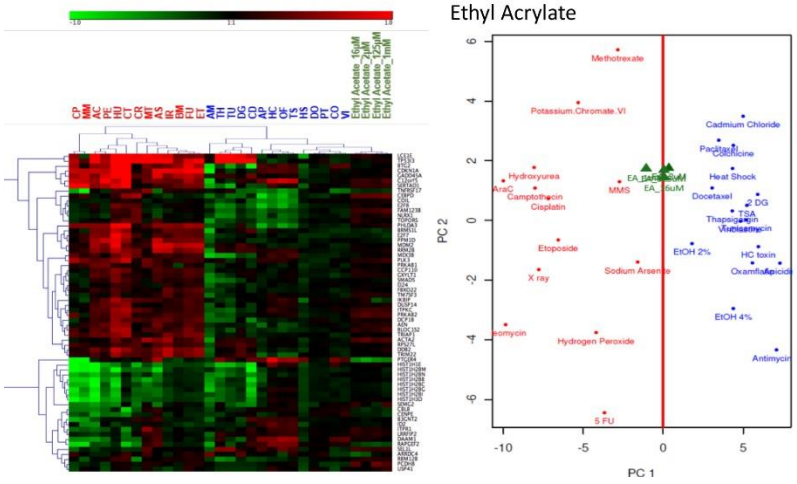
Colchicine
Heat Shock
Docetaxel
2 DG
TSA
Vinblastin
Thapsigargin
Hydroxyurea
AraC
Camptothecin
Cisplatin
MMS
Etoposide
EtOH 2%
HC toxin
X ray
Sodium Arsenite
Oxamflatin
Apicidin
EtOH 4%
Hydrogen Peroxide
Antimycin
5 FU
PC 1
PC 2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Vinblastin
Thapsigargin
Hydroxyurea
AraC
Camptothecin
Cisplatin
MMS
Etoposide
EtOH 2%
HC toxin
X ray
Sodium Arsenite
Oxamflatin
Apicidin
EtOH 4%
Hydrogen Peroxide
Antimycin
5 FU
PC 1
PC 2

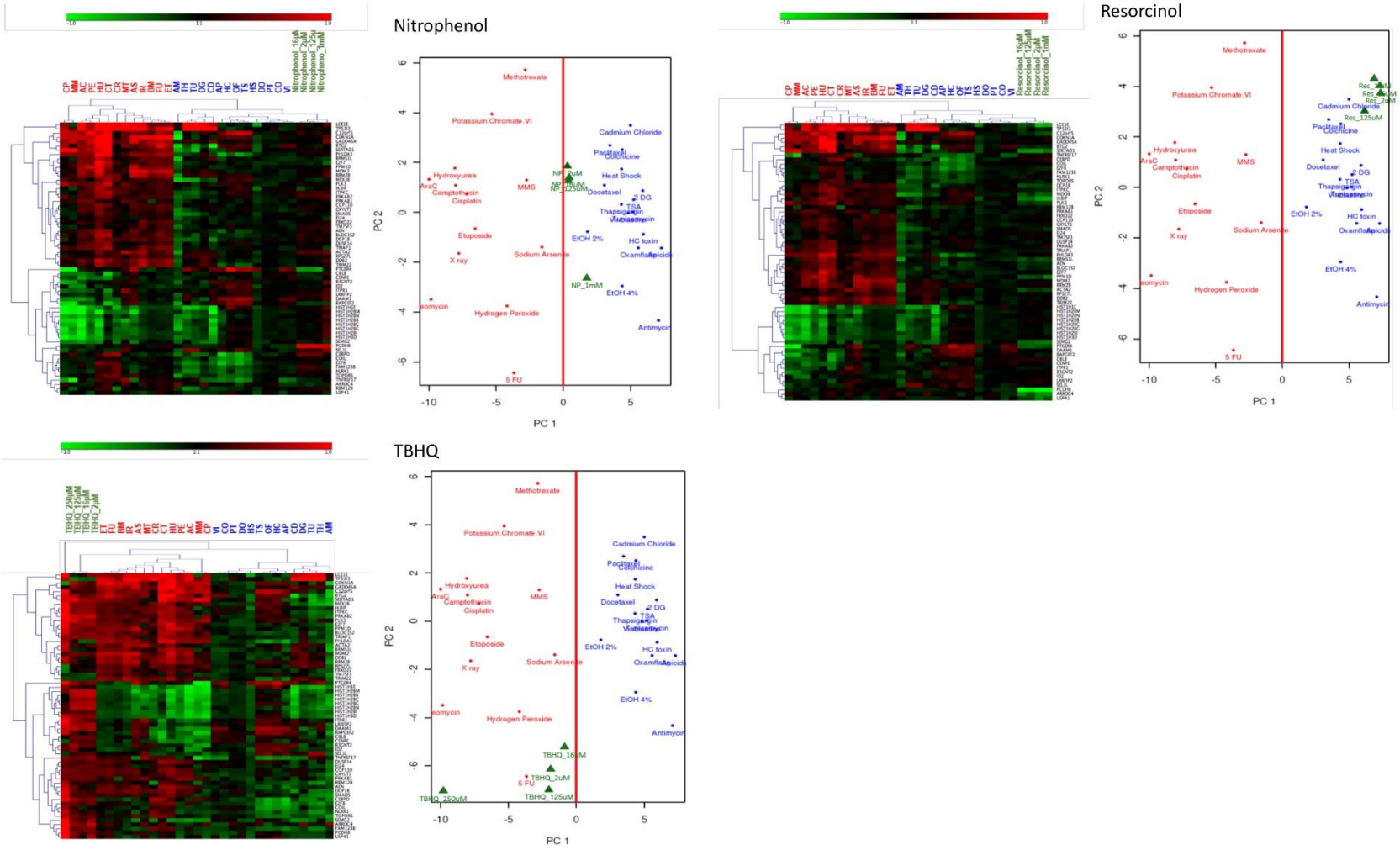

Fig. S2: Results of two DDI prediction methods using TGx-DDI, 2DC and PCA, for each agent

These two methods are described in detail in *Material and methods*. For each agent, the 2DC result is on the left and the PCA result is on the right. In both plots, the DDI compounds in the learning set are labeled in red while non-DDI compounds are labeled in blue. Agents in green are the test agents in each group. For 2DC plot, learning set compounds are labeled in two-letter short form. AC, AraC; AM, antimycin; AP, apicidin; AS, arsenite; BM, bleomycin; CD, cadmium; CO, colchicine; CP, cisplatin; CR, chromate; CT, camptothecin; DG, 2-DG; DO, docetaxel; ET, etoposide; FU, 5-FU; HC, HC-toxin; HS, heat shock; HU, hydroxyurea; IR, ionizing radiation; MM, MMS; MT, methotrexate; OF, oxamflatin; PE, peroxide; PT, paclitaxol; TH, thapsigargin; TS, Trichostatin A; TU, tunicamycin; VI, vinblastin. An agent clustering with the DDI branch in the 2DC plot is called DDI, and vice versa for non-DDI agents. In the PCA plot, agents with a negative first principal component (PC1) are classified as DDI, and those with a positive PC1 are classified as non-DDI. The plots of group 1-3 are displayed in A-C, respectively.

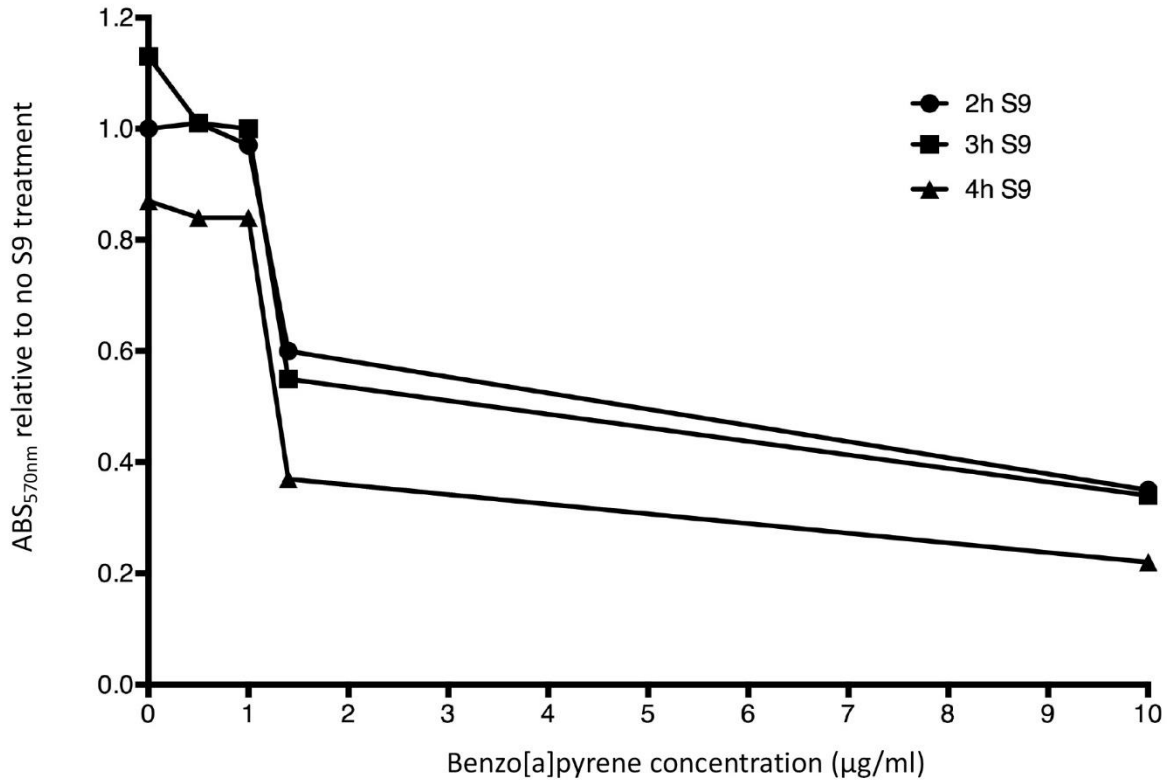

Fig. S3: MTT assay of benzo[a]pyrene (BaP)-treated TK6 cells
TK6 cells were exposed to BaP in the presence or absence of S9 rat liver extract for 2, 3 and 4 h. Cells were then washed with PBS to remove agent and S9, then incubated in fresh medium for 4 h. MTT assay was performed, and cell viability was determined. ABS_{570nm} for vehicle control with no S9 treatment at each time point was artificially set as 1, and the relative ABS_{570nm} were calculated. When ABS_{570nm} S9/ABS_{570nm} no S9 is less than 0.5, it is considered cytotoxic.

References

Buick, J. K., Moffat, I., Williams, A. et al. (2015). Integration of metabolic activation with a predictive toxicogenomics signature to classify genotoxic versus nongenotoxic chemicals in human TK6 cells. *Environ Mol Mutagen 56*, 520-534. doi:10.1002/em.21940