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 2uM to 1mM using 10 different concentrations with two-fold dilution for 4h 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 4h 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% CO₂ for 20h. Cell viability was measured at the end of recovery period using the MTT Assay Kit (Cayman Chemical) following the manufacturer’s instruction.

doi:10.14573/altex.2102121s
Fig S1: Cell viability for each agent was measured using the MTT assay. After the 4h treatment, cells were incubated in fresh medium for 20h. Cell viability was measured at the end of 20h 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.
ALTEX 39(x), SUPPLEMENTARY DATA
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, left is 2DC result and right is PCA result. In both plots, the compounds in the learning set were labeled as red indicating DDI and blue indicating non-DDI. Agents in dark green are the test agents in each group. For 2DC plot, learning set compounds were labeled in two-letter short form. The short-forms for the corresponding compounds are as follows: 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, paclitaxel; 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.
TK6 cells were exposed to BaP in the presence or absence of S9 rat liver extract for 2h, 3h, and 4h. Cells were then washed with PBS to remove agent and S9, then incubated in fresh medium for additional 4 hours. MTT assay was performed, and the cell viability was determined. \( \text{ABS}_{570\text{nm}} \) for vehicle control with no S9 treatment at each time point was artificially set as 1, and the relative \( \text{ABS}_{570\text{nm}} \) were calculated. When \( \text{ABS}_{570\text{nm}} \text{S9}/\text{ABS}_{570\text{nm}} \text{no S9} \) less than 0.5 we consider it is cytotoxic.