Supporting Information

Cyto- and Geno-Toxicity of 1,4-Dioxane and Its Transformation Products during Ultraviolet-Driven Advanced Oxidation Processes

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Text S1 Details of chemical analysis.

Chromatographic detection of 1,4-dioxane was achieved using an Agilent 1200 high performance liquid chromatograph equipped with a diode array detector. A Zorbax Eclipse Deltabond column (4.6×200mm, 5.0-μm particle size) was used. HPLC grade water and pure acetonitrile were used as eluents. The composition of mobile phase was 92% H_2O and 8% acetonitrile for 1,4-dioxane and ethylene glycol diformate. The retention times were 2.7 min and 1.9 min for 1,4-dioxane and ethylene glycol diformate, respectively. For formaldehyde and glycolaldehyde, mobile phase composition was 40% acetonitrile and 60% H_2O. The retention times were 3.5 min and 5.6 min, respectively. The sample injection volume was 100 μL and the flow rate was set at 1.0 mL/min for a total of 6 min run time.

Chromatographic detection of glycolic acid, formic acid and methoxyacetic acid was achieved using a Dionex 1000 Ion Chromatography equipped with a Dionex Ionpac AS22 column (4×250 mm, 11.0-μm particle size). A mixture of $NaHCO_3$ (1.4 mM) and Na_2CO_3 (4.5 mM) was used as the eluent. The sample injection volume was 10 μL and the flow rate was set at 0.8 mL/min for a total run time of 25 min. The retention time for methoxyacetic acid, glycolic acid and formic acid was 3.2, 3.7 and 4.2 min, respectively.

Text S2 Cyto- and Geno-toxicity bioassays

HCT-116 Human colorectal carcinoma cells were cultured in polystyrene culture flasks (Corning T-75) with McCoy's 5A Medium including 10% dialyzed fetal bovine serum (FBS), 100 μg/mL penicillin/streptomycin, and 5 μg/mL blasticidin. Cells were collected before reaching 80% confluence after the fourth passage and suspended with Opti-MEM Reduced Serum Assay Medium including 0.5% dialyzed fetal bovine serum (FBS), 1% 1 mM Sodium Pyruvate, 1% 0.1 mM non-essential amino acids (NEAA) and 1% of penicillin/streptomycin (100 U/mL and 100 mg/L) at a concentration of 5,5000 cells/mL. An aliquot of 90 μL of the cell suspension was added into each well of the black, clear-bottom, 96-well assay plate (Corning 3603). The cells were incubated overnight prior to sample exposures.

For cytotoxicity, a 10-μL aliquot of each concentration of the test compounds was dissolved in Assay medium with 5% DMSO and transferred to the assay plate. The final compound concentration in the 100-μL assay volume ranged from 0 μM to 500 uM over eight concentrations. Three replicates were tested for each concentration. The plate was allowed to settle for 30 min at room temperature and then incubated at 37 °C, 5% CO_2 for 16 hr. After 16 hr, 10-μL of MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) reagent was added to the wells, which was incubated at 37°C for 4 hr. The assay medium was then aspirated and 100-μL of DMSO detergent was added. Cells were then again incubated for 3 hr at room temperature in the dark. Cytotoxicity was assessed by recording the absorbance at 570 nm using the EnVision microplate reader (Perkin Elmer, Shelton, CT). All the readings in the test compounds were corrected with reference to the background reading, and percentage reduction in cell proliferation (cell viability) was normalized by that achieved in the growth medium controls (100% viable in growth medium). Positive controls were conducted with DMSO from 0

to 10%. 10% DMSO induced 100% cell death, whereas 0.5% DMSO resulted minimal cell loss (Figure S1). Dose response curves were plotted using GraphPad Prism 7.0, and the EC50 of each compound was calculated using the Four-Parameter Logistic nonlinear regression function.

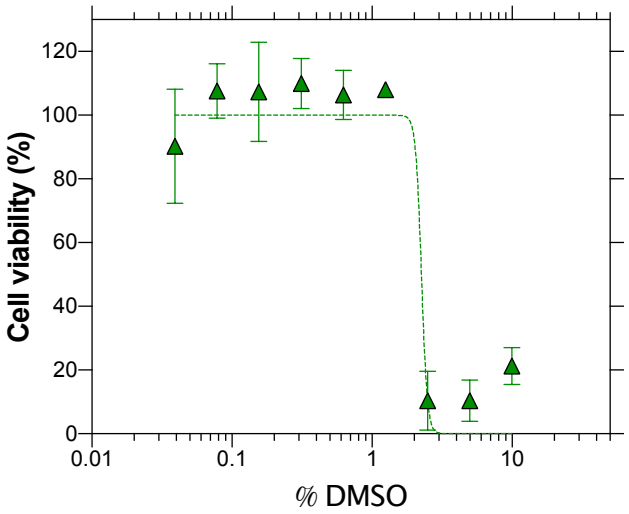


Figure S1 Cytotoxicity dose response curves of DMSO. Cell viability represented percent of viable cells compared to the controls based on the MTT assay (Text S2). Each value represents the mean of three replicates ± standard deviation.

For genotoxicity, 20 µL of LiveBLAzer™ - FRET B/G substrate mixture (CCF4-AM) was added to each well after 16-hr of incubation. The substrate mixture was prepared in the absence of direct light. The plates were then incubated at room temperature for 2 hr, and fluorescence intensity was measured at 460 and 530 nm emission and excitation at 409 nm. The two fluorescence readouts represent stimulated and unstimulated cells, respectively. Readings for each well were normalized by the background readings, and the response values (ratio of emissions at 460nm/530nm) was calculated by Excel. High ratio indicated high cell response to the chemicals. Finally, the dose response curves were plotted (GraphPad Prism 7.0) to compare to the positive control, mitomycin (Figure S2).

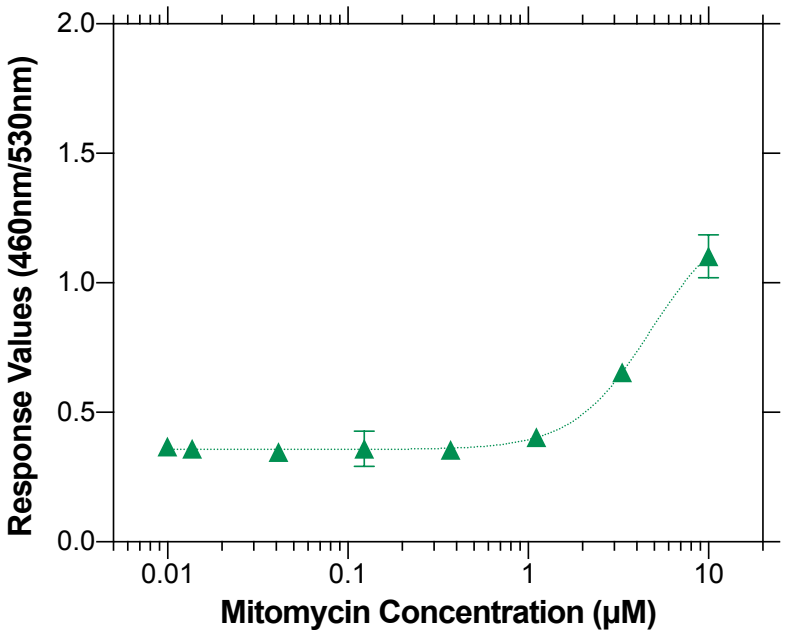


Figure S2 Genotoxicity dose response curves of Mitomycin. Response values was calculated based on the ratio of stimulated cells vs. unstimulated cells obtained from CellSensor p53RE-bla HCT-116 assay (Text S4). Each value represents the mean of three replicates ± standard deviation.

Text S3 Genotoxicity Mitomycin Equivalency Quotient (MEQ) calculations

First, the Relative Effect Potency (REP) value of a particular chemical *i* was calculated as the toxicity potency of the test compound relative to that of the standard, mitomycin, using the following equation:

$$REP_i = EC_{50(mitomycin)}/EC_{50(i)} \qquad \text{(Equation S1)}$$

where *i* is a specific oxidation transformation product from 1,4-D, $EC_{50(mitomycin)}$ is the concentration of mitomycin that gives the half-maximal response. $EC_{50(mitomycin)}$=4.77 μM, which was experimentally obtained from Figure S2. $EC_{50(i)}$ is the concentration of the target analyte (*e.g.,* 1,4-D, glycolaldehyde, formaldehyde, formic acid, methoxyacetic acid, and ethylene glycol diformate) that gives the half-maximal response, and its value for each transformation product was calculated based on experimental results from Figures 2B and S4B.

For example, REP for glycolaldehyde is calculated as:

$$REP = EC_{50(mitomycin)}/EC_{50(glycolaldehyde)} = 4.77\mu M /(7.1\times10^{1}\mu M) = 6.7\times10^{-2}$$

Second, the theoretical Mitomycin Equivalency Quotient (MEQ) of 1,4-D and its oxidation products was calculated as:

$$MEQ_{theoretical} = \Sigma(1 \times \%_i \times REP) \qquad \text{(Equation S2)}$$

$\%_i$ is the molar percentage of the transformation product *i* experimentally measured in a UV/AOP treatment (values are listed in Table S1), and REP is the Relative Effect Potency (REP) calculated from Equation S1.

For example, $MEQ_{theoretical}$ of the mixture resulting from $S_2O_8^{2-}$ photolysis of 1,4-D with a UV dosage of 720 mJ×cm^{-2} was calculated as:

$$MEQ_{theoretical} = \Sigma(1 \times \%_i \times REP)$$

$$= \%_{1,4\text{-}D} \times REP + \%_{Glycolaldehyde} \times REP + \%_{Formaldehyde} \times REP$$

$$=(20\% \times 2.4\times10^{-10}) + (26\% \times 6.7\times10^{-2}) + (4\% \times 1.2\times10^{-2})$$

$$= 1.79\times10^{-2}$$

$\%_i$ is the molar percentage of the transformation product *i* (Table S1). For example, after 10 minutes of reaction, the concentration of each compound in UV/$S_2O_8^{2-}$ treatment was 20% of 1,4-D, 20% of glycolaldehyde, 4% of gormaldehyde, 13% of formic acid, 13% of methoxyacetic acid, and 20% of ethylene glycol diformate (Figure 1, Table S1).

Third, the experimentally observed Mitomycin Equivalency Quotient (MEQ) of 1,4-D oxidation in a UV/AOP after 10 and 20 minutes of reaction was calculated as:

$$MEQ_{observed} = \frac{EC_{50(mitomycin)}}{EC_{50(720\ mJ\times cm^{-2})or(1440\ mJ\times cm^{-2})}} \qquad \text{(Equation S3)}$$

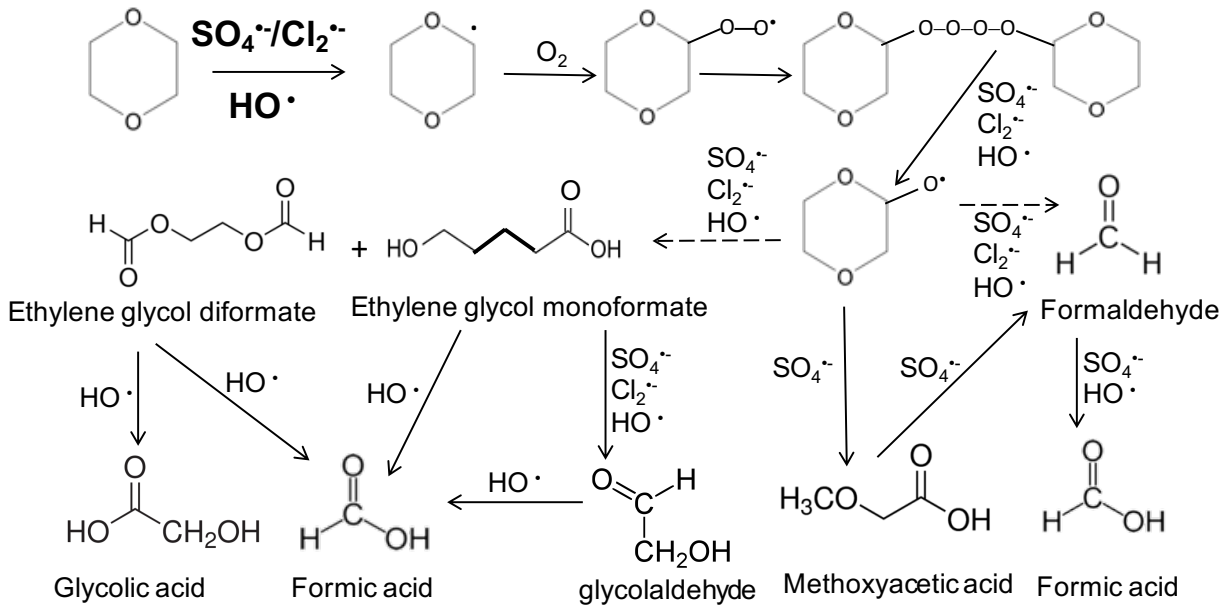
$EC_{50(720\ mJ\times cm^{-2})or(1440\ mJ\times cm^{-2})}$ is the EC_{50} of the mixed solution that contained 1,4-D and its transformation products (prepared according to the molar percentage specified in Table S1), under a UV dosage of 720 mJ×cm^{-2} or 1440 mJ×cm^{-2} (Figure S3). All six chemicals were mixed to reach a final total concentration of 1000 μM in the mixture based on the percentage listed in Table S1. A serial dilution was made from 0 μM to 100 μM over eight concentrations and the

genotoxicity and cytotoxicity assays were performed according to the procedure described in Text S2. The EC_{50} was calculated based on the dose-response curves in Figure S5.

For instance, the experimental observed MEQ value of a mixture solution resulting from UV/$S_2O_8^{2-}$ treatment of 1,4-D with a UV dosage of 720 mJ×cm^{-2} was calculated as:

$MEQ_{observed}$= 4.77μM/(2.48×10^2 μM) =1.92×10^{-2}

Using this approach, the theoretical and experimentally observed MEQ values of 1,4-D oxidation by three UV/AOPs after 10 and 20 minutes of reaction were calculated and plotted in Figure 3 in the main text. MEQ represented the toxicity evolution of 1,4-D during UV/$S_2O_8^{2-}$, UV/H_2O_2 and UV/NH_2Cl treatment.



Scheme S1 Proposed 1,4-dioxane degradation pathway under UV photolysis of $S_2O_8^{2-}$, H_2O_2, and NH_2Cl.

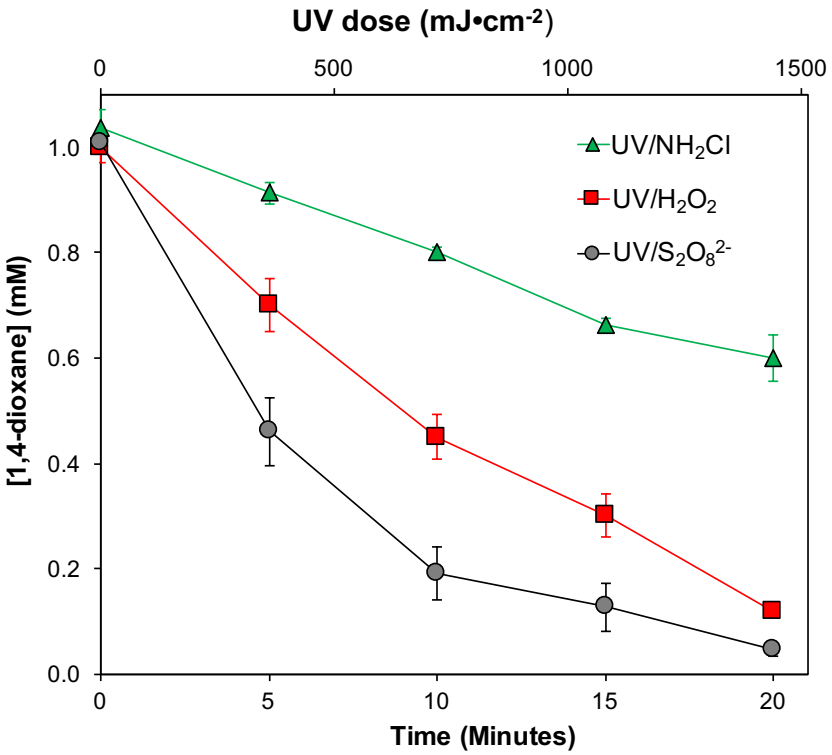


Figure S3 1,4-D degradation in $UV/S_2O_8^{2-}$, UV/H_2O_2 and UV/NH_2Cl. [Oxidants]=5 mM, [1,4-D]=1 mM, pH=8.

Table S1 Products distribution during UV/AOPs treatment.*

Chemical	$UV/S_2O_8^{2-}$		UV/H_2O_2		UV/NH_2Cl	
	UV dosage (mJ/cm^2)					
	720	**1440**	**720**	**1440**	**720**	**1440**
1,4-Dioxane	20%	4%	51%	14%	84%	65%
Glycolaldehyde	26%	18%	24%	46%	8%	21%
Formaldehyde	4%	7%	5%	9%	1%	2%
Formic acid	13%	16%	17%	22%	0%	0%
Methoxyacetic acid	17%	28%	2%	5%	0%	0%
Glycolic acid	0%	0%	1%	4%	0%	0%
Ethylene glycol diformate	20%	27%	0%	0%	7%	12%

* Experimental conditions: [Oxidants]=5mM, [1,4-dioxane]=1mM, pH=8. A reaction time of 10 minutes corresponds to a UV dosage of 720 mJ/cm^2, and a reaction time of 20 minutes corresponds to a UV dosage of 1440 mJ/cm^2.

The product distribution percentage is defined as:

$$\%_{analyte} = \frac{[analyte]}{[1,4-D]+[Glycolaldehyde]+[Formaldehyde]+[Formic\ acid]+[Methoxylacetic\ acid]+[Glycolic\ acid]+[Ethylene\ glycol\ diformate]} \times 100\%$$

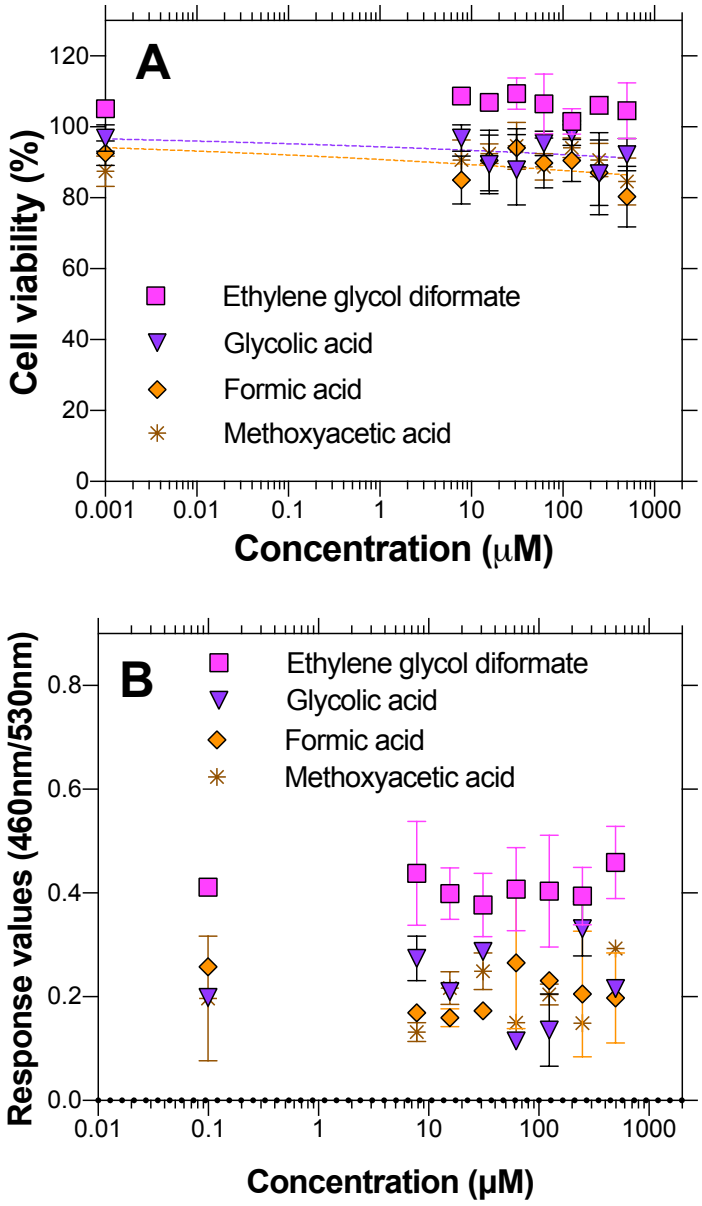


Figure S4 (A) Cytotoxicity and (B) Genotoxicity dose response curves of ethylenene glycol diformate, glycolic acid, formic acid and methoxyacetic acid. Cell viability represented percent of viable cells compared to the controls based on the MTT assay (Text S3). Response values was calculated based on the ratio of stimulated cells vs. unstimulated cells obtained from CellSensor p53RE-bla HCT-116 assay. Each value represents the mean of three replicates ± one standard deviation.

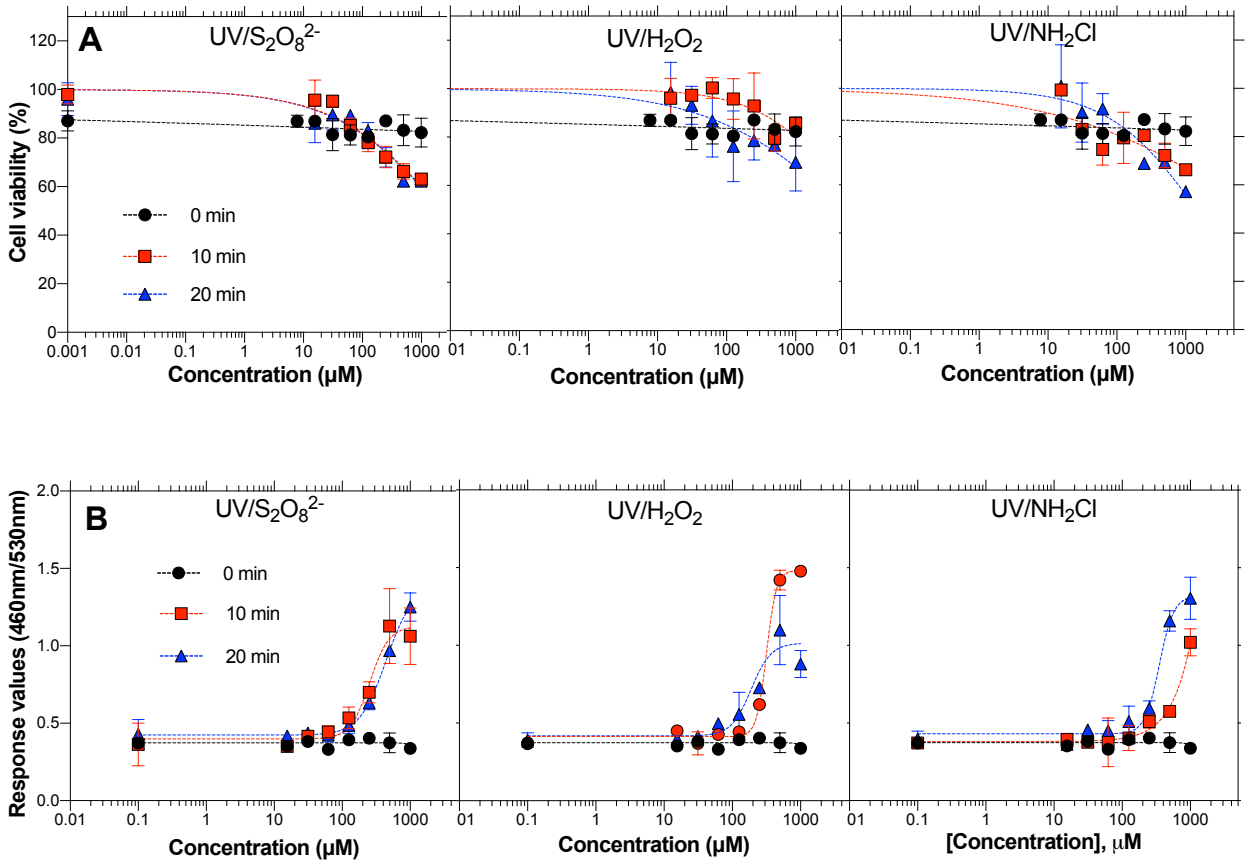


Figure S5 (A) Cytotoxicity and (B) Genotoxicity evolution of 1,4-D during UV/AOPs treatment. Cell viability represented percent of viable cells compared to the controls based on the MTT assay (Text S3). Response values was calculated based on the ratio of stimulated cells *vs.* unstimulated cells obtained from CellSensor p53RE-bla HCT-116 assay.