Characterization of Threshold Dose Response of Genotoxicity from Chemicals with Diverse Mechanisms of Damage

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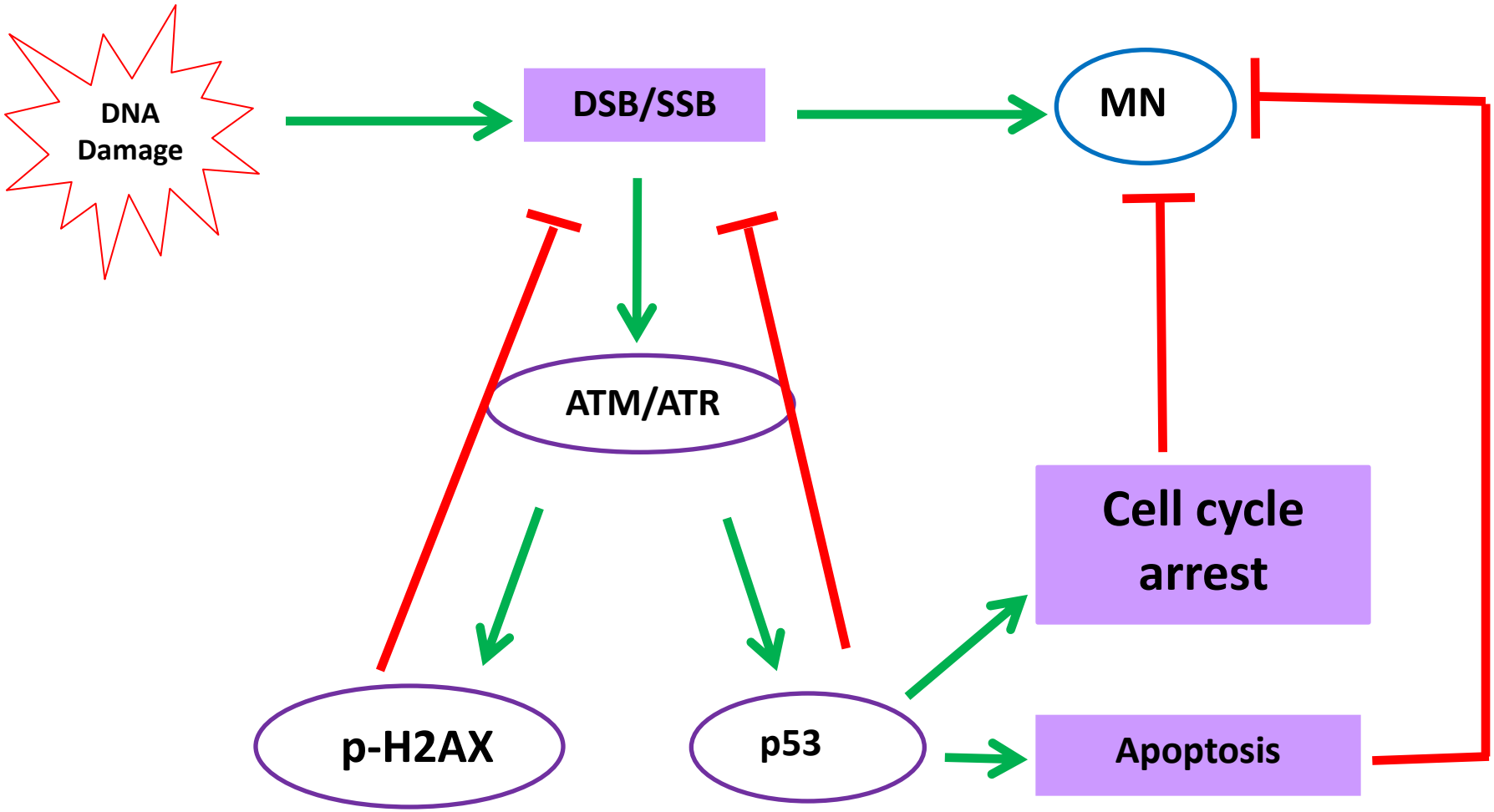
Research plan

1. Use high throughput assays to assess dose-response of DNA damage, p53 activation and cellular response

2. Use “hockey stick” statistical model to characterize threshold dose-response of DNA damage and p53

3. Development of more sensitive assays to assess DNA damage and repair

Working hypothesis



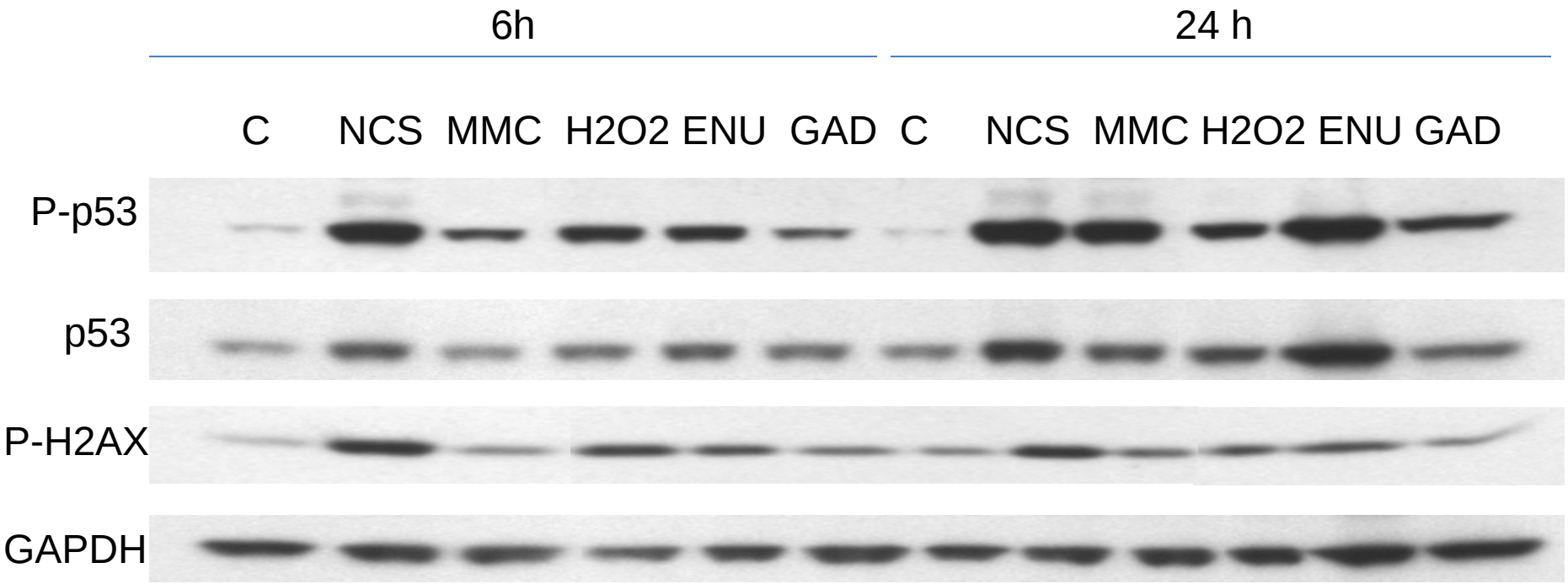
Chemicals with different DNA damage mechanisms

- Neocarzinostatin (NCS)
  - DSB, γ-irradiation mimic
- Etoposide (ETP)
  - DSB, topoisomerase II inhibitor
- Mitomycin C (MMC)
  - DSB, DNA crosslinking
- Methyl methanesulfonate (MMS) and ethyl nitrosourea (ENU)
  - SSB, DNA adduct, alkylating agents
- Glycidamide (GAD)
  - SSB, DNA adduct
- Hydrogen peroxide (H_2O_2,)
  - SSB, oxidative damage
- Curcumin (CUR) and quercetin (QUE)
  - SSB, oxidative damage, polyphenols

Tools and Methods

- **HT1080 cells**
  - Human fibrosarcoma cells
  - Wild-type p53
- **Micronucleus (MN) assay**
  - Litron In Vitro MN assay
  - High-throughput flow cytometry (24hrs -1.5-fold doubling time)
- **Protein characterization**
  - DNA damage (p-H2AX), p53 activation
  - Multiplex high-throughput flow cytometry
- **Dose-response characterization**
  - MN and protein
- **Transcriptomics – chemical comparison**
  - Whole genome array
  - Affymetrix Titan platform

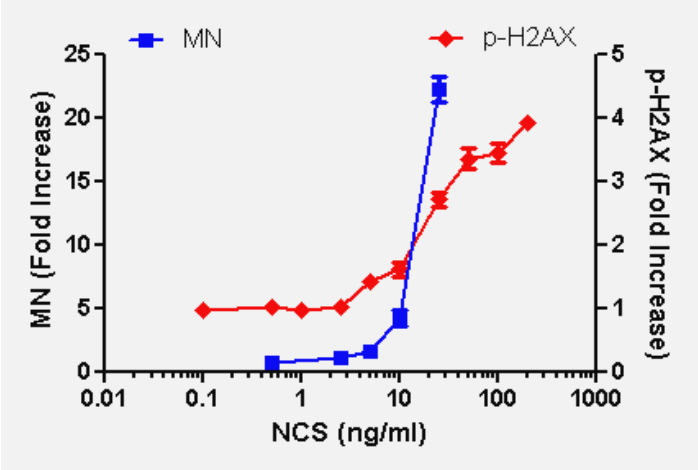
Validation of optimal time points for p53 and pH2AX

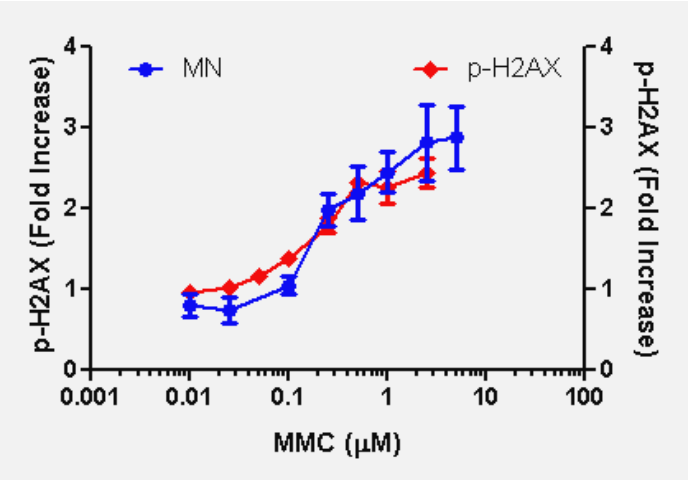


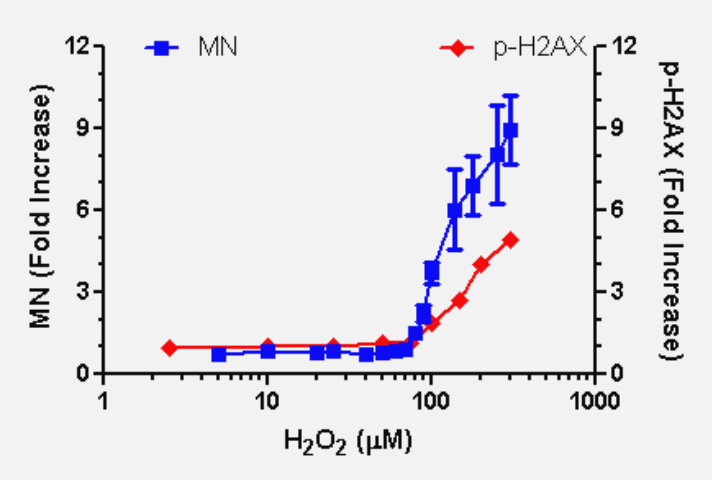
NCS – 200 ng/mL
MMC – 500 nM
H_2O_2 – 200 μM
ENU – 2 mM
GAD – 2 mM

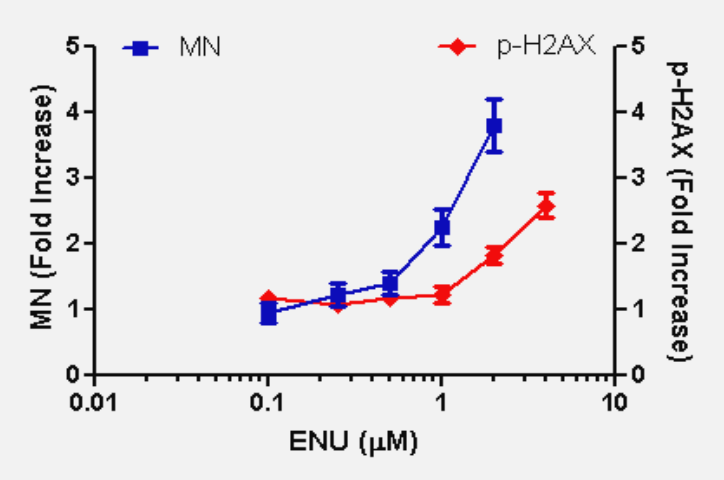
Previous studies:
24 hr time point was optimal for ETP, MMS, QUE, CUR

DNA damage and MN response

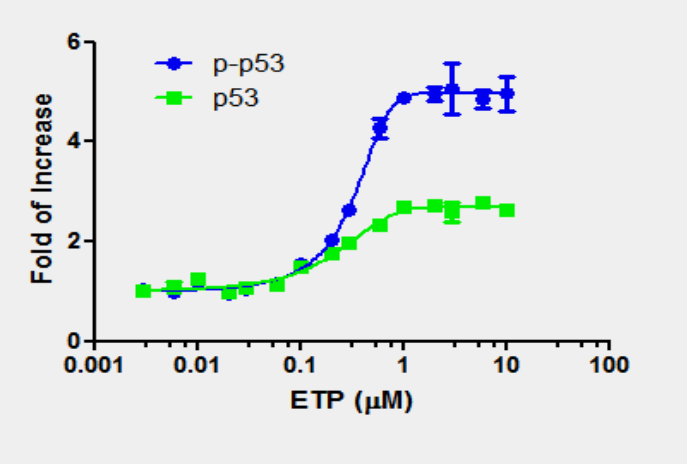


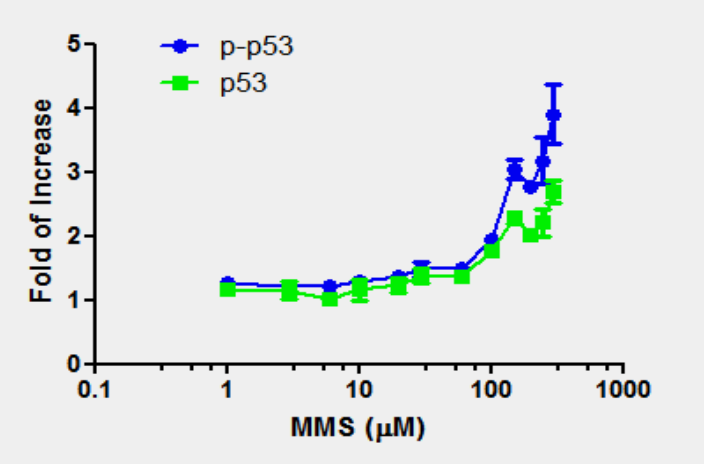


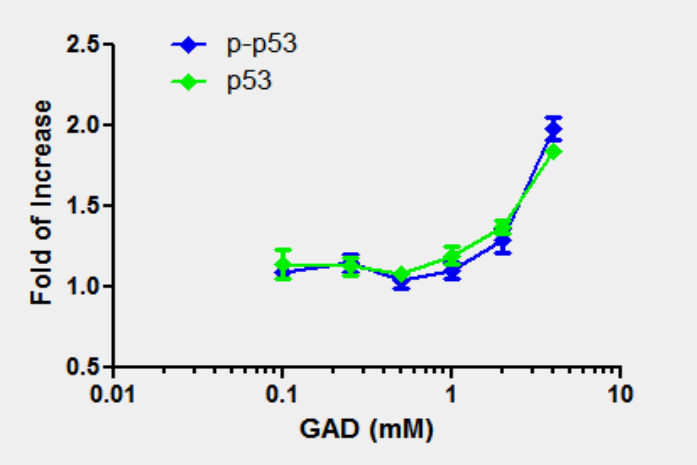


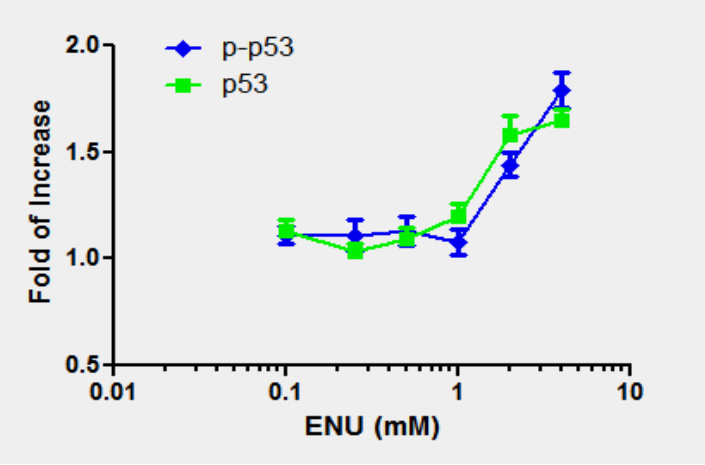


p53 response to DNA damage

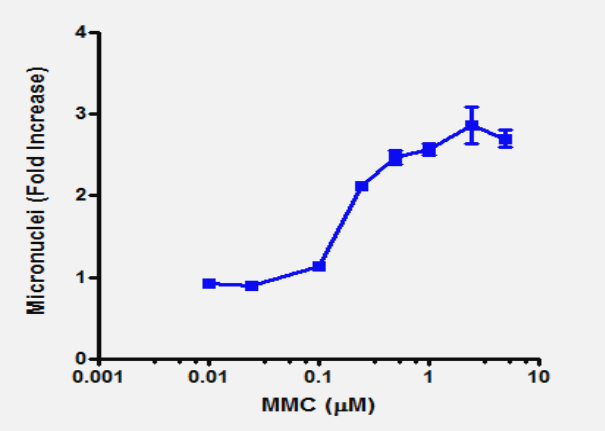


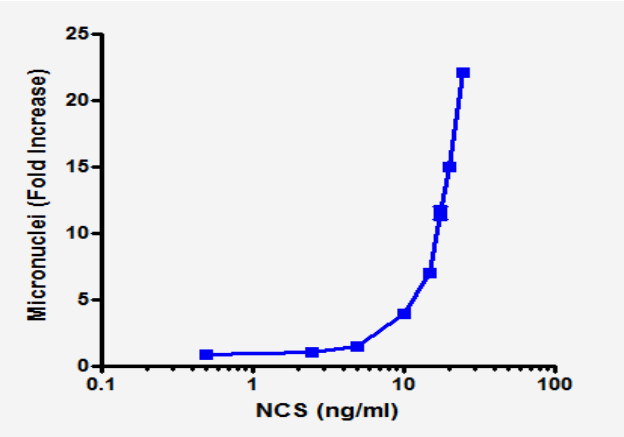


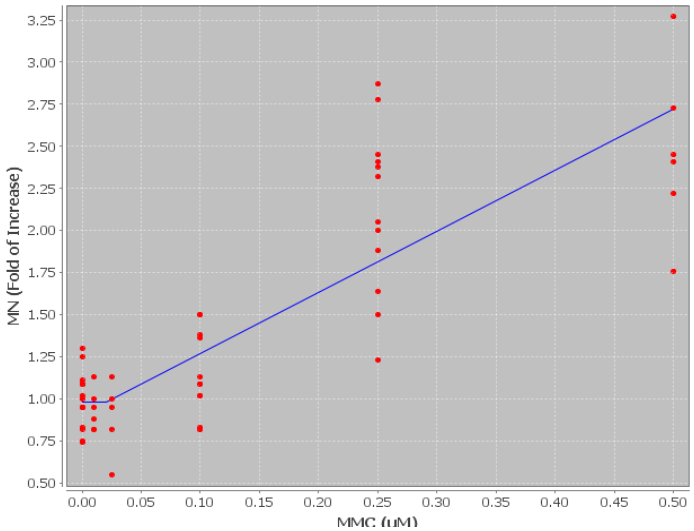


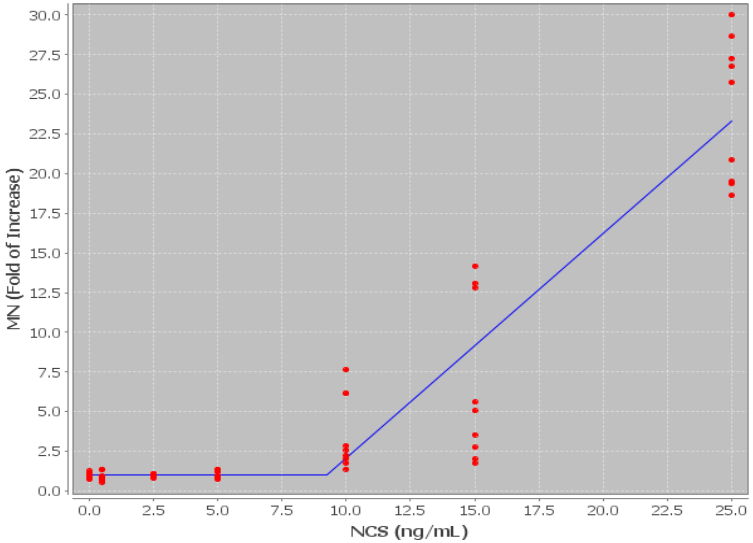


Lutz threshold model to test linearity





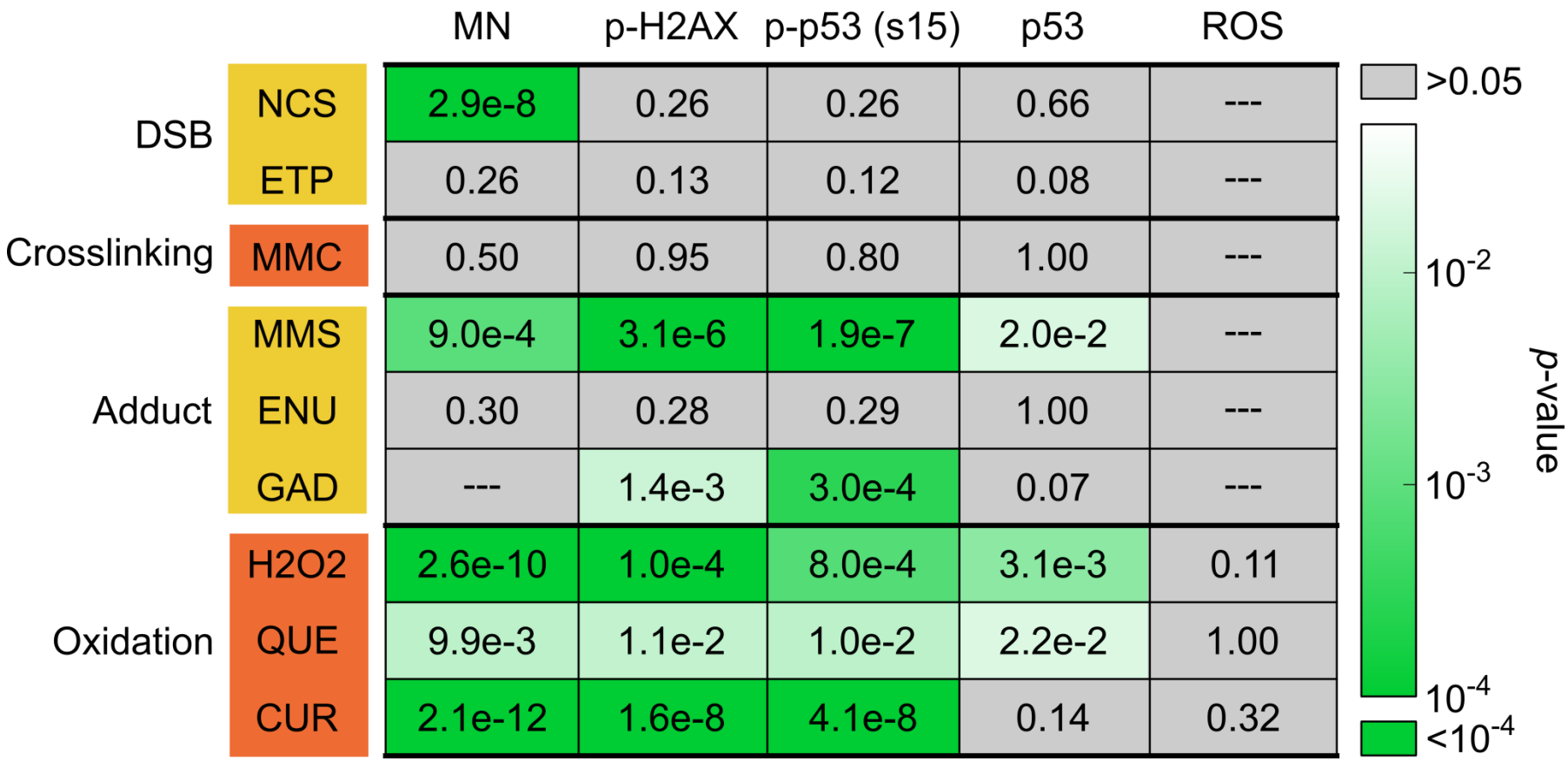




p = 0.503

p = 2.89e-11

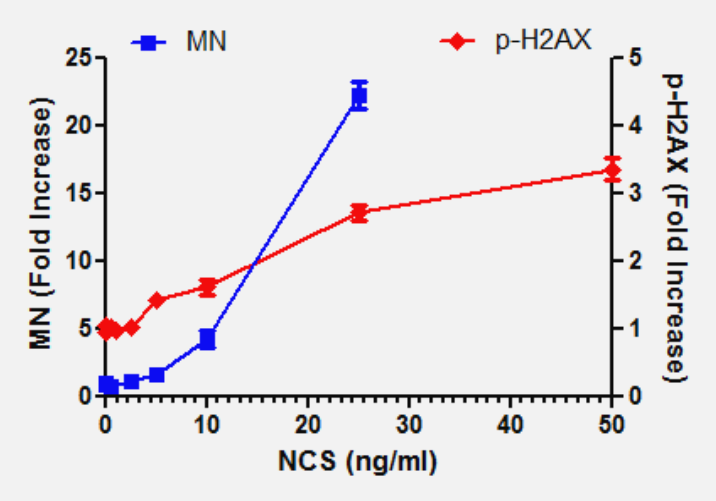
Chemical comparison of dose-response shape



Examining mechanistic underpinning for thresholds

"If the hockey stick model fits the data significantly better than linearity, the threshold-like appearance of the dose–response curve will have to be corroborated by mechanistic considerations and experimental testing of the respective hypothesis."

- Lutz & Lutz, Mut. Res., 2012



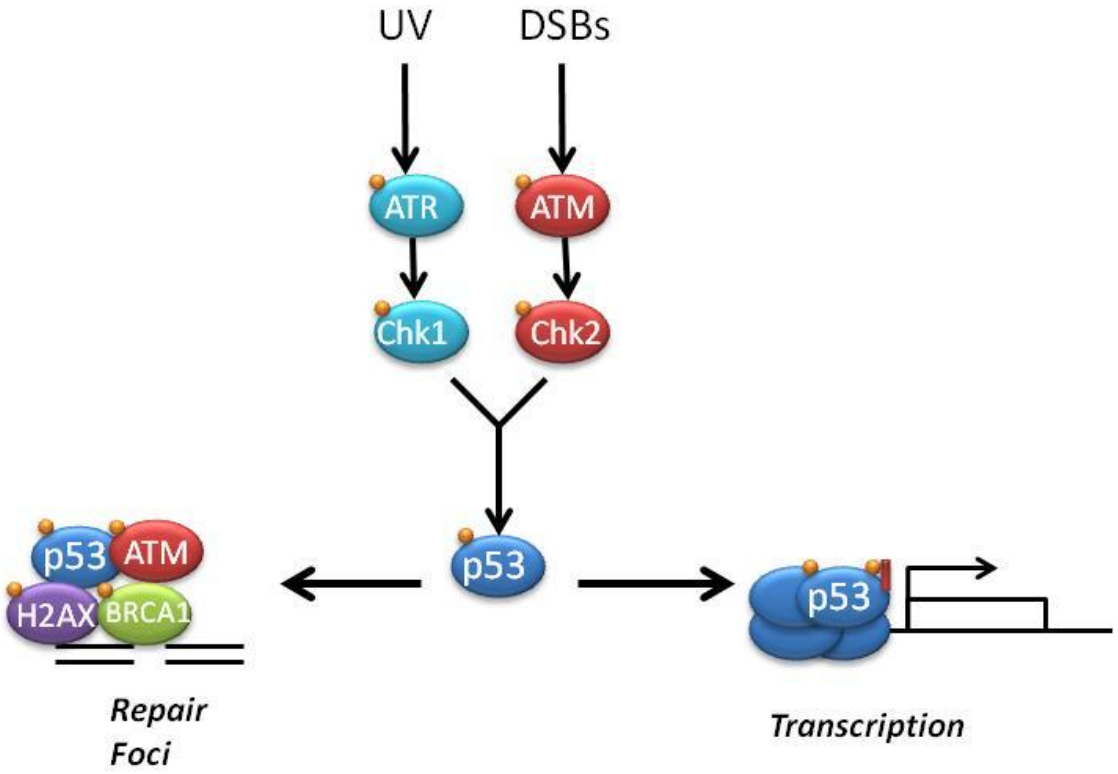
- **Repair may prevent MN at low doses**

• Work with γ-irradiation, indicates repair is more efficient at lower levels of damage
(Neumaier et al., PNAS, 2011)

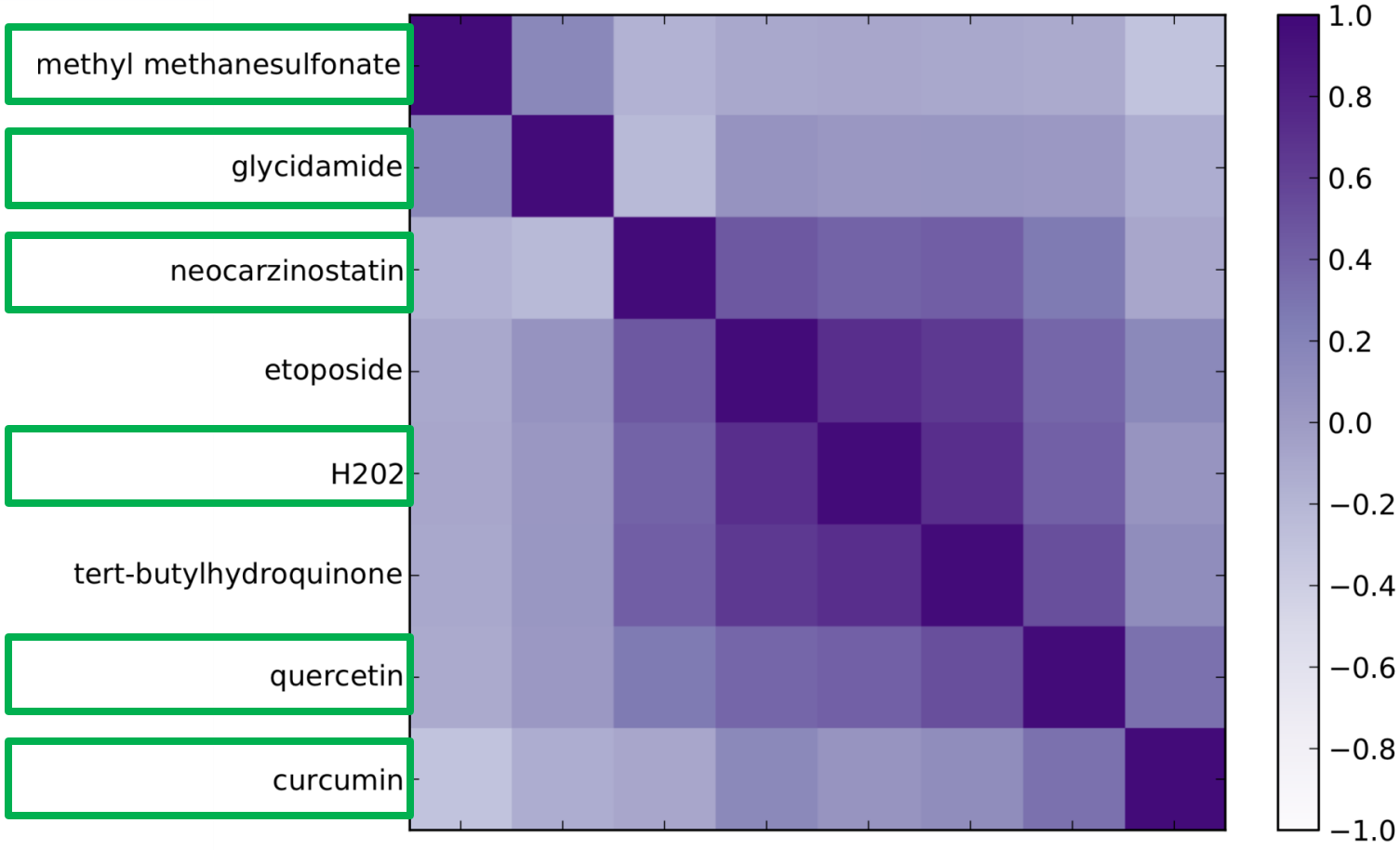
Examining mechanistic underpinning for thresholds

Possible explanation for threshold behavior:

- DNA damage repair process
  - Transcriptional up-regulation of repair pathways
  - Post-translational repair processes

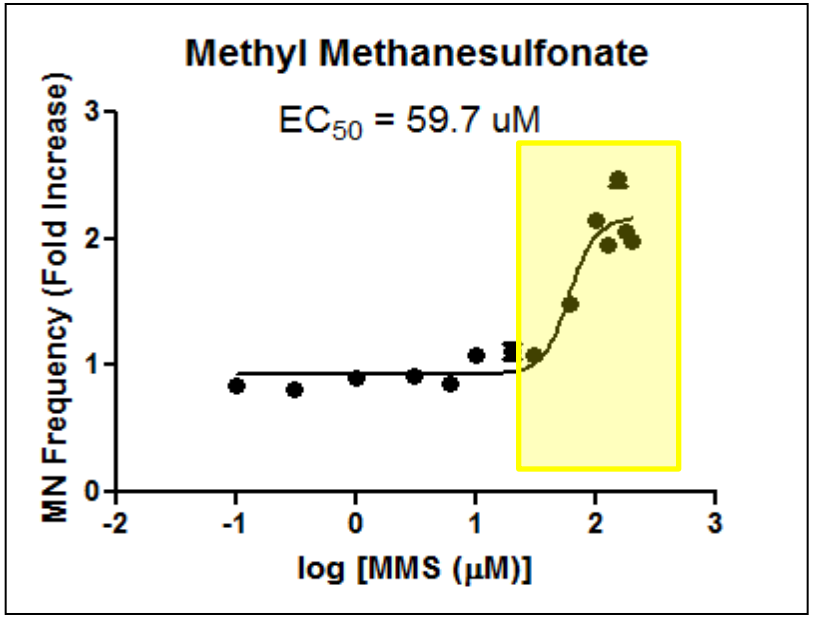


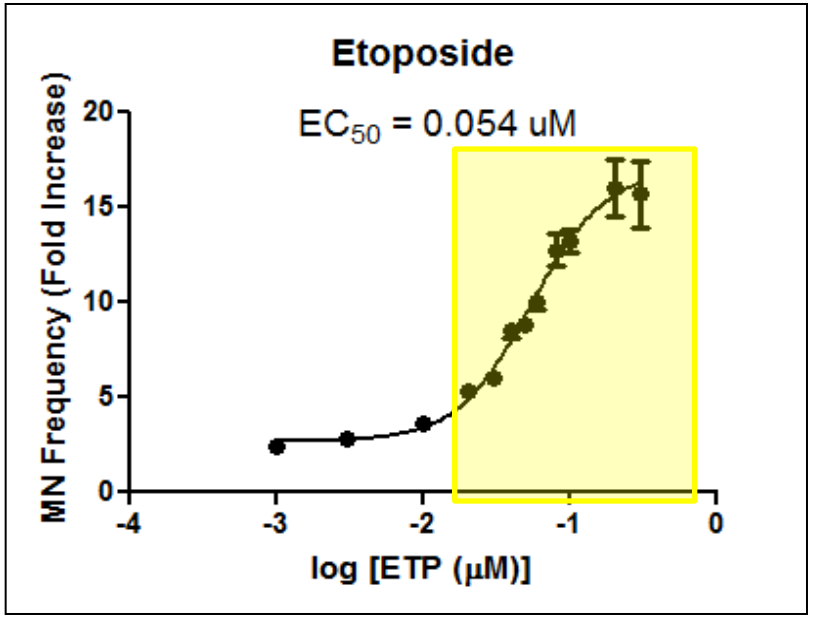
Whole genome transcriptomics – Chemical comparison



- Gene responses group by chemical class
  - More closely grouped by gene signature than linearity of dose-response
  - Signature may be dominated by genes not related to DNA repair

Gene array – dose response with selected chemicals

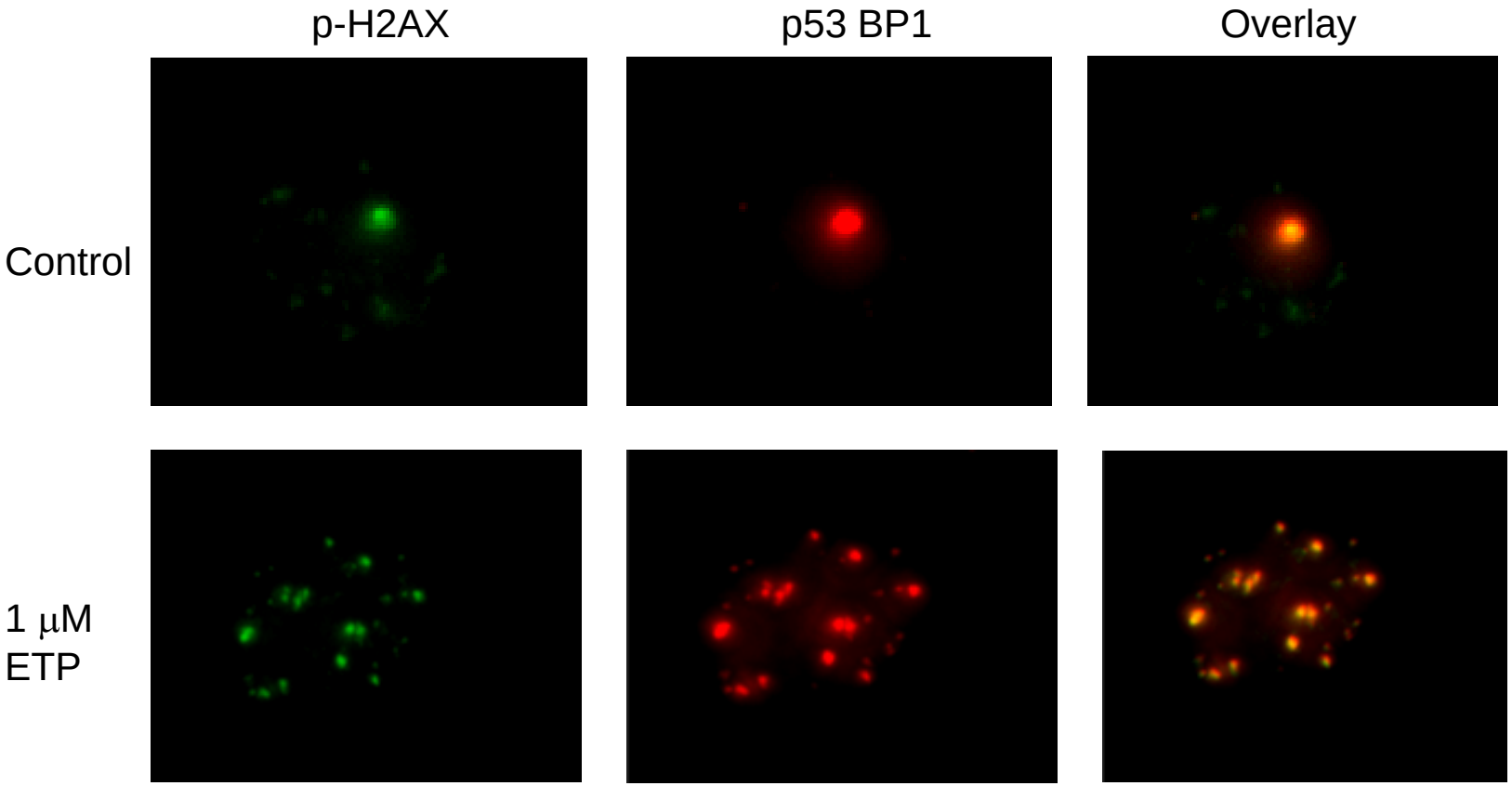




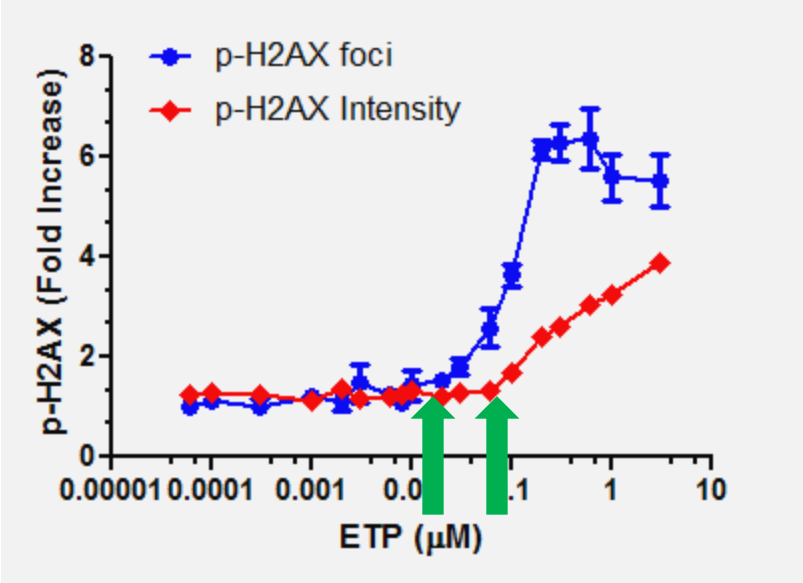
- 1st gene changes occure at same concentrations as MN
  - 1st gene changes are not associated with repair

- Post-translational repair may be more important for low dose response

DNA repair centers – a better assay to quantitate DSBs



Quantitation of repair centers



- Foci counting is a more sensitive measure of cellular response than total protein increase
- Increased ability to measure effects at low levels of damage

Future Directions

- DNA repair centers
  - Better quantitation of DNA damage at lower doses
- Real-time measures of DNA repair
- Gene array and protein analyses to identify specific repair pathway inititation

The DNA damage pathway team:

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