A DNA Damage Pathway
Case Study at an intermediate Stage

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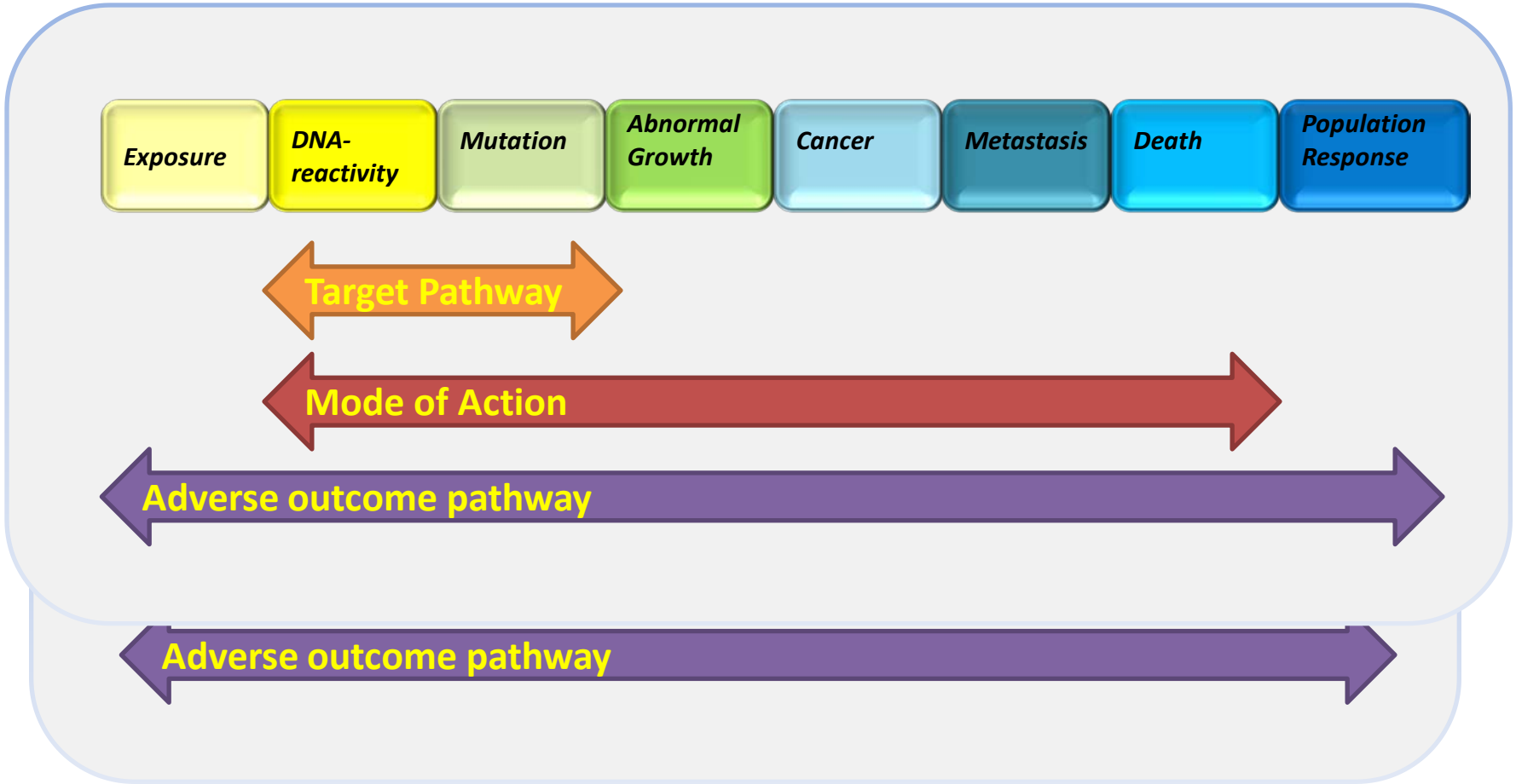
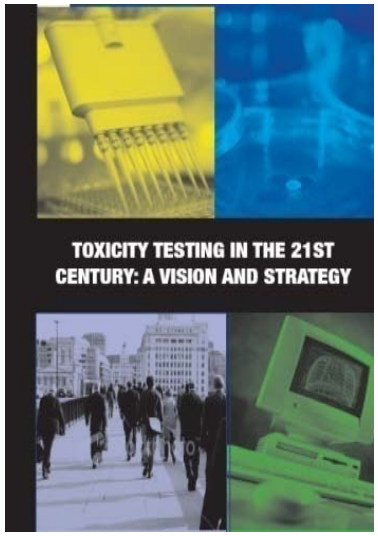
Dealing with mutagens in the AOP framework





Biological Signaling or Toxicity Pathways *in vitro*

in vitro exposure

Concentration

Biologic Interaction

Perturbation Level

Higher yet

Biologic Inputs

Normal Biologic Function

Early Cellular Changes

Adaptive Stress Responses

DNA changes

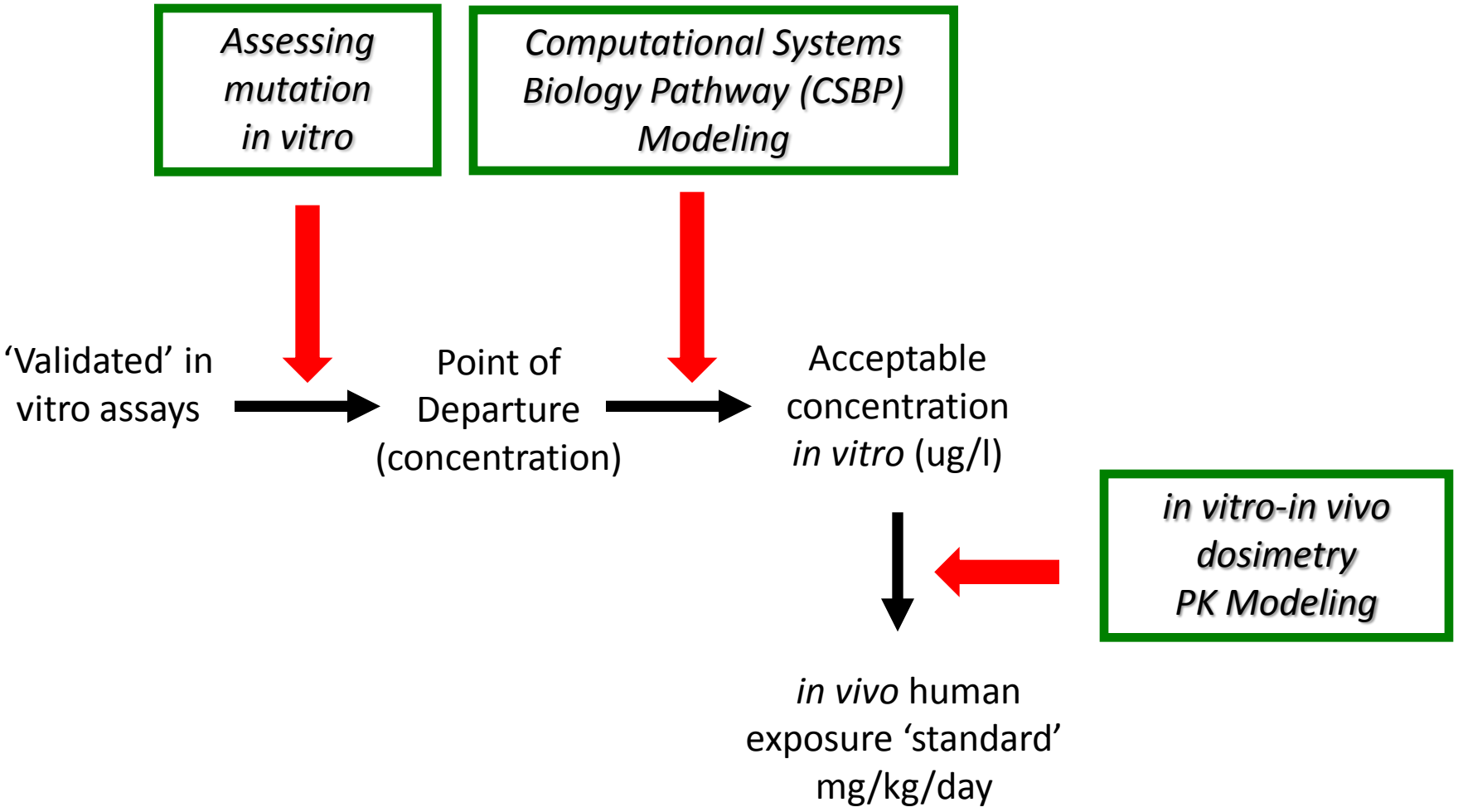
Mutation

Project Goals

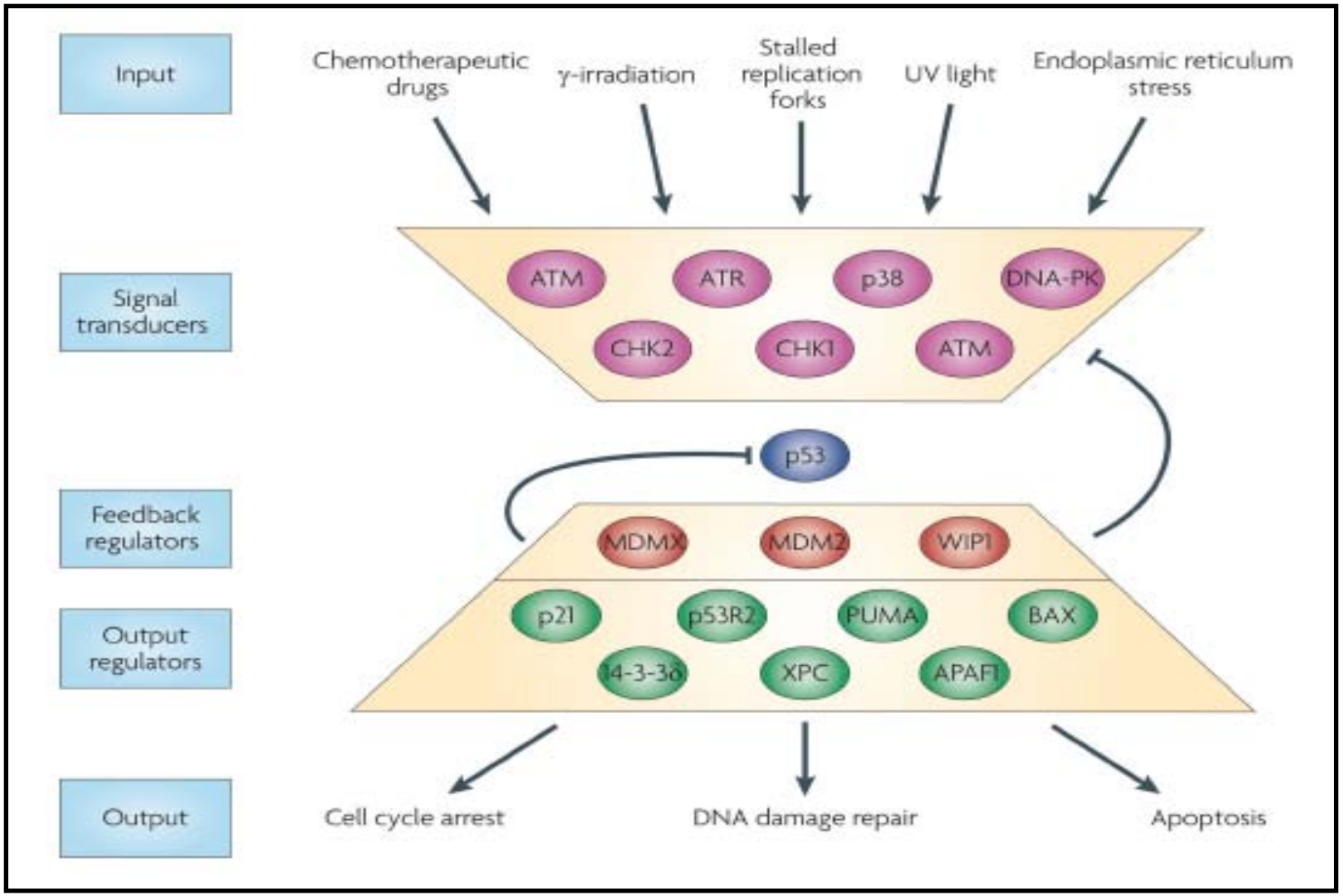
- Collect necessary data to complete a computational systems biology pathway model for the p53-DNA-damage stress pathway with different prototypical test compounds

- Develop a safety assessment case study based on the CSBP model and IVIVE approaches for specific compounds of interest

Hypothesis – By understanding the dynamics of the DNA-damage repair pathway, we can use results from cellular assays to estimate safe exposure levels



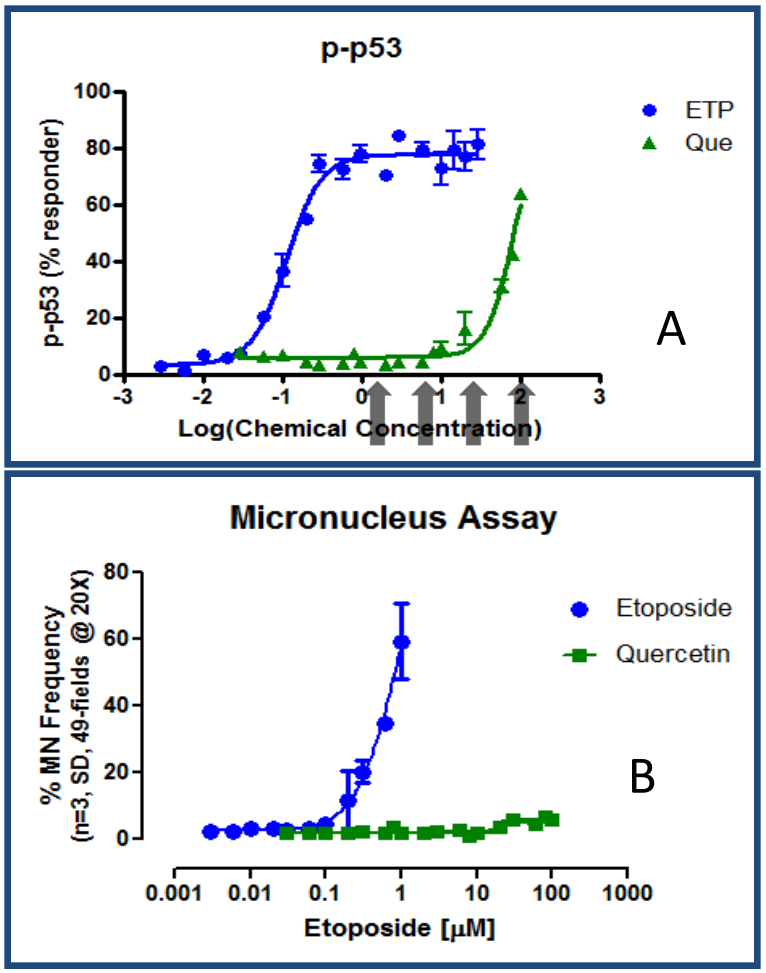
DNA-damage stress pathway components…



Chemical Selection

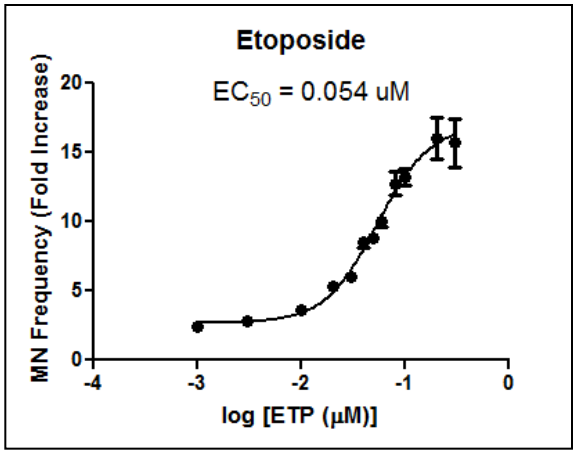
- Methyl methanesulfonate (MMS)
  - Methylating agent
- Etoposide (ETP)
  - Topoisomerase II inhibitor
- Quercetin (QUE)
  - Metal-dependent oxidative damage
  - Kinase inhibition and others

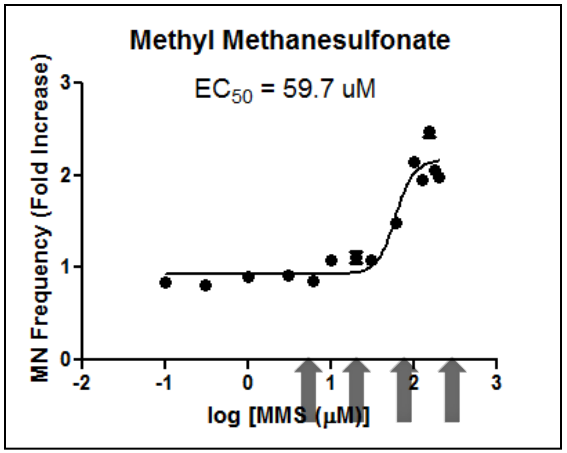
Unilever/Hamner Project – Data Collection

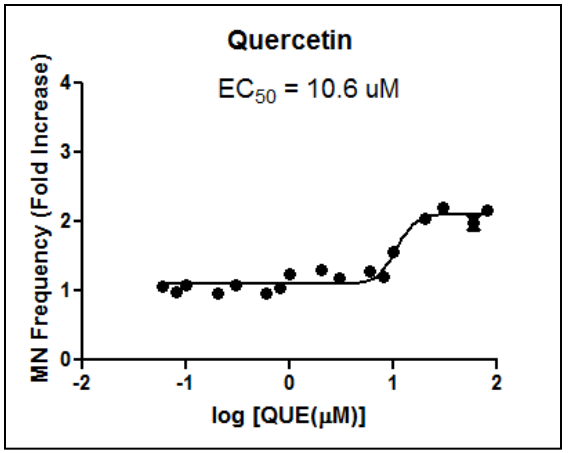


- Dose-dependent transcriptomics
- Dose dependencies in p53, H2AX, mdm2, wip1, kinase activation, etc.
- Pathway inference from gene expression, phosphoproteins and TF analysis for p53, bioinformatics
- Create dose-dependent model for DNA-repair circuitry
- Confirm pathway structure with siRNA , kinase inhibitors, or cell lines with stable knock-outs of key genes

Micronucleus Assay*



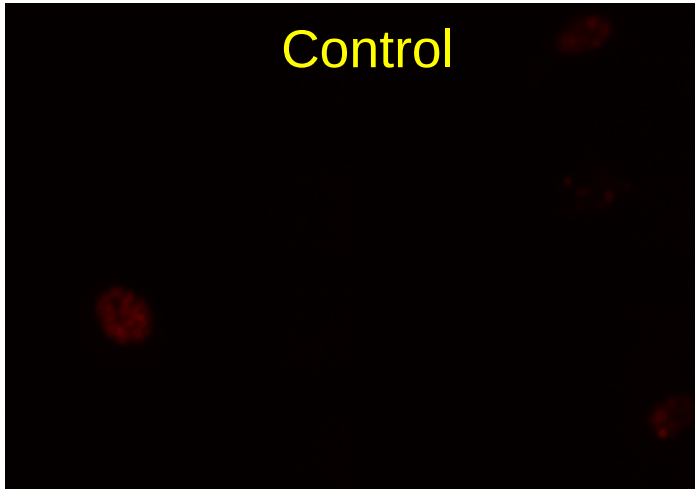


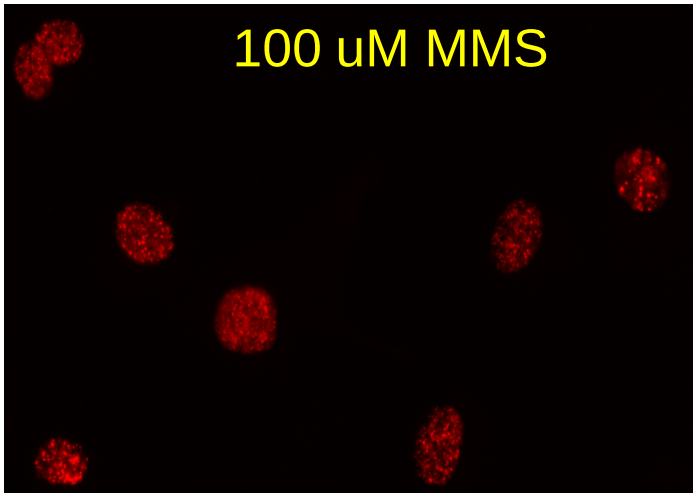


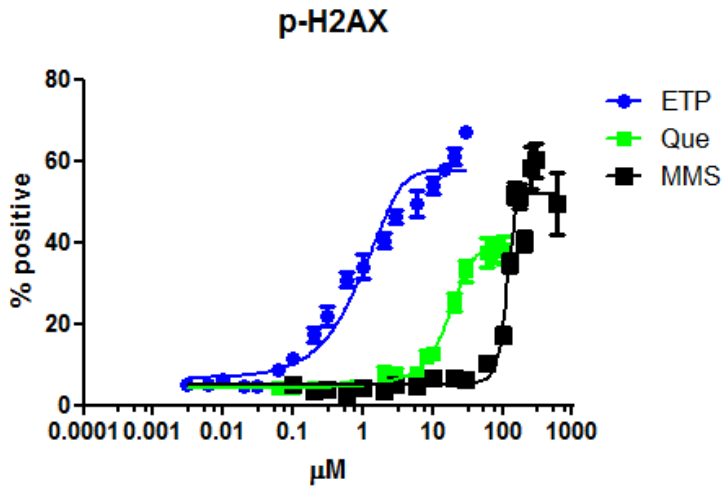
	Max Fold Change	Lowest Conc. w/ 3-fold change (µM)
ETP	20	0.04
MMS	3	150
QUE	3	100

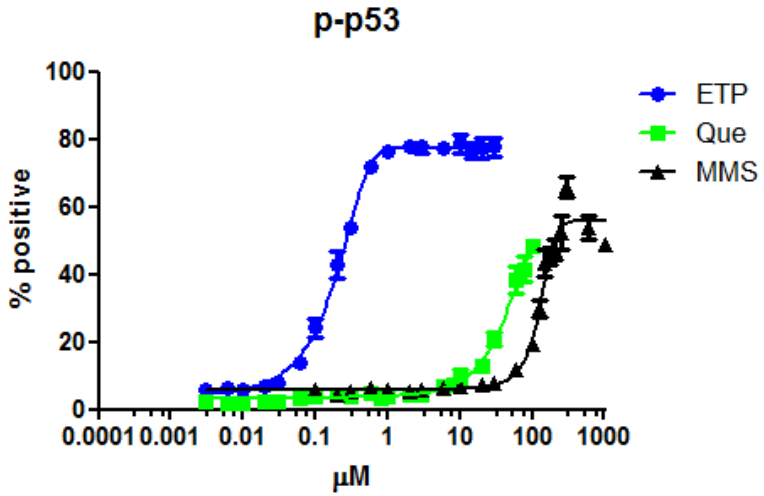
*Performed using Litron MicroFlow MN assay.

DNA Damage – pH2AX

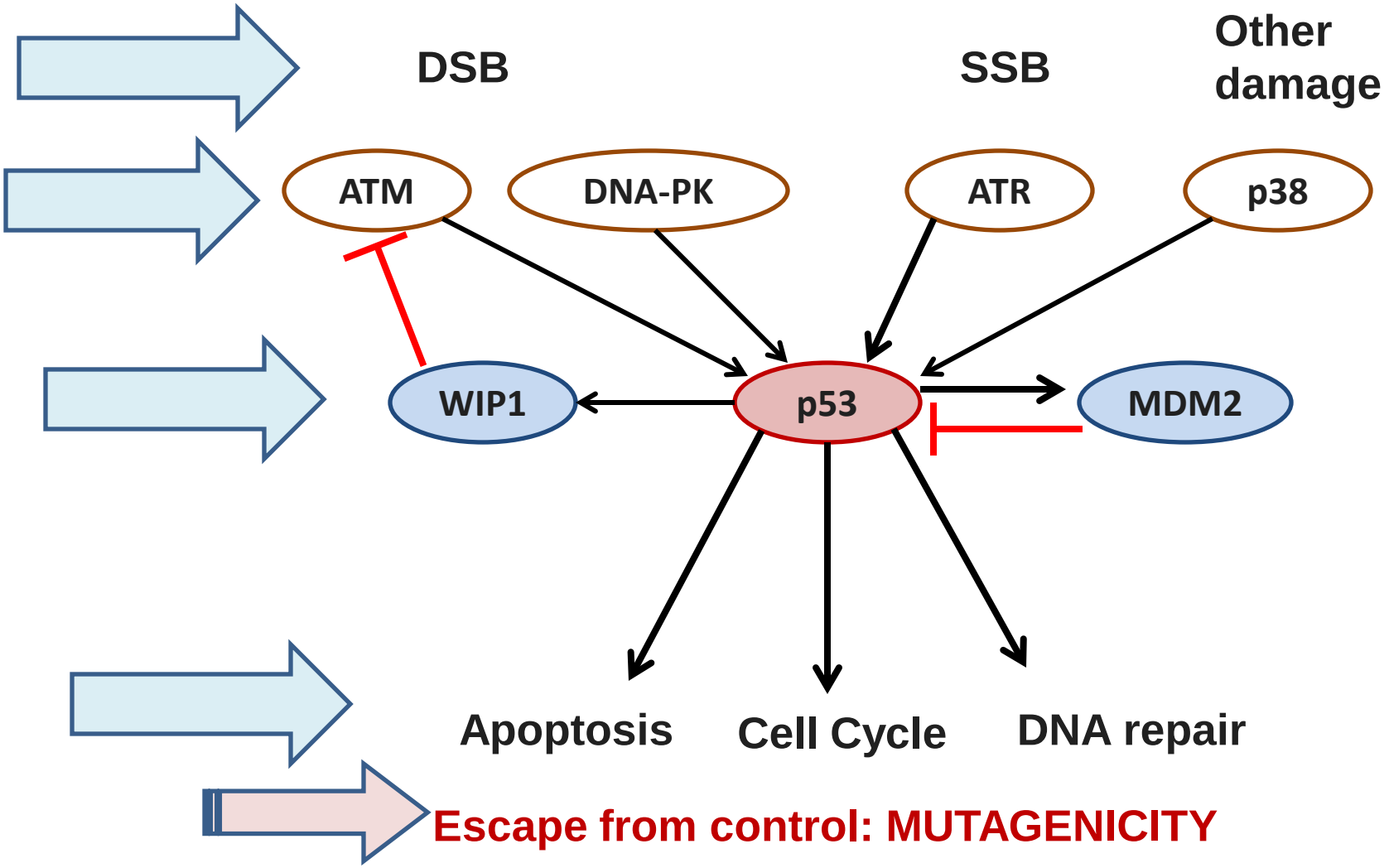




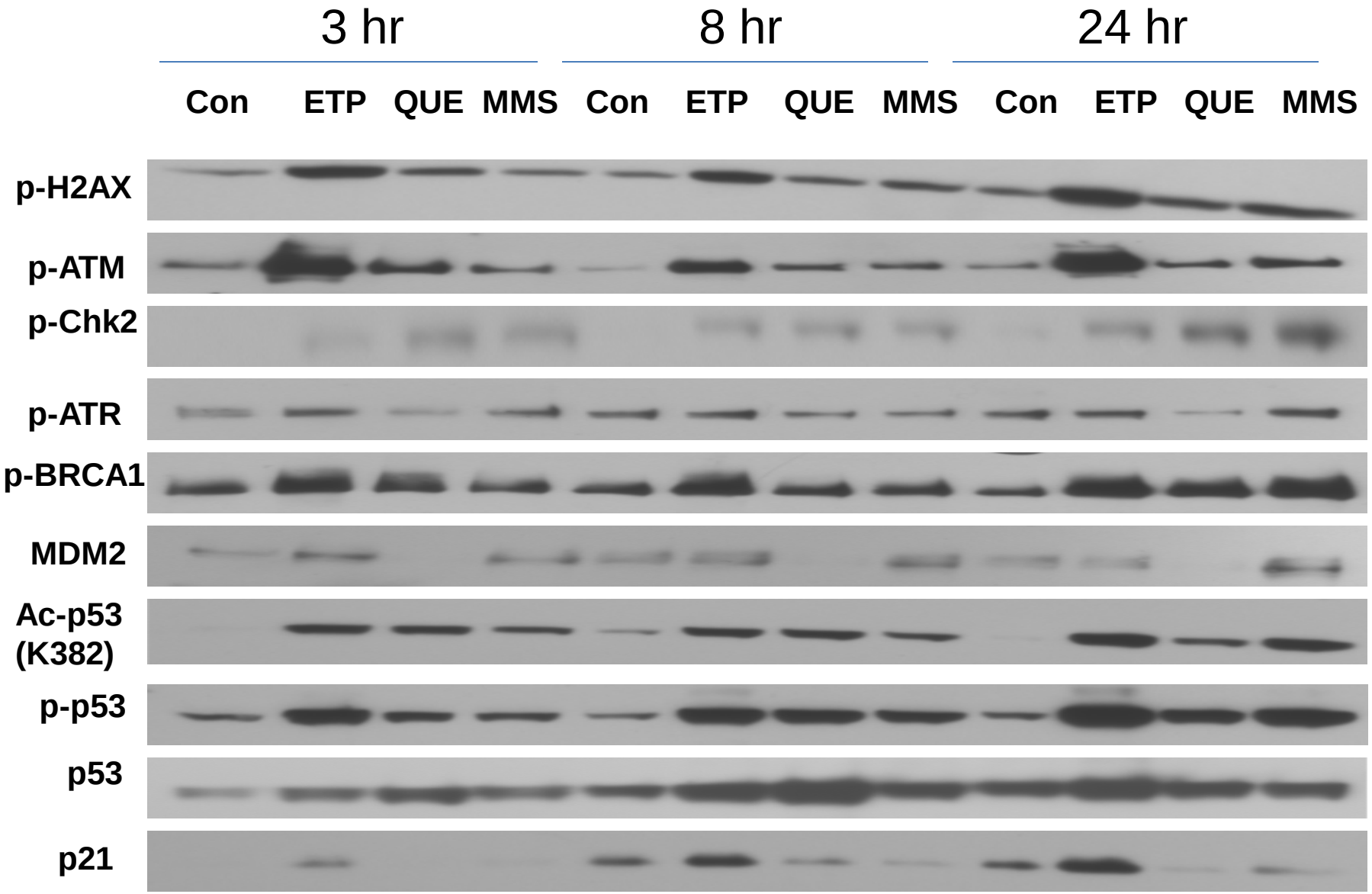




Measured responses



HT1080



ETP: 1uM; Que: 30 uM; MMS 200 uM

Altered Genes in p53 Pathway

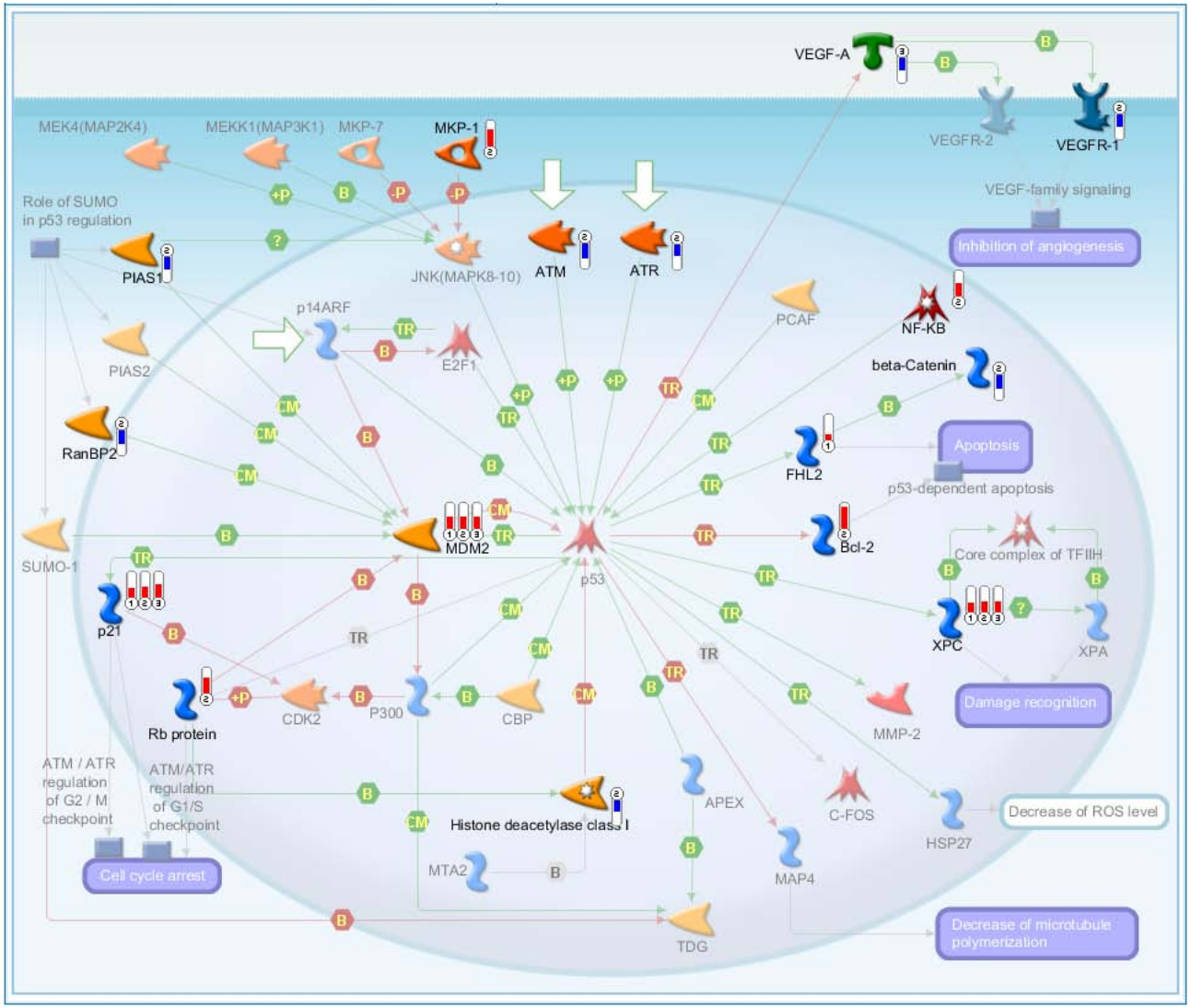
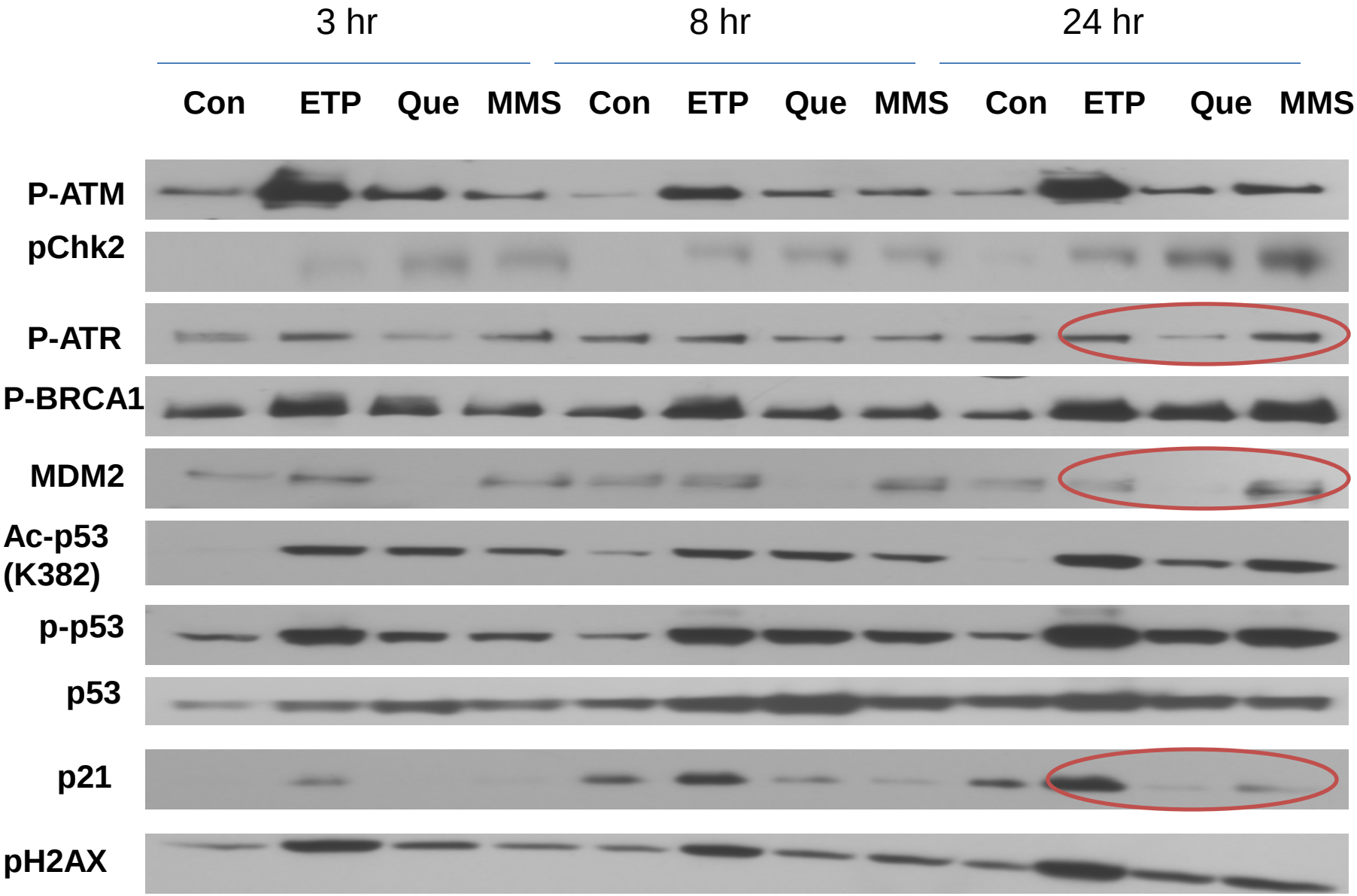
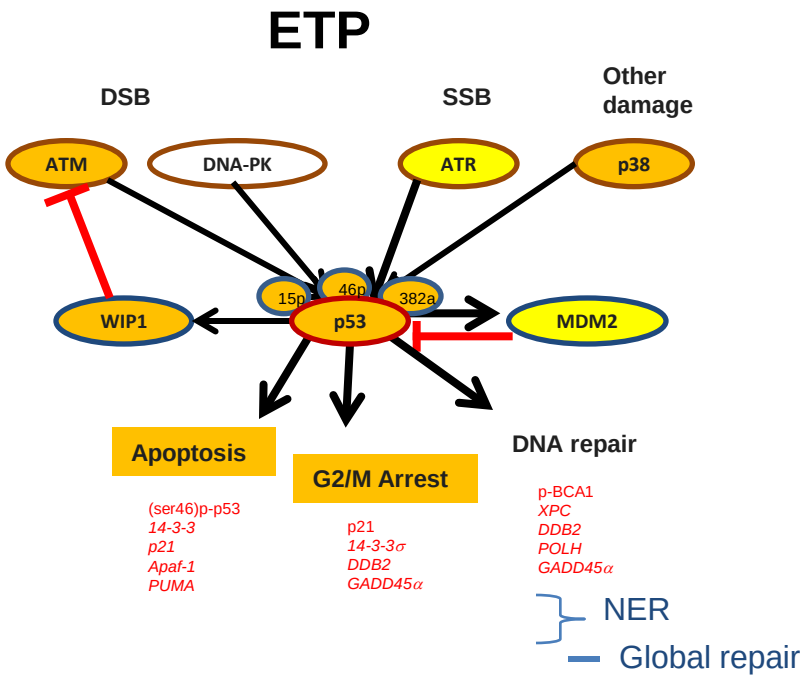


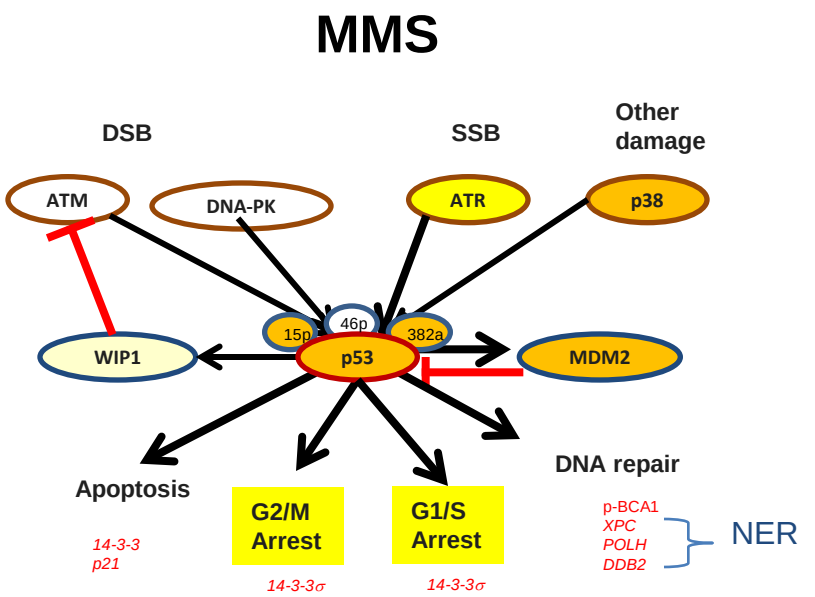
Image created in GeneGo (Metacore v 6.9)

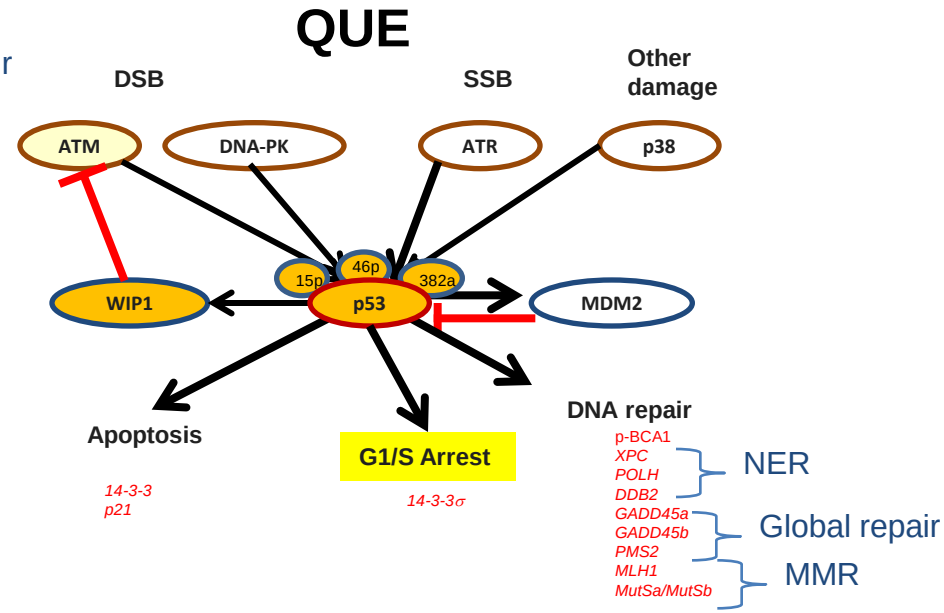
HT1080



ETP: 1uM; Que: 30 uM; MMS 200 uM





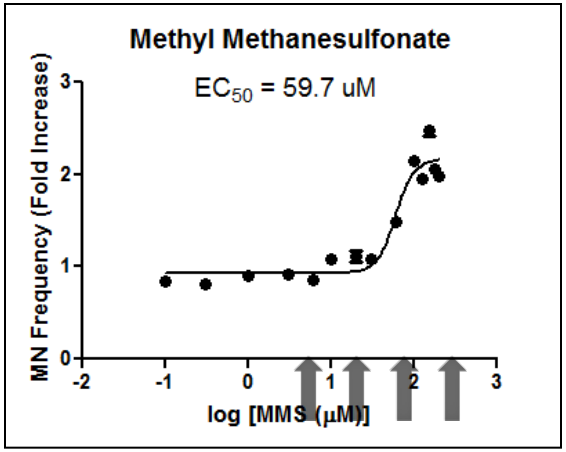


Activation of DNA-damage stress pathway differs for the test compounds

Comparison of Dose-Response across Endpoints
No differential dose responses with these endpoints

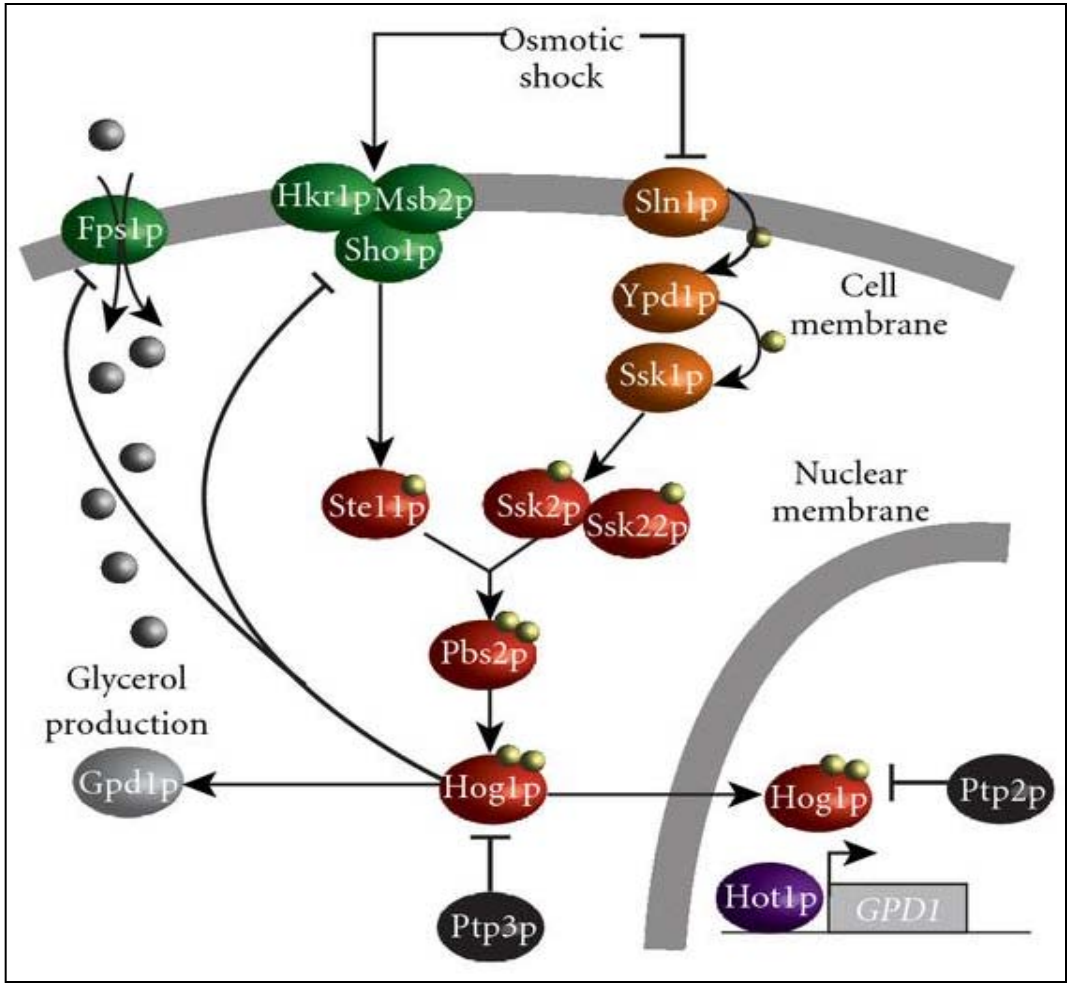
Endpoint	LOEL		
	ETP	QUE	MMS
MN	0.06	20	100
P-p53(s15)	0.1	20	100
p53	0.2	30	125
P-H2AX	0.2	20	100
Cell cycle	0.06	8/60	100
Apoptosis	2	60	450
P-p53(s46)	0.6	20	300
Necrosis	0.2	100	600

Need to reorient some of the data collection and thinking about stress pathway function



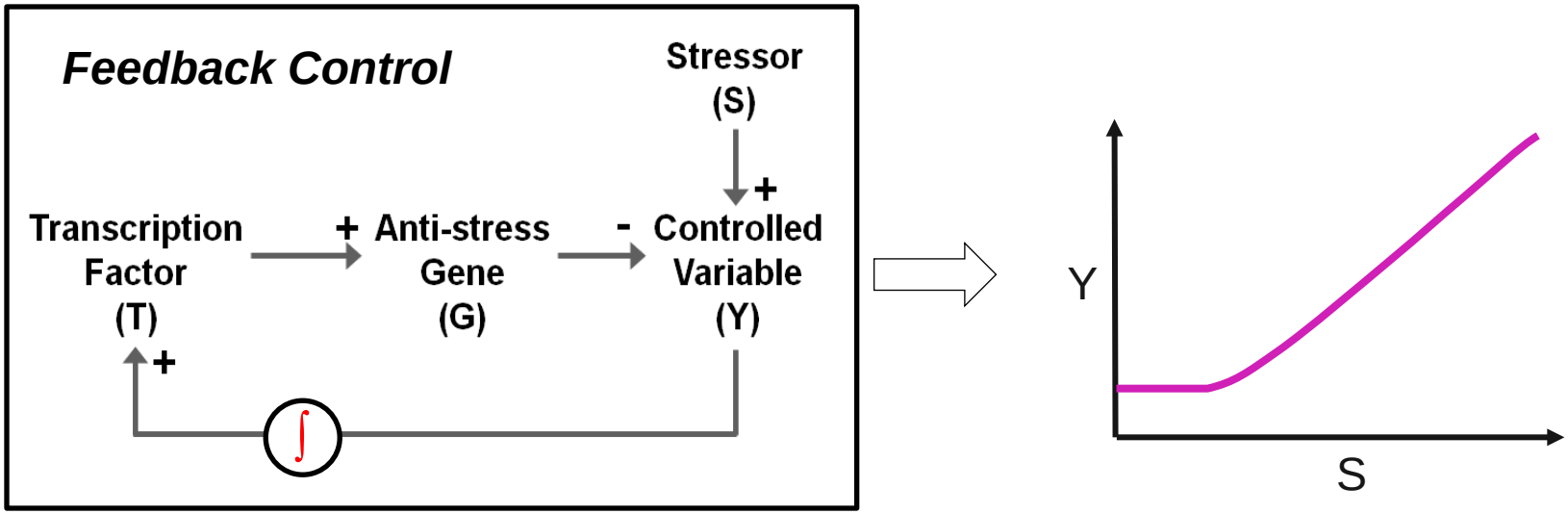
- Pathway homeostasis likely to include post-translational control of p53 to insure rapid adjustments in face of low level stressor
- Data acquisition and computational models need to move in these directions

Common structures associate with common control processes in controlling cellular stress – a model from yeast

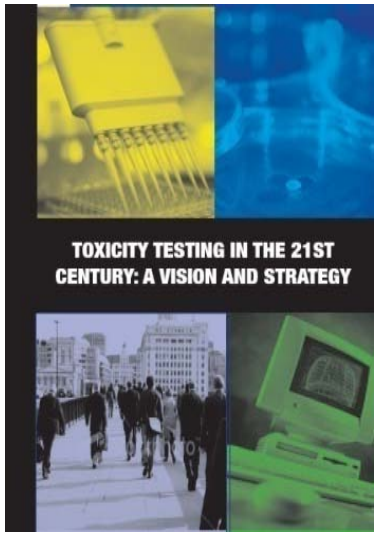


Adapted from Miermont et al Signal Trans 2011, Muzzey et all Cell 2009, and Mettetal et all Science 2008

Assessing mechanistic basis for homeostasis, threshold behaviors and overall dose response



Homeostasis requires perfect adaptation of rapidly acting pathways (post-translational modification) and perfect adaptation of slower acting pathways (transcriptional). Integral feedback underlies perfect adaptation in multiple signaling pathways. Look at the post-translational components maintaining homeostasis.



Biological Signaling or Toxicity Pathways *in vitro*

A dollop of chemical

⬇

Concentration

⬇

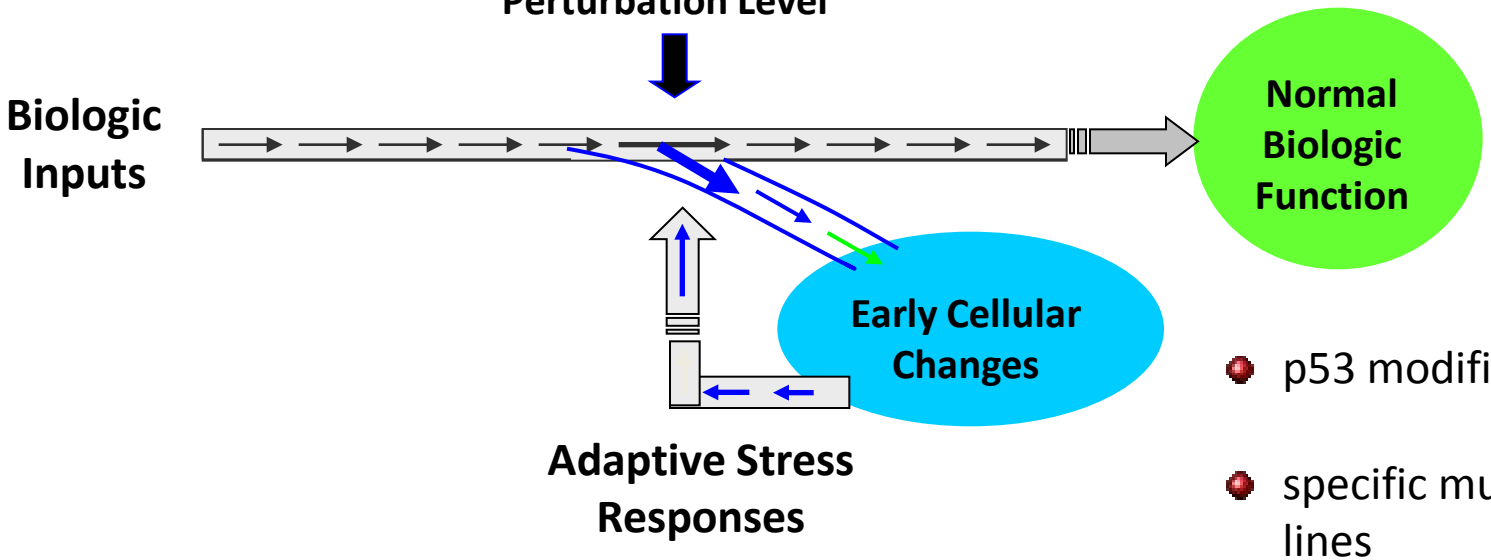
Biologic Interaction

⬇

Perturbation Level

⬇

Low Dose



- p53 modifications
- specific mutant cell lines

Status

- Stepped along to examine higher dose region and see differential pathway activation with the test compounds
- Need to reorient to adaptive control by post-translational mechanisms with these stress pathways
- CSBP models for dose dependent transitions also need to focus more on these processes

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