# What's in a Tipping Point?

## Using Systems Biology to Characterize Adverse Oxidative Responses in Human Lung Cells

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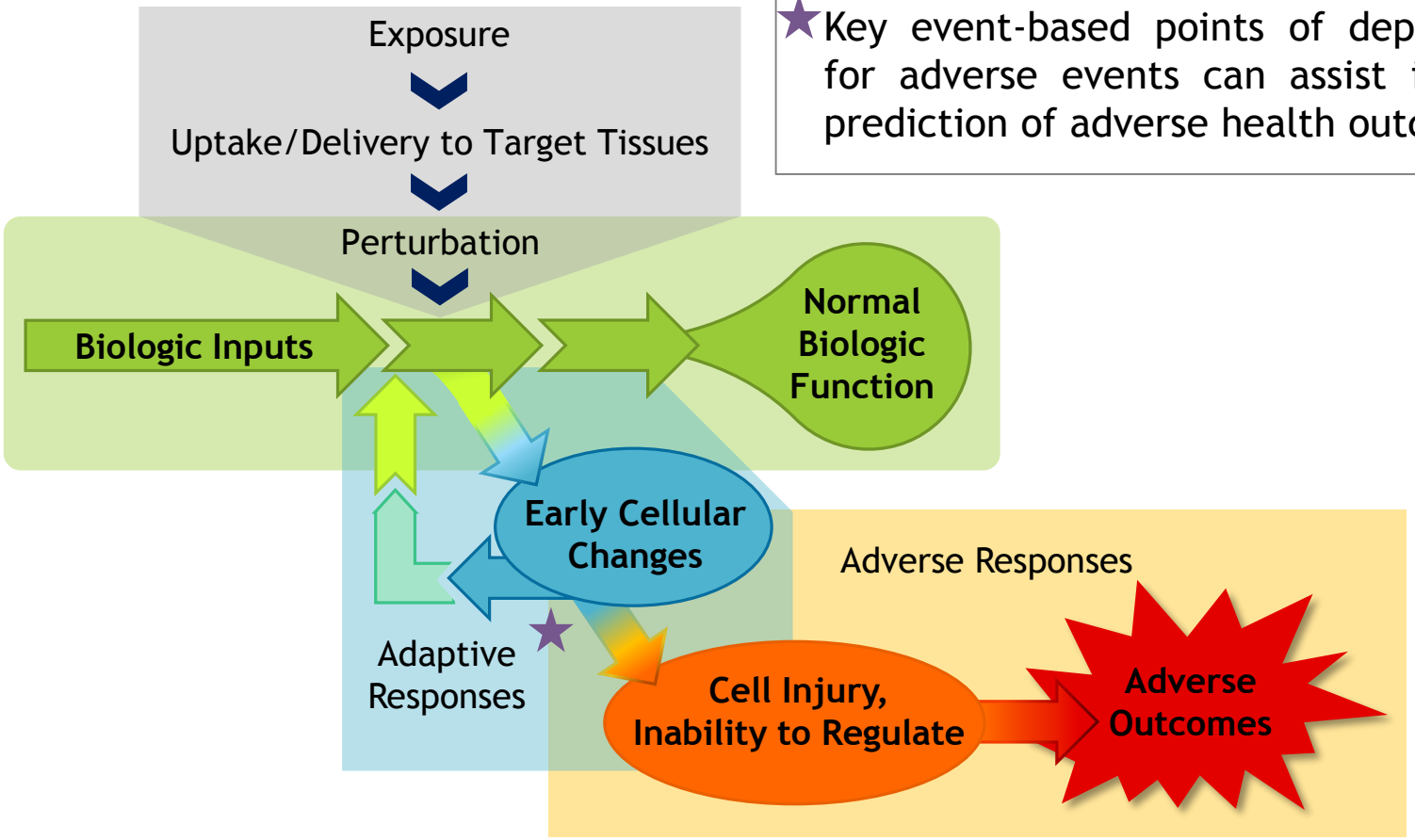
# Why study tipping points?

★Key event-based points of departure for adverse events can assist in the prediction of adverse health outcomes

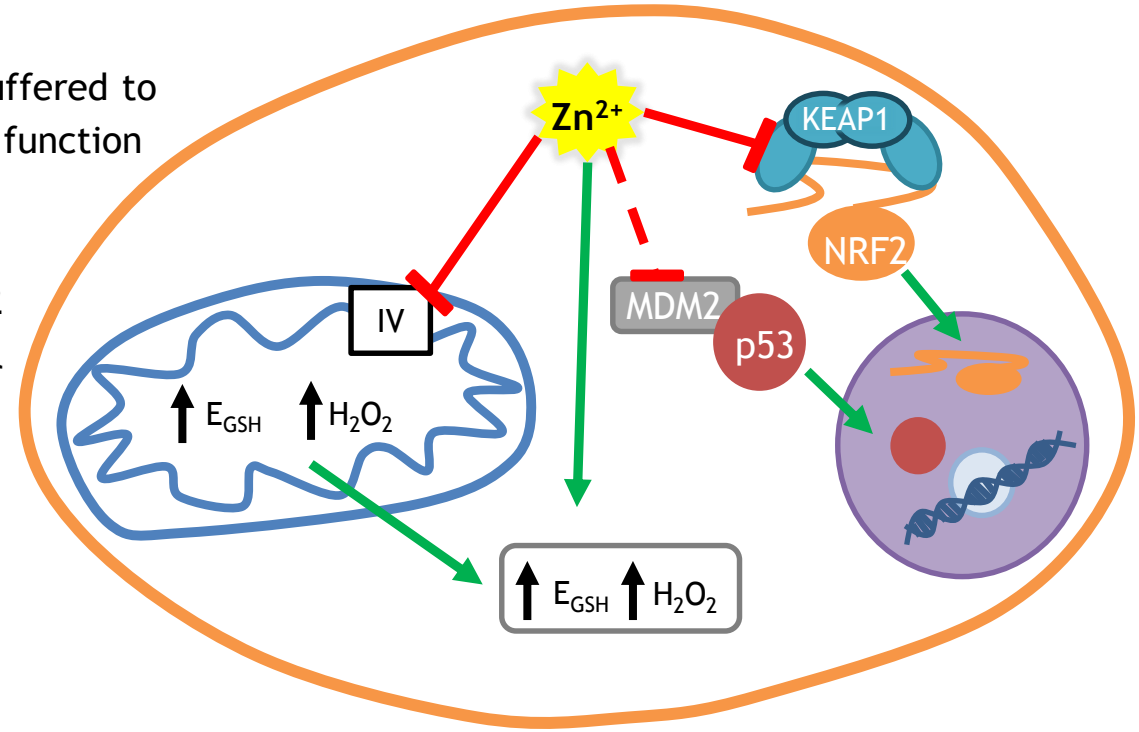



Modified from: Andersen, M. E. and D. Krewski (2009) Toxicol Sci **107(2): 324-330.**

# What mediates the switch between adaptive and adverse responses?

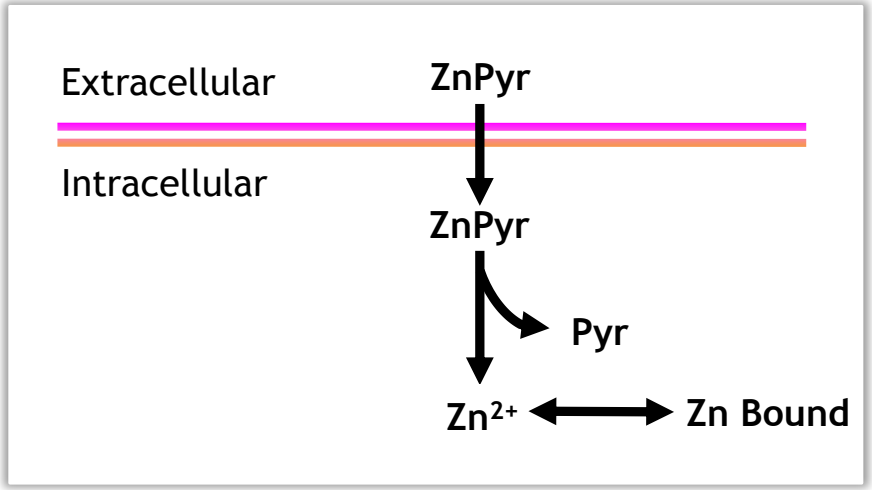
- Zn is sequestered and buffered to maintain normal cellular function

- Crosstalk between p53 and NRF2 coordinate the p53-initiated cell survival response

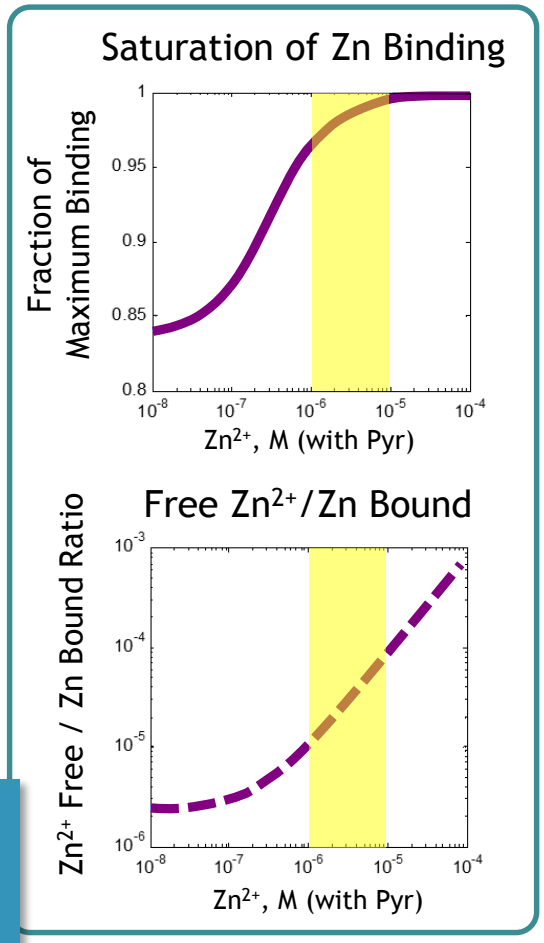



Mechanisms of the shift from p53-mediated cellular adaption to programmed cell death are less understood

# Using biological modeling to define the tipping point

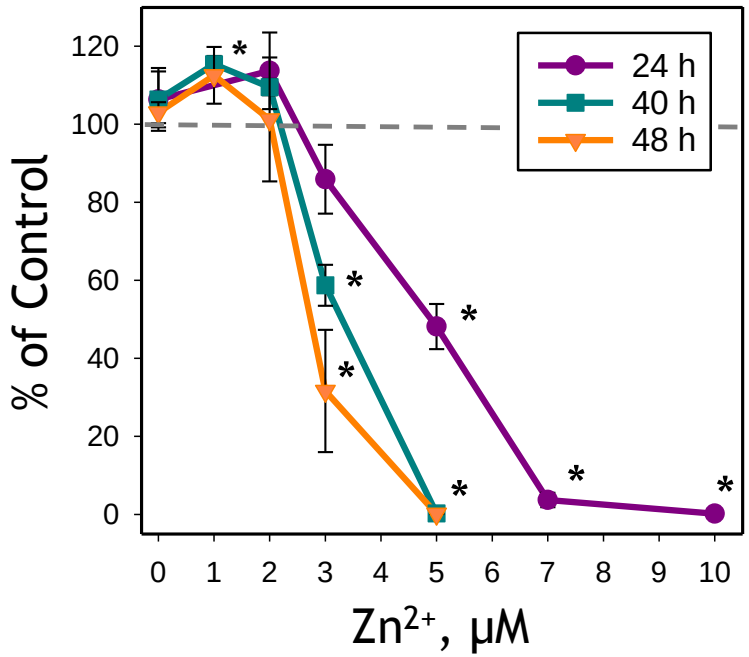



Pyrithione (Pyr) – a zinc specific ionophore that facilitates transport




**Pharmacodynamic model predicts saturation of intracellular $Zn^{2+}$ binding**

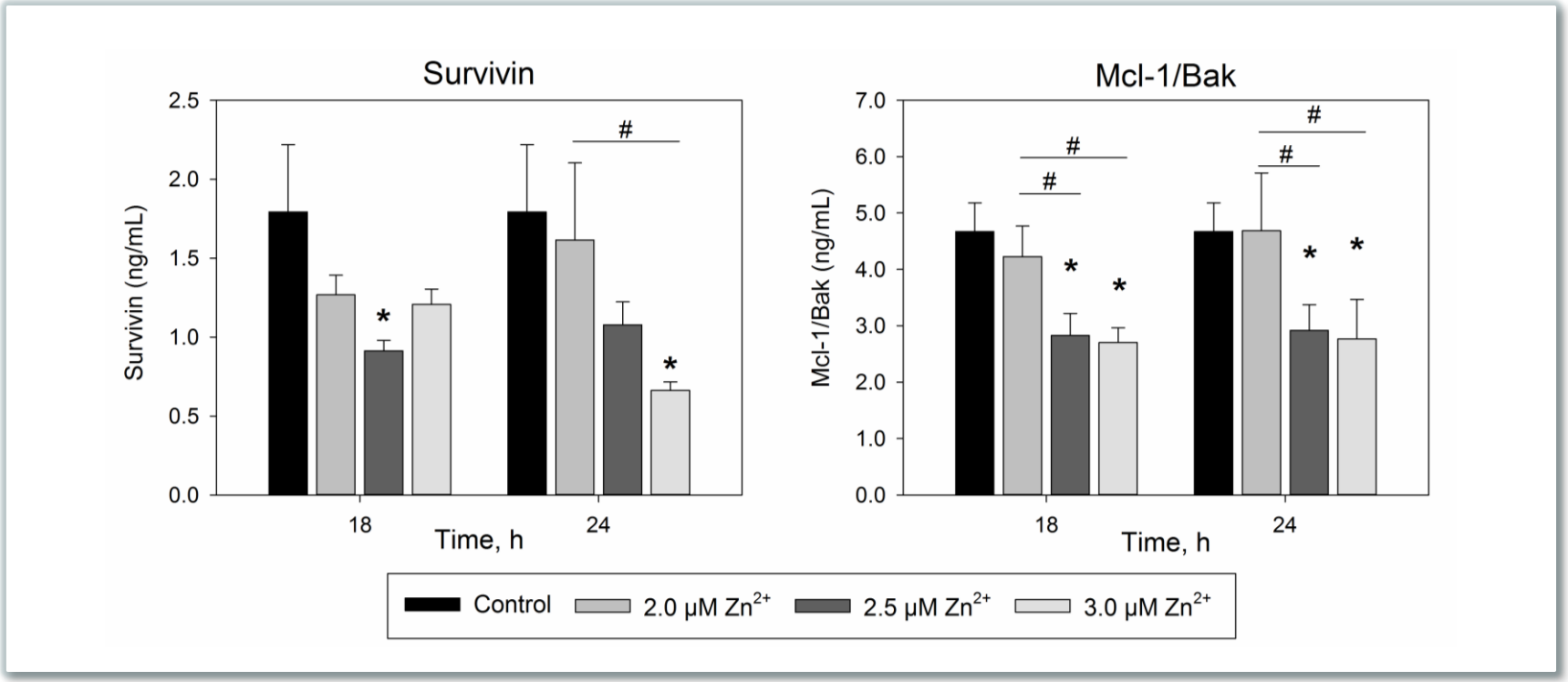
# Does *in vitro* cytotoxicity correspond to computational predictions?




- BEAS-2B cells exposed to $Zn^{2+}$ in the presence of 1 μM pyrithione
- ATP content measured by luminescent CellTiter-Glo assay

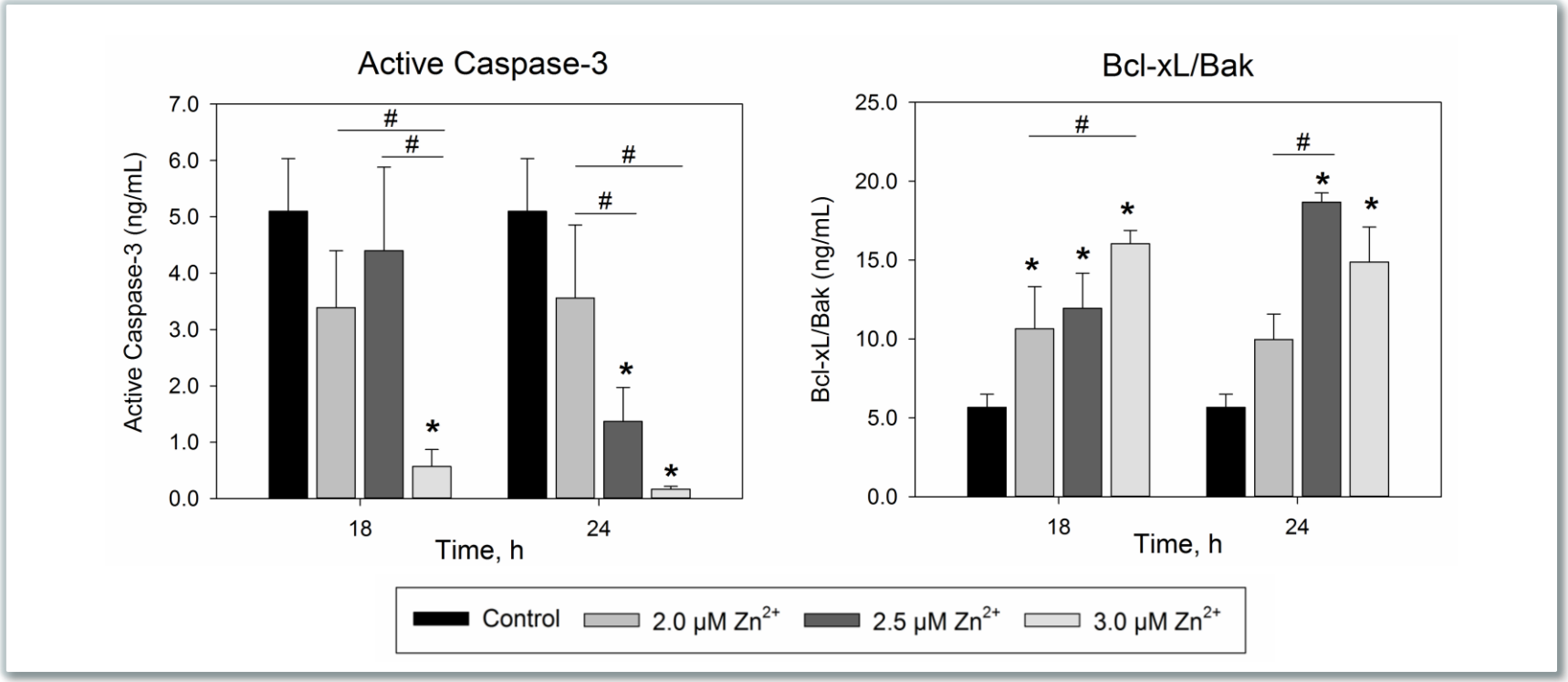
Cytotoxicity corresponds to concentrations predicted to saturate intracellular $Zn^{2+}$ buffering

# Are markers of apoptosis altered after $Zn^{2+}$ exposure?




Mean ± SD, $n$=3. $p$<0.01 compared with control (*) or each other (#) by two-way ANOVA and Holm-Sidak post hoc test.

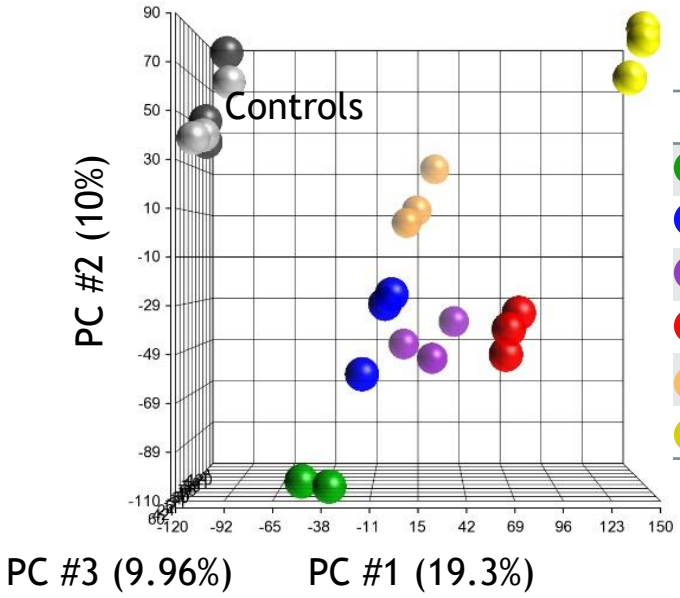
# Are markers of apoptosis altered after $Zn^{2+}$ exposure?




Tipping point from adaptive, recoverable cellular processes to an unrecoverable, cytotoxic response with exposures ≥2.5 μM $Zn^{2+}$

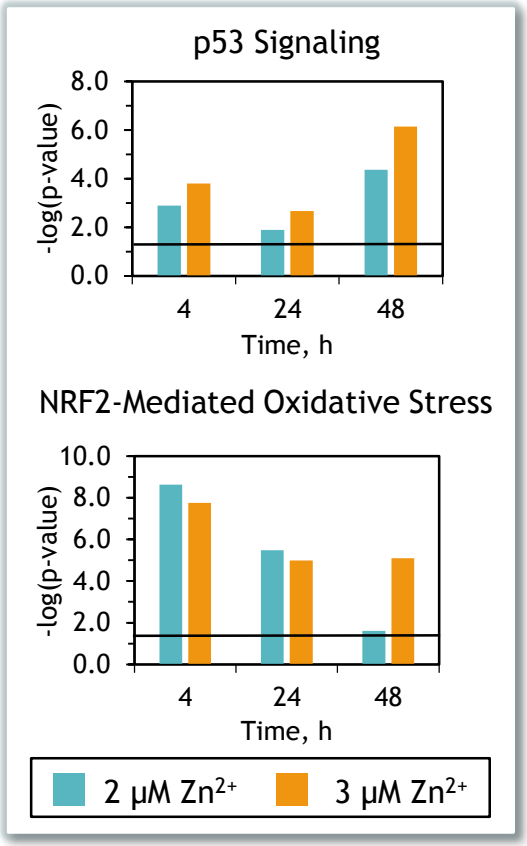
# Exploring adaptive and apoptotic gene expression changes

PCA Mapping (39.2%)




| [$Zn^{2+}$] | Time | DEGs |
|---|---|---|
| 2 µM | 4 h | 620 |
| 3 µM | 4 h | 585 |
| 2 µM | 24 h | 860 |
| 3 µM | 24 h | 1134 |
| 2 µM | 48 h | 797 |
| 3 µM | 48 h | 1864 |

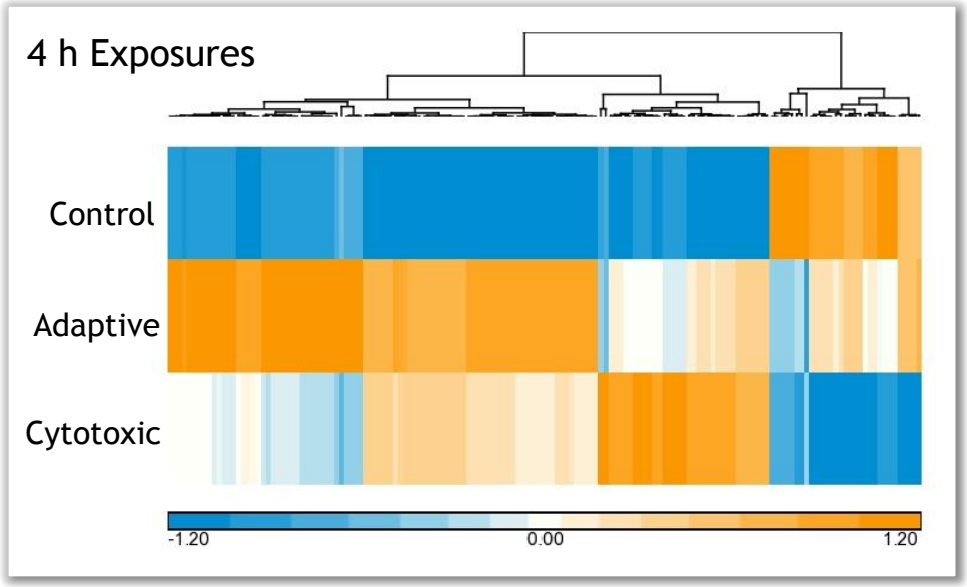
Pathway Enrichment



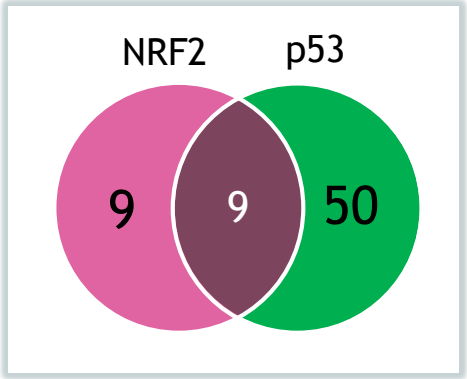

Concentration and duration of $Zn^{2+}$ exposure mediate significant gene expression changes

# Can early gene expression changes differentiate cellular responses?

Hierarchical Clustering

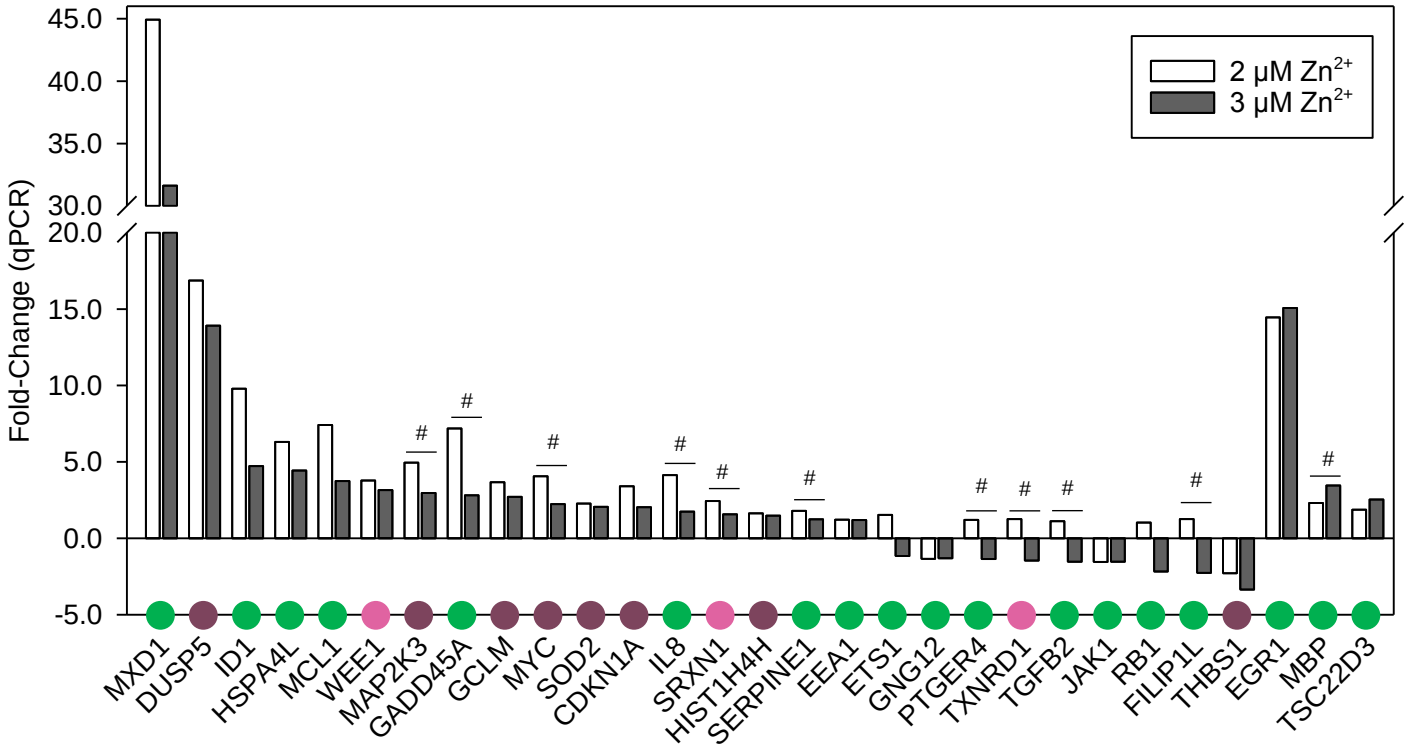



68 genes related to p53 and/or NRF2




Gene expression changes are evident earlier than typically measured phenotypic endpoints of cytotoxicity or apoptosis

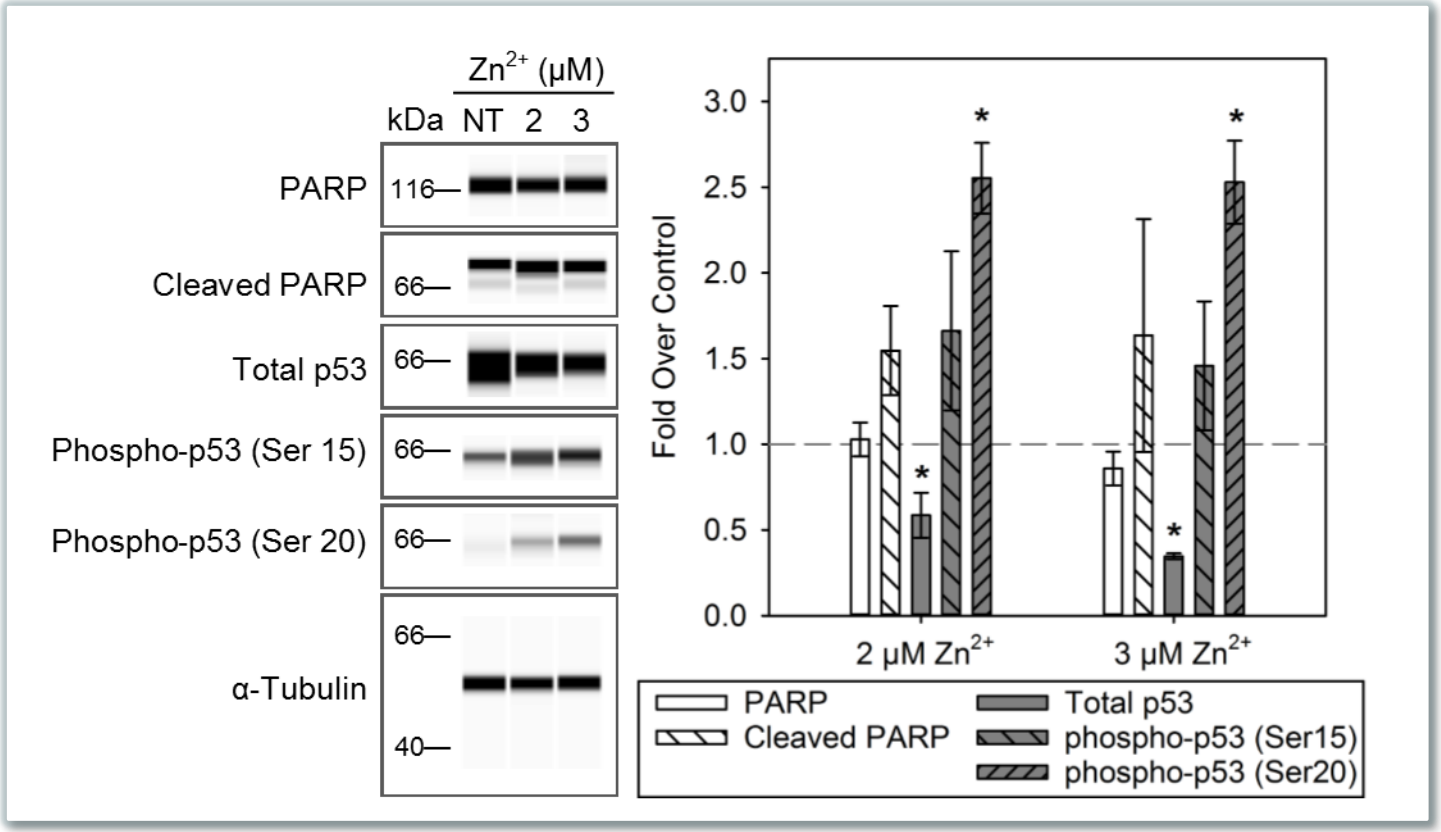
# Can early gene expression changes differentiate cellular responses?





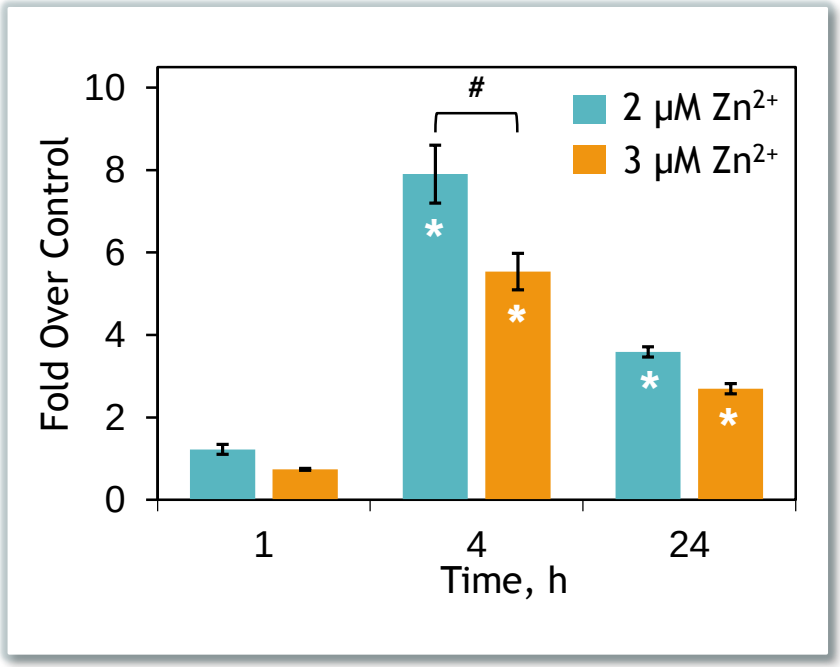
p53- and NRF2-related genes may mediate the switch between adaptation and apoptosis

# Can p53 activation distinguish the tipping point?



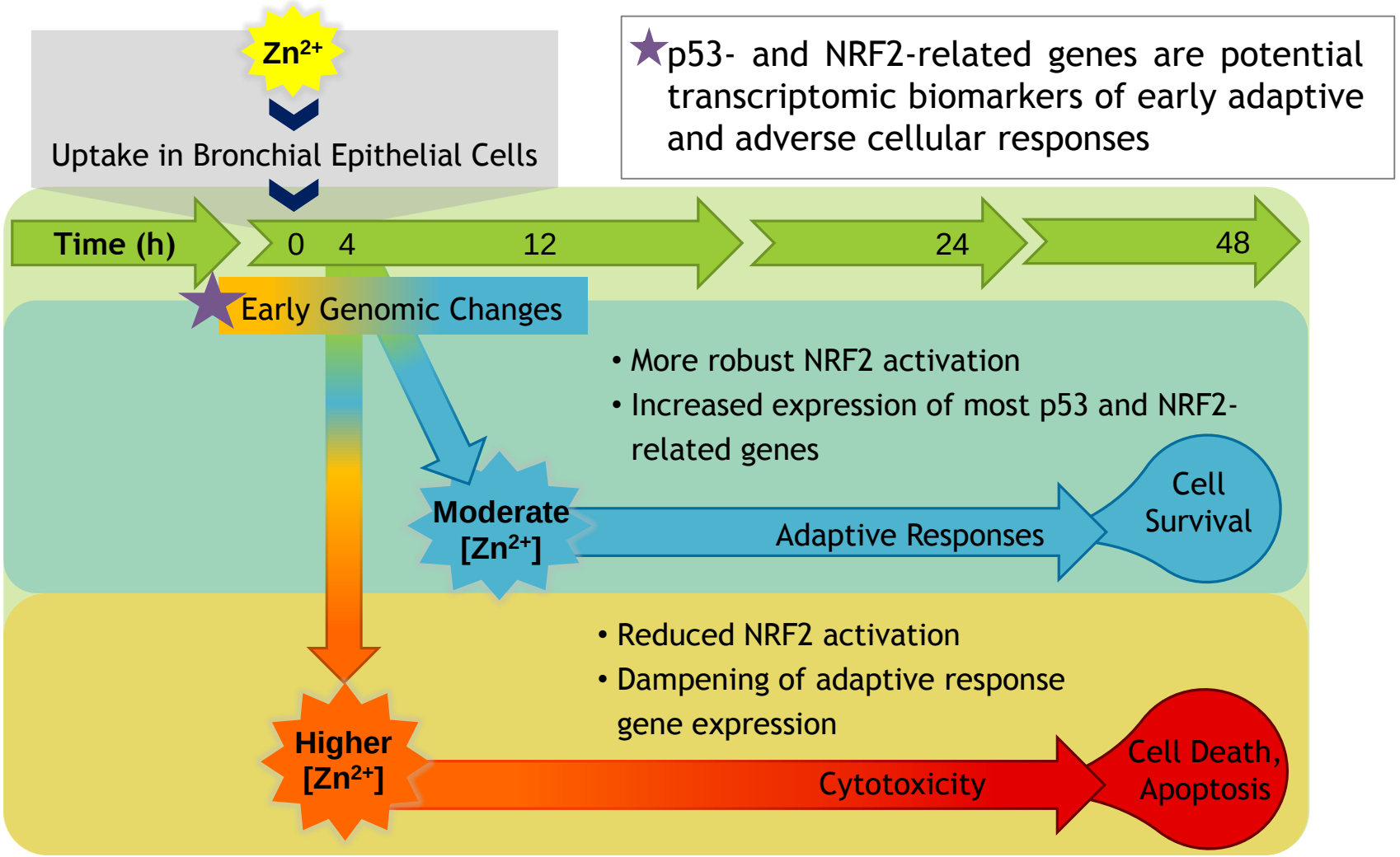

p53 activation, but no early distinction around tipping point

# Can NRF2 activation distinguish the tipping point?

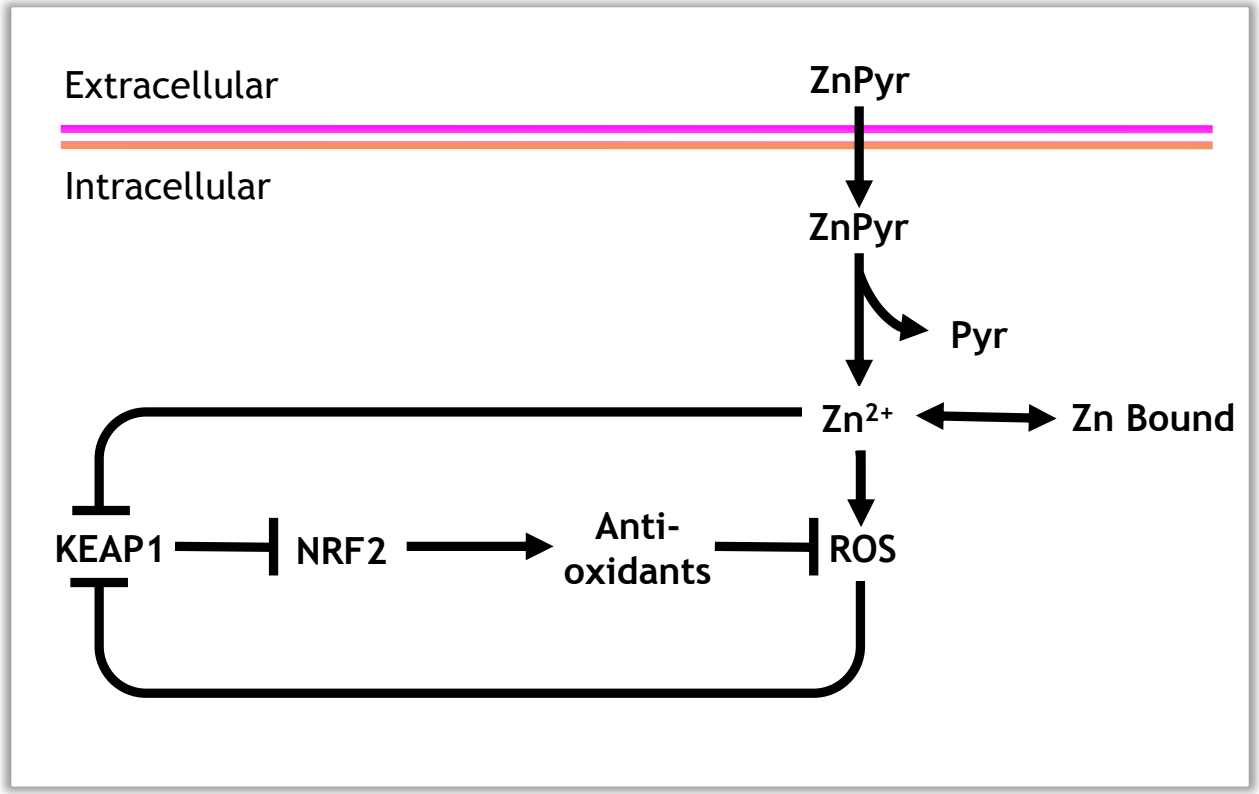



Increased activation of NRF2 at the adaptive, 2 μM $Zn^{2+}$ exposure

# What's the tipping point?



# Extending the computational model



# Future Applications

- Identify transcriptomic-based biomarkers for screening and prioritization of chemical and environmental-based exposures
  - Utilize high-throughput *in vitro* model systems
- Develop a computational model to predict adverse outcomes
- Assess complex chemical mixtures using both paradigms

# Acknowledgements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