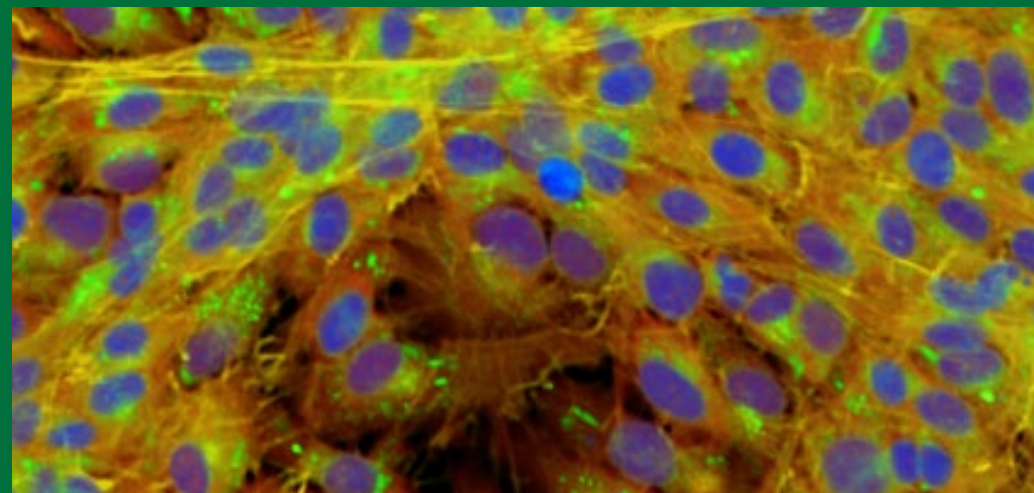
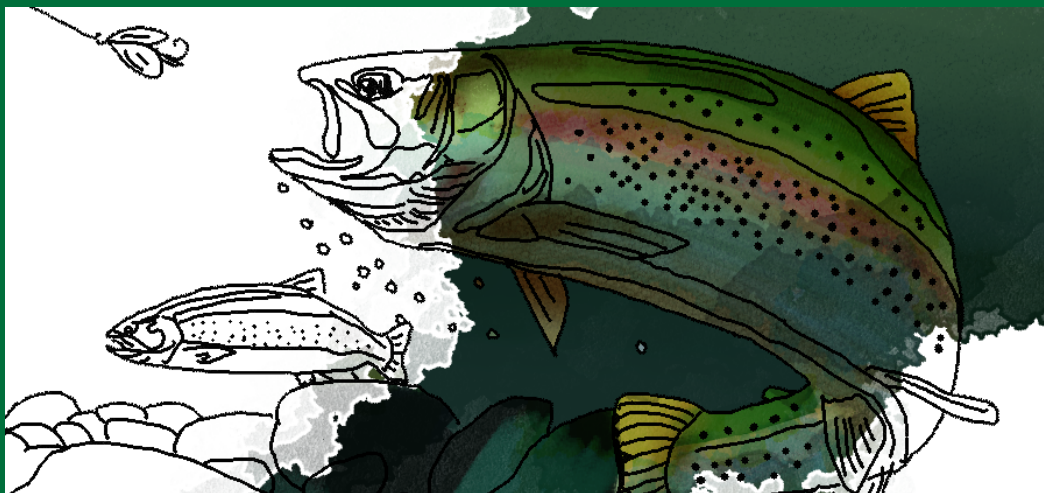


Painting the Rainbow: Expanding high-throughput chemical hazard evaluation to ecotoxicology-relevant species with a rainbow trout gill cell line

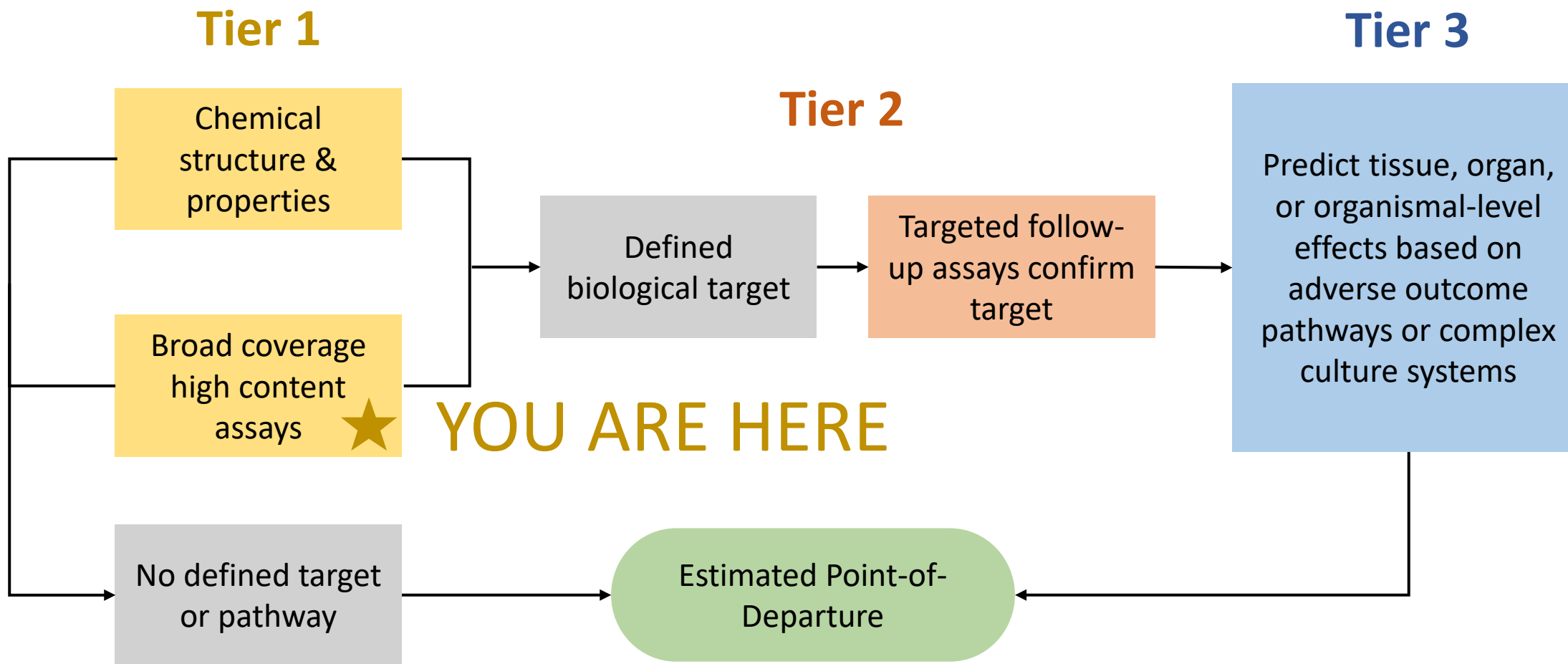
Felix Harris

Contractor for Oak Ridge Associated Universities in EPA's Center for Computational Toxicology and Exposure



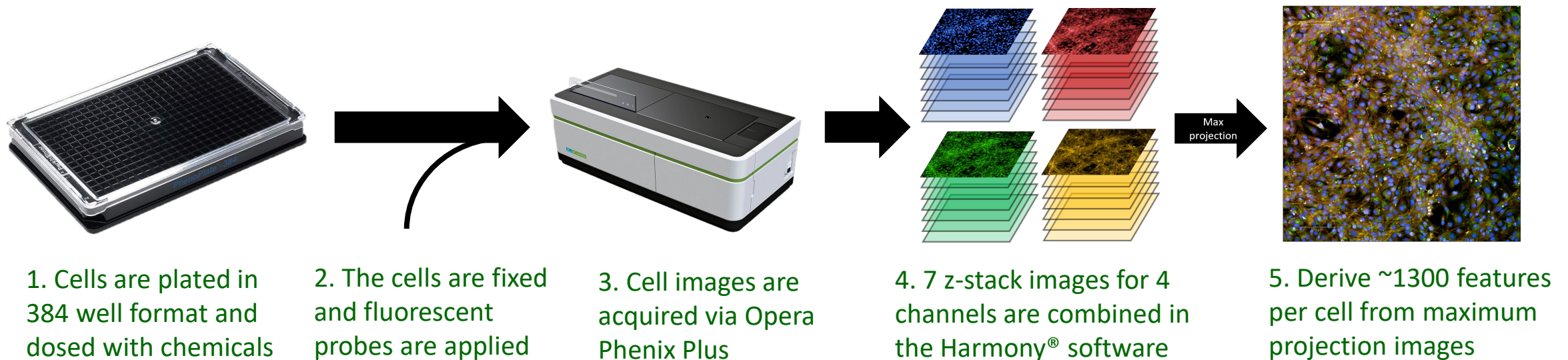
The views expressed in this presentation are those of the author(s) and do not necessarily represent the views or the policies of the U.S. Environmental Protection Agency. Any mention of trade names, manufacturers or products does not imply an endorsement by the U.S. Government or the EPA. EPA and its employees do not endorse any commercial products, services, or enterprises.

EPA Computational Toxicology Blueprint for Hazard Evaluation



High Throughput Phenotypic Profiling (HTPP)

- First tier hazard evaluation
- Based on the Cell Painting method (Bray et al. 2016)
- Fluorescent probes label cellular structures and organelles
- Used to screen chemicals in concentration/response format

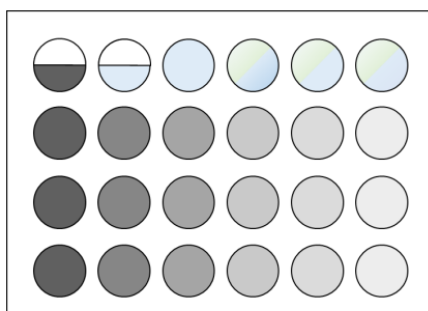


**What do YOU have in
common with a rainbow
trout?**

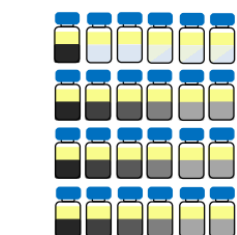
RTgill-W1 Cell Line

- Rainbow Trout gill line
- ATCC recommended for *in vitro* toxicology
- OECD TG 249 to predict acute toxicity in a plate reader assay

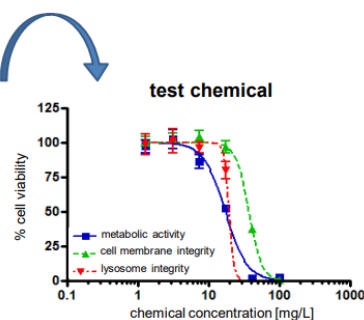
day 3
sampling and measurement
of cell viability




sampling at end of exposure
500 µL sample per well and vial



measurement of cell viability




 Section 2
Effects on Biotic Systems

Test Guideline No. 249
Fish Cell Line Acute Toxicity:
The RTgill-W1 cell line assay

14 June 2021

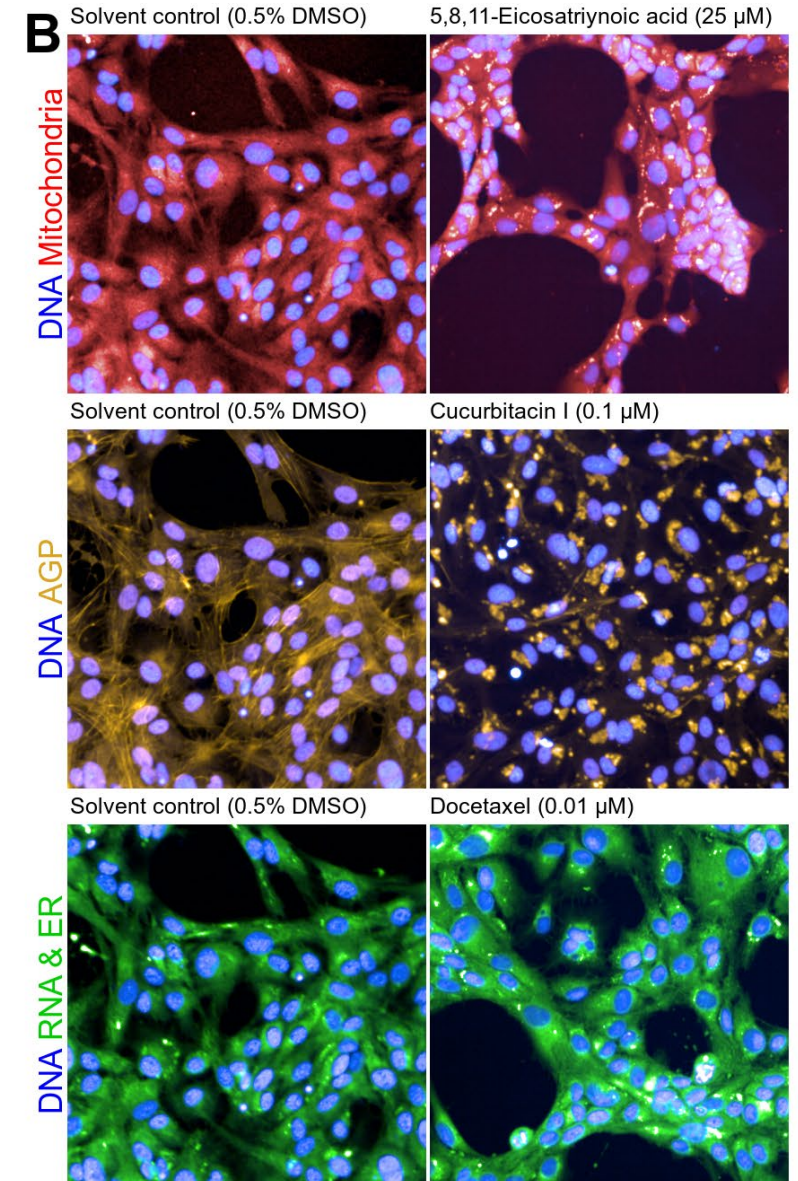
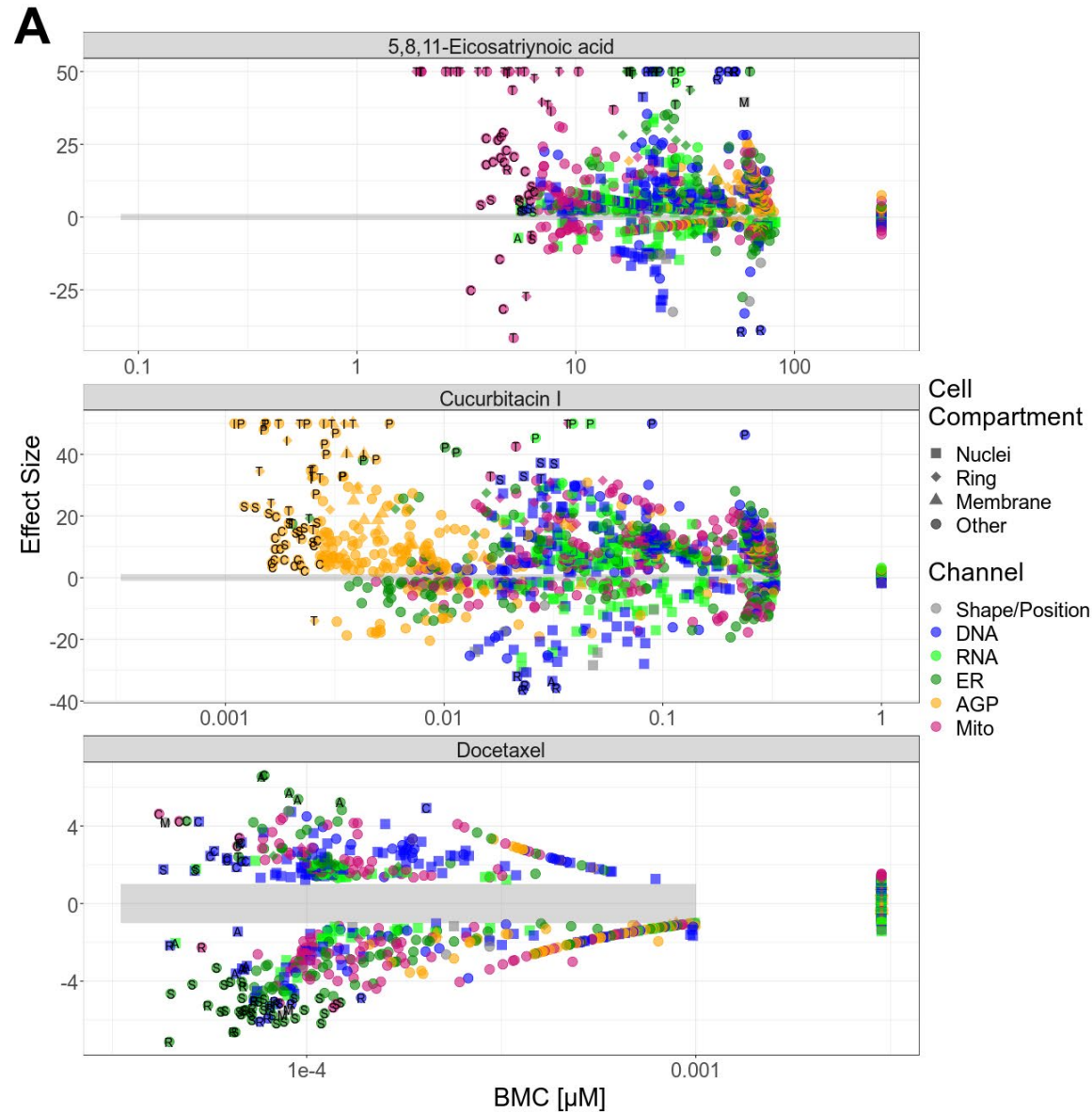
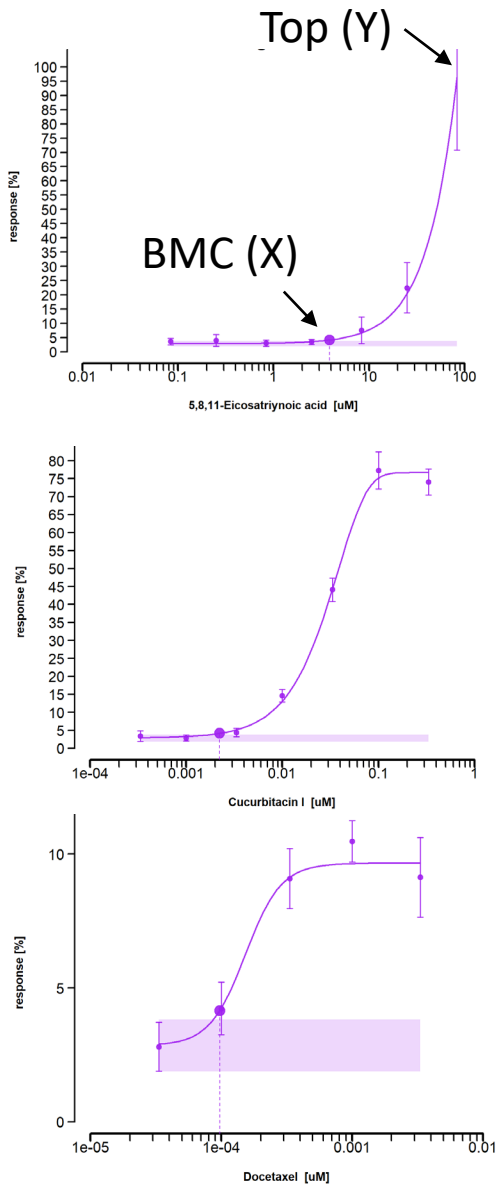
OECD Guidelines for the Testing of Chemicals



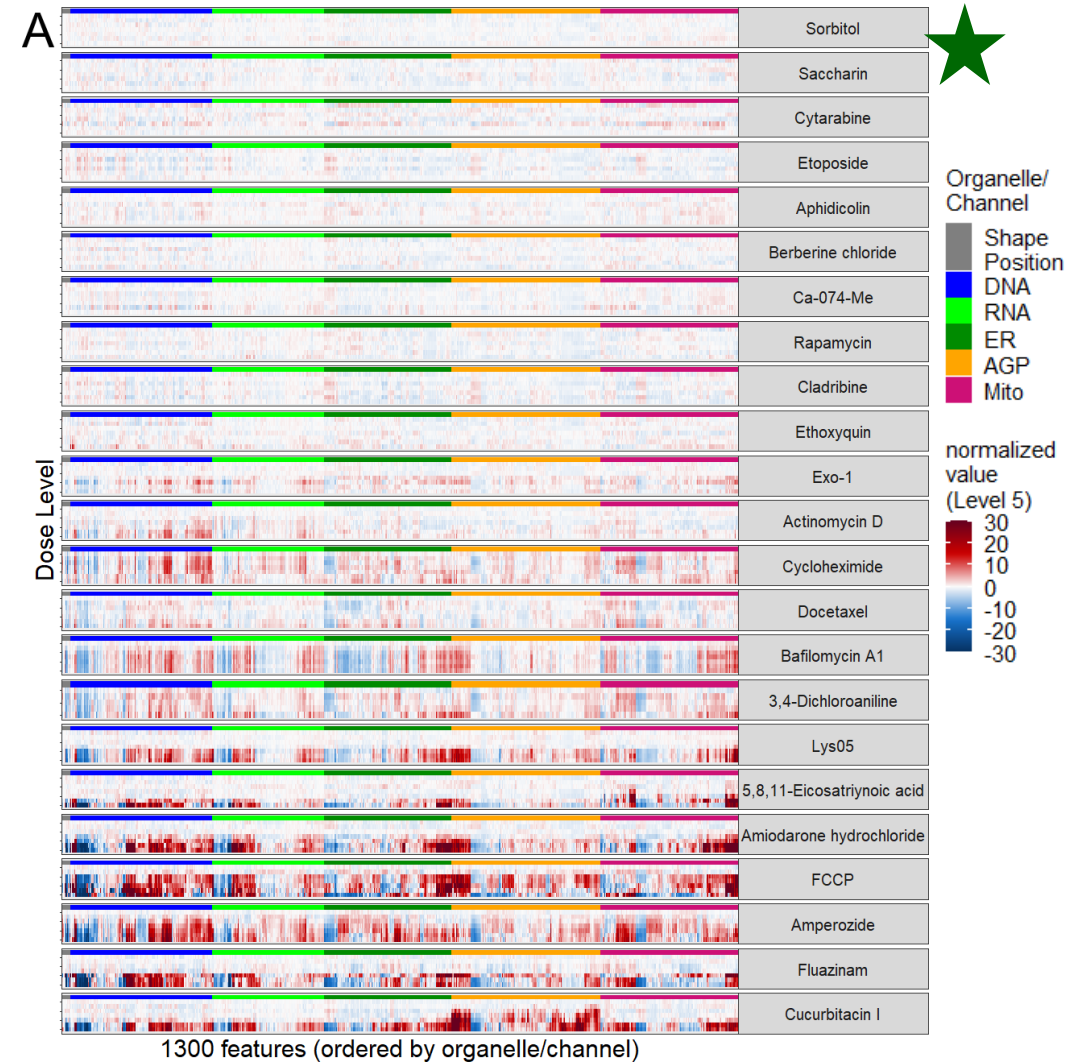
Aims

1. Adapt existing Cell Painting (CP) and image-based cell viability protocols to RTgill-W1 cells and identify suitable reference chemicals
2. Miniaturize OECD TG 249 from 24 well to 384 well format and run in tandem with Cell Painting
3. Screen 231 chemicals in concentration-response format, including:
 - 129 with *in vivo* rainbow trout data, 69 with rainbow trout *in vitro* data
 - 29 detected in Great Lakes water
 - 110 tested at EPA in human U-2 OS osteosarcoma cells
4. Compare data to relevant rainbow trout *in vivo* toxicity data and relevant Cell Painting data from other cell lines

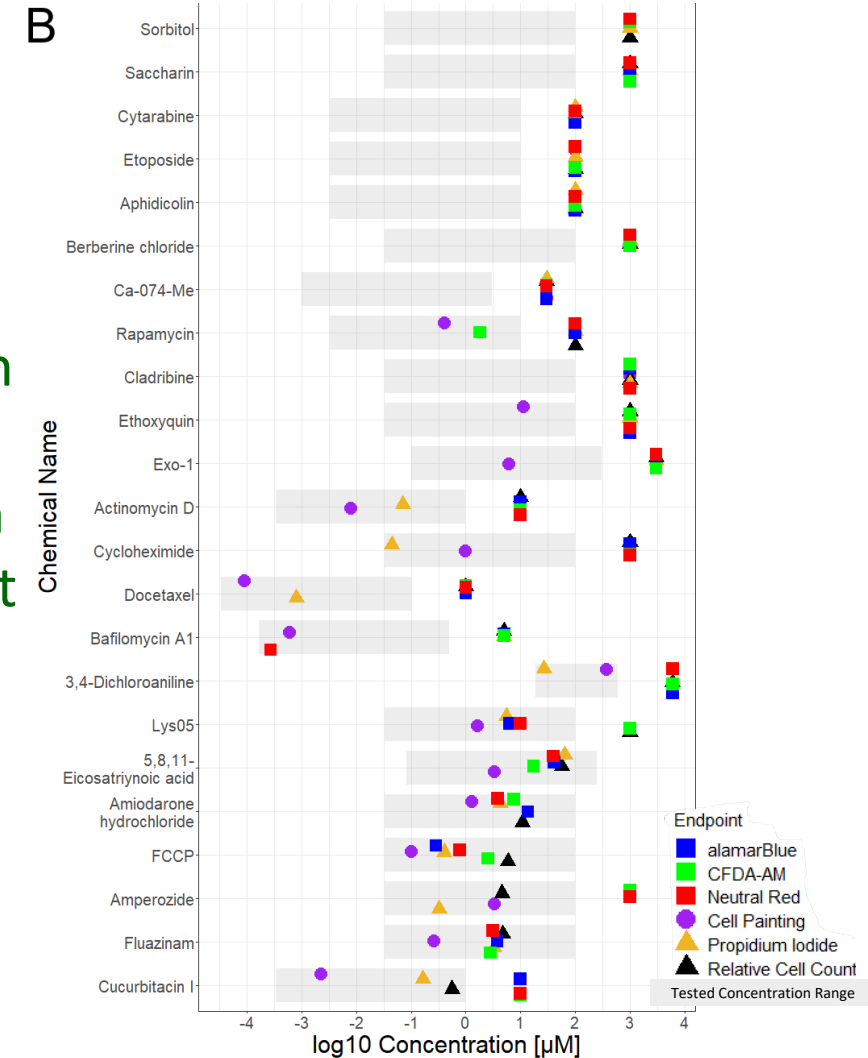
Cell Painting in RTgill



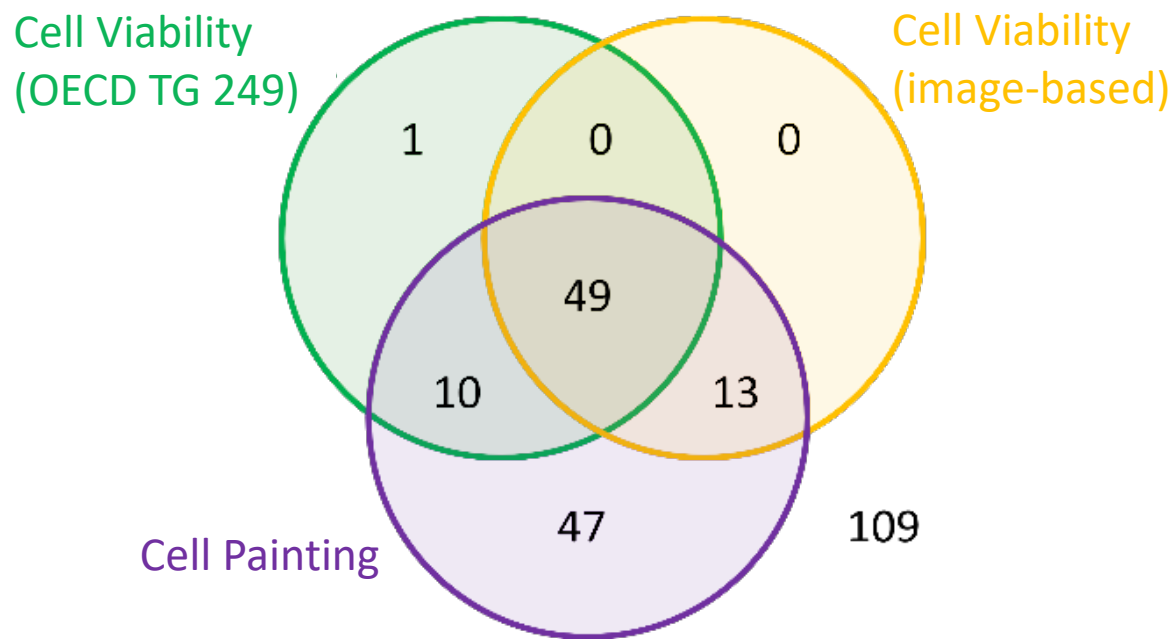
Cell Painting in RTgill



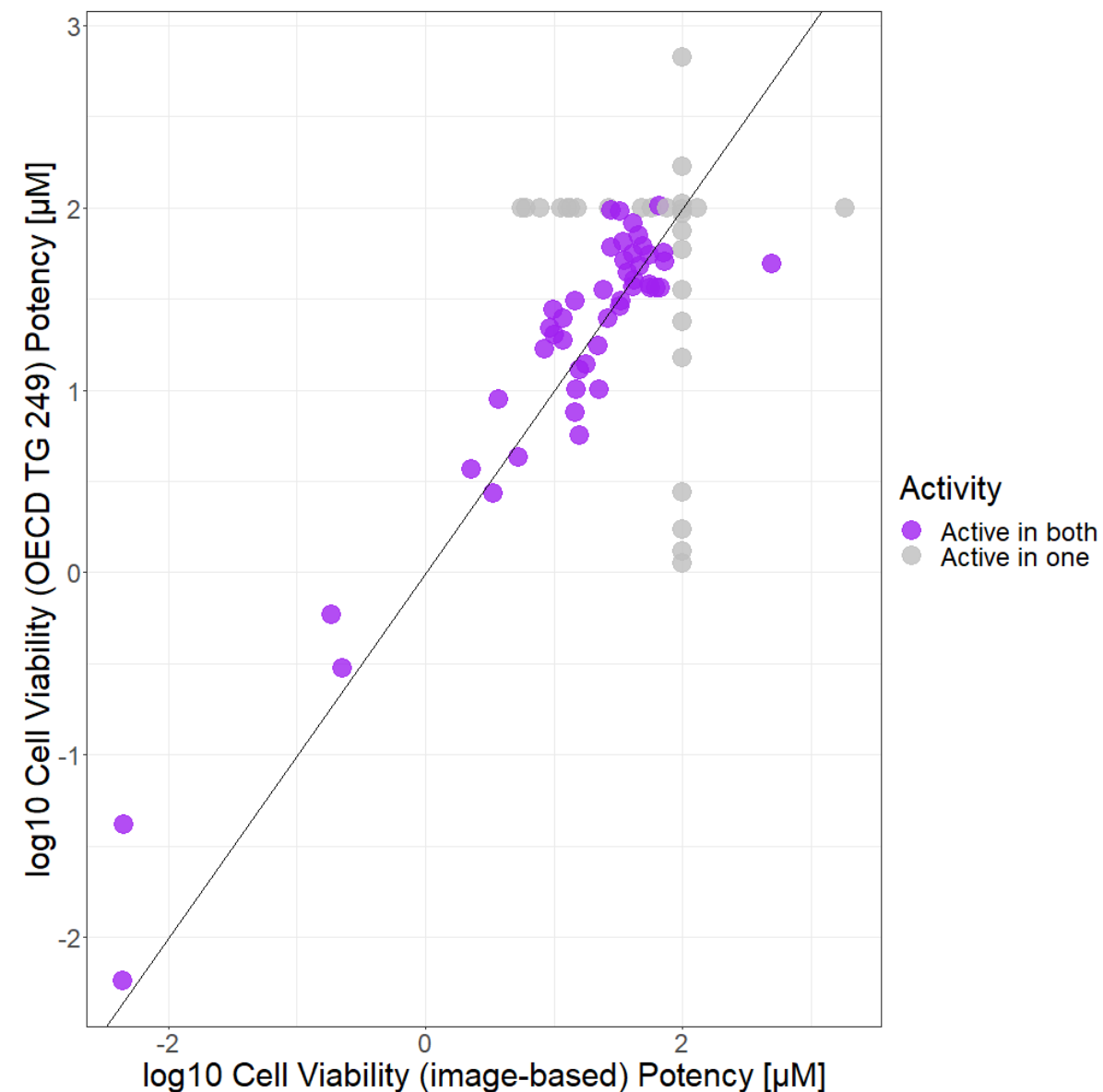
- Pilot studies profiling candidate reference chemicals were successful!
- ★ Sorbitol and saccharin do not produce detectable changes in phenotype, consistent among cell types
- Most phenotypic effects occur below cytotoxic concentrations



Miniaturization of OECD TG 249 for Cell Viability



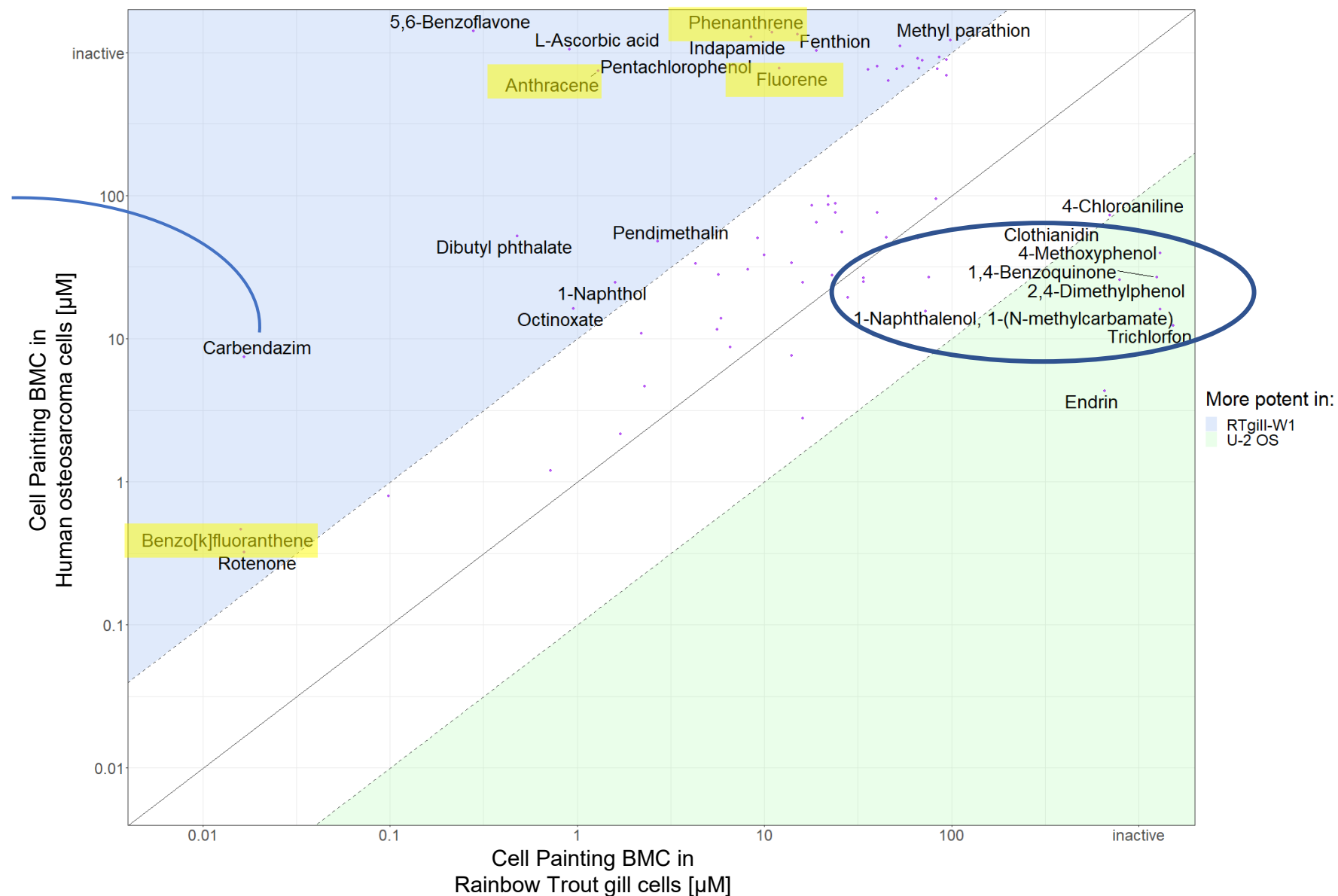
- CP identifies a larger number of chemicals than CV assays, implying CP assay marks positive at sub-cytotoxic concentrations
- OECD TG 249 cell viability results (CV-PR) were comparable to imaging-based cell viability measurements (CV-IB)



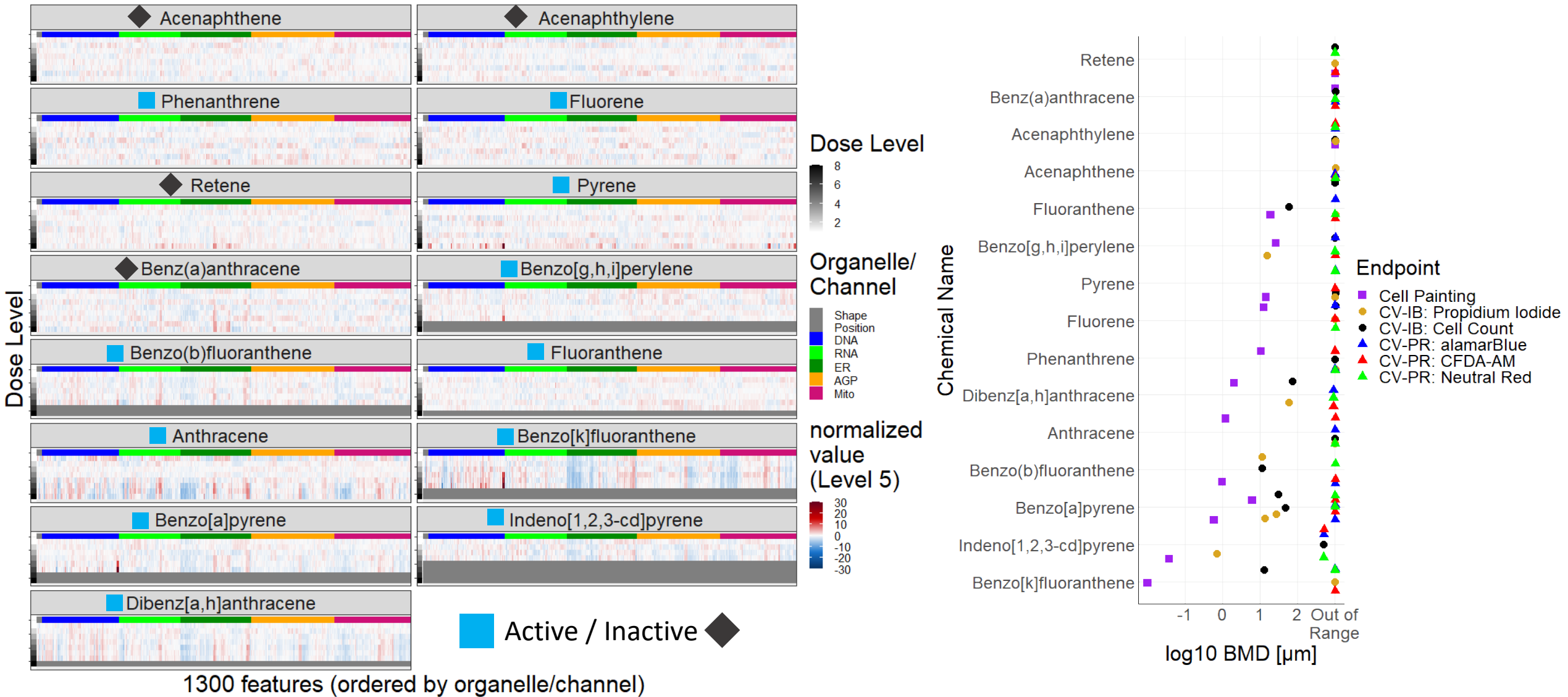


RTgill-W1 vs. U-2 OS

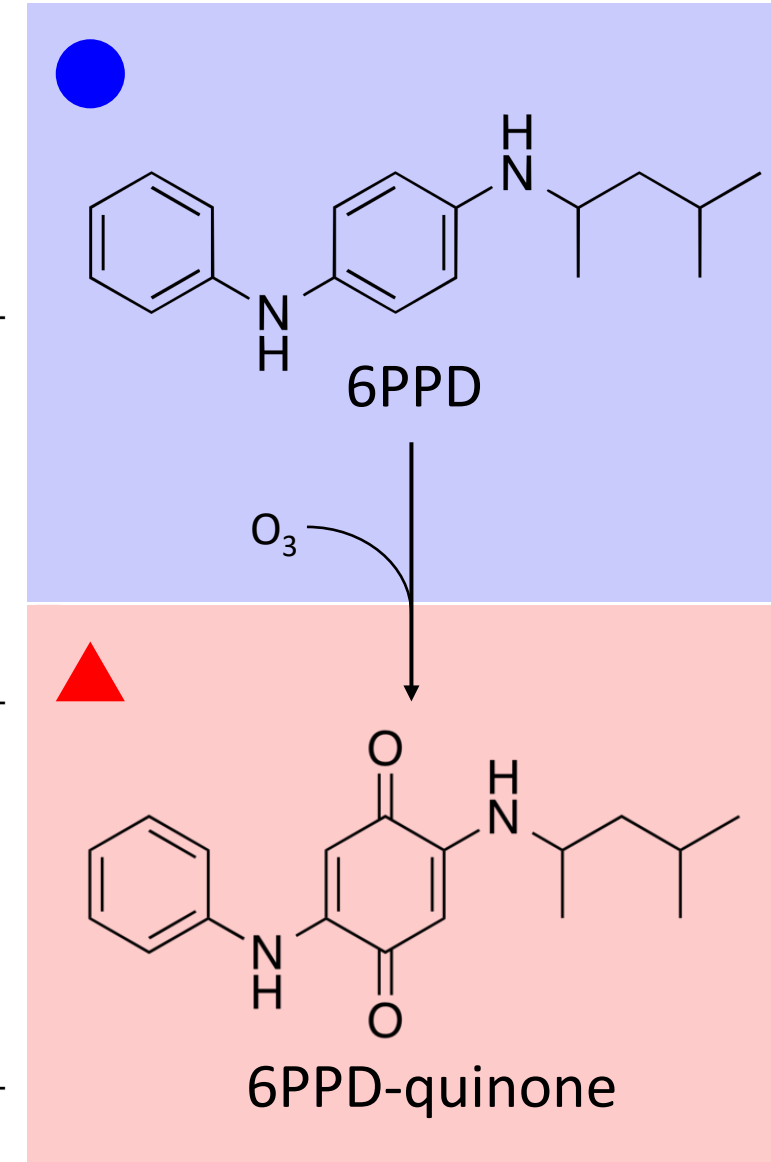
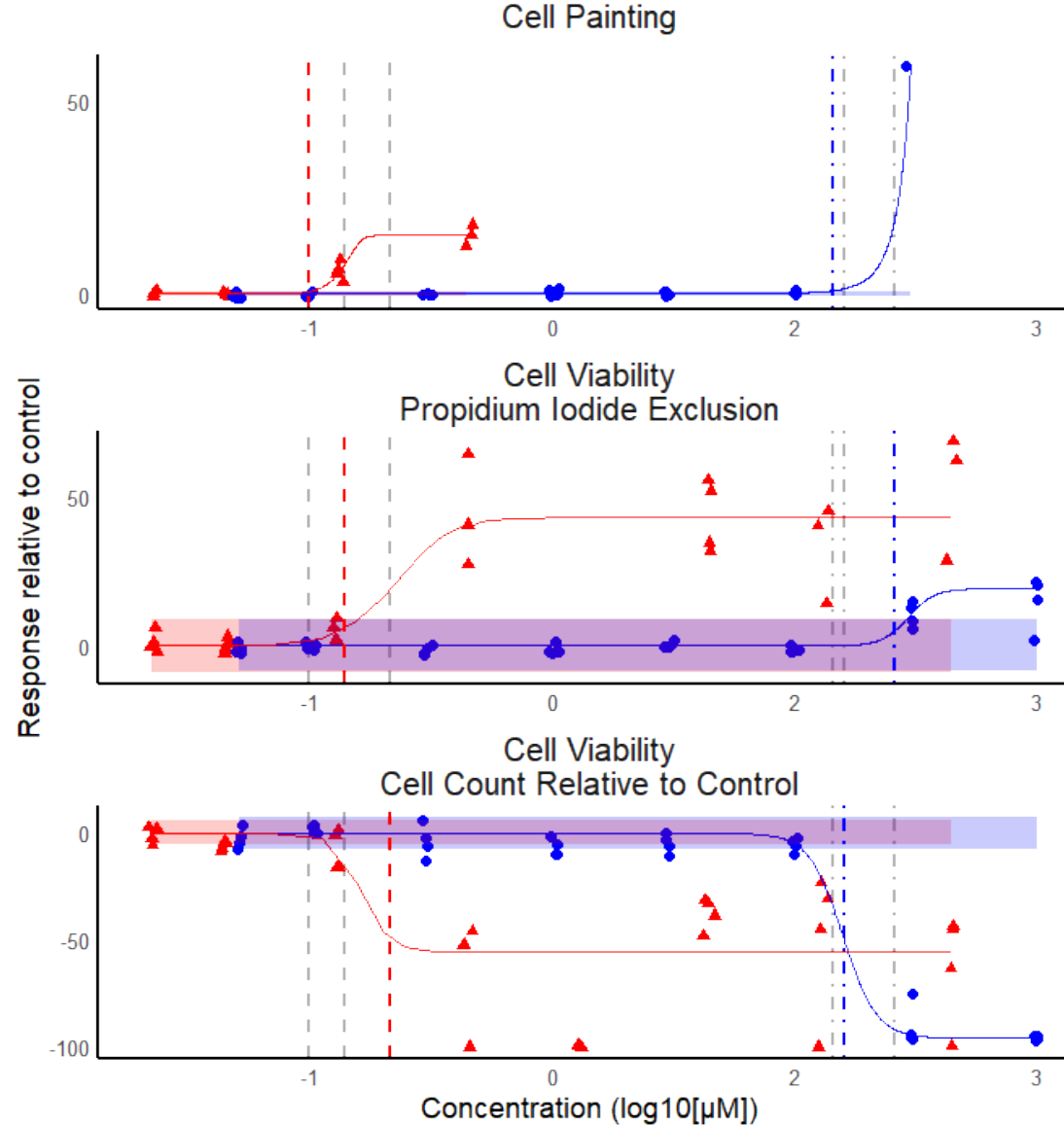
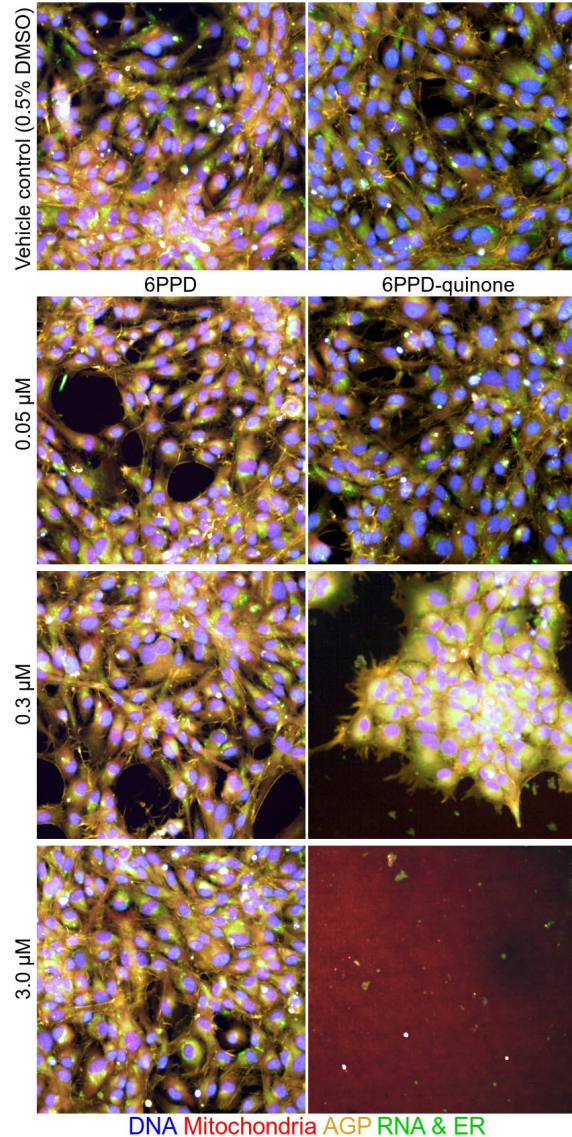
- RTgill-W1 cells were more sensitive to a wider range of compounds than U2-OS
- Many polycyclic aromatic hydrocarbons (PAHs) inactive in U2-OS were active in RTgill-W1 cells
- Many phenolic compounds were inactive in RTgill-W1 cells



Many PAHs produce potent phenotypic profiles in RTgill cells



6PPD-quinone, the oxidation product of 6PPD, is more toxic in RTgill-W1 cells



Future Directions

- *In vitro* to *in vivo* extrapolation to compare Cell Painting data to *in vivo* literature data
 - Ongoing experiments include *in vitro* disposition with a subset of diverse chemicals
- Further comparison to other cell types previously screened with Cell Painting
- Currently pending Regional / Office of Research and Development Applied Research Program proposal with Region 10 to use RTgill-W1 cells to test alternative antiozonants to 6PPD

Acknowledgements & Questions?

- Johanna Nyffeler
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