In vitro developmental neurotoxicity (DNT) dosing vehicles nominally impact growth, viability, and phenotypic profile of human neural progenitor cells

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United States Environmental Protection Agency
Office of Research and Development

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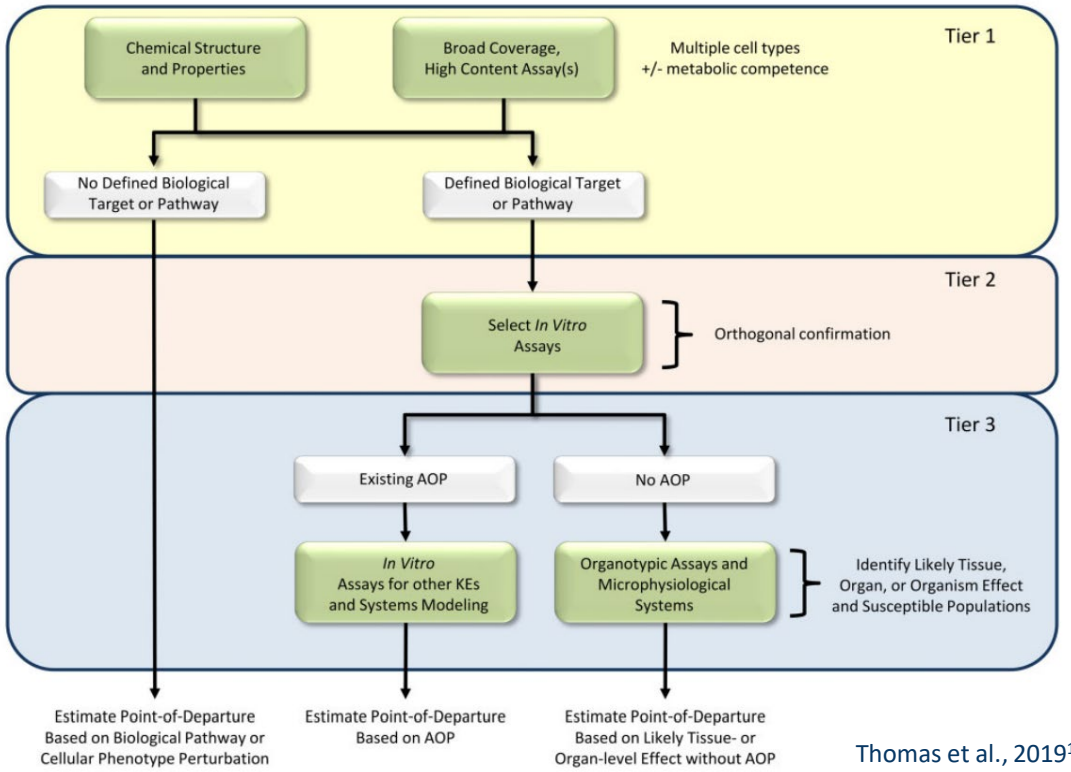
Developmental Neurotoxicity (DNT)

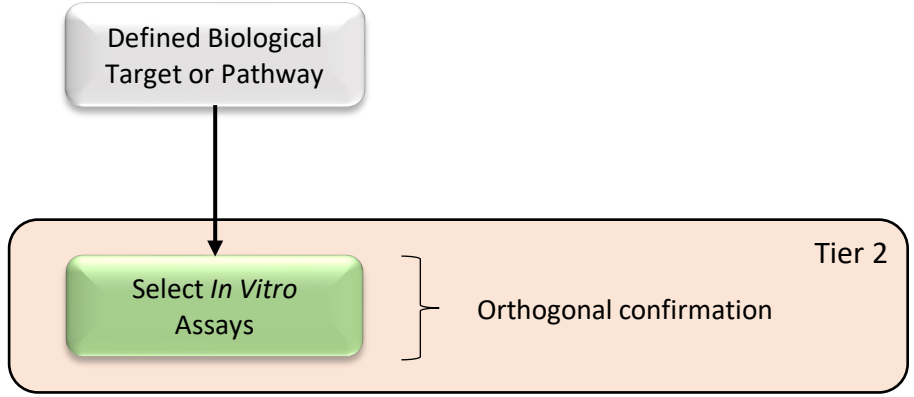
'an adverse change in the structure or function of the central and/or peripheral nervous system following exposure to a chemical, physical, or biological agent' U.S. EPA 1998

- Regulatory organizations (e.g., U.S. EPA, OECD) presently still rely on guideline *in vivo* studies to assess potential DNT chemical hazard
- These studies are now requested less frequently, however, due to ambiguous results and the lack of mechanistic insight provided
- As such, reliable and efficient new approach methods (NAMs) are needed to evaluate the many chemicals without DNT data

U.S. EPA Tiered Chemical Testing Strategy

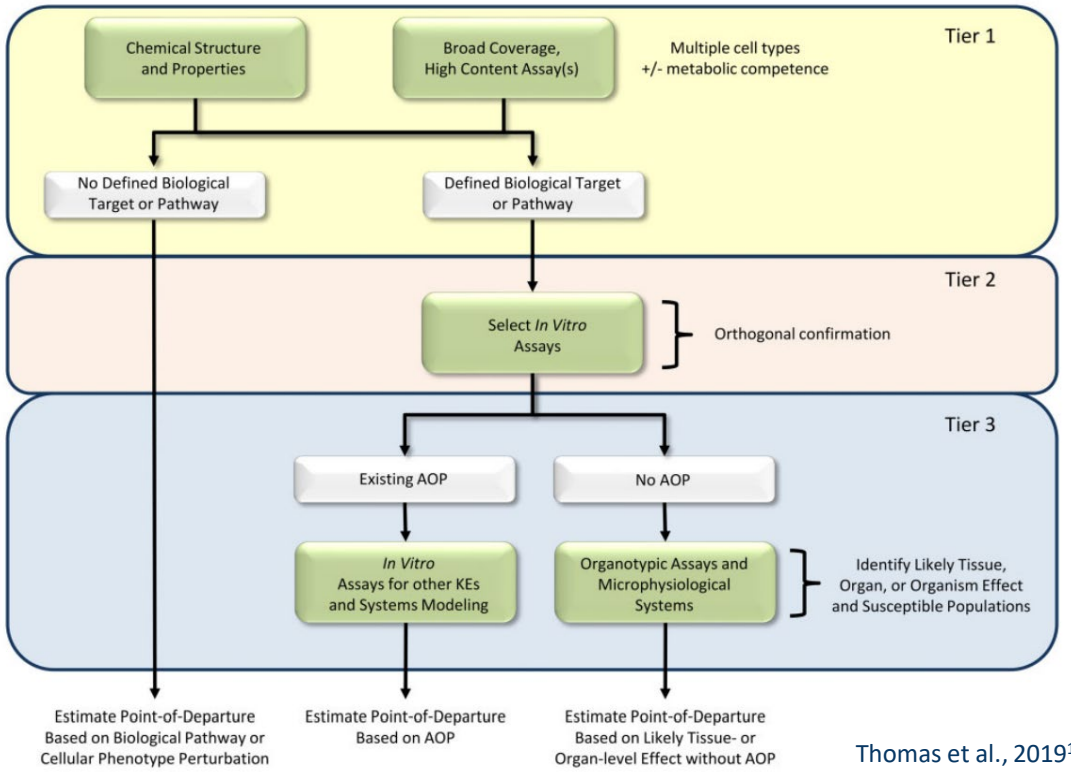


Thomas et al., 2019[1]



[1]doi: 10.1093/toxsci/kfz058

Current *in vitro* DNT NAMs are all Tier 2 assays



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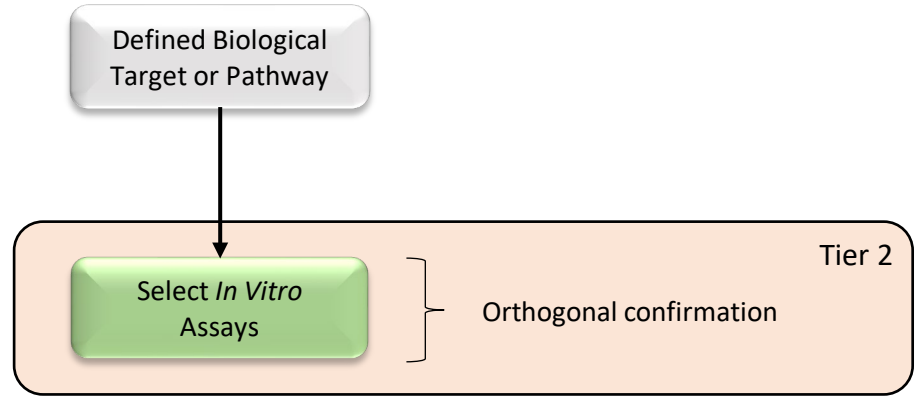

Assay	Species	Cell Type
Proliferation	human	neural progenitors (hNP1)
Apoptosis	human	neural progenitors (hNP1)
Neurite Outgrowth	human and rodent	neurons (iCell Gluta; primary rat cortical)
Synaptogenesis	rodent	neurons (primary rat cortical)
Network Formation and Function	rodent	neurons (primary rat cortical)

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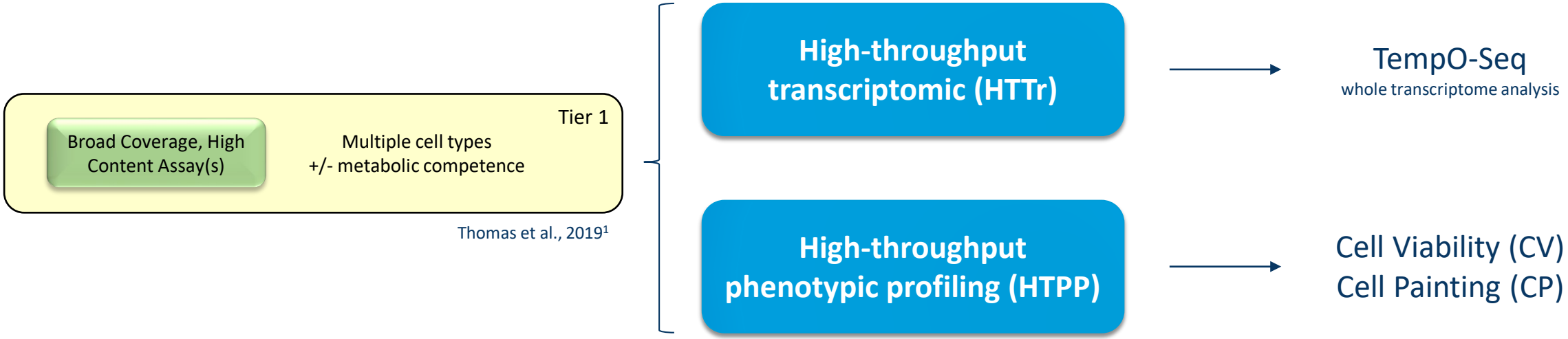
Tier 1 approaches for DNT hazard evaluation and prioritization



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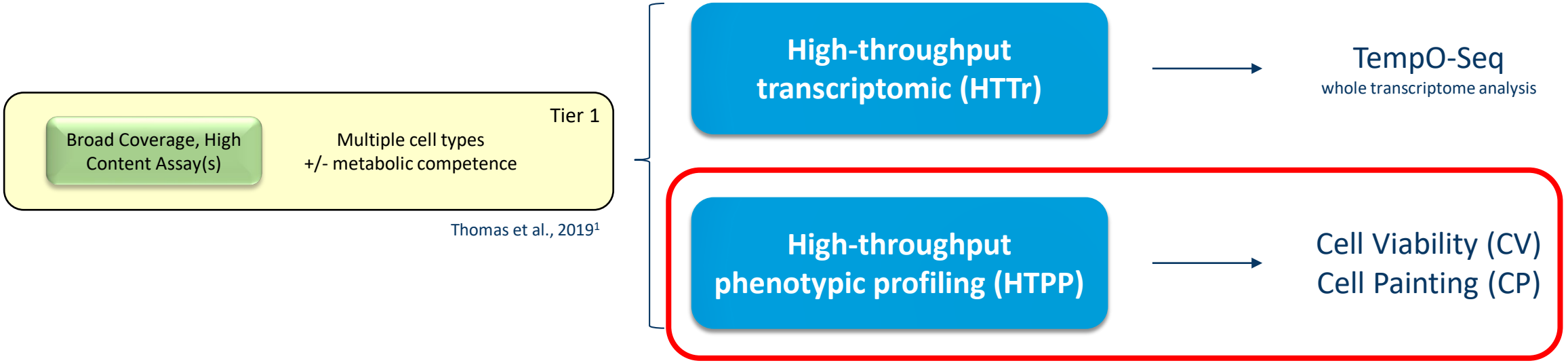
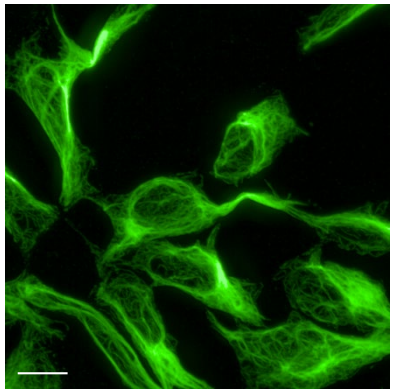
Tier 1 approaches for DNT hazard evaluation and prioritization



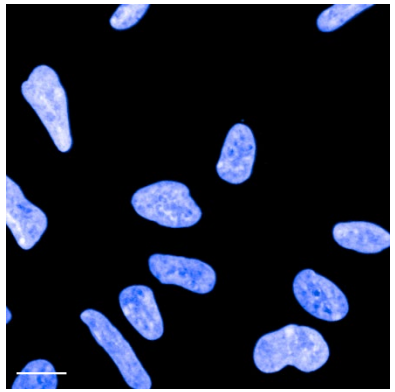
[1]doi: 10.1093/toxsci/kfz058

hNP1 human neural progenitor cells selected as initial model to optimize for HTPP

Nestin



Hoechst



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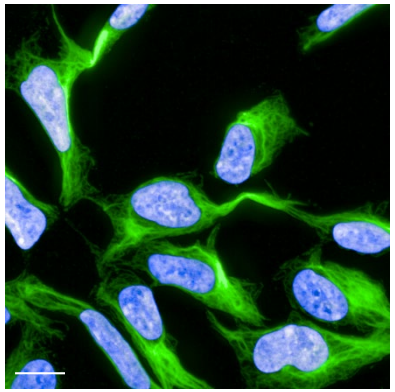
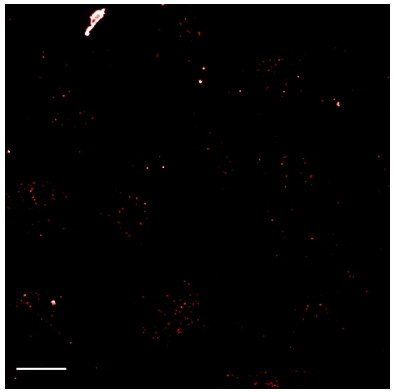


MAP2



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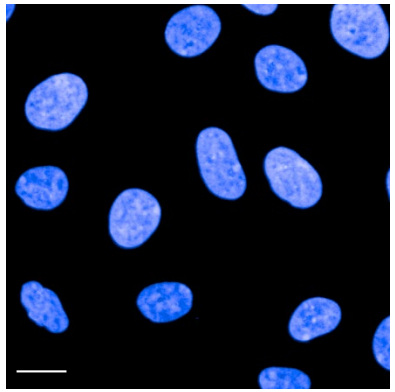

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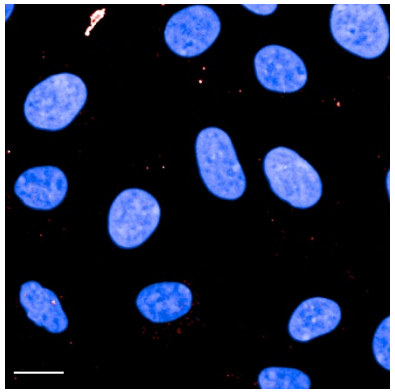
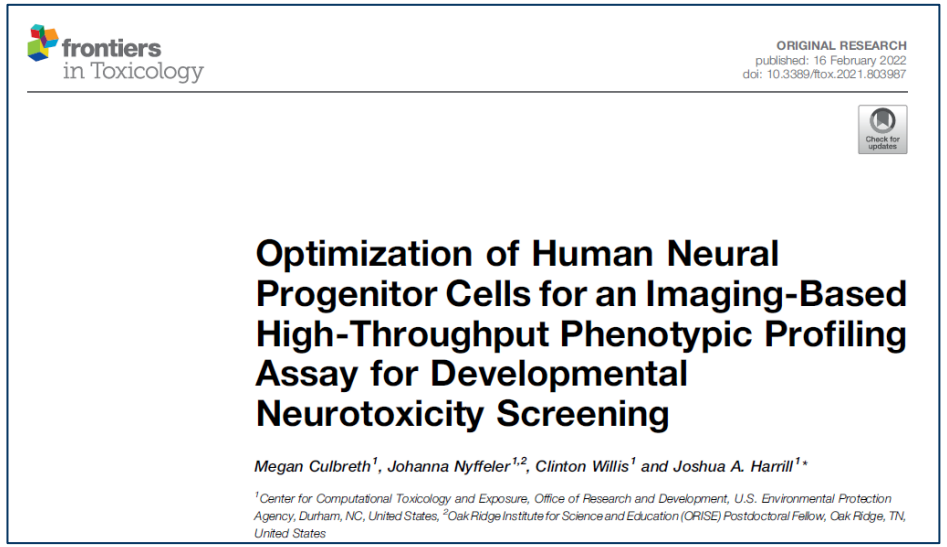


The method for HTPP of hNP1 cells has been established



frontiers in Toxicology

ORIGINAL RESEARCH
published: 16 February 2022
doi: 10.3389/ftox.2021.803987

Optimization of Human Neural Progenitor Cells for an Imaging-Based High-Throughput Phenotypic Profiling Assay for Developmental Neurotoxicity Screening

*Megan Culbreth[1], Johanna Nyffeler[1,2], Clinton Willis[1] and Joshua A. Harrill[1]**

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HTPP Approach

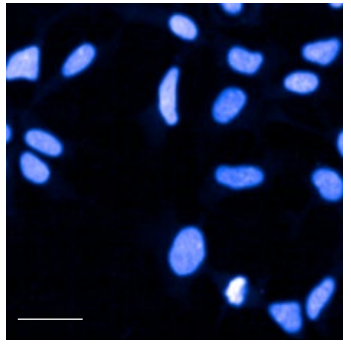
Cell Viability (CV)

Live-cell Labeling → Fixation → Image

Cell Painting (CP)

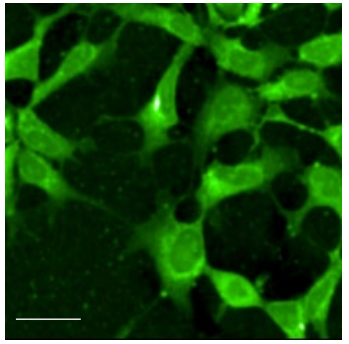
Live-cell Labeling → Fixation → Permeabilization → Fluorescent Labeling → Image

Nuclei (DNA)



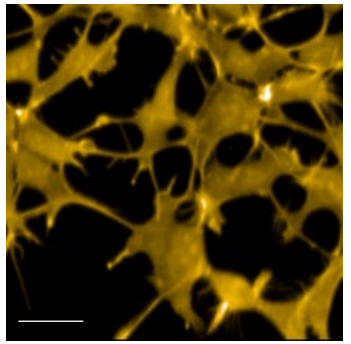
Hoechst 33342

Nucleoli (RNA) + Endoplasmic Reticulum (ER)



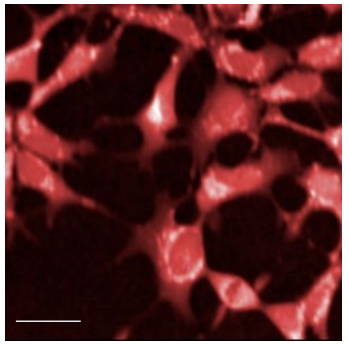
SYTO14/Alexa 488

Actin cytoskeleton + Golgi apparatus + Plasma membrane (AGP)



Alexa 555/568

Mitochondria (Mito)



MitoTracker

Measure
Individual Features

↓

Evaluate
Qualitative & Quantitative
Phenotypic Profiles

Phenotypic Altering
Concentrations (PACs)

What potential affect might a dosing vehicle have on *in vitro* DNT NAM endpoints?

Dosing vehicle = solvent control

- *In vitro* models do not fully recapitulate human biology; therefore, any deviation from baseline further limits translation of an identified hazard
- Most chemicals are diluted in a dosing vehicle (e.g., dimethyl sulfoxide (DMSO), ethanol (EtOH)) for screening; however, these dosing vehicles are not necessarily inert
- Media-only (untreated) wells often are not included as an assay-level control

There has yet to be a systematic evaluation of potential dosing vehicle effects relative to baseline (untreated) in U.S. EPA DNT NAMs

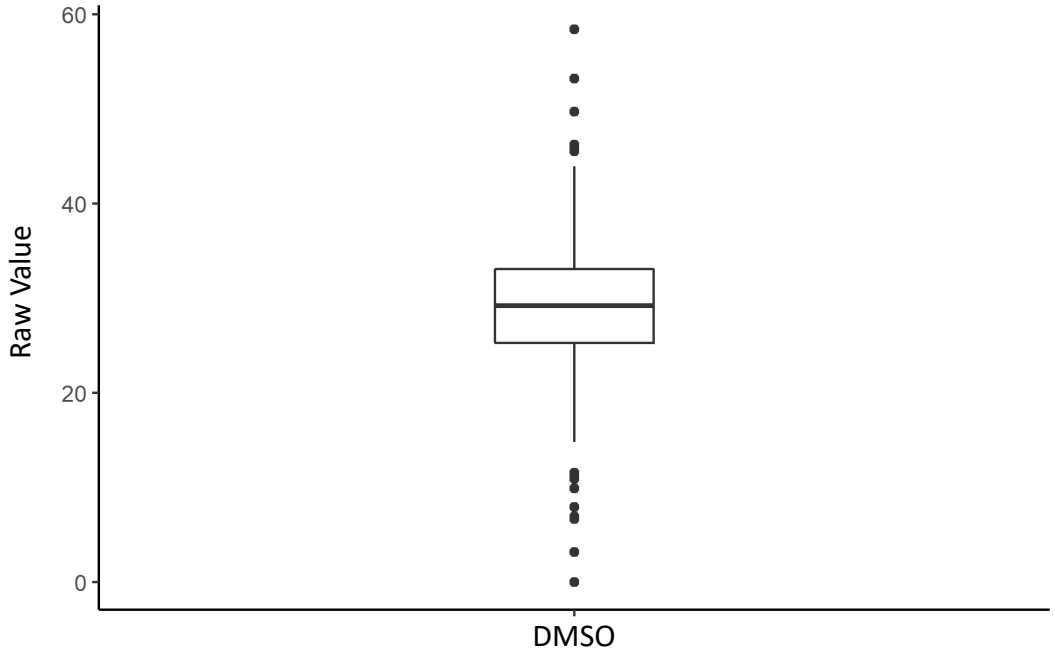
In vitro DNT NAMs incorporate multiple dosing vehicles

Dosing vehicle = solvent control

Assay	Species	Cell Type	Dosing Vehicles
Proliferation	human	neural progenitors (hNP1)	DMSO, EtOH, water
Apoptosis	human	neural progenitors (hNP1)	DMSO, EtOH, water
Neurite Outgrowth	human and rodent	neurons (iCell Gluta; primary rat cortical)	DMSO, EtOH, water
Synaptogenesis	rodent	neurons (primary rat cortical)	DMSO, EtOH, water
Network Formation and Function	rodent	neurons (primary rat cortical)	DMSO, DMSO-EtOH, EtOH, water

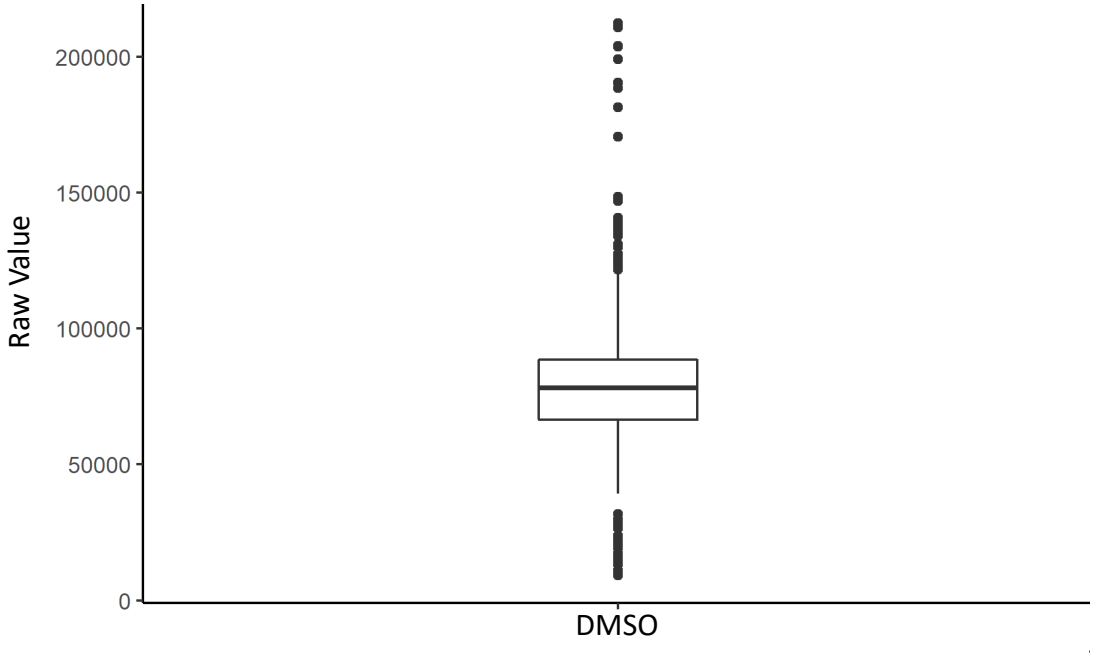
Dosing vehicle response can vary within assay endpoints

hNP1 Proliferation



Endpoint: Responder Average Intensity

hNP1 Apoptosis



Endpoint: Caspase 3/7

Data provided by Shafer Lab (T. Freudenrich, K. Wallace, S. Choo)

Will the dosing vehicles produce distinct effects in the HTPP approach?

- hNP1 human neural progenitor cells plated in 384-well format were allowed to attach and grow for 24 hours

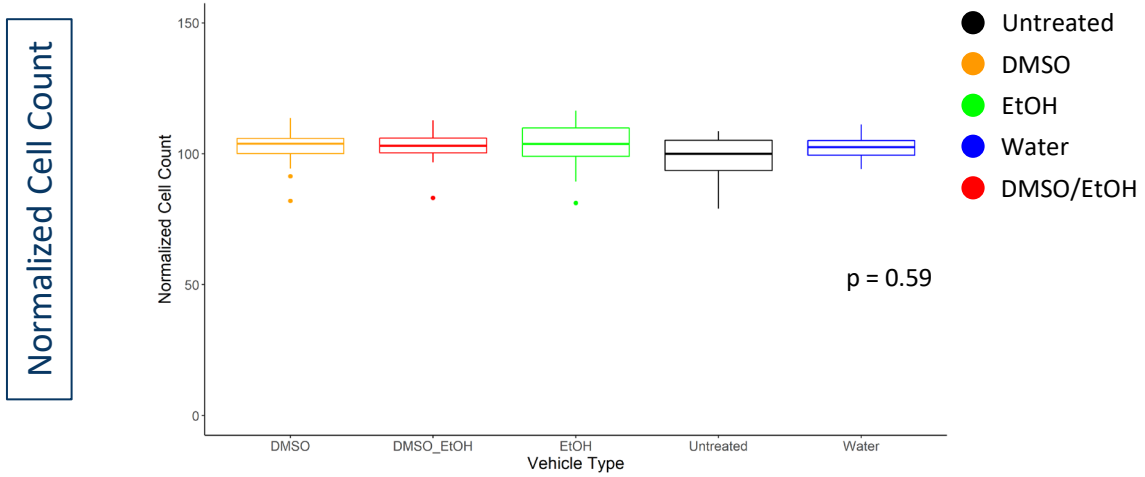
- Cells were then exposed to culture media-only (untreated), DMSO, EtOH, water, or DMSO-EtOH (1:1) for an additional 24 hours
  - Final in-plate concentration 0.1% (v/v)
  - 16 technical replicates/dosing vehicle

- After exposure, cells were live-labelled for cell viability (CV) or cell painting (CP) assays, fixed, stained, and imaged
  - 0.1 μM Staurosporine – CV assay positive-control
  - 10 μM Aphidicolin – CP assay positive-control

- Finally, effects on hNP1 cell health, growth, and phenotype were evaluated

The dosing vehicles did not affect cell health endpoints

A



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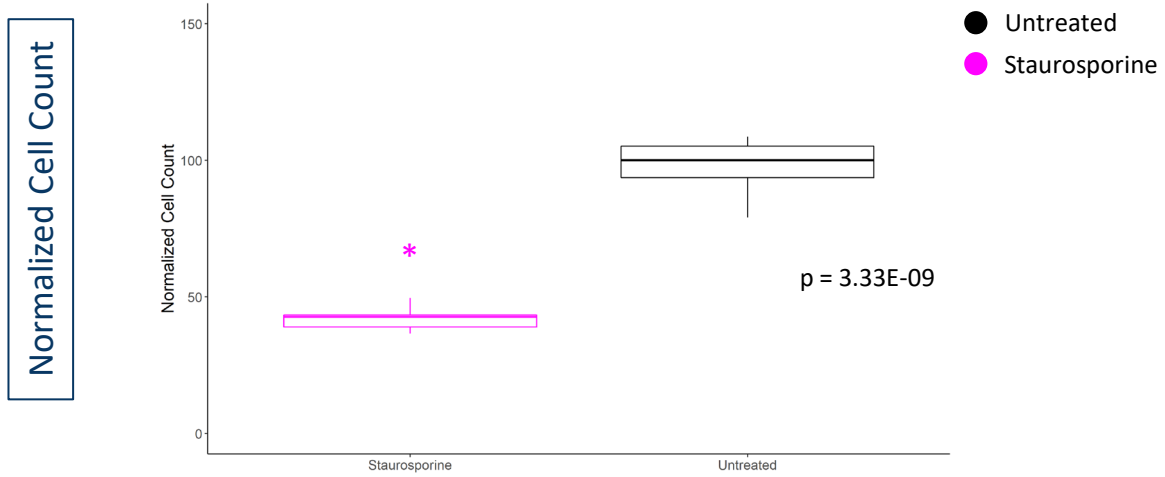

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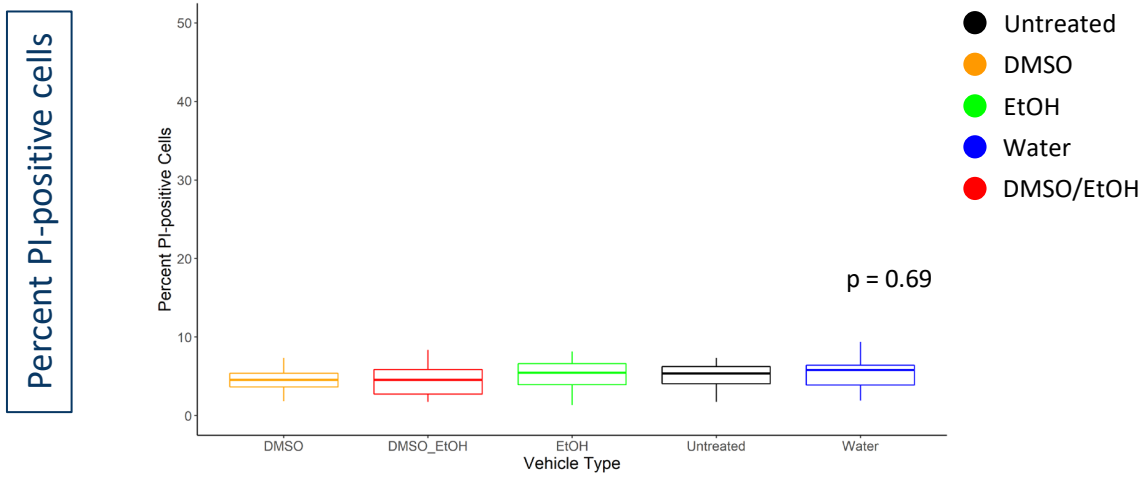

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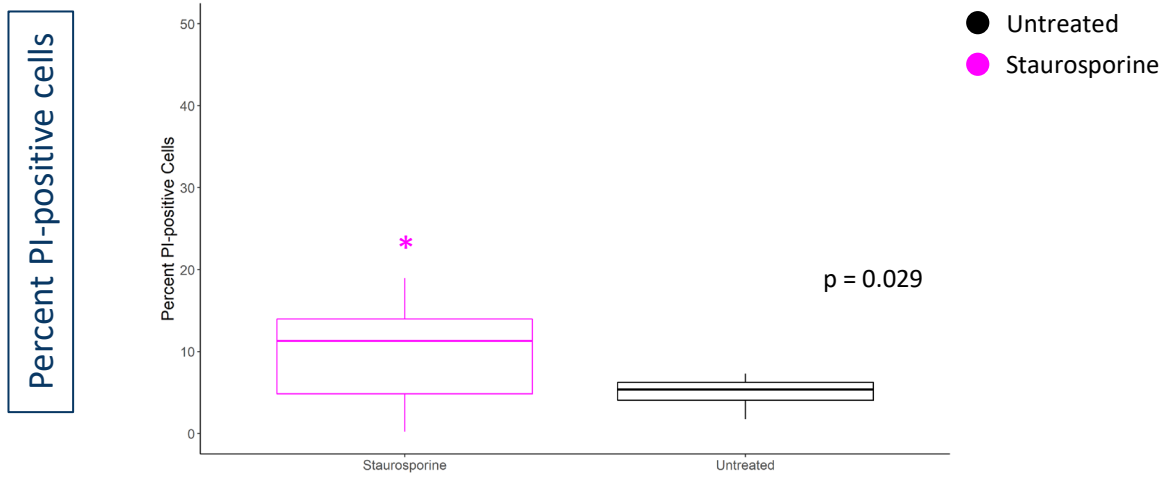
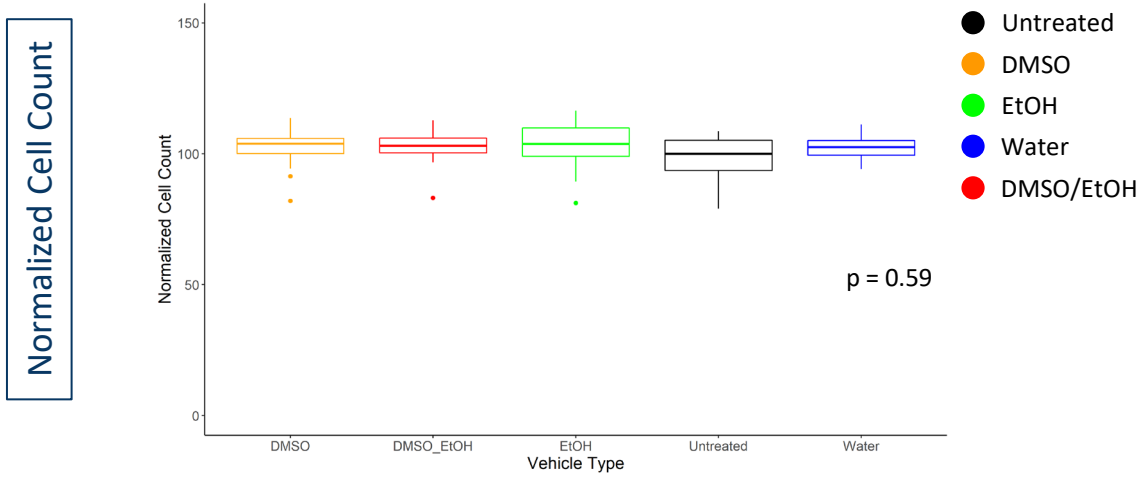


Staurosporine induced significant effects on cell health endpoints

A



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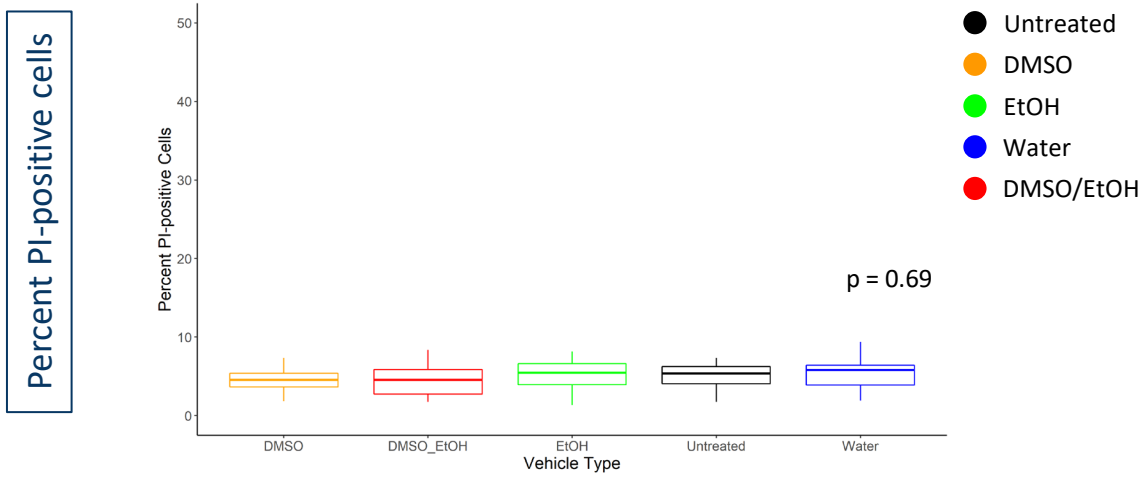

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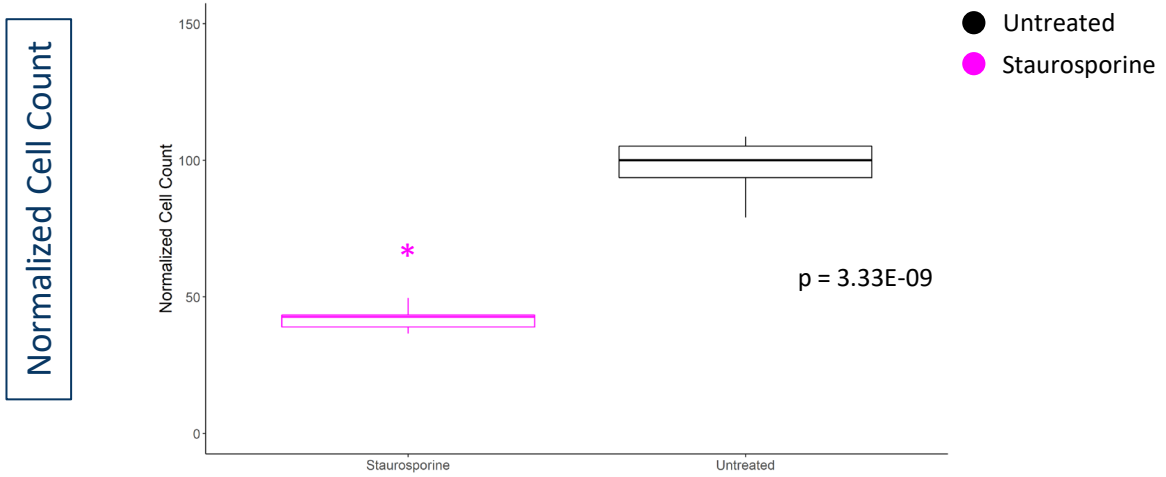

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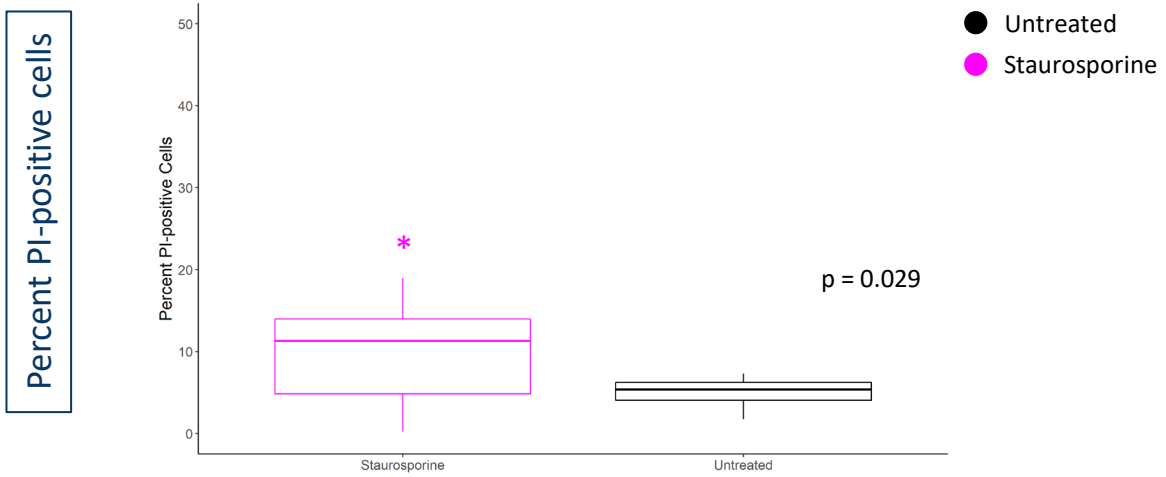
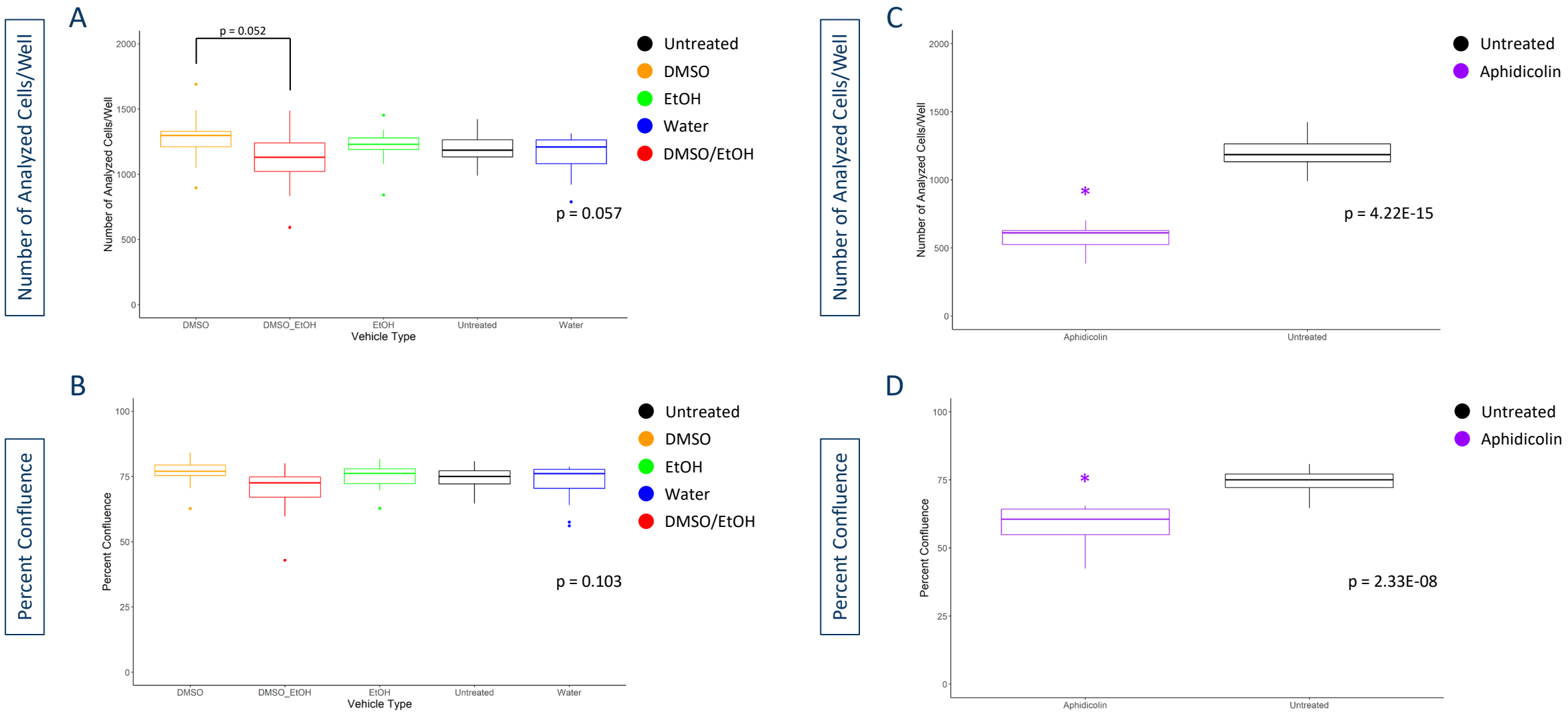
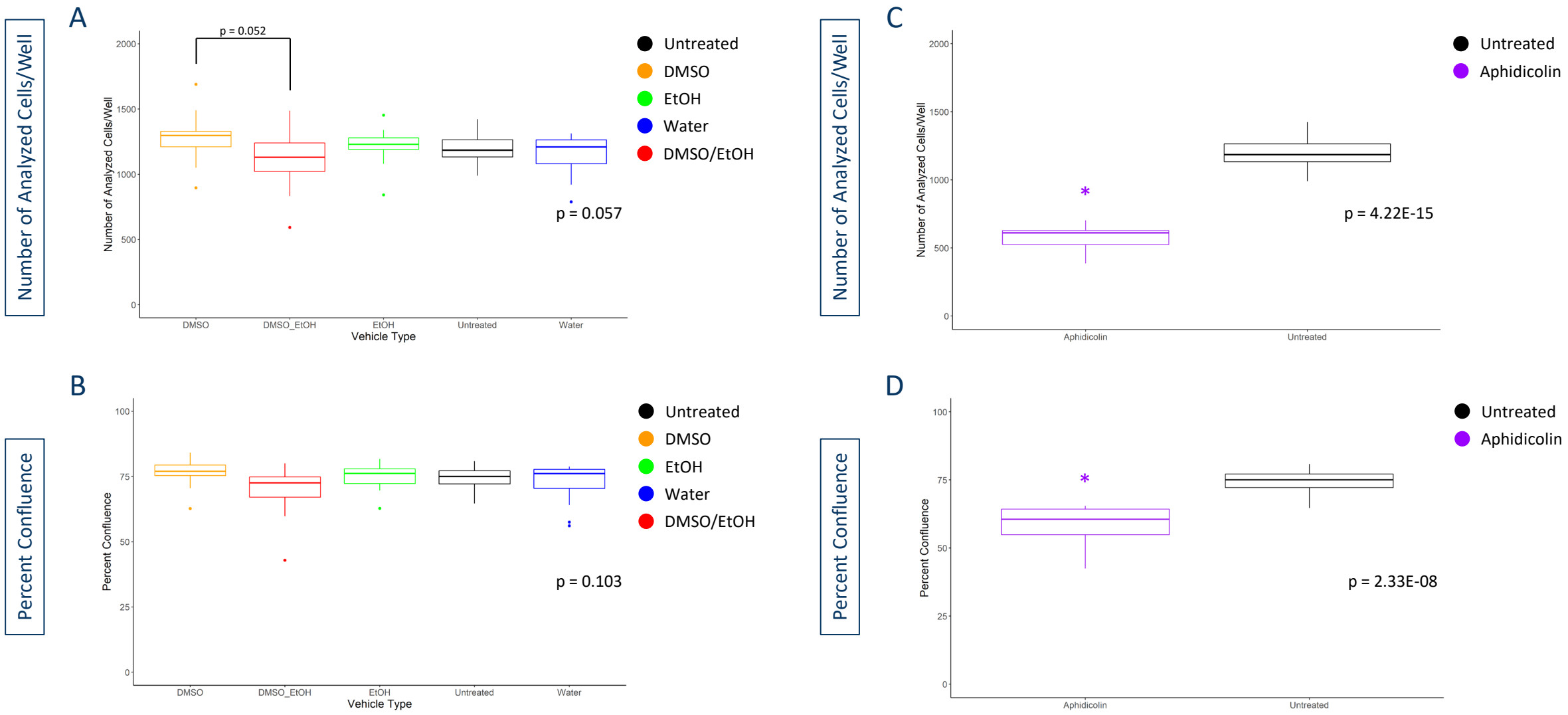


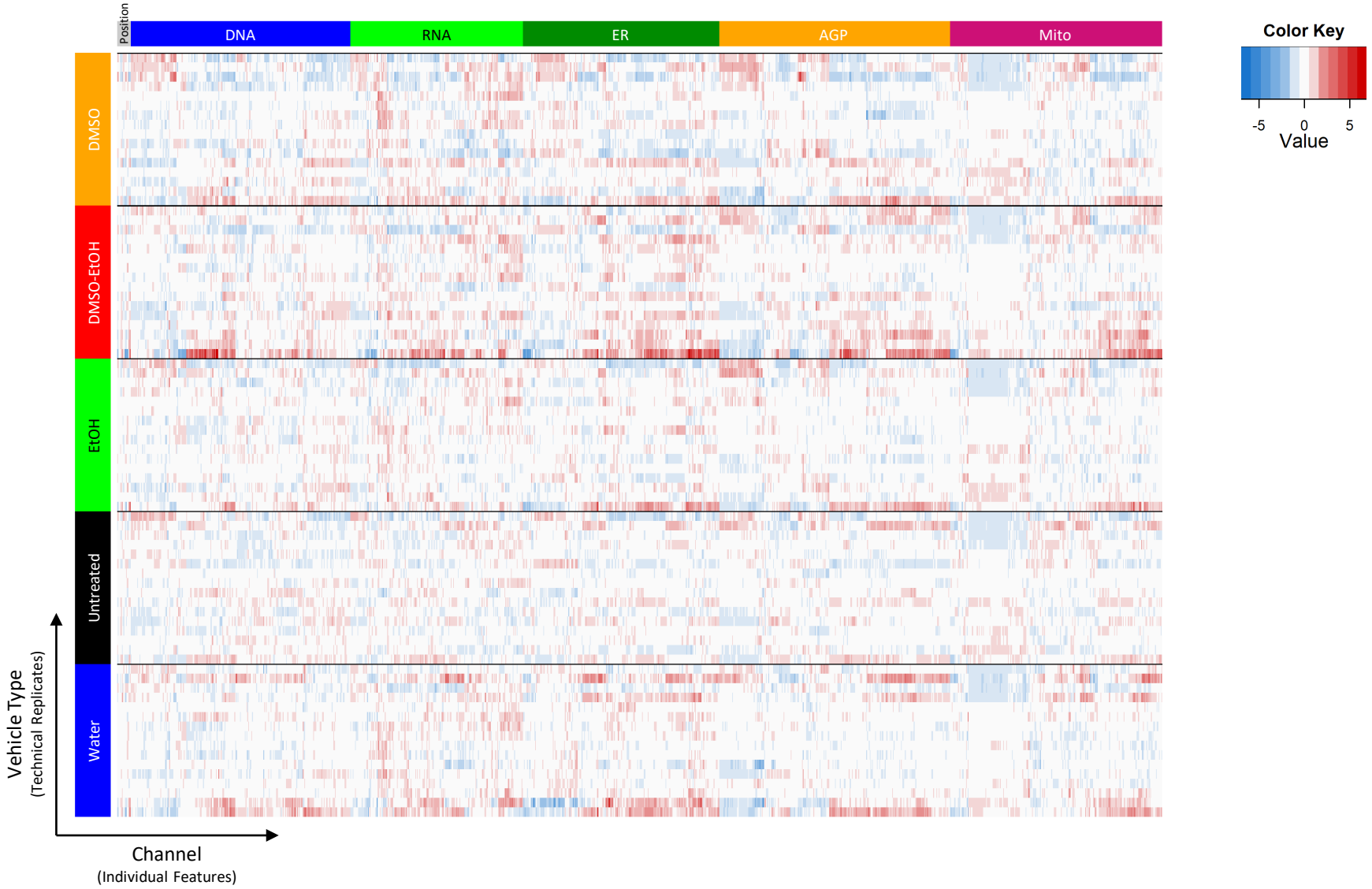
The dosing vehicles did not affect cell growth parameters



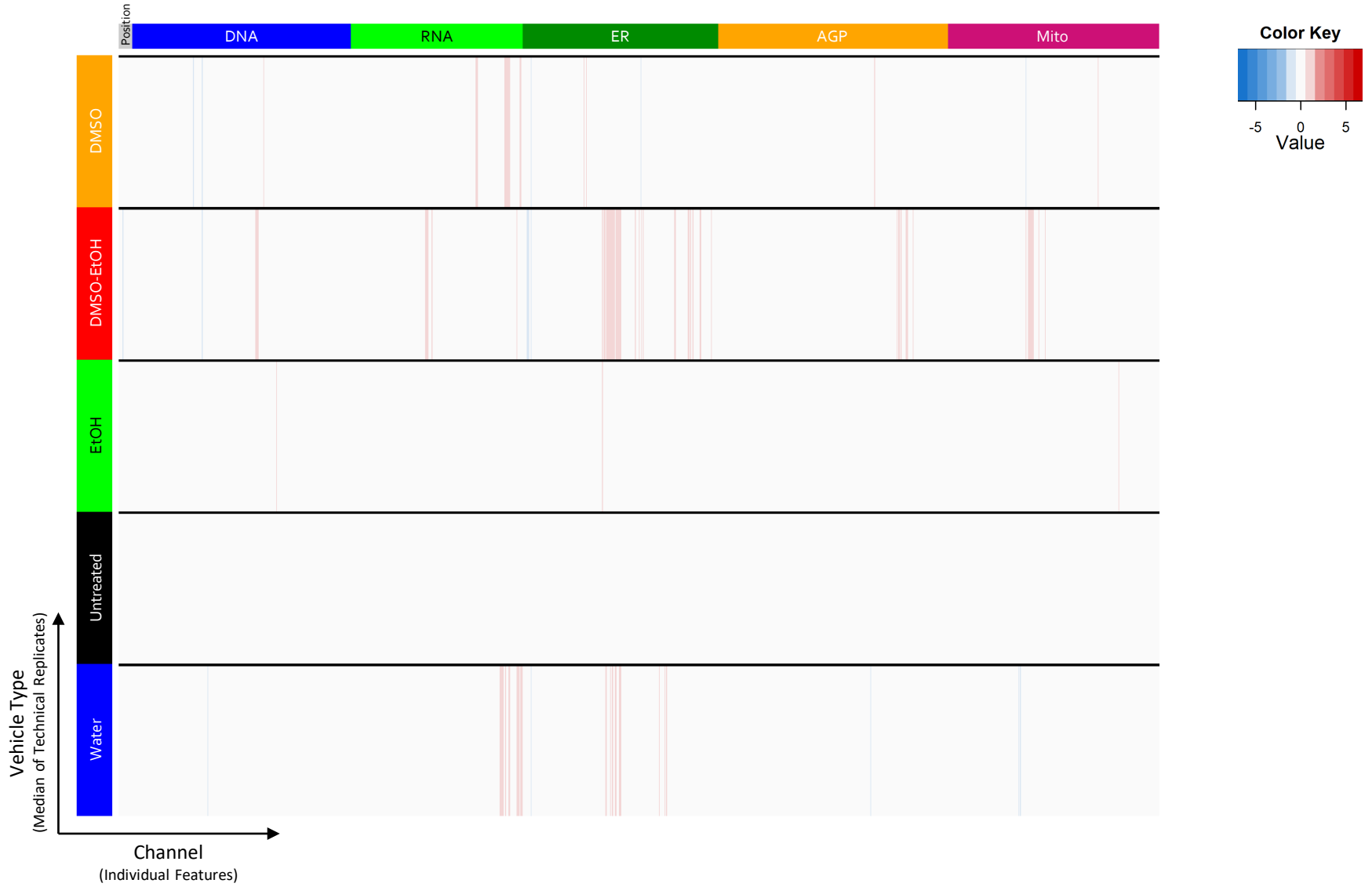
Aphidicolin elicited a marked decrease on cell growth parameters



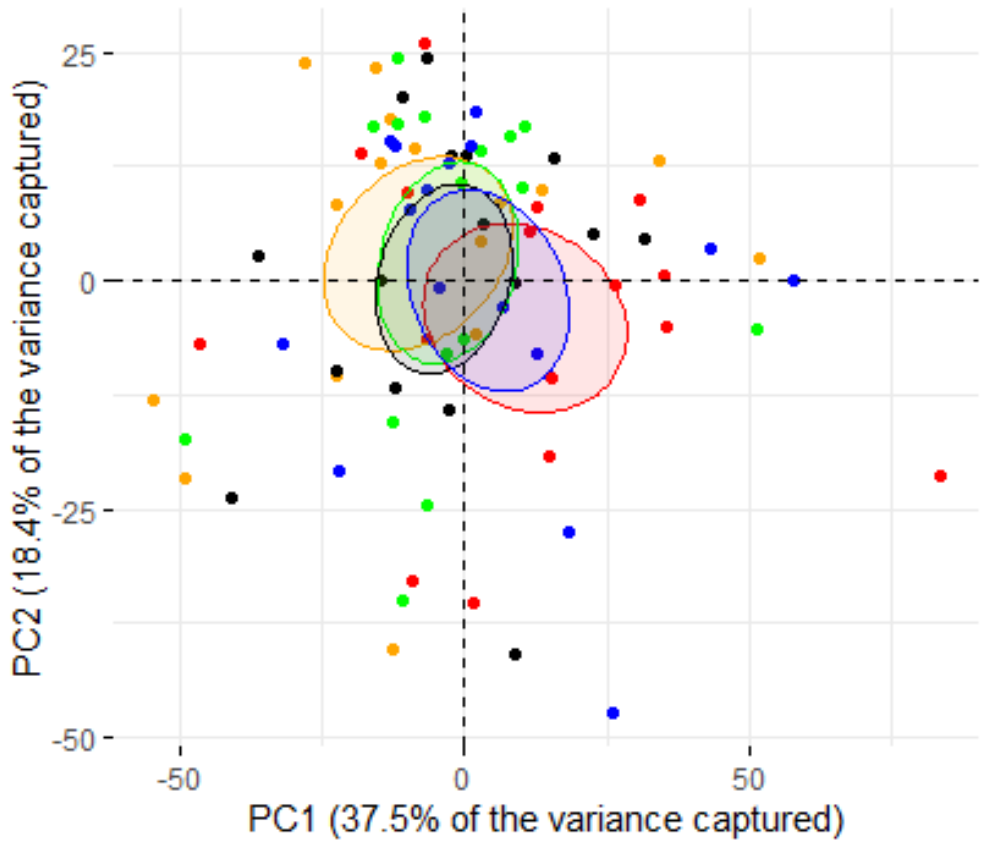
Dosing vehicle phenotypic profiles were qualitatively similar to untreated controls



No one individual feature was uniquely affected by all dosing vehicles



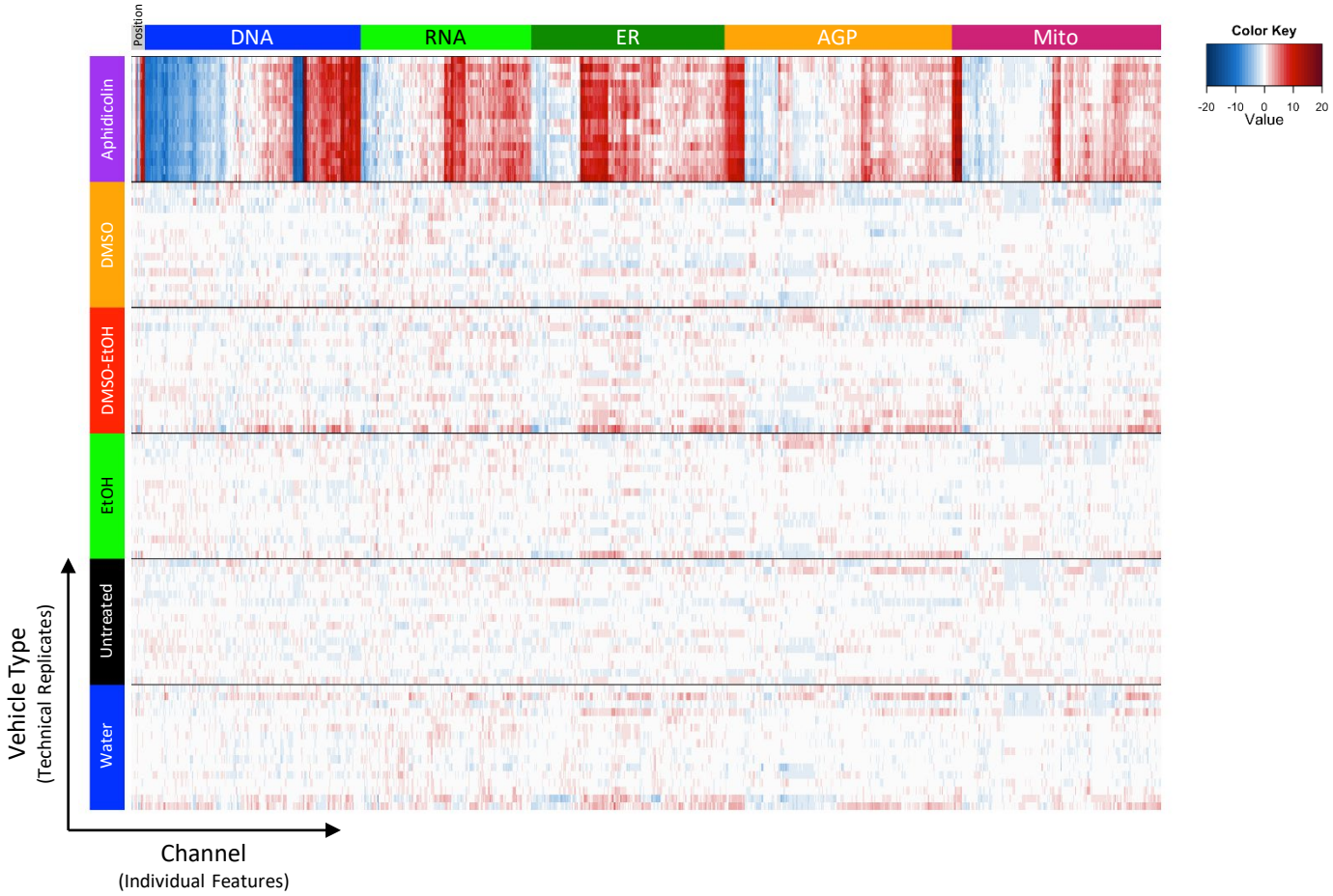
DMSO-EtOH had the more unique phenotypic profile relative to untreated controls

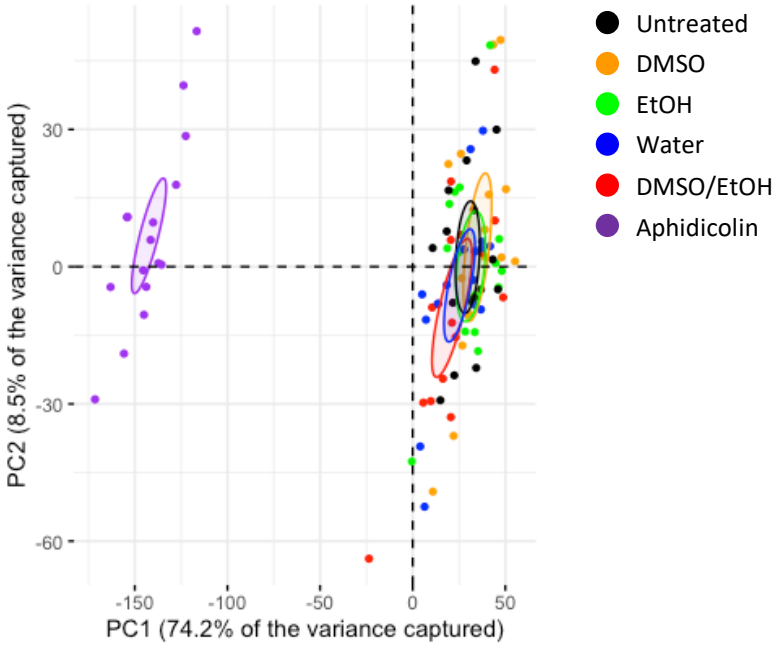


- Untreated
- DMSO
- EtOH
- Water
- DMSO/EtOH

Vehicle Type	Centroid Distance
Untreated	-
DMSO	5.3
EtOH	1.9
Water	7.8
DMSO/EtOH	14.9

Aphidicolin significantly altered the phenotypic profile of the hNP1 cells





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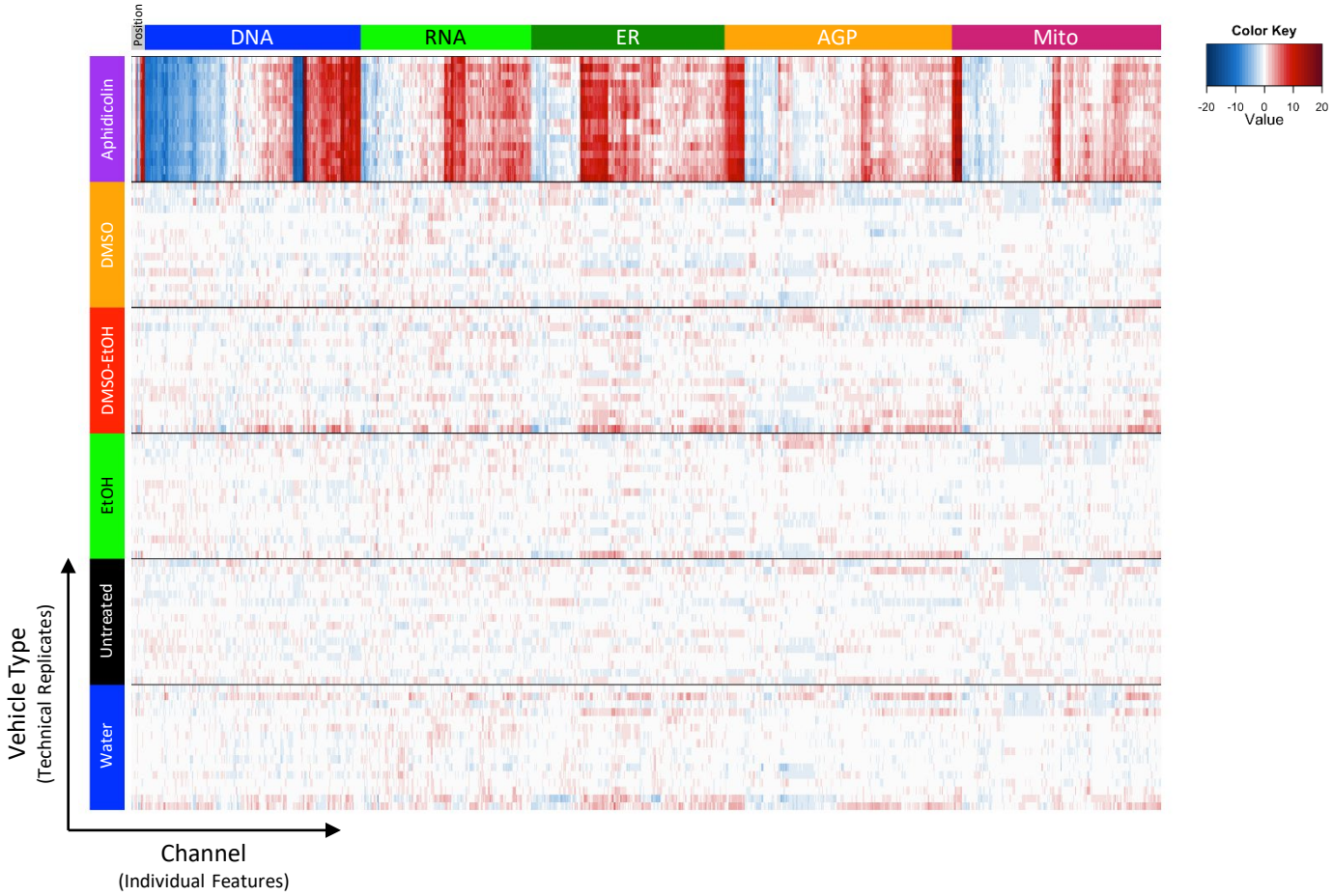
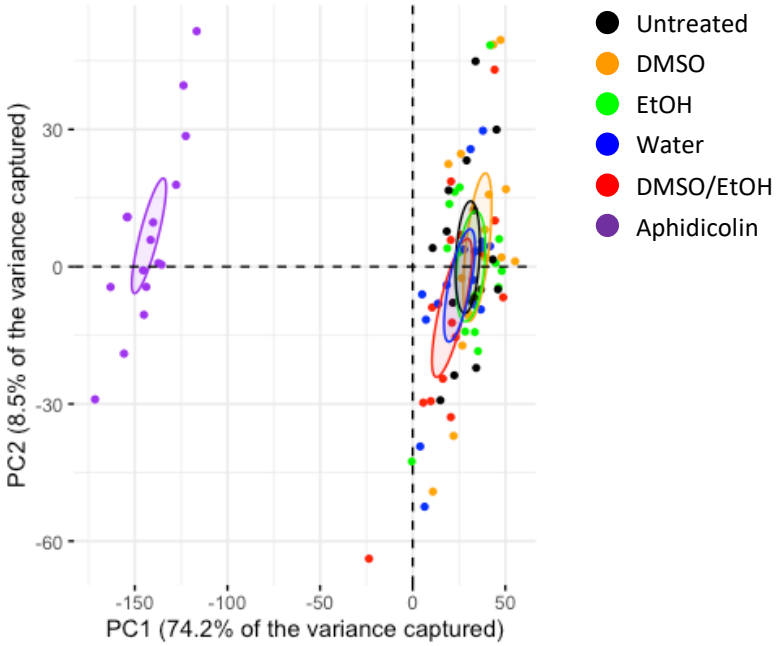




Vehicle Type	Centroid Distance
Untreated	-
DMSO	5.7
EtOH	2.6
Water	7.6
DMSO/EtOH	13.9
Aphidicolin	172.1

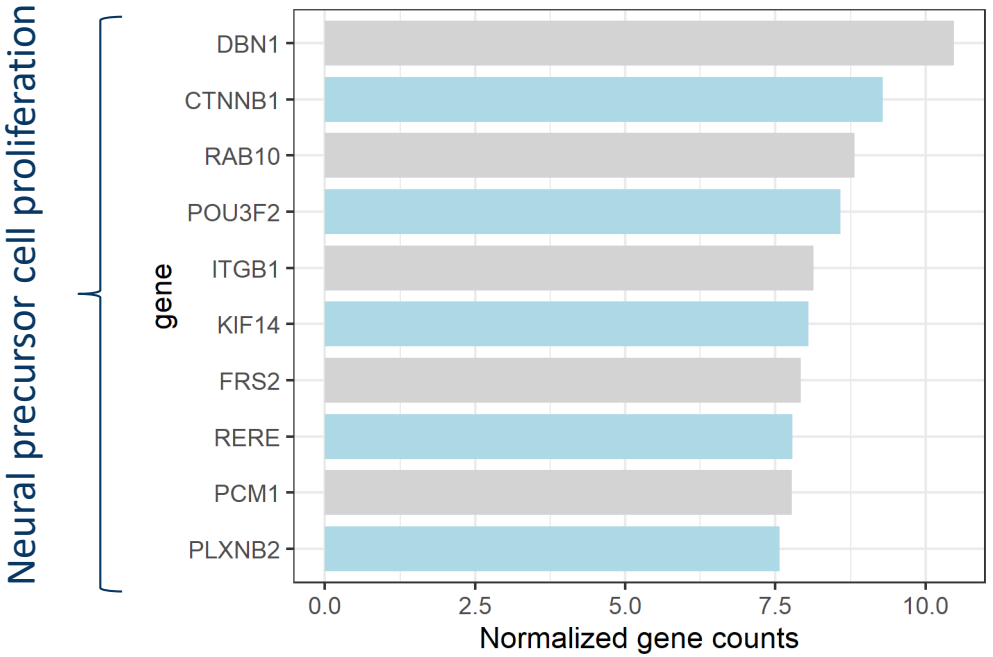
Conclusions

- Dosing vehicle responses were not identical to untreated in the HTPP approach; however, differences were marginal compared to assay positive-controls
- As HTPP is a more broad-based approach, it remains to be determined whether variances observed here would persist across neurodevelopmental-specific DNT NAM endpoints
- Differences cannot yet be distinguished from potential technical variability, but must be characterized to validate any assay at the level of the cell model
- Inclusion of untreated control wells as a “static negative” control may aide the assessment of within plate variability and possibly biological variability as well

Future Directions

1. Evaluate the whole transcriptome profile of each dosing vehicle, as potentially a more sensitive indicator of effect

High-throughput transcriptomic (HTTr) → TempO-Seq
whole transcriptome analysis



Each bar represents 2 biological replicates
(3 technical replicates/biological replicate)

Future Directions

1. Evaluate the whole transcriptome profile of each dosing vehicle, as potentially a more sensitive indicator of effects

2. Generate baseline whole transcriptome profiles for *in vitro* DNT NAMs cell models

High-throughput transcriptomic (HTTr) → TempO-Seq
whole transcriptome analysis

Assay	Species	Cell Type
HTPP (CV and CP)	human and rodent	neural progenitors (hNP1; mCNS)
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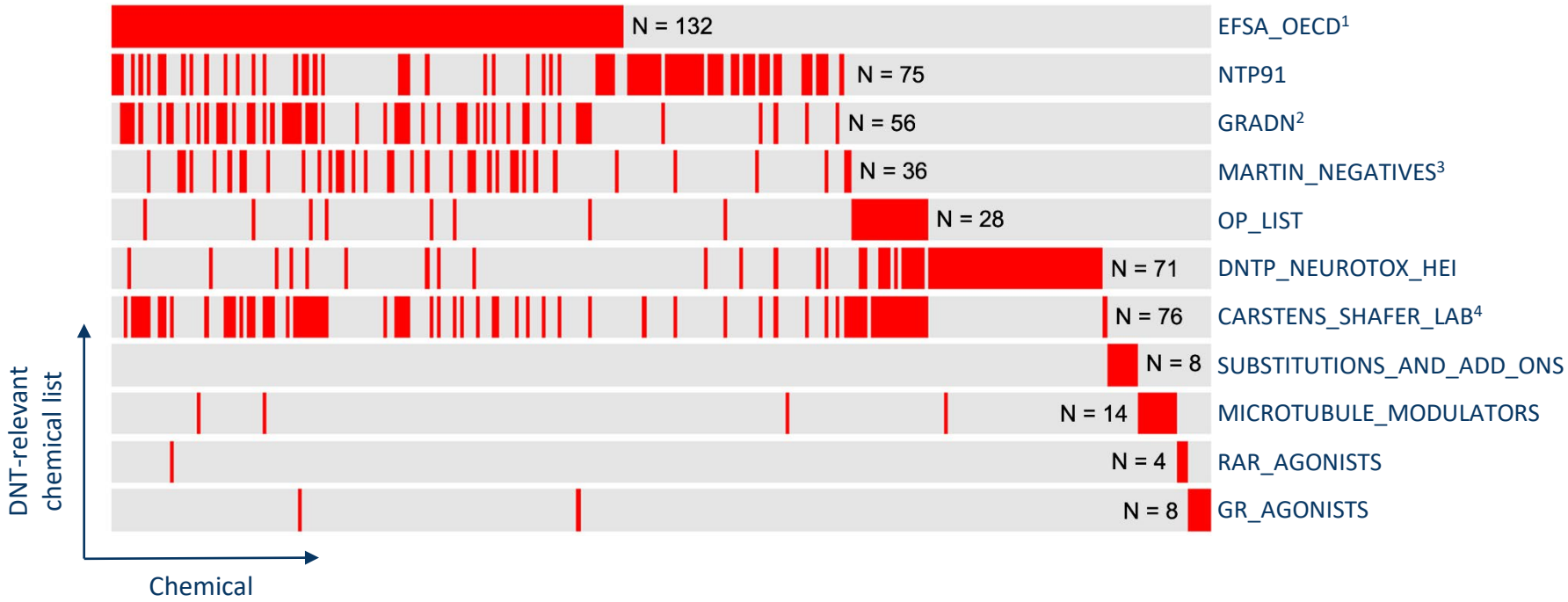
Apoptosis	human	neural progenitors (hNP1)
Neurite Outgrowth	human and rodent	neurons (iCell Gluta; primary rat cortical)
Synaptogenesis	rodent	neurons (primary rat cortical)
Network Formation and Function	human and rodent	neurons (SynFire; primary rat cortical)

Future Directions

1. Evaluate the whole transcriptome profile of each dosing vehicle, as potentially a more sensitive indicator of effects

2. Generate baseline whole transcriptome profiles for *in vitro* DNT NAMs cell models

3. Screen 284 DNT-relevant compounds in the HTPP approach



[1]Masjosthusmann et al., 2020 (doi: 10.2903/sp.efsa.2020.EN-1938); [2]Mundy et al., 2015 (doi: 10.1016/j.ntt.2015.10.001); [3]Martin et al., 2022 (doi: 10.1016/j.ntt.2022.107117); [4]Carstens et al., 2022 (doi: 10.1093/toxsci/kfac018)

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Seline Choo
Theresa Freudenrich
Kathleen Wallace