High-throughput phenotypic profiling (HTPP) of chemical-induced changes in human neural progenitor cell morphology for developmental neurotoxicity (DNT) hazard assessment

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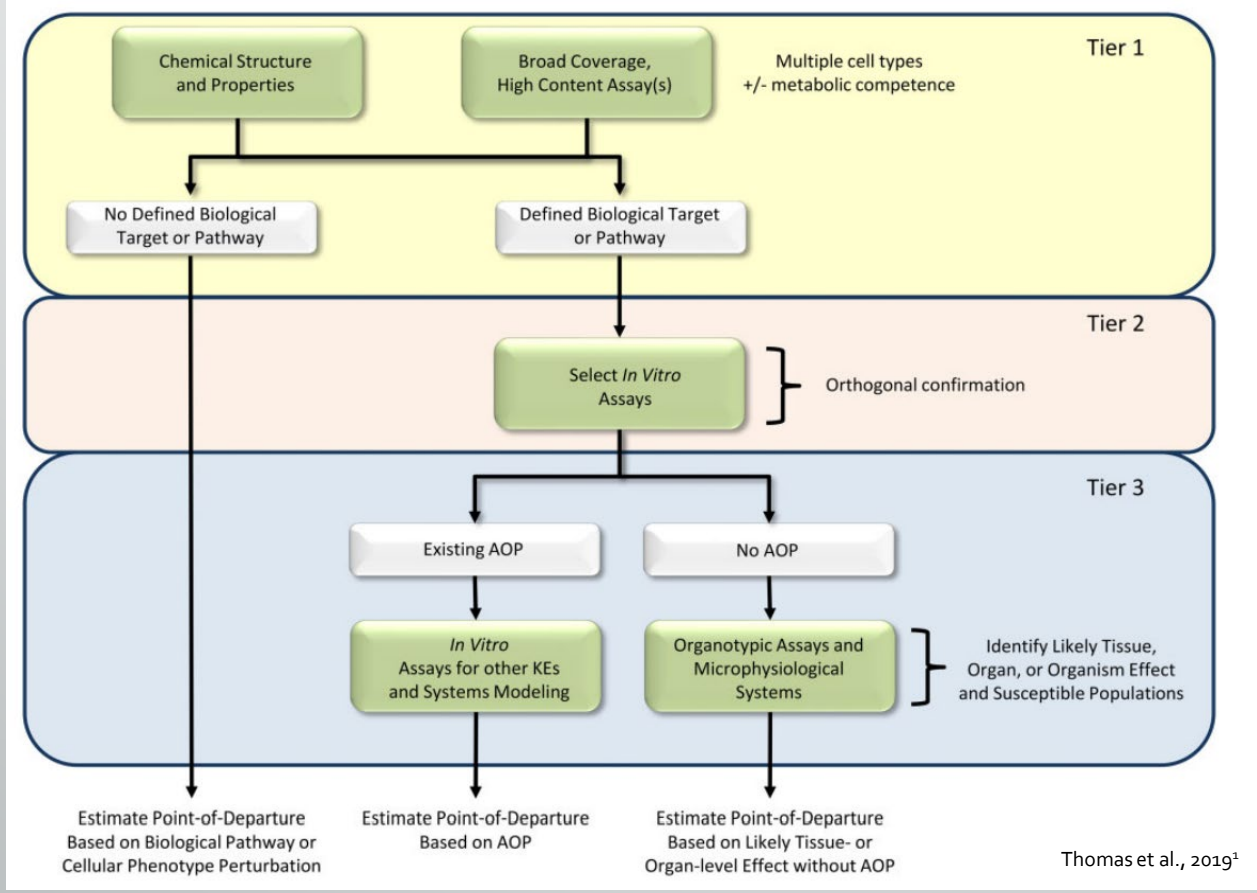
Office of Research and Development
Center for Computational Toxicology & Exposure

Disclaimer

The views expressed in this presentation are those of the author and do not necessarily reflect U.S. EPA policy. Mention of any trade names does not constitute endorsement.

The current DNT *in vitro* battery (IVB) only includes Tier 2 assays



Thomas et al., 2019[1]

Process	Cell Type	Species	Reference
Proliferation	hNP1	human	Harrill et al., 2018
	NPC1	human and rodent	Baumann et al., 2016
	UKN1	human	Balmer et al., 2012
Apoptosis	hNP1	human	Harrill et al., 2018
Migration	NPC2	human and rodent	Baumann et al., 2016
	UKN2	human	Nyffeler et al., 2017
Neuron differentiation	NPC3	human and rodent	Baumann et al., 2016
Oligodendrocyte differentiation & maturation	NPC5/6	human and rodent	Baumann et al., 2016
Neurite outgrowth	iCell Gluta	human	Druwe et al., 2016
	UKN 4 & 5	human	Krug et al., 2013
	NPC4	human and rodent	Baumann et al., 2016
Synaptogenesis	Primary rat cortical	rodent	Harrill et al., 2018
Network Formation (MEA-NFA)	Primary rat cortical	rodent	Brown et al., 2016 and Frank et al., 2018

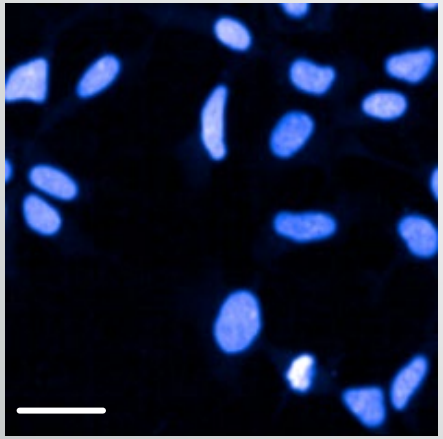
Modified from Sachana et al., 2019[2]

Tier 1 approaches are higher-throughput and do not require a known biological target

[1]doi: 10.1093/toxsci/kfz058; [2] doi: 10.193/toxsci/kfy211

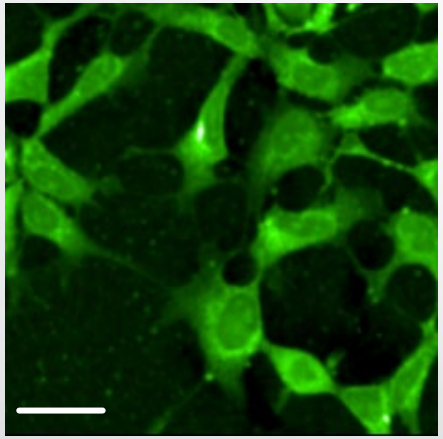
High-Throughput Phenotypic Profiling (HTPP)

Nuclei (DNA)



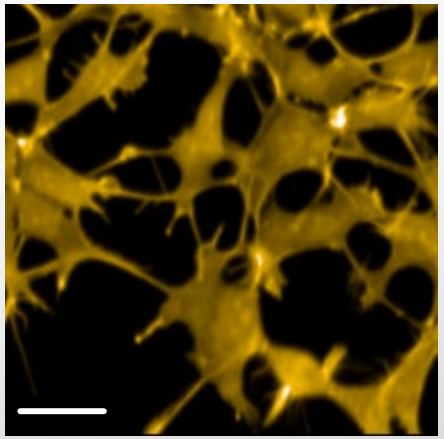
Hoechst 33342

Nucleoli (RNA) +
Endoplasmic reticulum (ER)



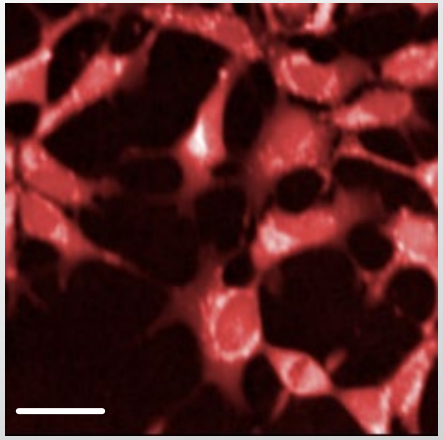
SYTO14/Alexa 488

Actin cytoskeleton,
Golgi apparatus,
plasma membrane
(AGP)



Alexa 568/555

Mitochondria

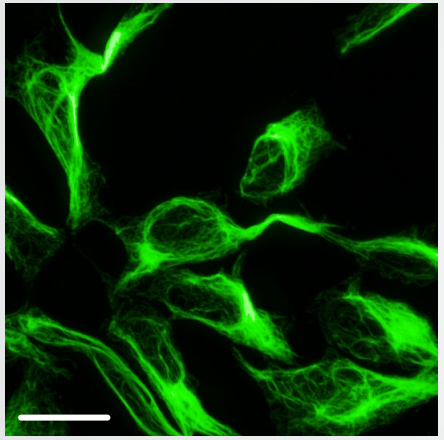


MitoTracker

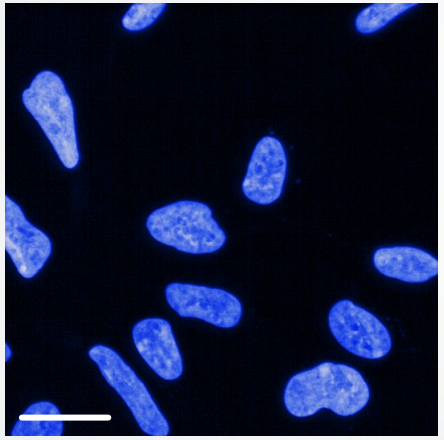
Cell-level feature extraction

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

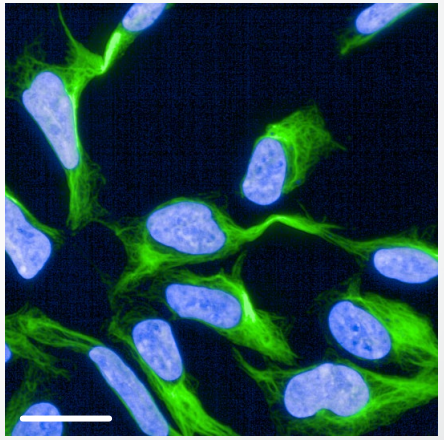
Nestin



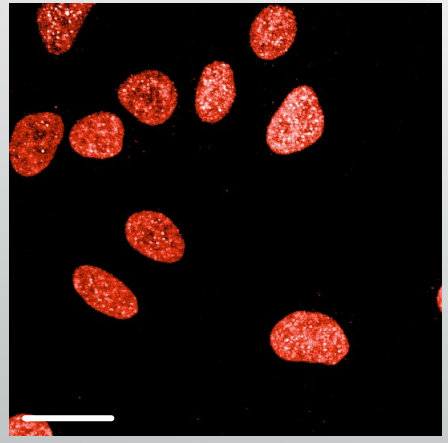
Hoechst



Composite



Sox2



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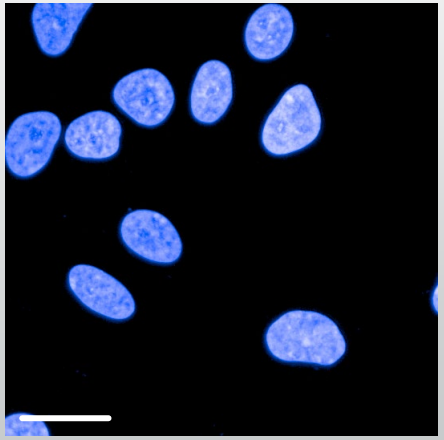

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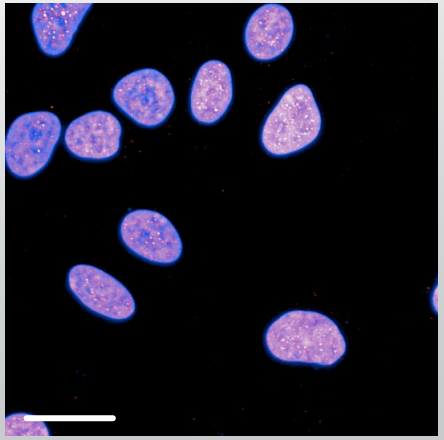
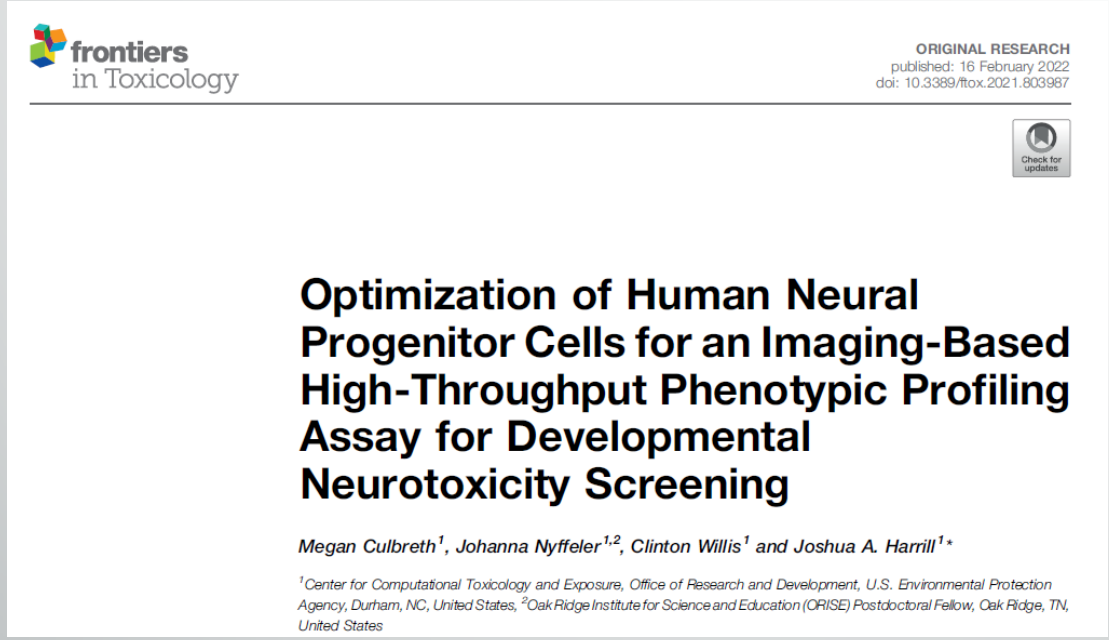


The method for HTPP of hNP1 cells has been established



frontiers in Toxicology

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

Check for updates

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]**

[1]Center for Computational Toxicology and Exposure, Office of Research and Development, U.S. Environmental Protection Agency, Durham, NC, United States, [2]Oak Ridge Institute for Science and Education (ORISE) Postdoctoral Fellow, Oak Ridge, TN, United States

- Optimized high-throughput automation
  - 384-well plate coating
- Determined an appropriate plating density in 384-well plates
- Identified the minimum laminin concentration required for optimal cell growth and attachment
- Demonstrated antibiotic addition to the culture medium does not alter cellular morphology
- Selected applicable in-plate assay controls

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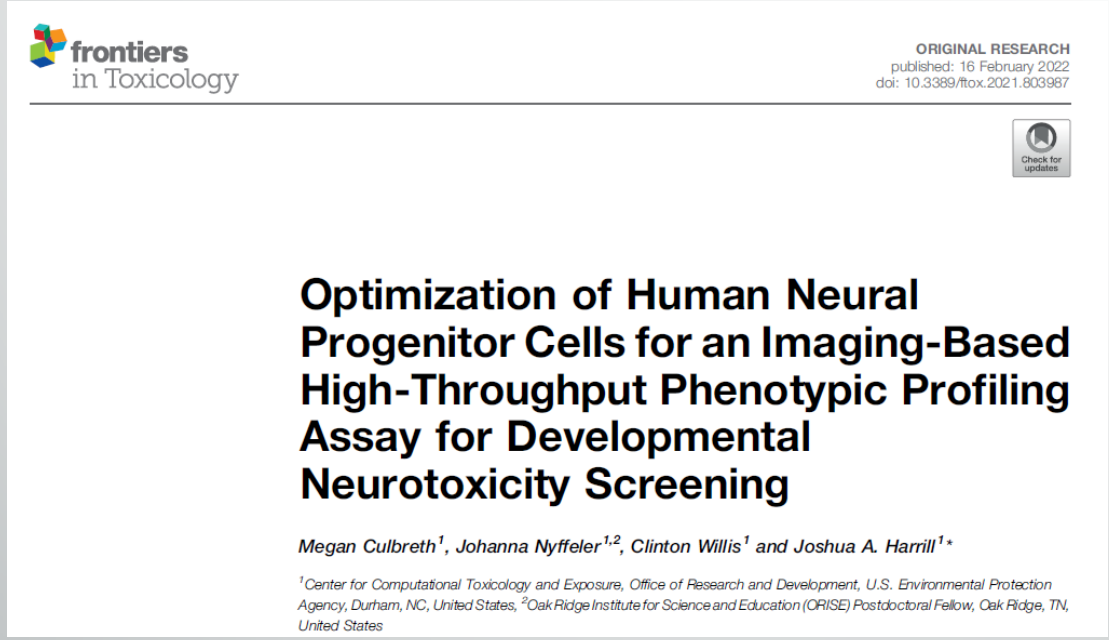

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Some HTPP reference chemicals evaluated as potential in-plate assay controls were previously assessed in DNT-IVB assays

Reference chemical	Mechanism of action (MoA)	DNT-IVB	Anticipated phenotype	Reference
Aphidicolin	DNA polymerase inhibitor	Proliferation	Modification to DNA compartment	Unpublished data
Actinomycin D	Transcription inhibitor	Apoptosis	Modification to RNA compartment	Unpublished data
Etoposide	DNA Topoisomerase II inhibitor	Neurite outgrowth	Increased cell size	Willis et al., 2020[1]

Modified from Culbreth et al., 2022

[1]doi: 10.1177/2472555220928004

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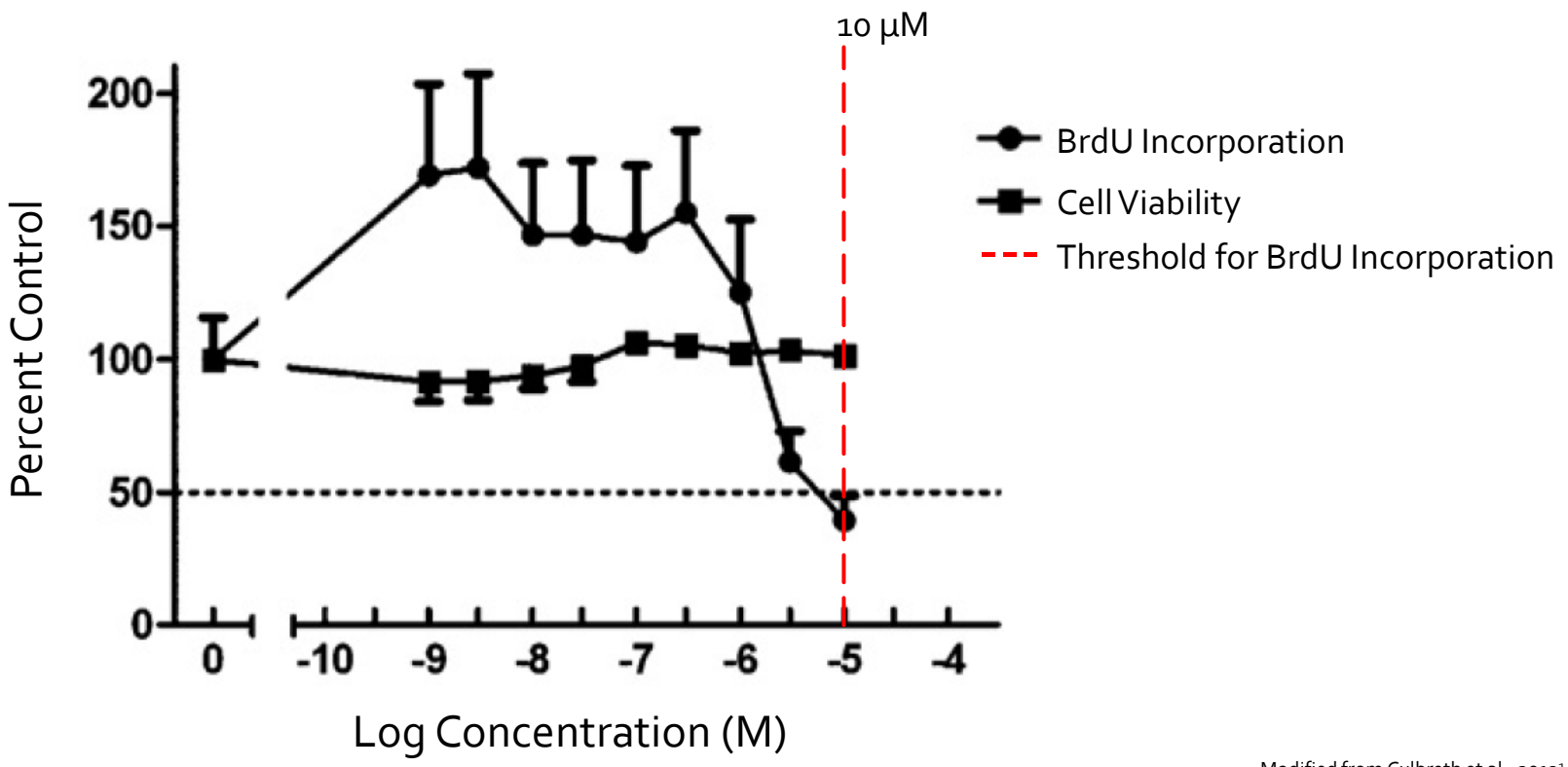
Aphidicolin has historically been utilized as a proliferation assay positive control in human neural progenitor cells



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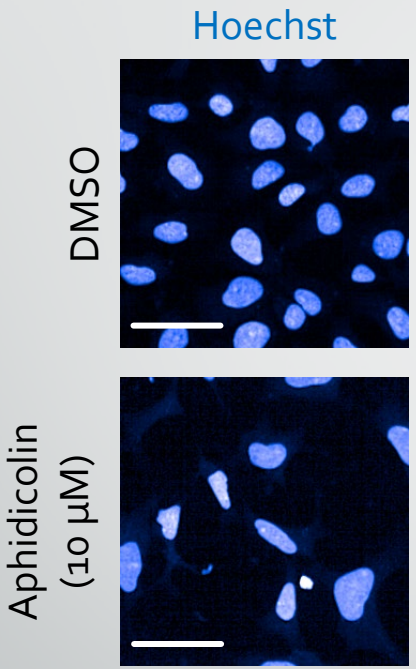
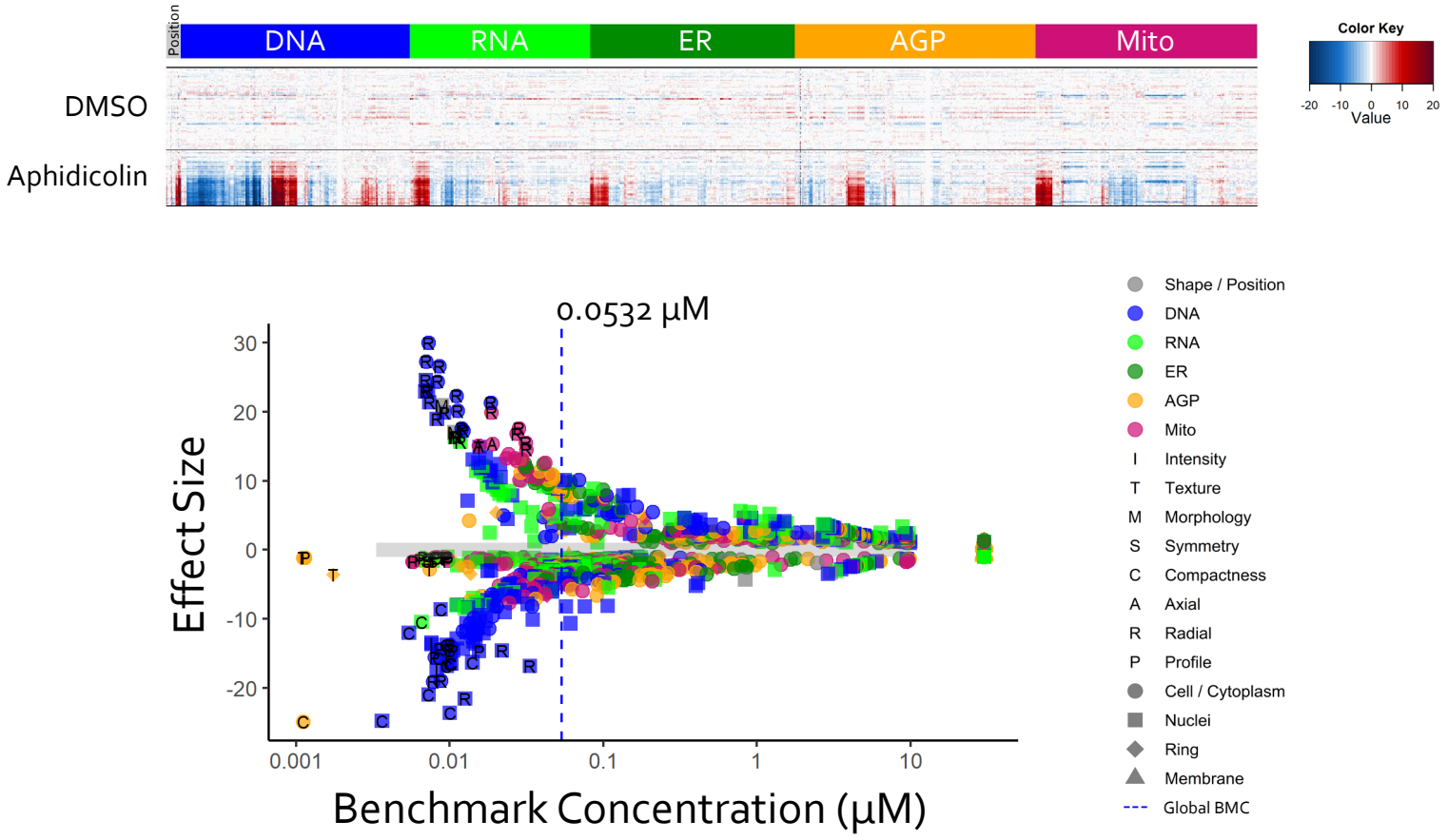
[1]doi: 10.1016/j.neuro.2012.05.012

Aphidicolin selected as an HTPP in-plate assay control for hNP1 cells





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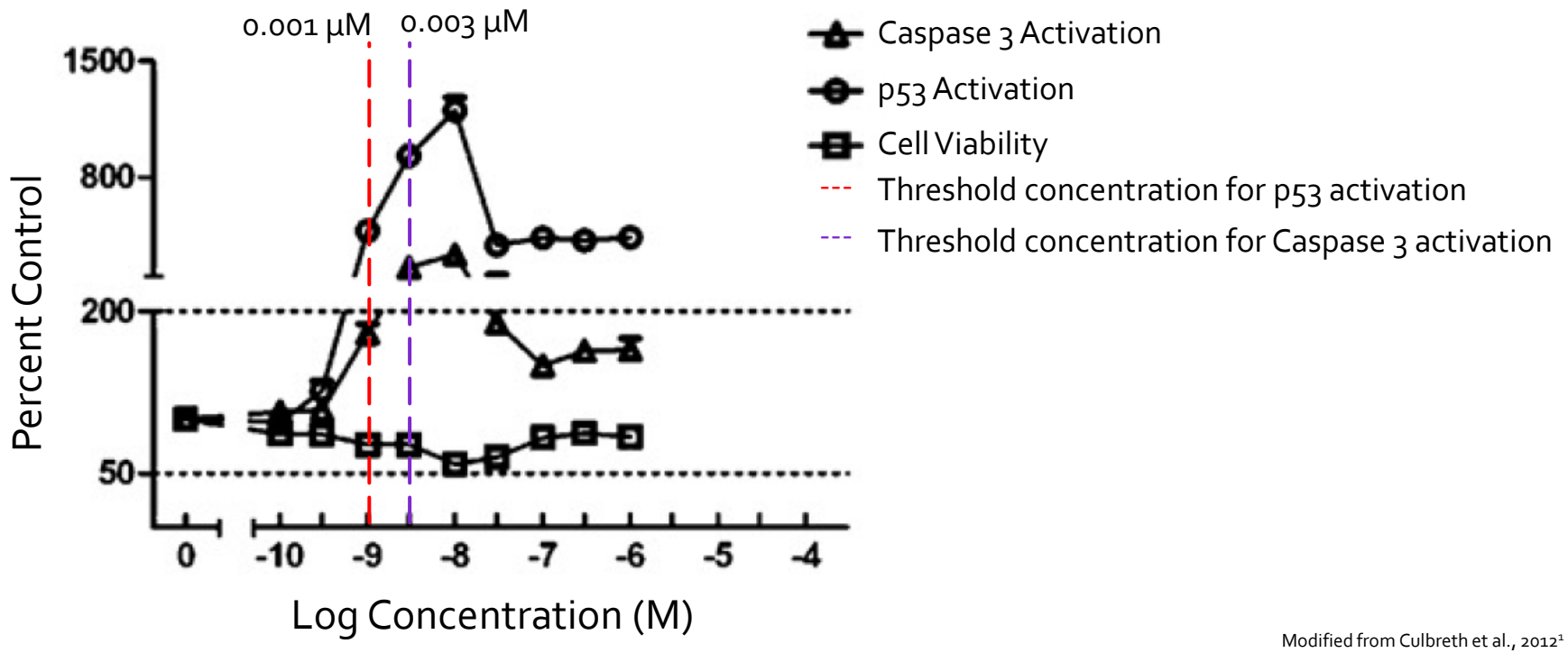
Actinomycin D was previously utilized as an apoptosis assay positive control in human neural progenitor cells



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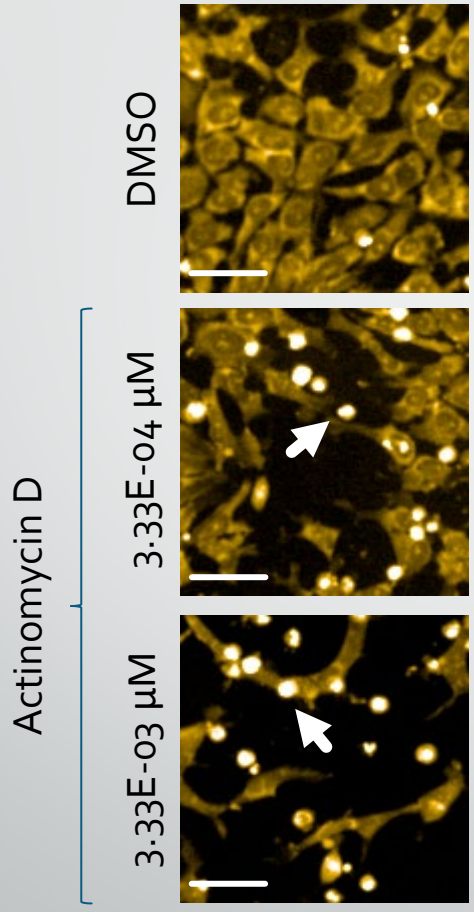
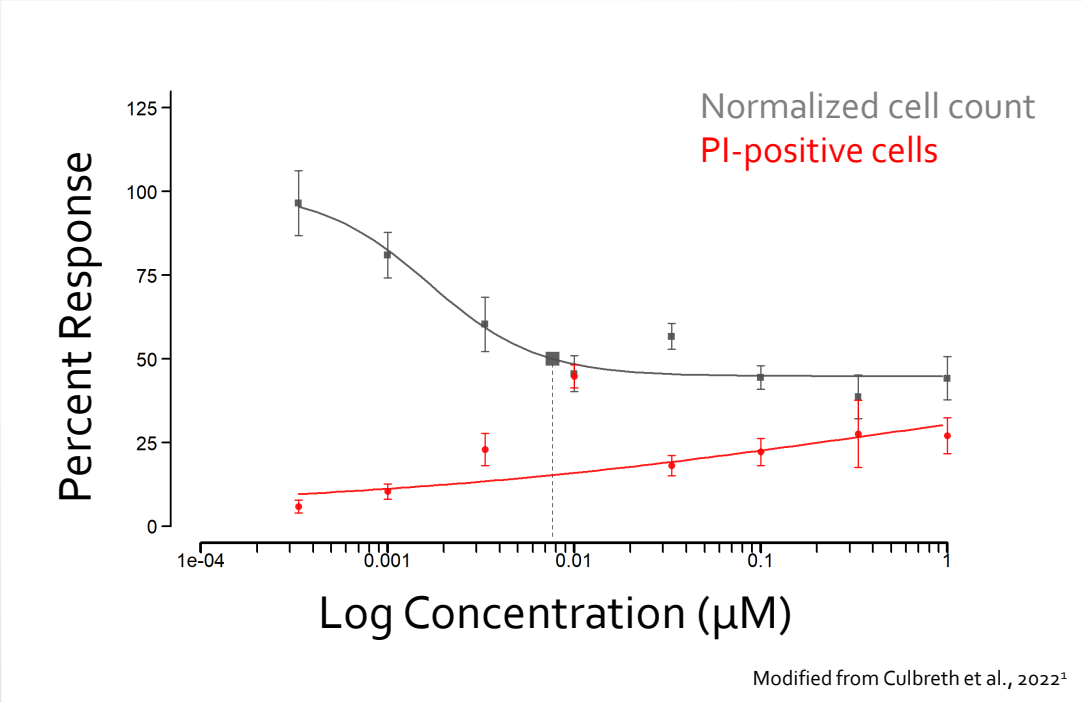
Phenotypic effects of Actinomycin D could not be modeled due to overt cytotoxicity

Propidium iodide (PI)



p53 and Caspase 3 activated in apoptosis assay



Modified from Culbreth et al., 2022[1]

	Benchmark Concentration (µM)
Normalized cell count	0.00767
PI-positive cells	0.0000496

Below tested concentration range

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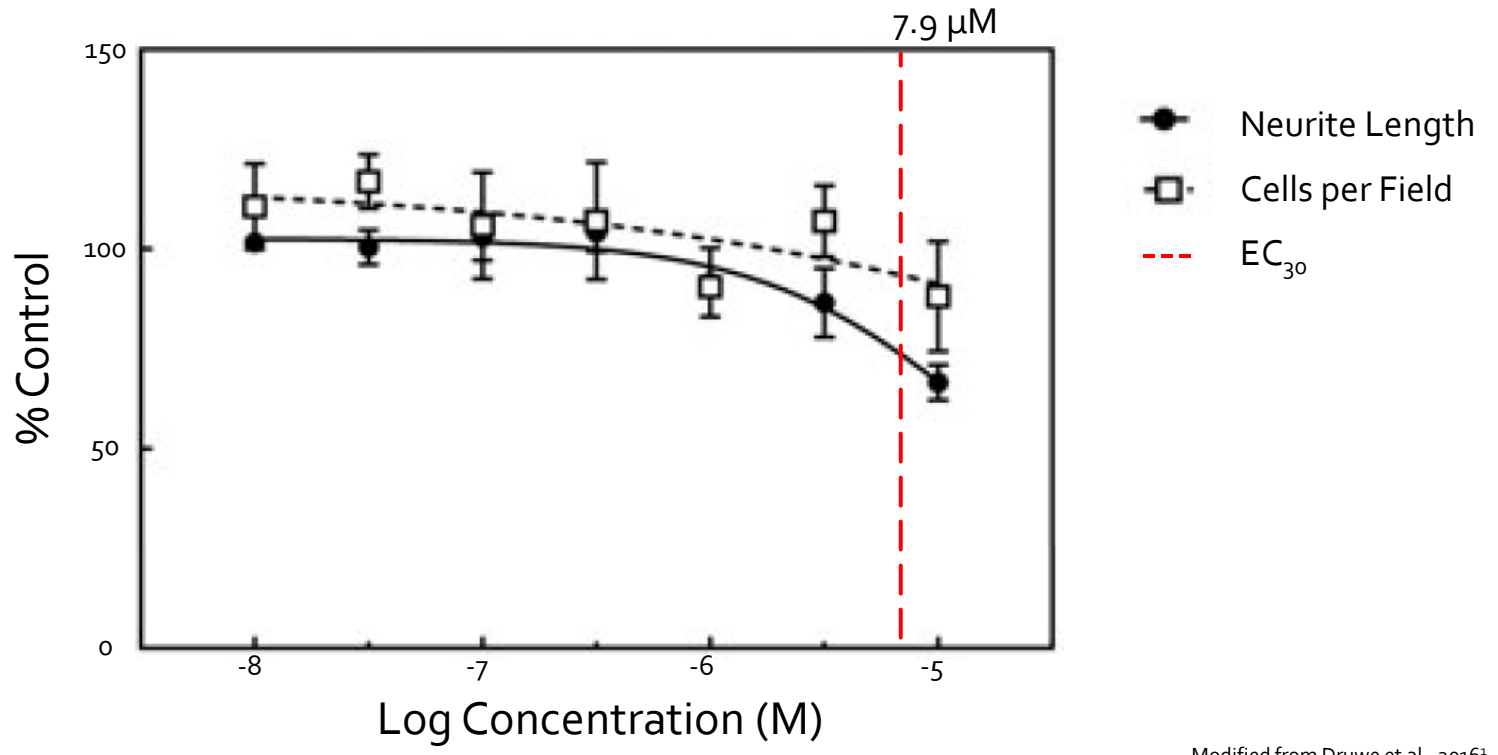
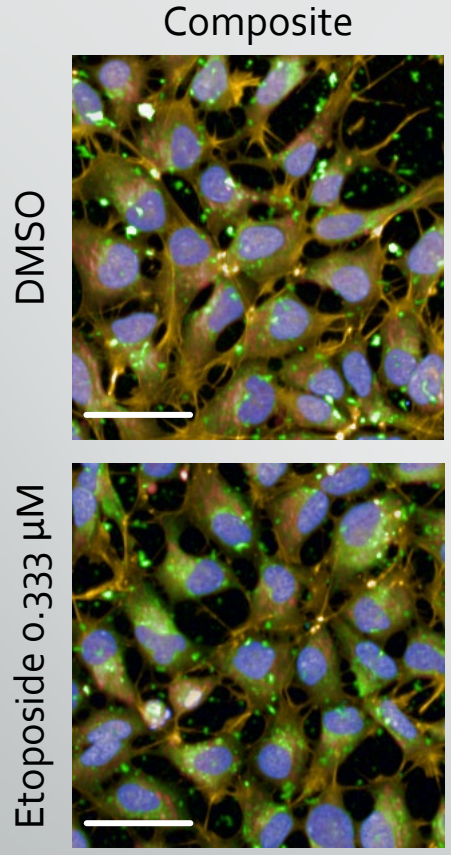
Etoposide inhibited neurite outgrowth of human IPSC-derived neurons

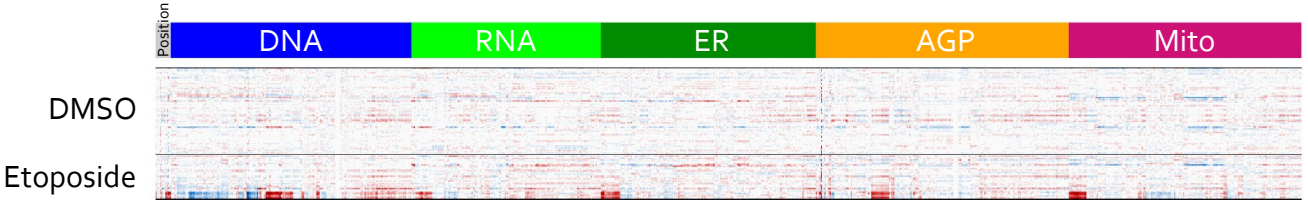


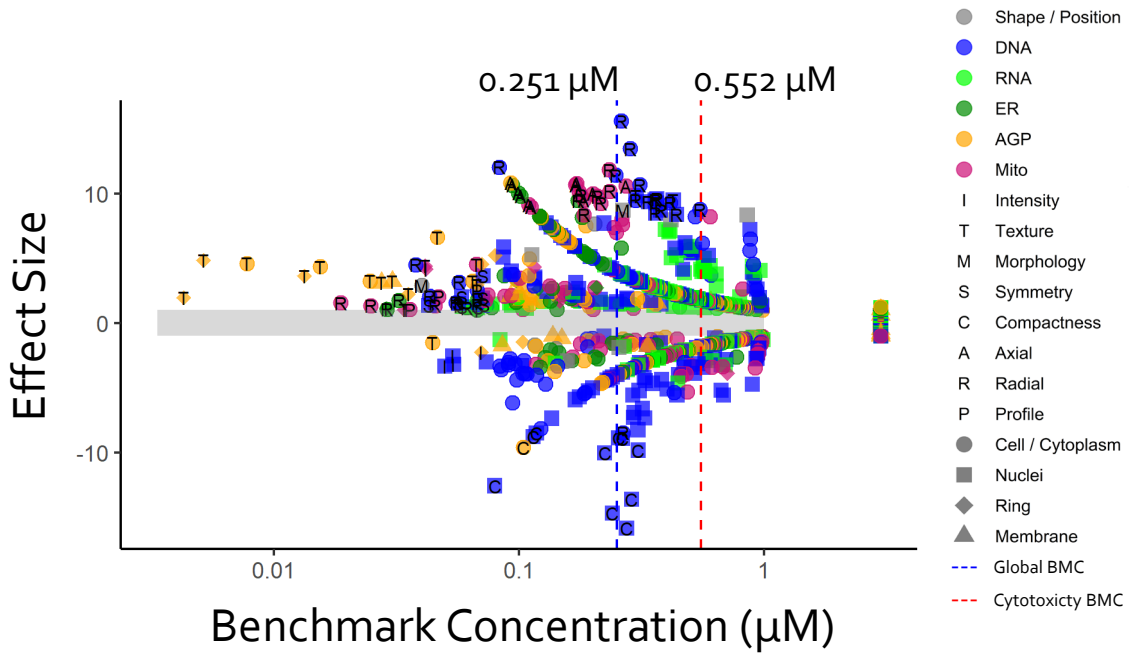
Modified from Druwe et al., 2016[1]

[1]doi: 10.1089/aivt.2015.0025

Etoposide altered hNP1 cellular phenotype below the threshold of effect on neurite outgrowth







EC_{30} for neurite outgrowth = 7.9 µM

Modified from Culbreth et al., 2022[1]

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Conclusions

- hNP1 human neural progenitor cells are amenable to an HTPP approach for chemical prioritization
- Historic DNT-IVB positive control compounds exhibited variable phenotypic effects
  - Aphidicolin altered the DNA compartment below the threshold for effects on cell proliferation
  - Actinomycin D was overtly cytotoxic relative to previous results
- An HTPP approach potentially can be utilized to identify compounds previously detected in DNT-IVB assays with other cell types
  - Etoposide induced phenotypic effects below the EC_{30} for neurite outgrowth

Future Directions

- Screen approximately 300 DNT-relevant compounds in the HTPP approach

DNT-relevant chemical list	Number of compounds
EFSA_OECD	132
NTP91	75
GRADN	56
MARTIN_NEGATIVES	36
OP_LIST	28
DNTP_NEUROTOX_HEI	71
CARSTENS_SHAFER_LAB	76
SUBSTITUTIONS_AND_ADD_ONS	8
MICROTUBULE_MODULATORS	14
RAR_AGONISTS	4
GR_AGONISTS	8

- Determine HTPP approach sensitivity and specificity relative to DNT-IVB assays
- Extrapolate phenotypic effects to historical *in vivo* and human DNT data

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Kathleen Wallace

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Sid Hunter

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