INTEGRATED -OMIC ANALYSIS FOLLOWING CHEMICAL-INDUCED ALTERATIONS OF NEURAL NETWORK FORMATION IN VITRO

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Background of the Study

- Early-life environmental exposures to developmental neurotoxicants carries a risk for children's health.
  - Neurotoxicant exposure is associated with increased risk of neurodevelopmental disorders (NDD)
  - NDDs have been linked with altered neuronal gene expression and metabolism

Problem

Thousands of chemicals in the environment have not been characterized for developmental neurotoxicity (DNT) hazard.

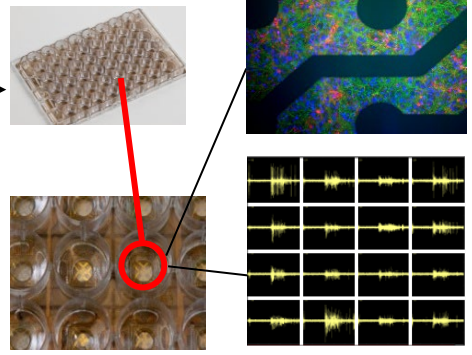
Objective

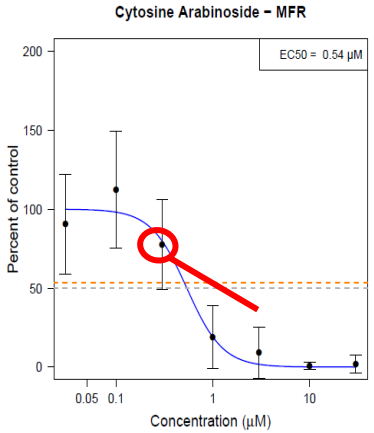
Identify nervous systems signaling pathways and upstream physiological regulators that may contribute to chemically-induced neural network dysfunction associated with altered neurophysiological development in vitro.

Summary of Data Analysis Approach:

Step 1: Chemical Dose Identification with MEA Assay

Compound exposure in rat cortical cells

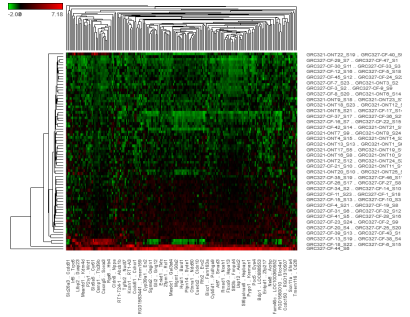


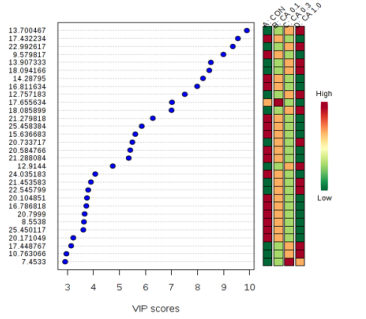


- Grid of extracellular electrodes
- Each electrode can record event rates and patterns of any electrically excitable tissue in “contact” with it.
- **Spontaneous** or **Evoked** activity in cortical networks

Critical concentration determined

Step 2: Identifying Key Pathways & Regulators Involved in Neurodevelopment





Transcriptomics

Metabolomics

Disease & Function Analysis

Impact:

Identified pathways and regulators will add to the integrated-omics strategy of resolving toxicological problems linked to the human health burden of NDD following chemical exposure.

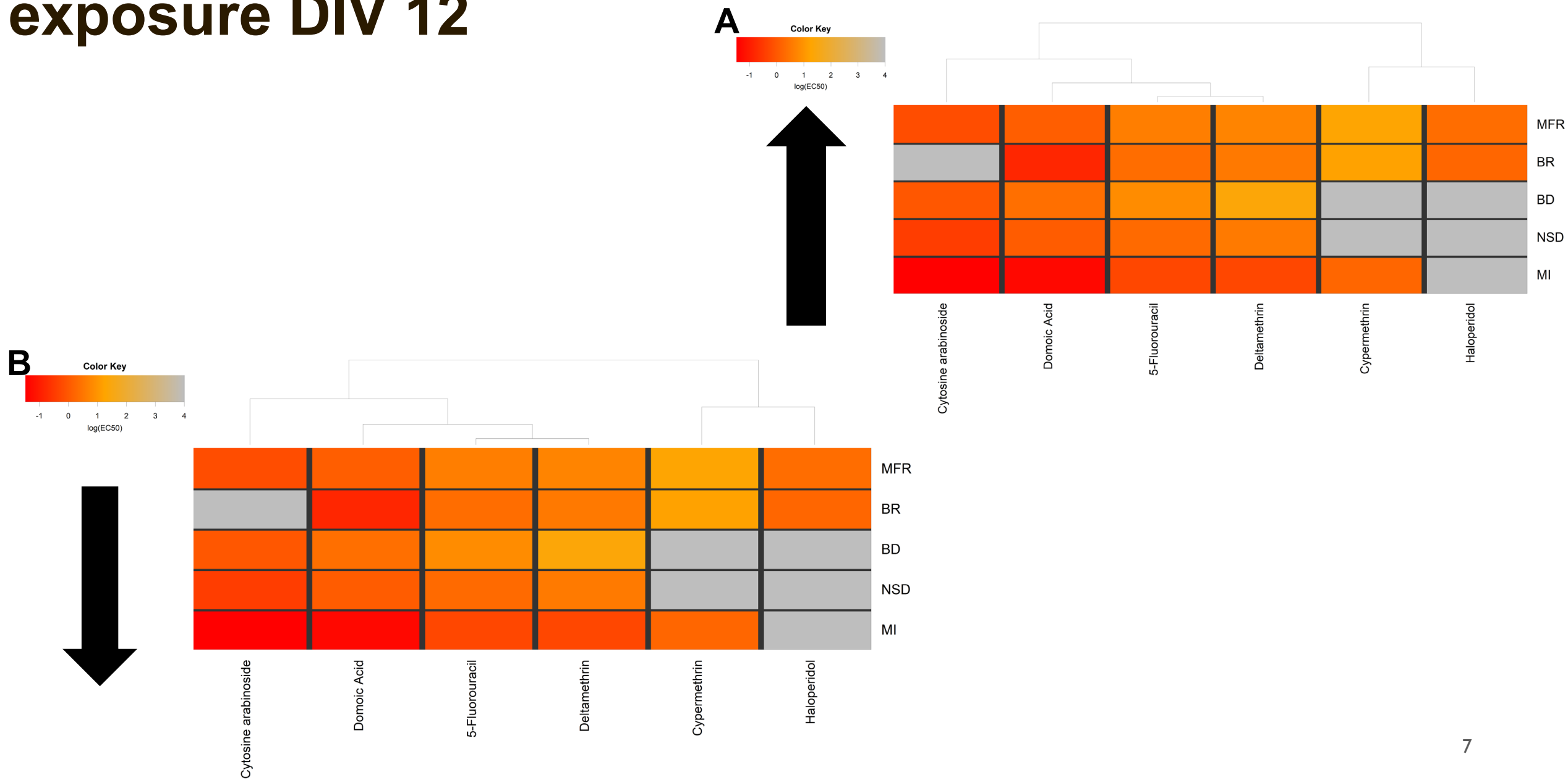
Neurotoxicant	Concentration (uM)	Exp fold change Direction for genes	Exp fold change Direction for metabolites
5 Fluorouracil	1	↓	↑
Domoic Acid	0.3	↑	↑
Cytosine Arabinoside	1	↓	↑
Deltamethrin	10	↑	
Cypermethrin	10	↑	
Haloperidol	3	↓	↑

*Notable candidate transcriptomic biomarkers DAO, BDNF, DISC1, GRIN2B, NRG1, COMT implicated in neurodevelopmental diseases.

Preliminary Results

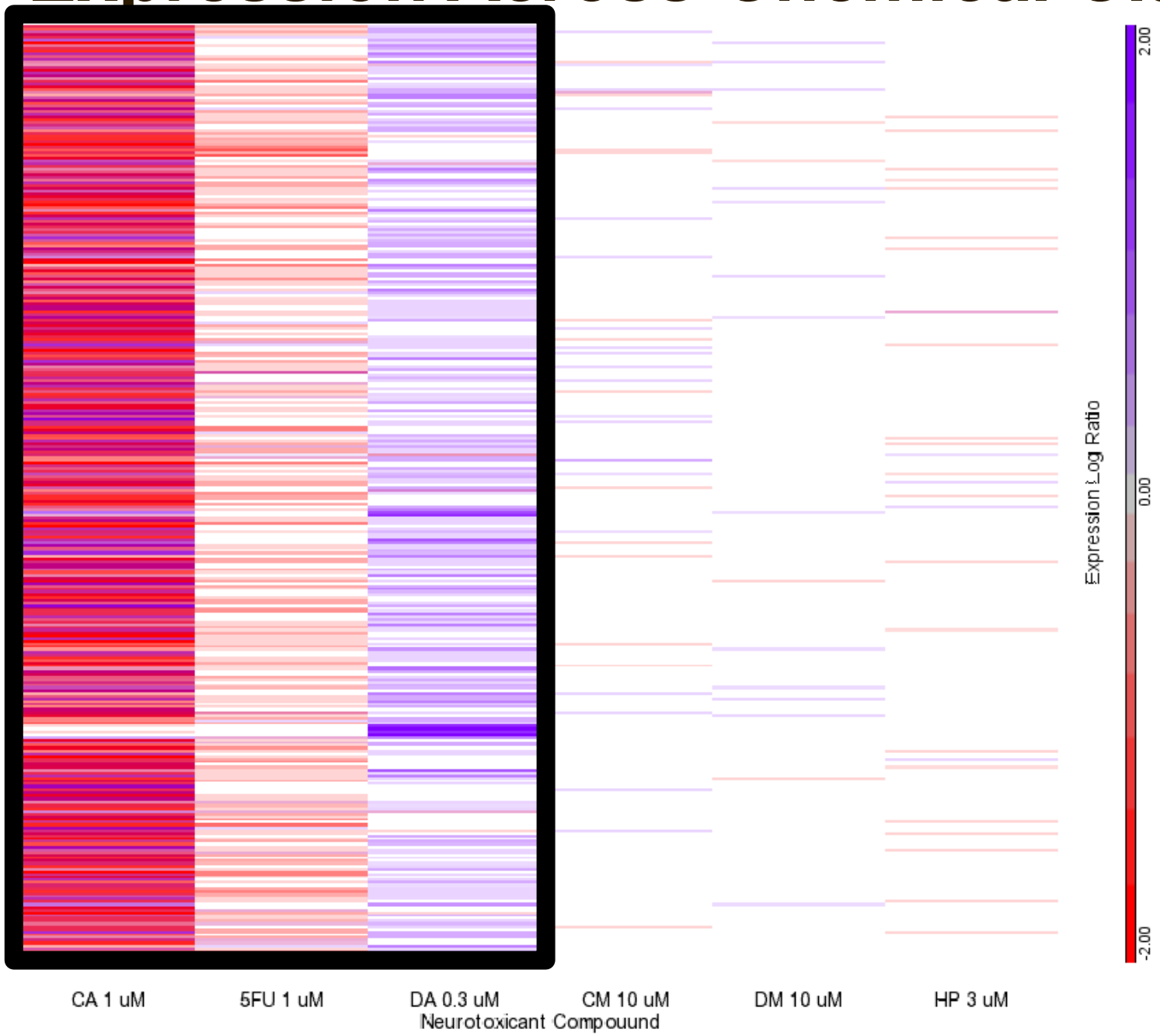
- MEA data has successfully shown disruption of neuronal network development.
- The selected compounds for this study have been shown to produce changes in neuronal gene expression and DNA methylation patterns and alter neural network formation.

MEA characterization of primary cortical neurotoxicant exposure DIV 12

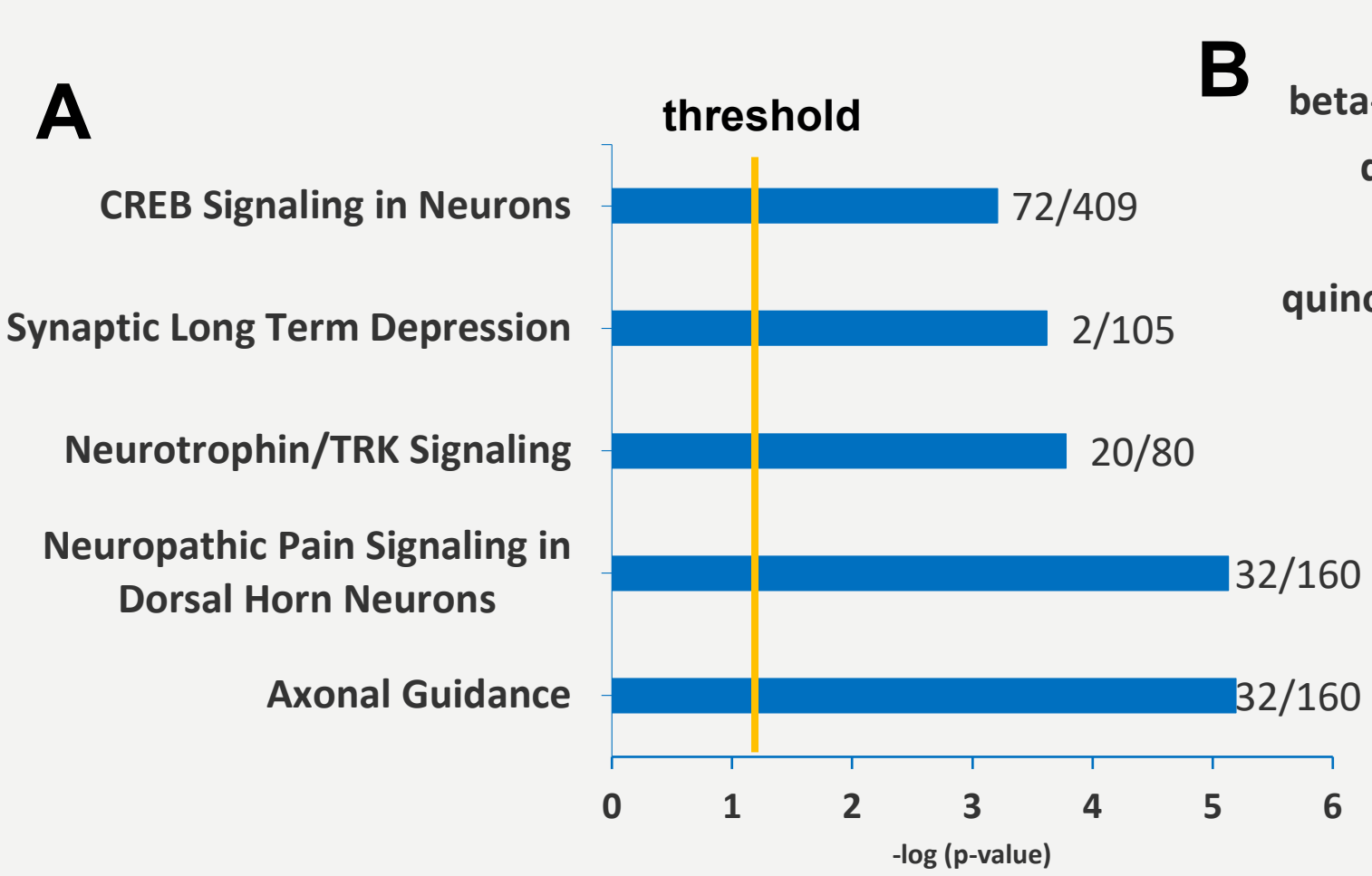


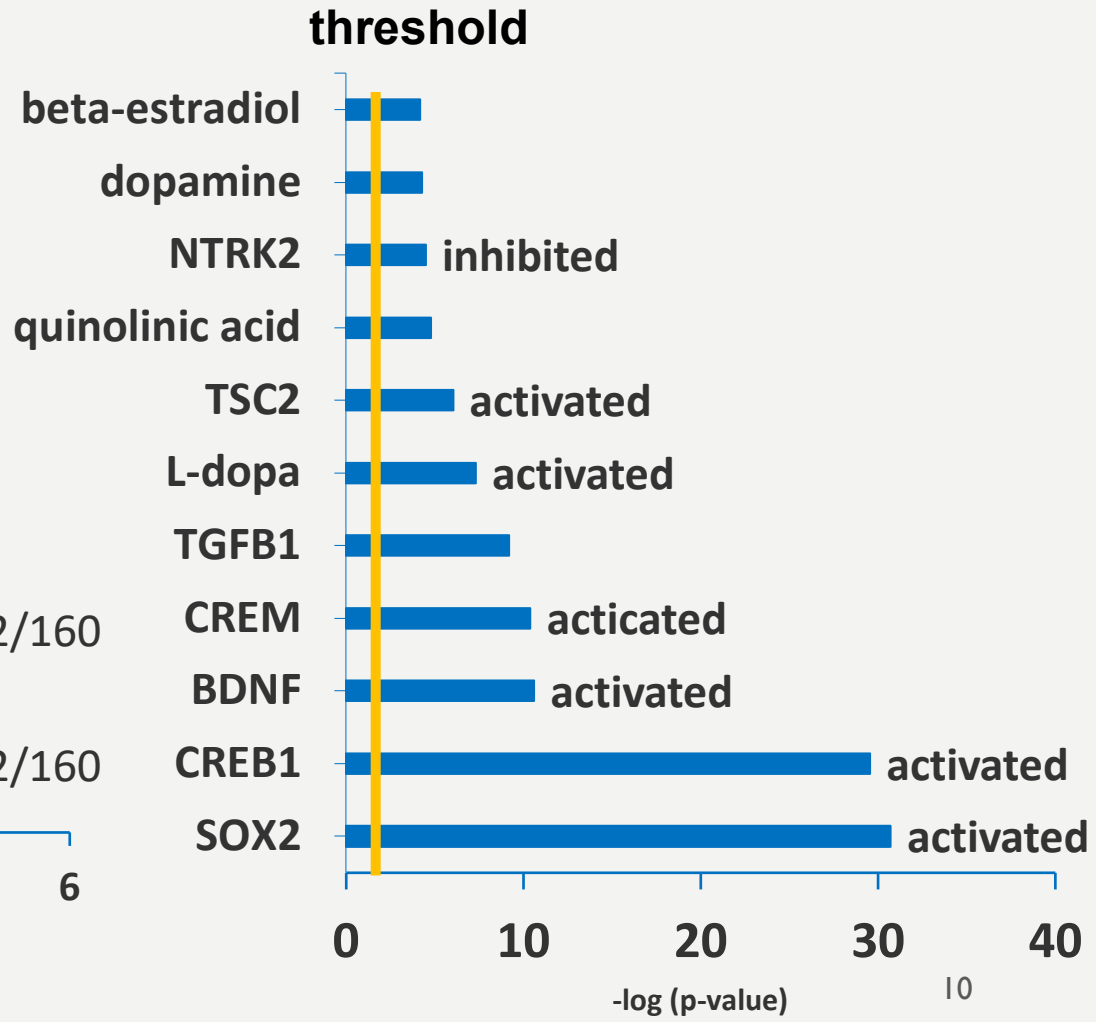
Metabolomic visualization of treatment differences and compound class clustering

Gene Expression Across Chemical Class

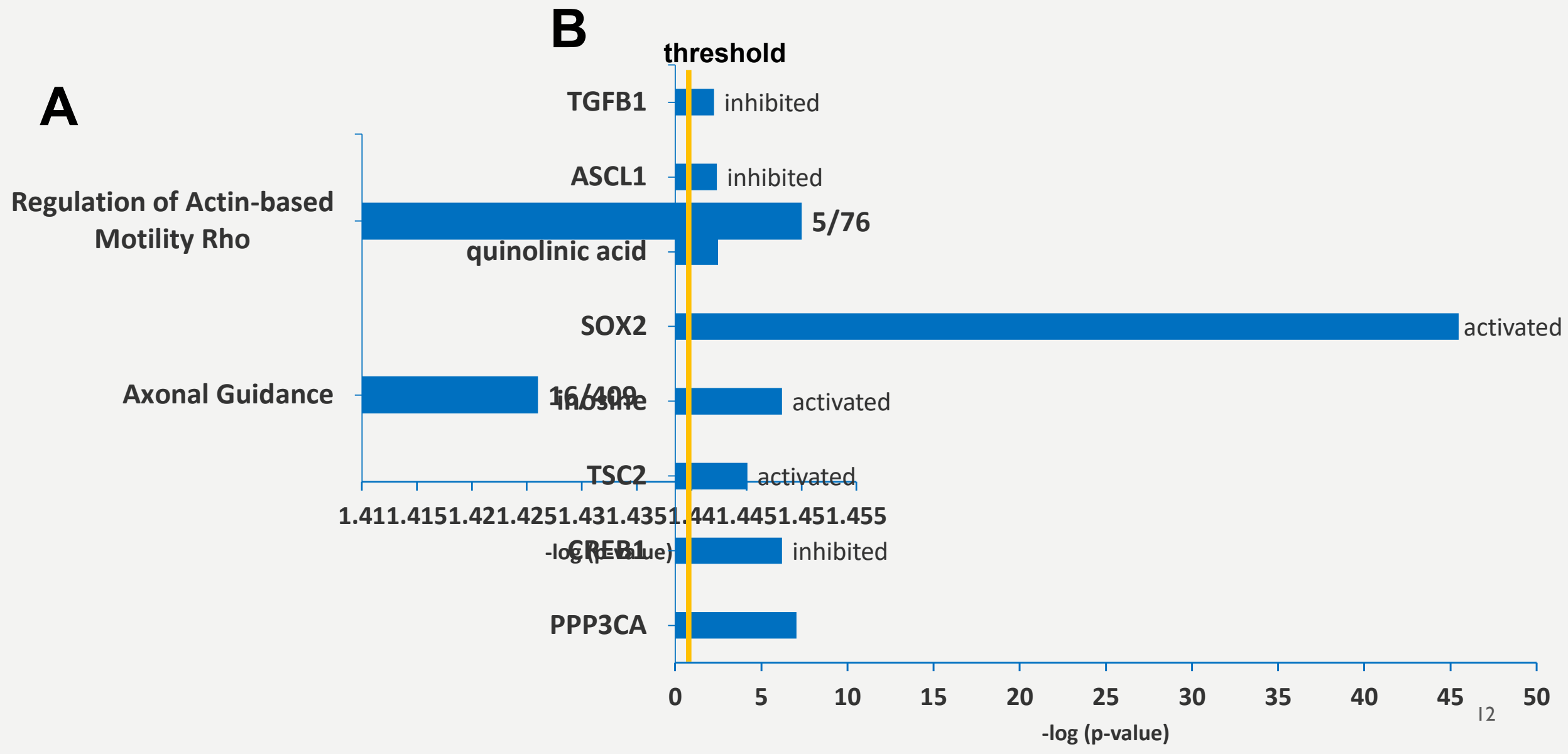


Integrated genomic and metabolomic characterization of primary cortical CA 1uM exposure

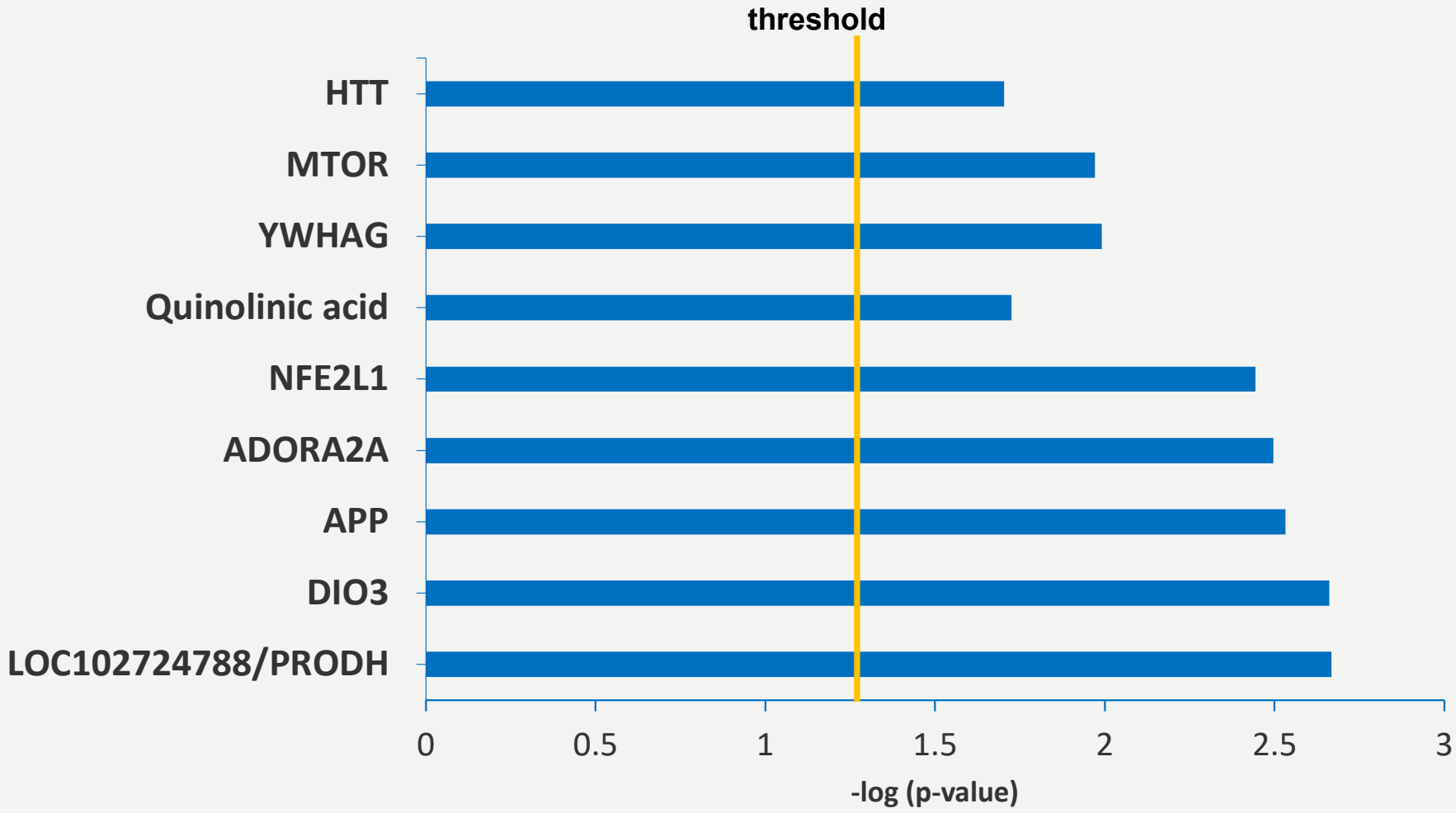




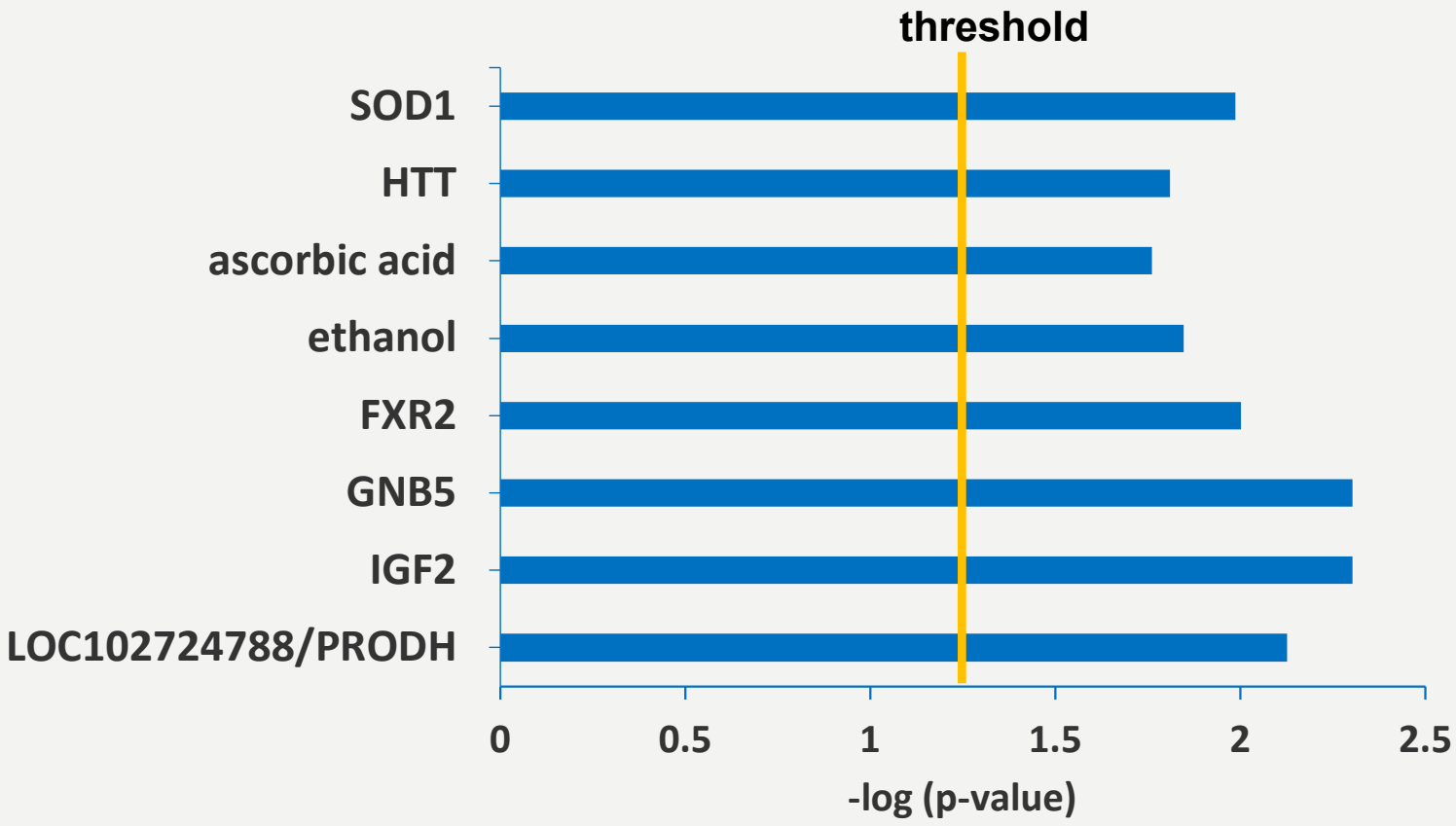
Integrated genomic and metabolomic characterization of primary cortical 5FU 1uM exposure



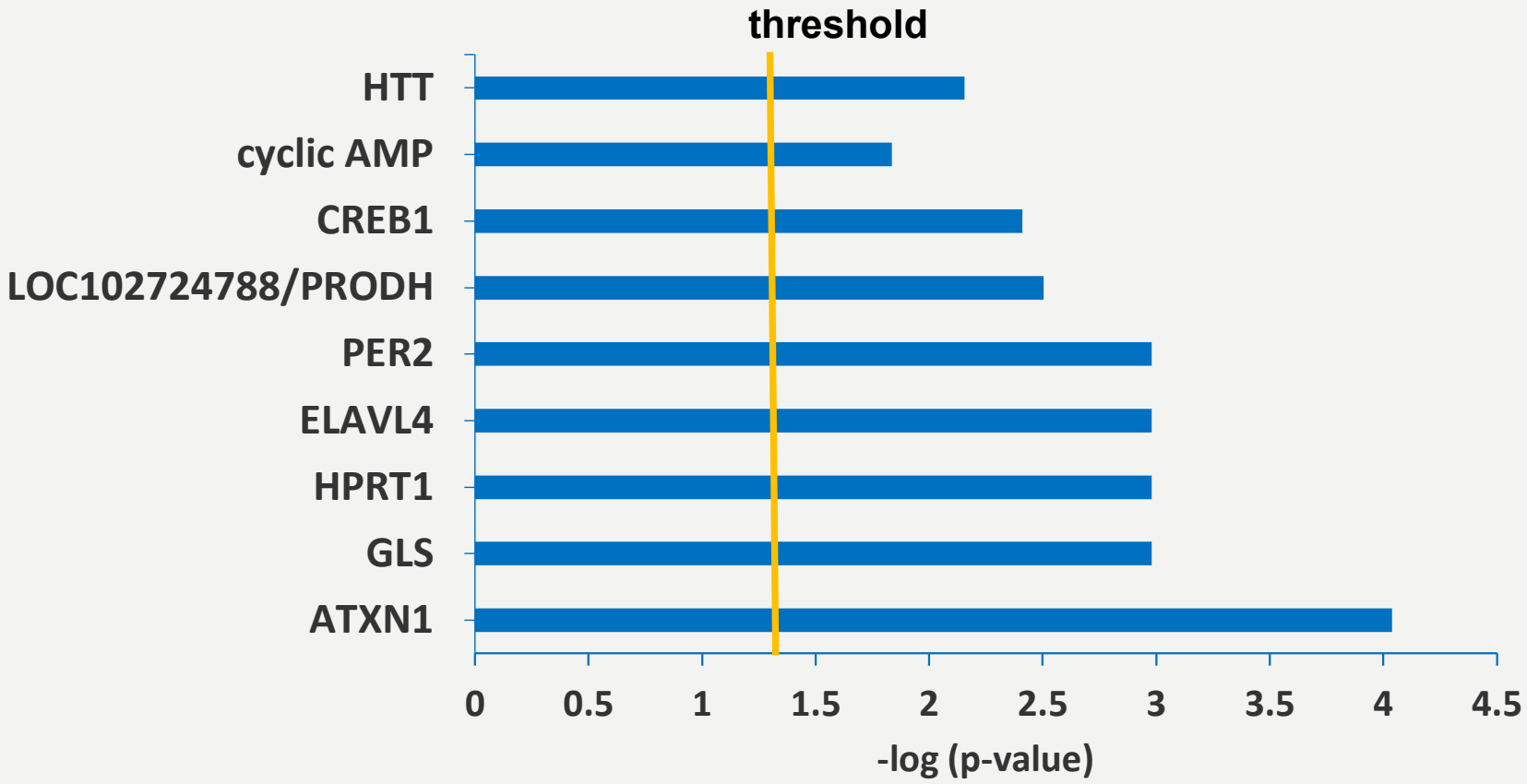
Integrated genomic and metabolomic characterization of primary cortical DA 0.3uM exposure



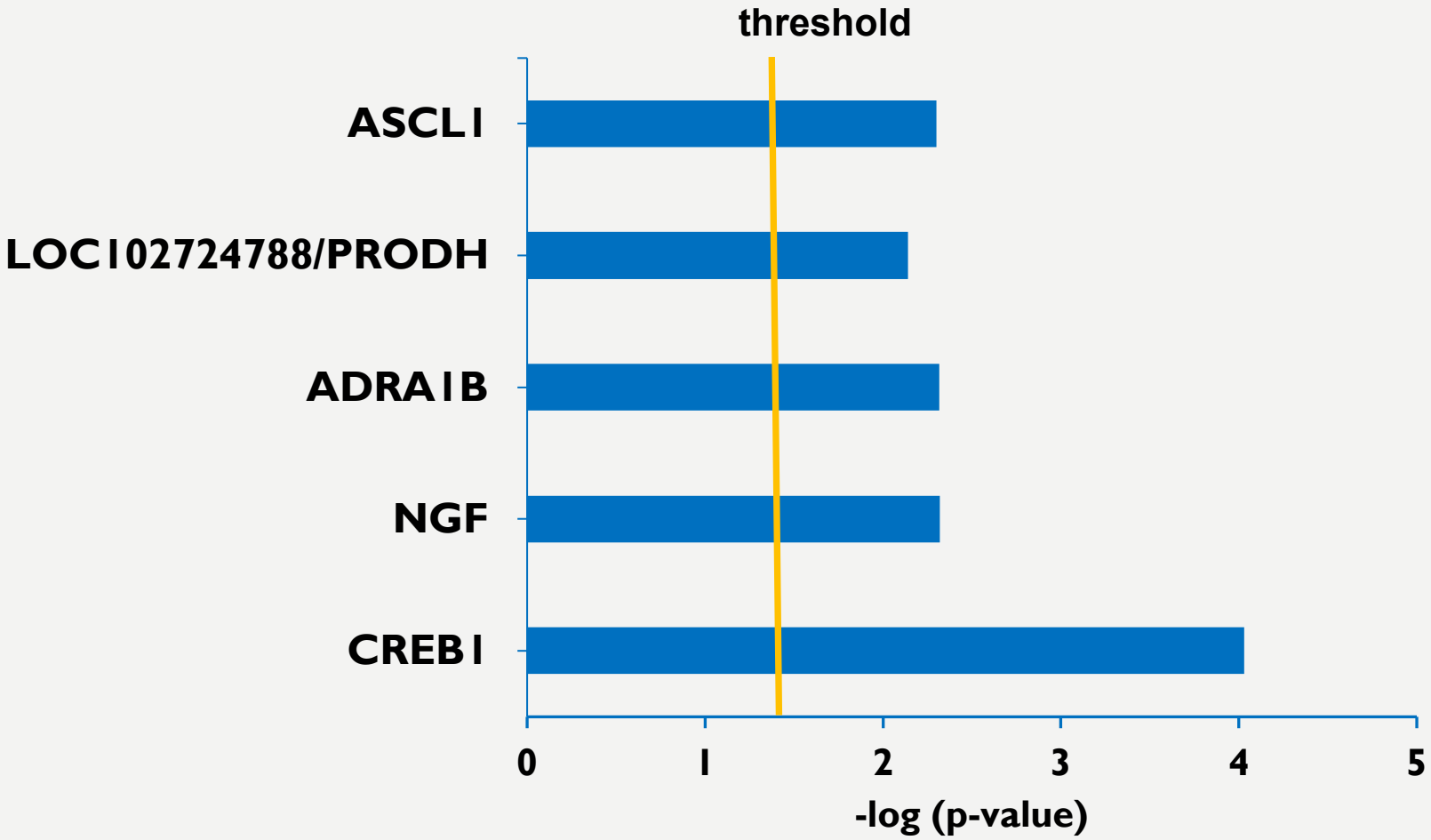
Integrated genomic and metabolomic characterization of primary cortical CM 10uM exposure



Integrated genomic and metabolomic characterization of primary cortical DM 10uM exposure



Integrated genomic and metabolomic characterization of primary cortical HP uM exposure



Summary

- Integrated transcriptomic and metabolomic signatures for DNA synthesis inhibitors, CA and 5FU, were most correlated with MEA network activity loss as well as IPA's database of genes, pathways, and metabolites associated with NDD of interest.
- Key markers of the axonal guidance signaling, ephrin receptor signaling and GDNF family ligand-receptor interactions, neuroinflammation signaling, neuregulin signaling, and NGF signaling pathways. Upstream regulator prediction in IPA identified CREB1, beta-estradiol, BDNF, TGFB1, SOX2, Levodopa, NTRK2, dopamine, quinolinic acid, TSC2, and PRODH as significantly associated with altered neurophysiological function; these regulators are also associated with NDD in vivo.
- These findings suggest that observed pathway-level effects in vitro are informative of in vivo responses. Further research is needed to determine the complex mechanistic interactions underlying the MEA detected neurotoxicants, identified transcriptomic and metabolic signatures, and neural developmental disorders.

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ORISE