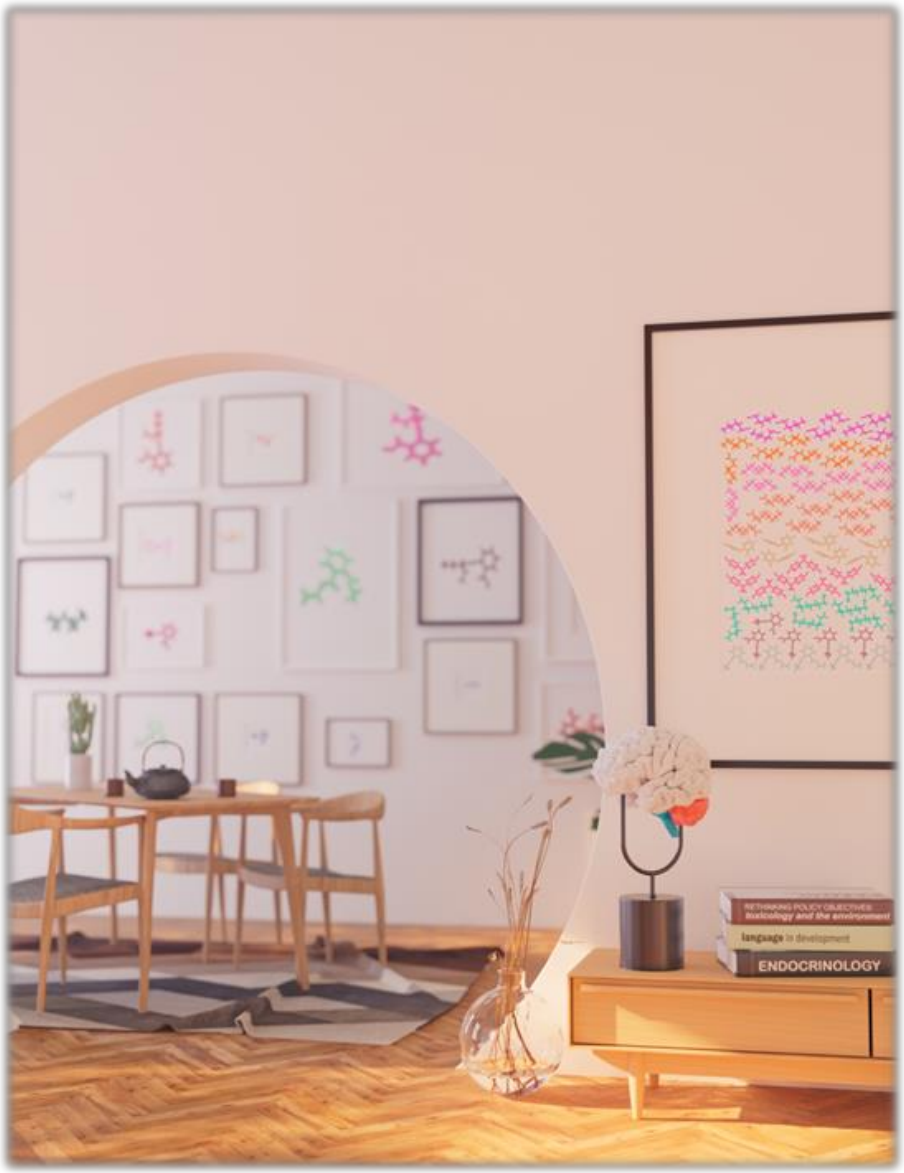


RESEARCH ARTICLE

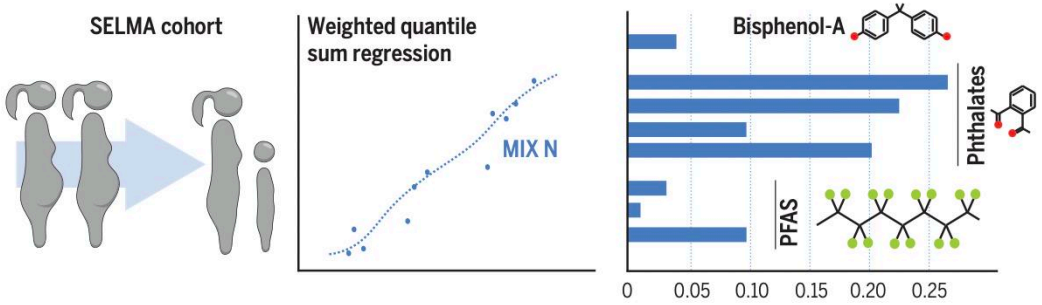
ENVIRONMENTAL TOXINS

From cohorts to molecules: Adverse impacts of endocrine disrupting mixtures

Epidemiology
EDC levels in urine, blood and clinical data

Biostatistics
Identification of EDCs of concern

Chemistry
EDC mixture and synthesis

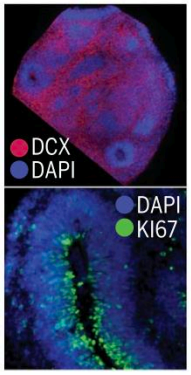


Experimental biology

Identification of molecular mechanisms of action

Fetal progenitors

Brain organoids



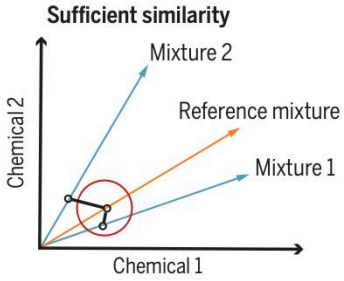
Dose-response modeling for benchmark dose estimation

Xenopus laevis

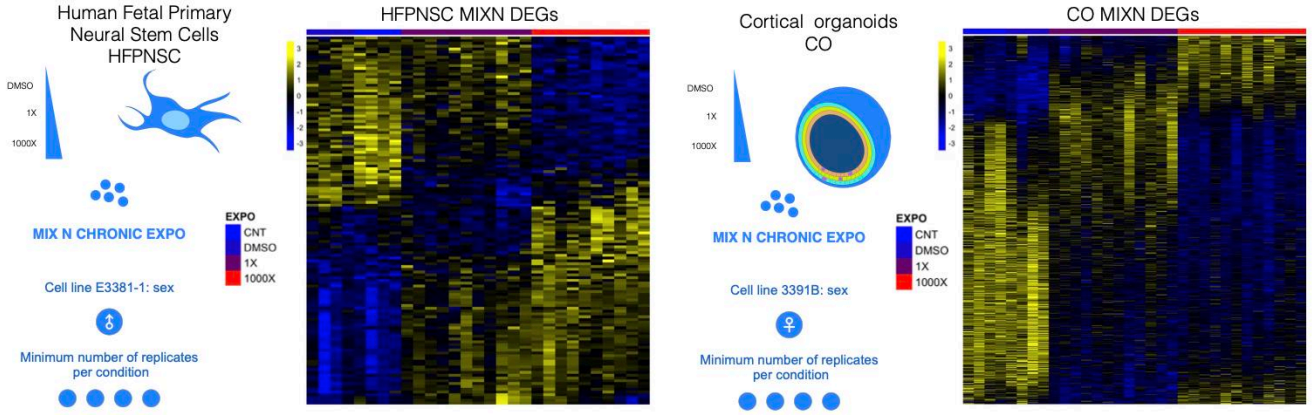
Danio rerio

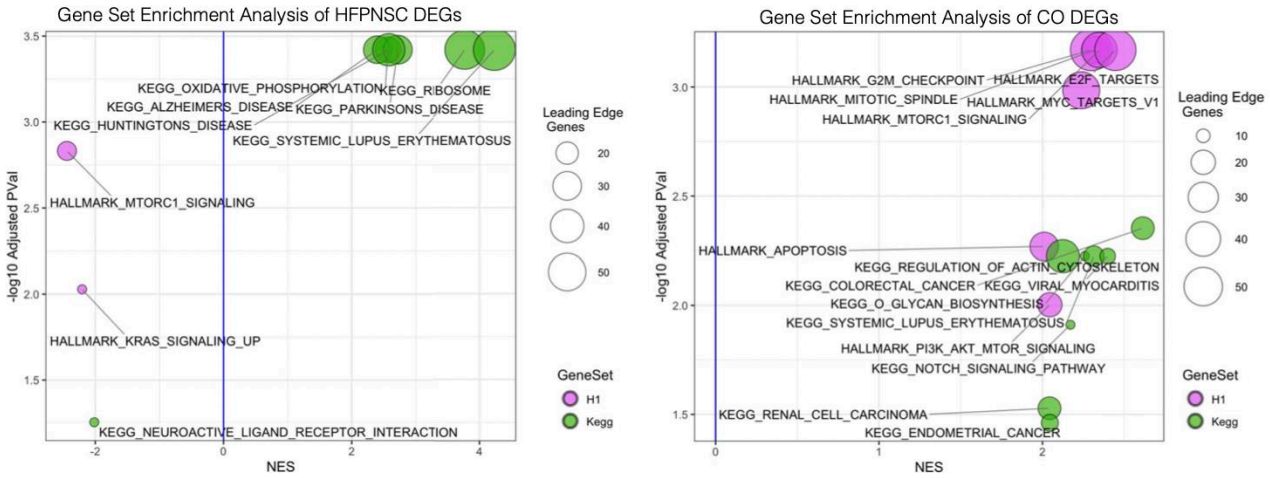
Similar mixture approach

Determination of the human population with exposure ranges of concern



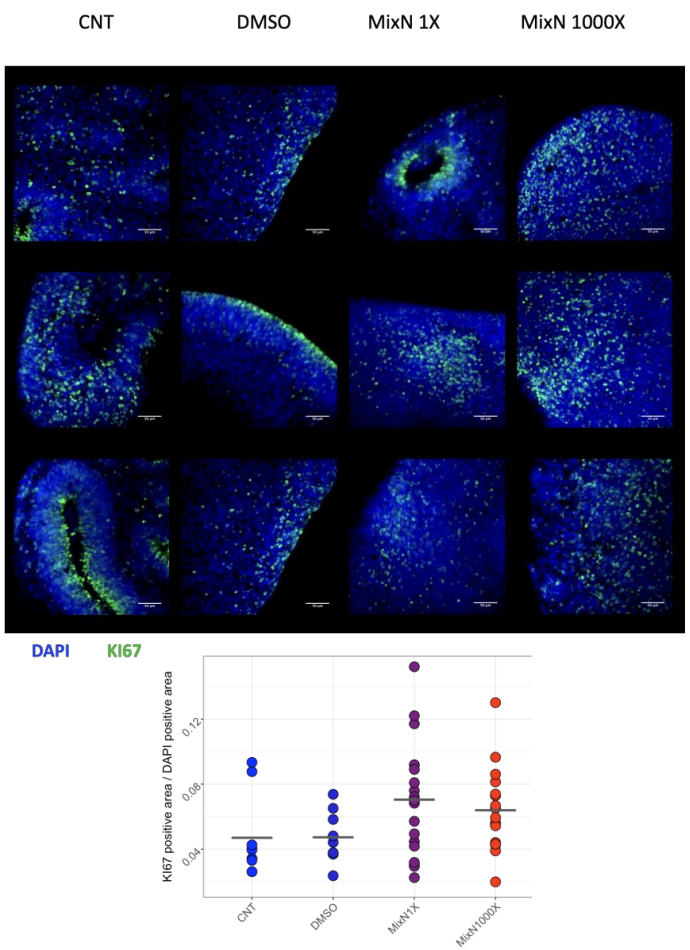
MIX N was tested in vitro on human fetal progenitors and brain organoids. Exposure disrupts human neurodevelopmental molecular pathways

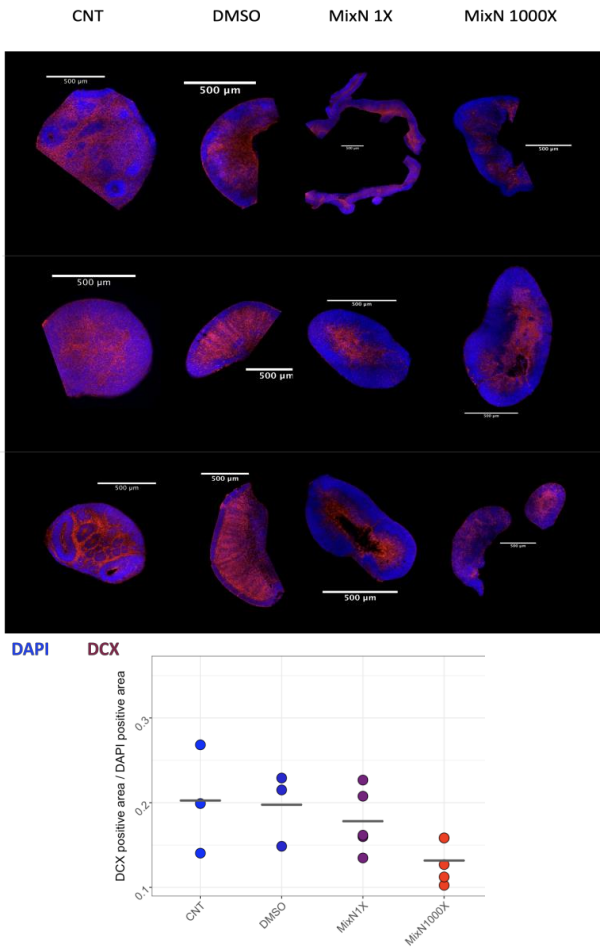




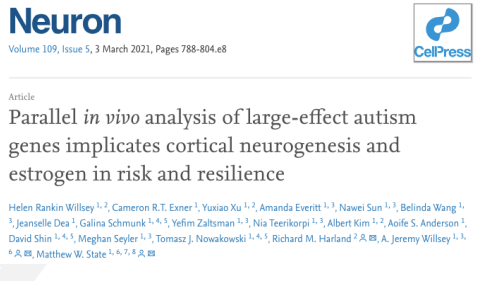
- Chronic exposure of Human Fetal Primary Neural Stem Cells and Cortical Organoids to MIX N, at both real-life concentration (1X) and higher doses (1000X) impacts heavily on gene expression profiles
- Pathways related to neurodegenerative disorders and cell cycle dynamics are among those impacted by MIX N

MIX N exposure disrupts human neurodevelopmental cellular pathways

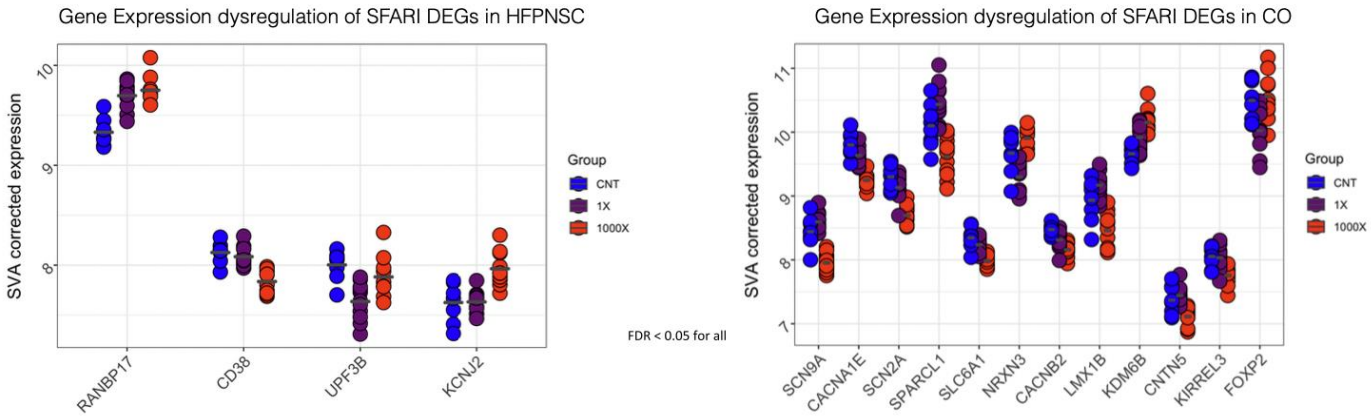


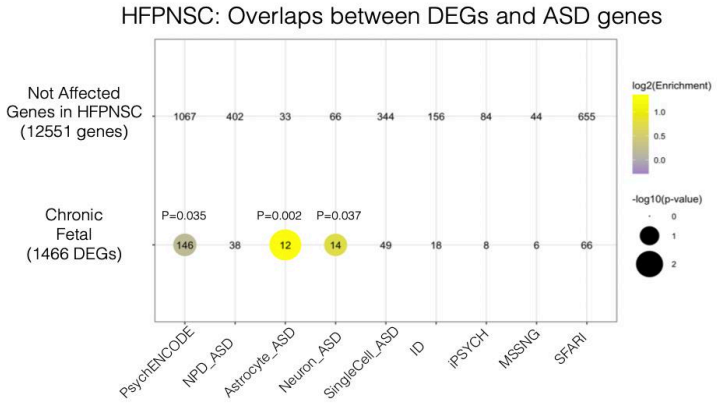


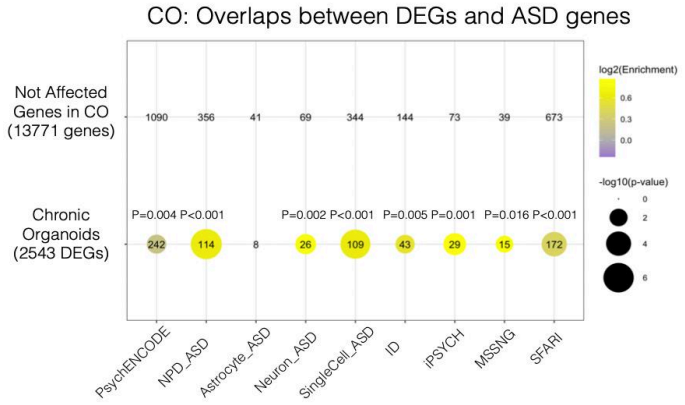
- Increase of KI67-positive cells for MIX N exposed organoids coupled with a decrease of DCX-expressing cells, suggesting an effect favoring neural progenitor proliferation while hindering neuronal differentiation
- Results in line with recent observations that hormonal exposure affects the same developmental processes that regulate neuronal progenitor proliferation and neuronal maturation of genetic mutations, increasing ASD vulnerability


Neuron
Volume 109, Issue 5, 3 March 2021, Pages 788-804.e8
CellPress
Article
Parallel *in vivo* analysis of large-effect autism genes implicates cortical neurogenesis and estrogen in risk and resilience
Helen Rankin Willsey [1, 2], Cameron R.T. Exner [1], Yuxiao Xu [1, 2], Amanda Everitt [1, 3], Nawei Sun [1, 3], Belinda Wang [1, 3], Jeanselle Dea [1], Galina Schmunk [1, 4, 5], Yefim Zaltsman [1, 3], Nia Teerikorpi [1, 3], Albert Kim [1, 2], Aoife S. Anderson [1], David Shin [1, 4, 5], Meghan Seyler [1, 3], Tomasz J. Nowakowski [1, 4, 5], Richard M. Harland [2], A. Jeremy Willsey [1, 3, 6], Matthew W. State [1, 6, 7, #]

MIX N exposure converges with genetic causes of ASD

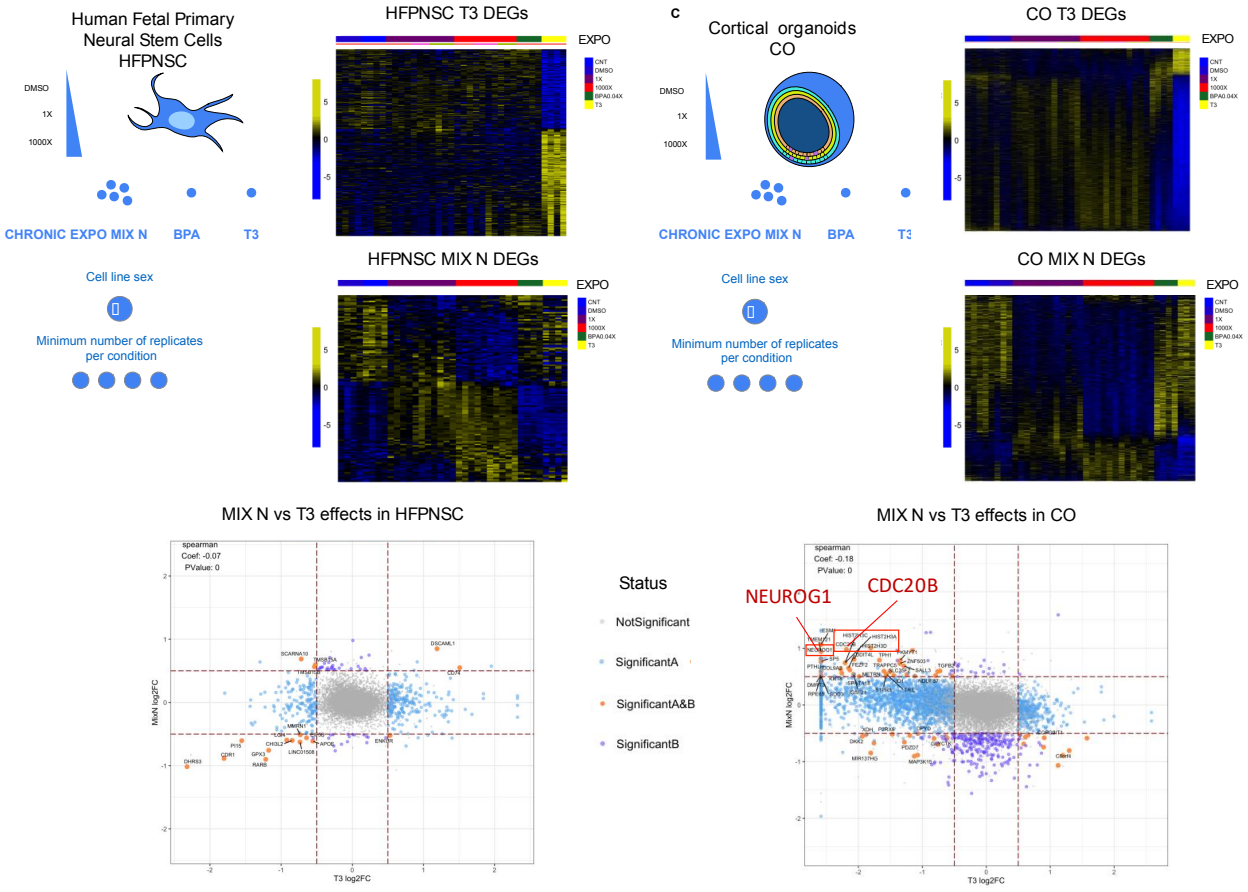






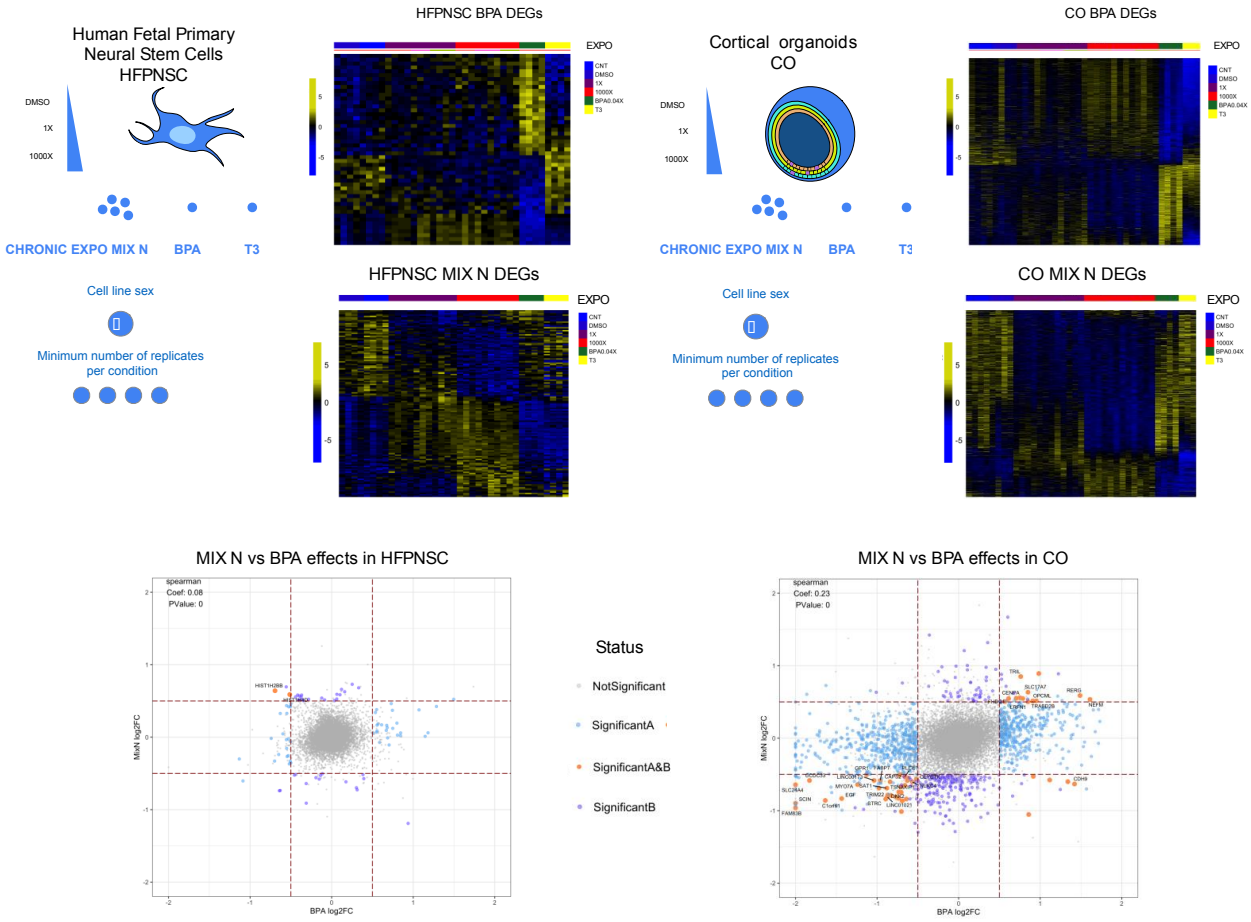
- Simon Foundation Autism Research Initiative genes are found among the DEGs in both cellular systems
- When assessing the presence of CO and HFPNSC DEGs among well-established databases for NDD-related genes, a significant overlap was found for several of them

MIX N effects are specific and different from single compound exposure: Thyroid hormone



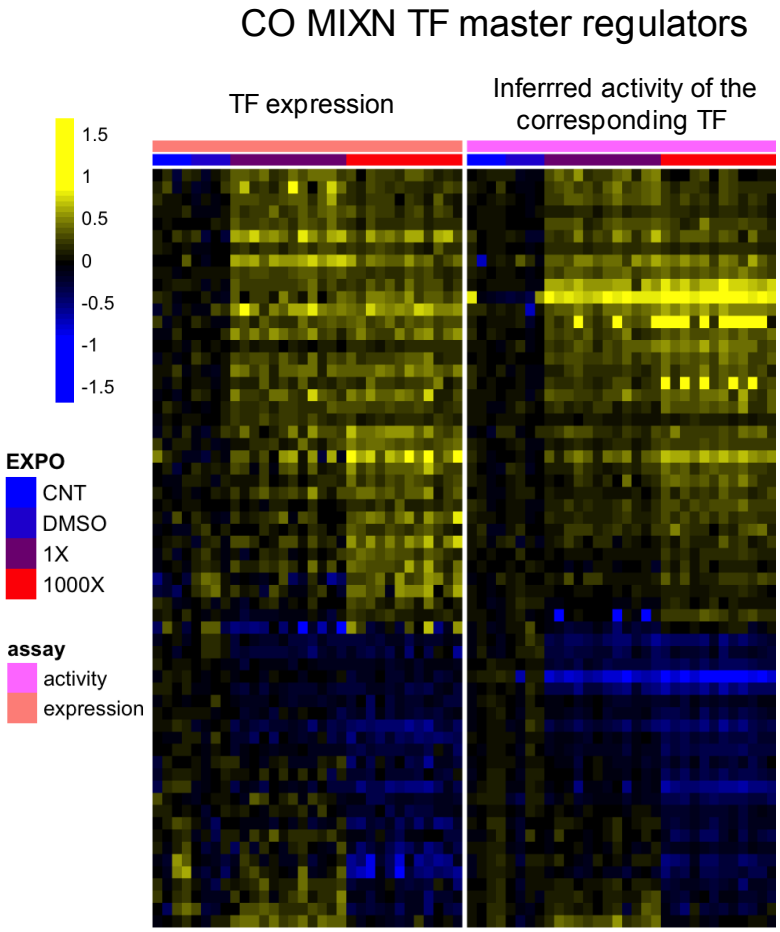
- We also tested the effect of thyroid hormone T3, given its essential role in brain development and identified, as expected, a major transcriptional impact and observed different patterns of dysregulation relative to MIX N.
- In organoids, T3 and MIX N exposures showed an opposite effect for cell proliferation-related genes (CDC20B and histone-related genes) as well as NEUROG1, which was shown to act as a negative regulator of neocortical neurogenesis

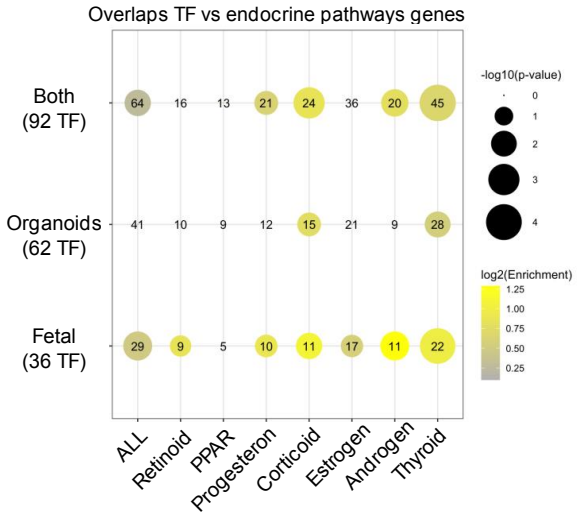
MIX N effects are specific and different from single compound exposure: Bisphenol A



- BPA has been reported to affect human brain development and behavior by epidemiological, in vitro, and in vivo evidence
- We also probed its impact as a single compound at the same concentration at which it is present in 1X MIX N
- For both fetal progenitors and organoids the effect of MIX N, although showing an expected partial overlap, extended well beyond that of BPA alone

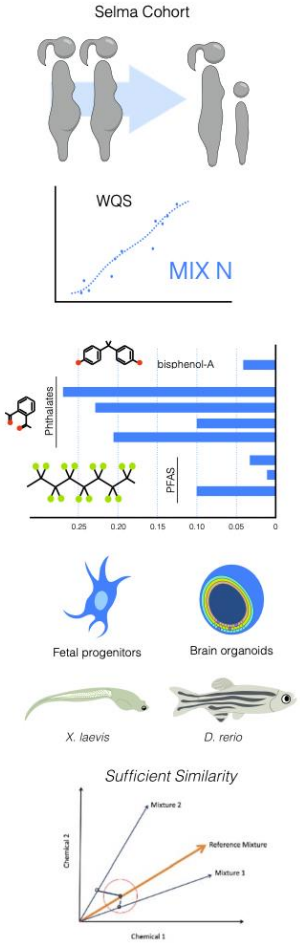
Master regulator analysis on MIX N targets identify





- To identify the key regulators for the transcriptomic phenotypes, we performed a master regulator analysis using recently released data from the PsychENCODE consortium integrated into a human brain–specific gene regulatory network
- The 92 transcription factors whose altered activity most likely mediated the impact of MIX N include upregulation of SOX9, known to control neurogenesis and downregulation of the TH–dependent factor KLF9, which plays a key role in neurogenesis
- Intersecting TFs with genes related to hormonal pathways, thyroid-related genes were the most enriched

A novel Risk Assessment framework integrating epidemiological and experimental evidence



Motivation: Epidemiological data and human biomonitoring should guide experimental toxicology to include the concentrations of chemical mixtures relevant for real-life exposure and for which there is evidence of associations between exposures and health outcomes of concern. EPIDEMIOLOGY

Step 1: Biostatistical methods for characterizing environmental mixtures such as weighted quantile sum (WQS) regression should be used to identify combinations of chemicals that are associated with health outcomes in humans. The selection of chemicals should be both sensitive (identifying chemicals of concern) and specific (identifying chemicals that are not of concern). BIOSTATISTICS

Step 2: One or more human-relevant typical mixtures (relative proportions and total concentrations) should be identified, synthesized and experimentally tested. CHEMISTRY

Step 3: Experimental evidence should identify the molecular mechanisms of action of the mixtures associated with adverse outcomes and dose-response experiments should be performed for estimation of a Point of Departure (POD), through the integration of:

- human experimental systems, especially induced pluripotent stem cell (iPSC) derived organoids that recapitulate the tissue-level complexity and developmental timings of human exposure
- in vivo models validated by the OECD

EXPERIMENTAL BIOLOGY

Step 4: similar mixture approach (SMACH) should be applied to compare human exposure (determined to be sufficiently similar to the experimental mixture(s)) to experimental evidence of the mixture POD using the similar mixture risk index (SMRI) and determine the proportion of the human population with exposure ranges of concern (SMRI>1). SMACH

Conclusion: Based on the integration of evidence from Step 1 through 4 standard single chemical risk assessment strategies should be benchmarked against the backdrop of relevant exposure to chemical mixtures of concern. EDCMixRisk