



Bypassing the Brain Barriers: Serum microRNAs Reflective of Developmental Neurotoxicity Induced by Thyroid Disruption

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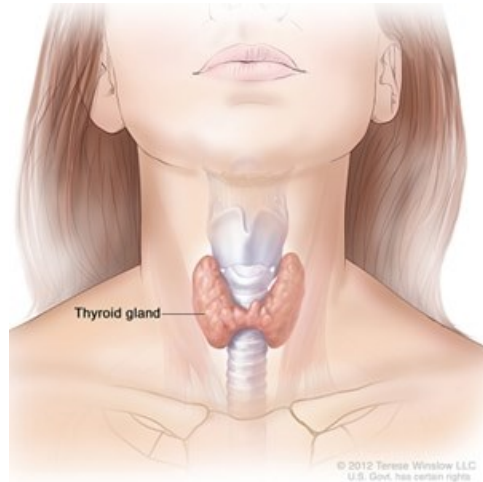
Public Health and Integrated Toxicology Division

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Thyroid Hormones Are Required For Brain Development



The thyroid gland, NIDDK.

<https://www.nidDK.nih.gov/health-information/diagnostic-tests/thyroid>



Infant with cretinism.

Young W. Collection of Medical Illustrations, Endocrine - Volume 2

Tetraiodothyronine (T4) and Triiodothyronine (T3)

- Both T4 and T3 control cell signaling
- Actively transported across the placenta and brain barriers

Congenital hypothyroidism (cretinism)

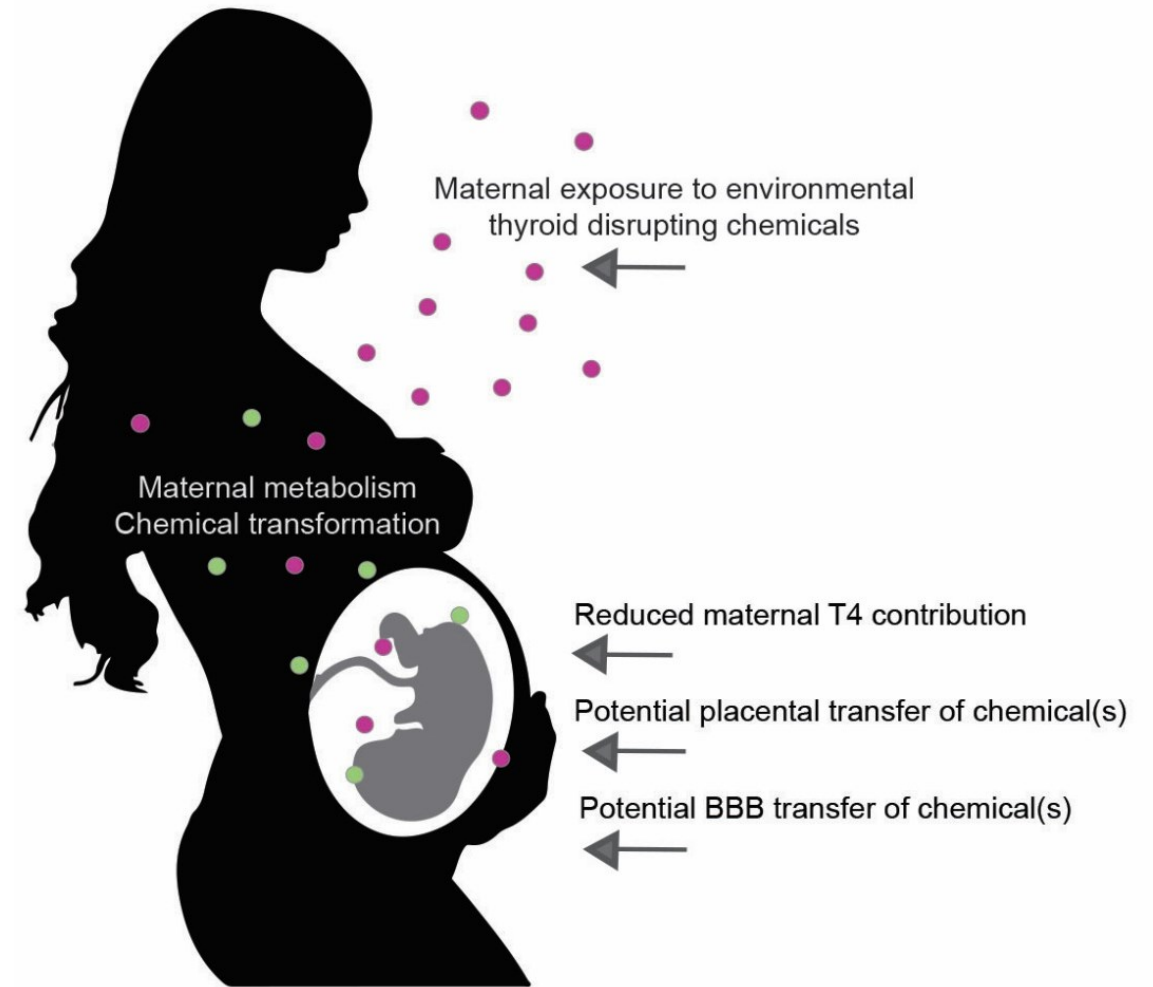
- Associated with low birth weight, altered brain structure, and severe intellectual disability

Thyroid hormone (TH) insufficiency during pregnancy is also correlated to lower IQ and structural defects

- Maternal iodine deficiency, disease mismanagement
- Environmental endocrine disrupting chemicals (EDCs)*

Some Environmental EDCs Interrupt Thyroid Homeostasis

Chemicals like perchlorate, pesticides, and per-and polyfluoroalkyl substances (PFAS)



O'Shaughnessy and Gilbert, *Molecular and Cellular Endo*, 2020

Newborn Screening is a Successful Public Health Initiative

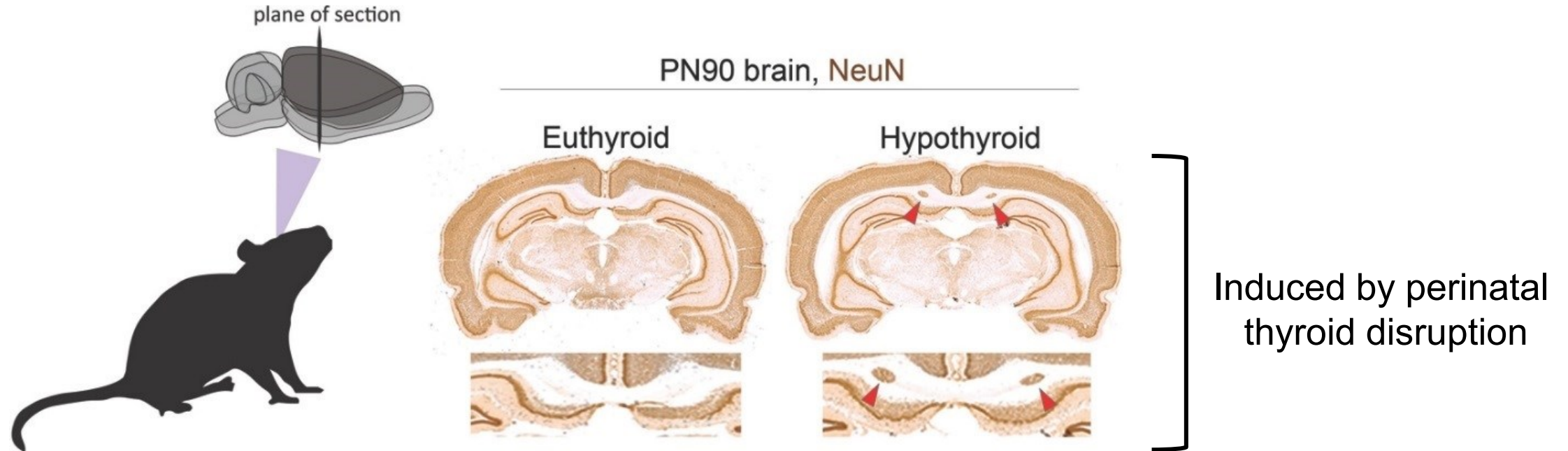


Image courtesy of <https://www.perkinelmer.com/corporate/stories/china-newborn-screening.html>

- Genetic disorders - phenylketonuria, cystic fibrosis, sickle cell disease
- Thyroid function, liver function

What if we could identify *predictive* biomarkers of neurodevelopmental disorders?

A Rat Model of Complex Neurodevelopmental Disorders



Periventricular heterotopia (pictured, morphological)

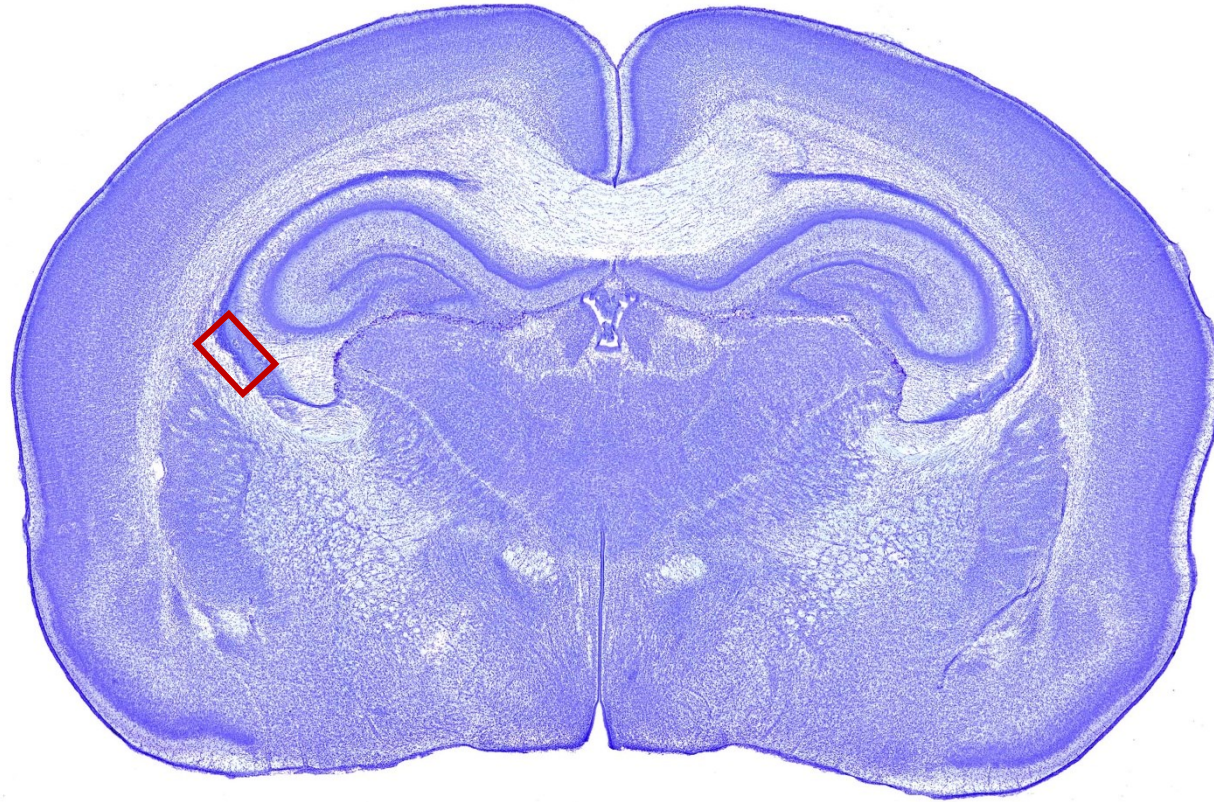
Increased seizures

Learning deficits

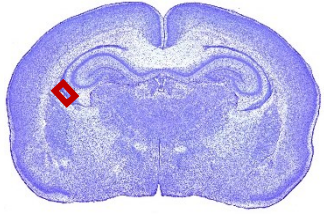
Shifts in the balance of neuronal excitation/inhibition

Thyroid Insufficiency Affects a Stem Cell Niche in the Brain

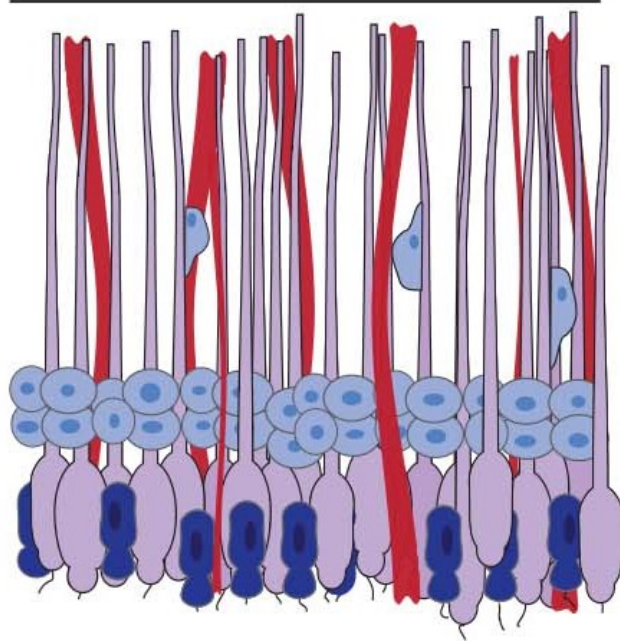
Postnatal Day 8 (PN8) Coronal Section, Nissl



Thyroid Insufficiency Affects a Stem Cell Niche in the Brain

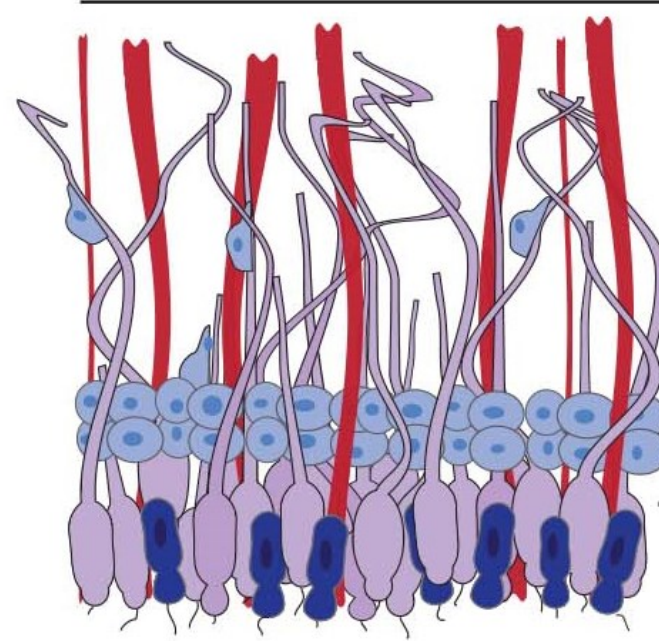


Euthyroid Neonatal Ventricle
(CSF-Brain Barrier)




Cerebrospinal Fluid (CSF)

Hypothyroid neonatal ventricle
(CSF-Brain Barrier)

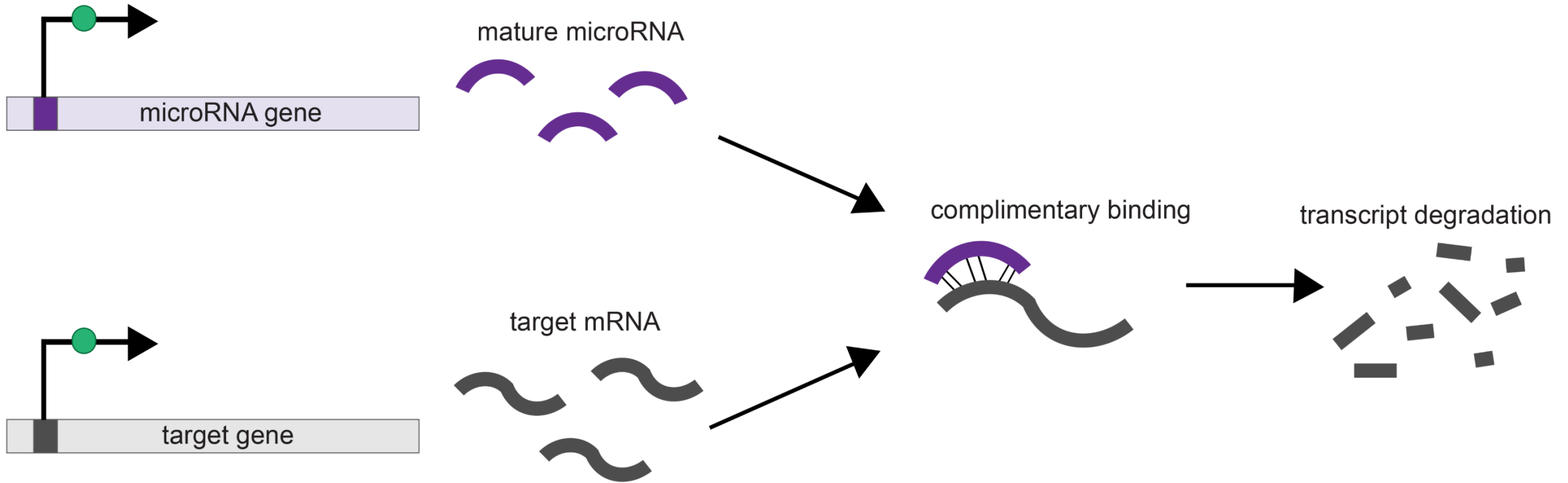


Cerebrospinal Fluid (CSF)

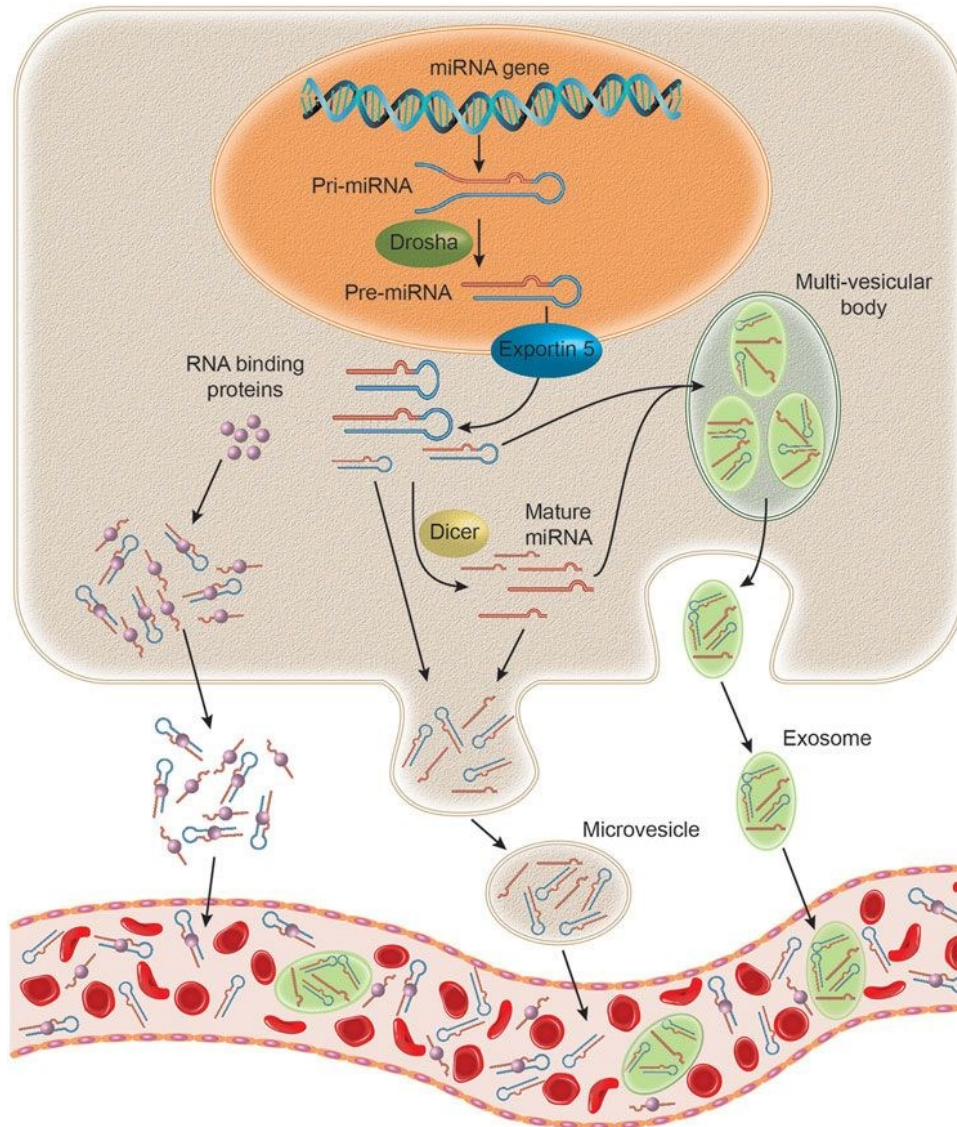
microRNA (18-24 nt)


Hypothesis: Could small molecules escape from this affected region and into general circulation?

microRNAs Regulate Gene Expression



microRNAs Regulate Gene Expression



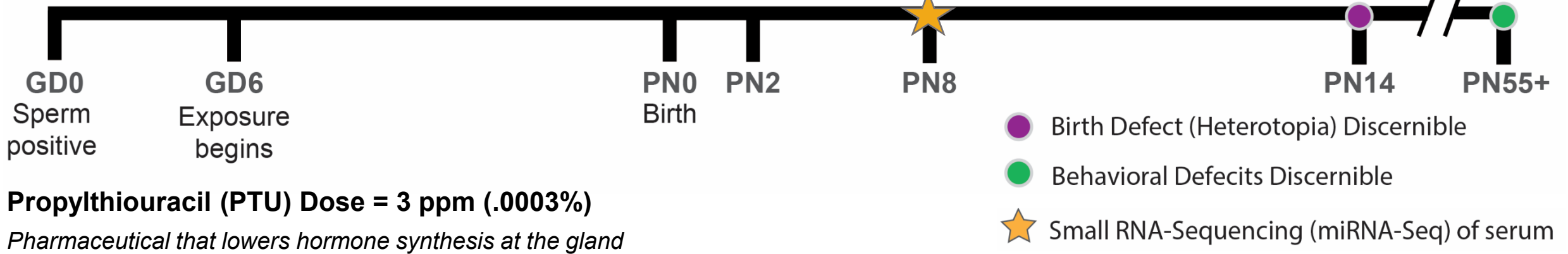
Extracellular microRNAs are present in biofluids

- Uniquely stable
- Resistant to degradation

Currently being investigated as biomarkers of complex diseases in patients

- Renal cancer (urine)
- Traumatic brain injury (serum, saliva)
- Lung cancer (serum)

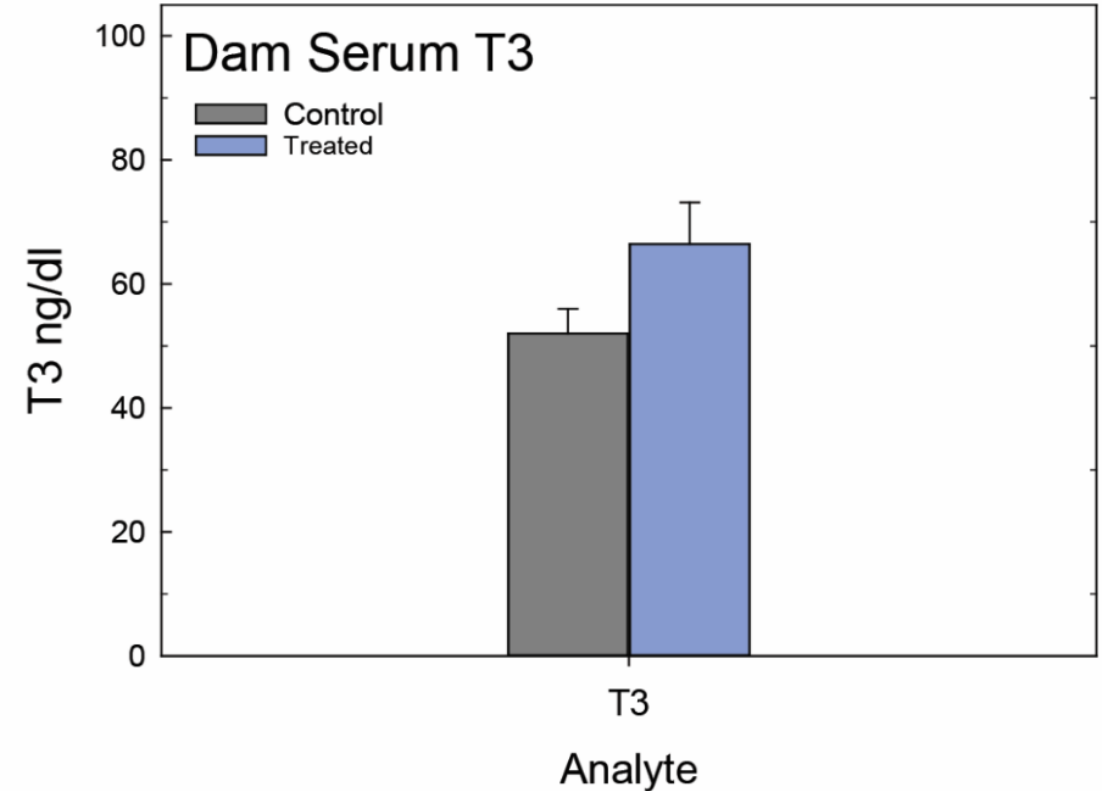
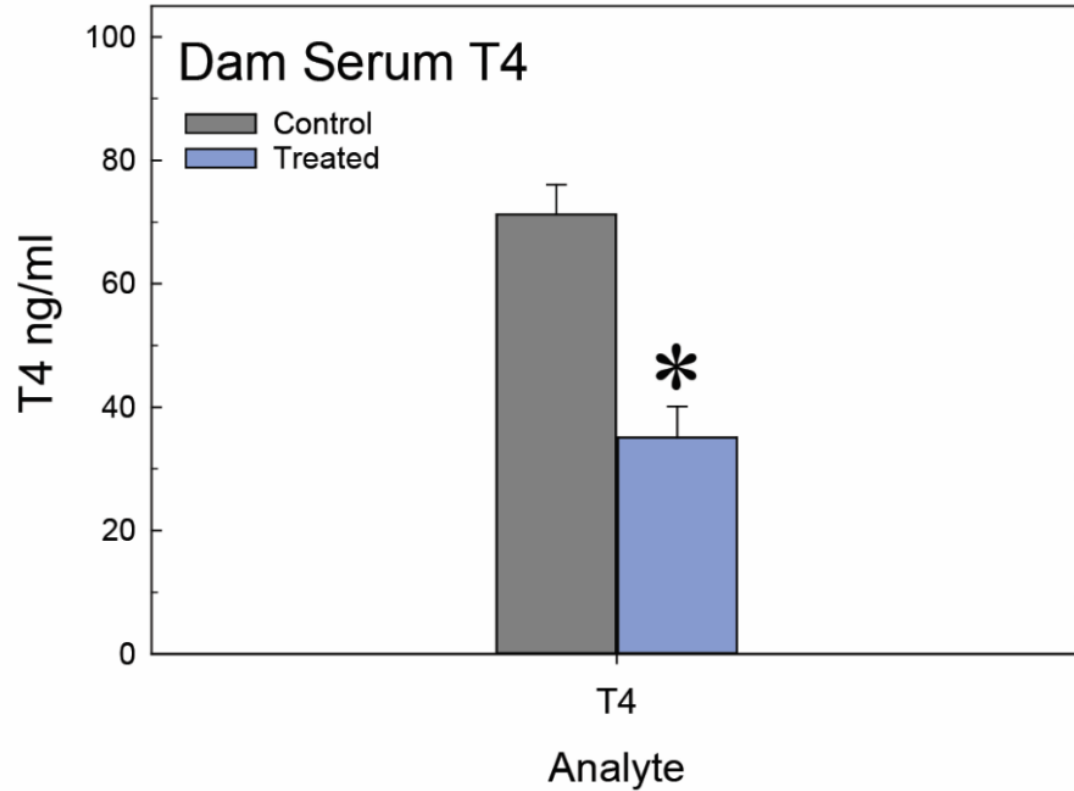
Experiment



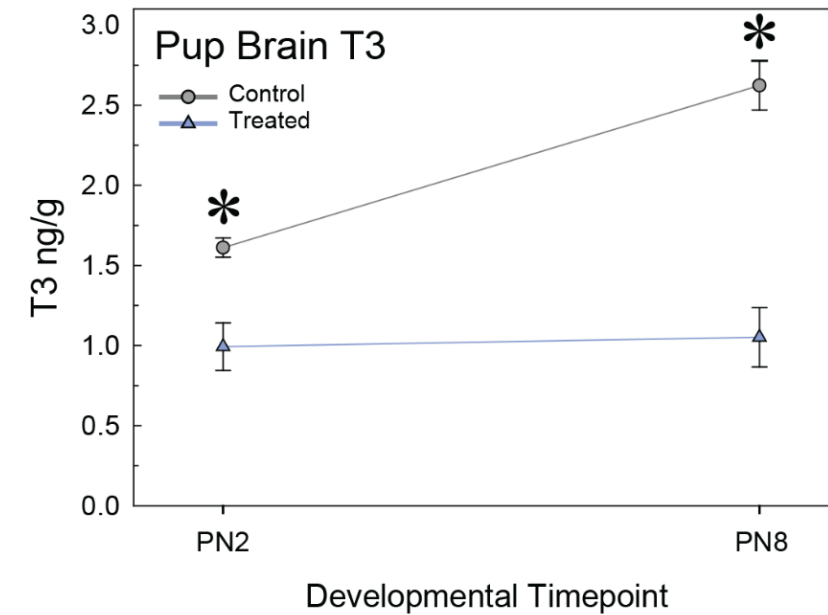
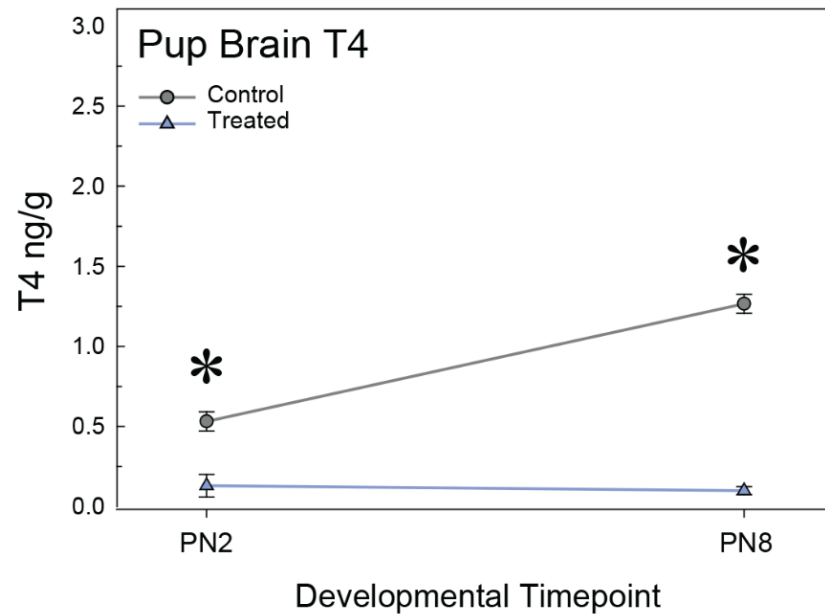
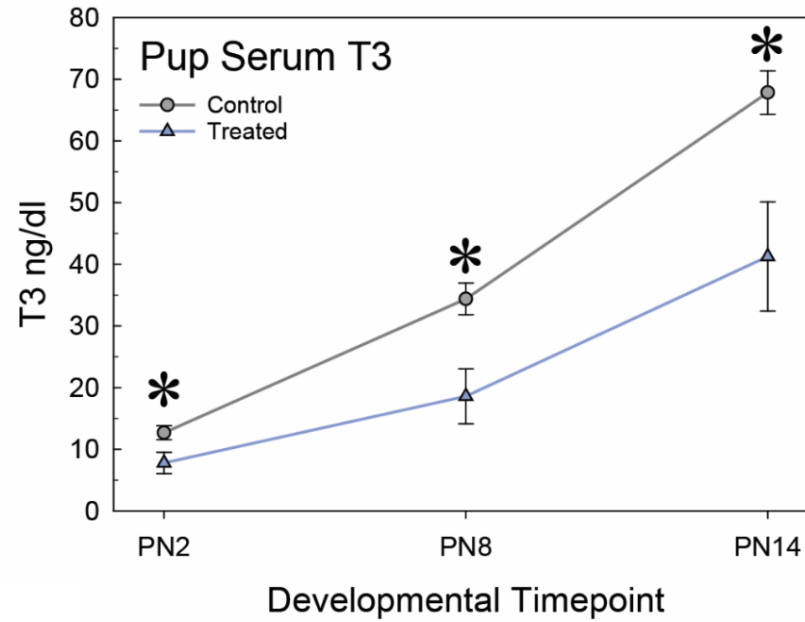
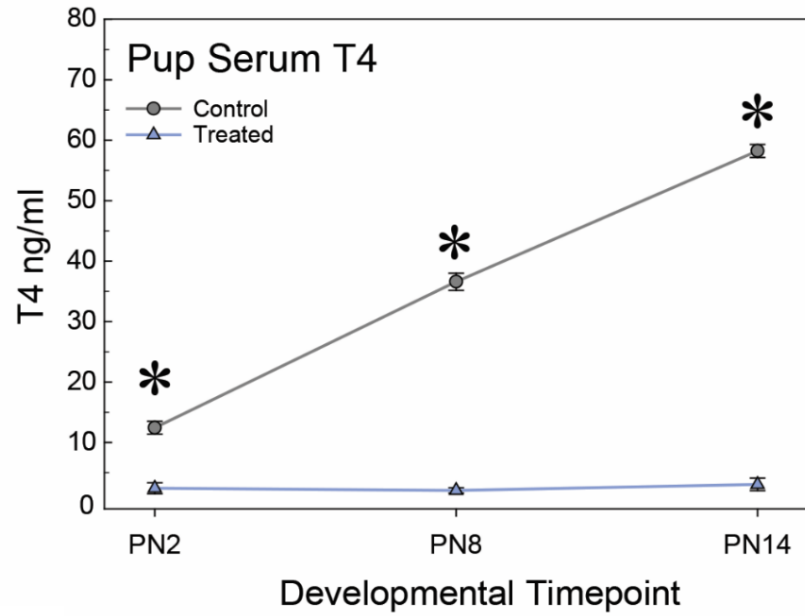
Propylthiouracil (PTU) Dose = 3 ppm (.0003%)

Pharmaceutical that lowers hormone synthesis at the gland

Maternal Serum Thyroid Hormones



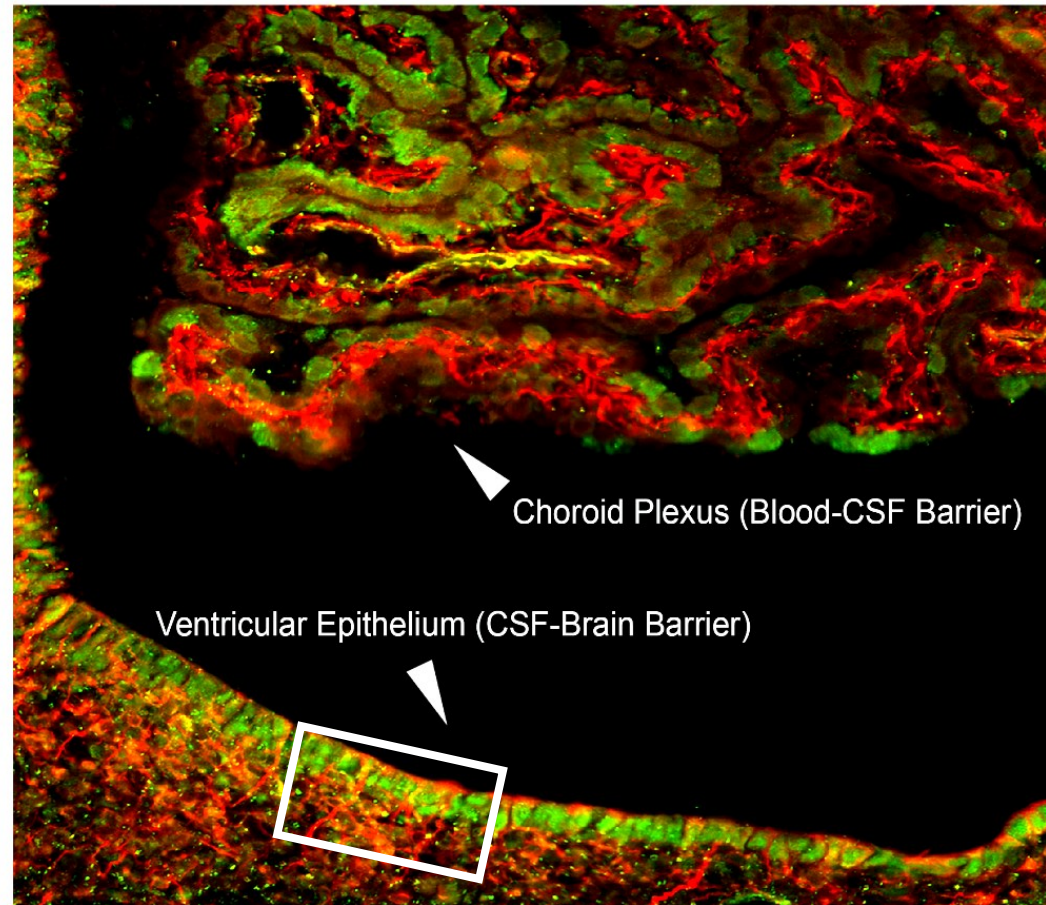
Neonatal Serum and Brain Thyroid Hormones are Reduced



The Brain Barriers are Compromised in Neonates

PECAM-1 (blood vessels), CLD5 (tight junctions)

Neonatal (PN8) Ventricle



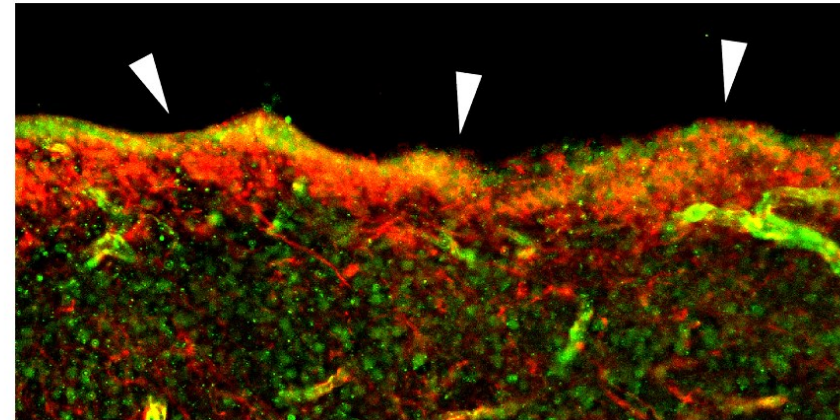
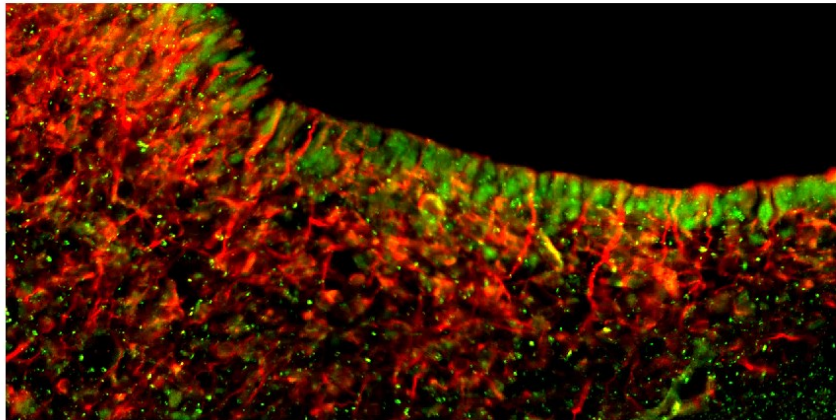
The Brain Barriers are Compromised in Neonates

PECAM-1 (blood vessels), CLD5 (tight junctions)

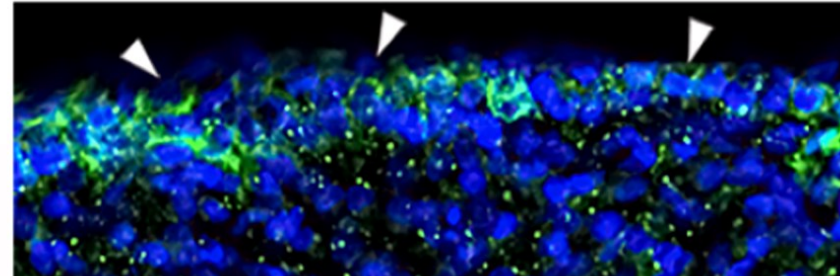
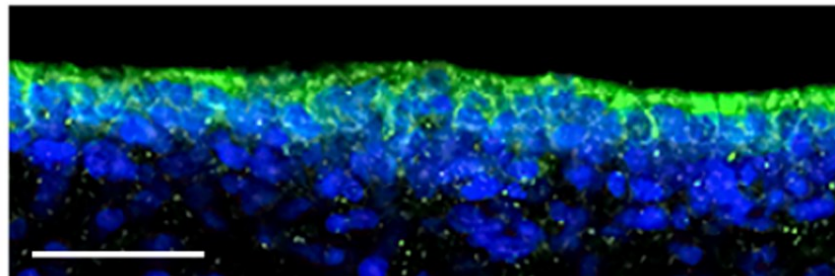
Ventricular Epithelium

Control

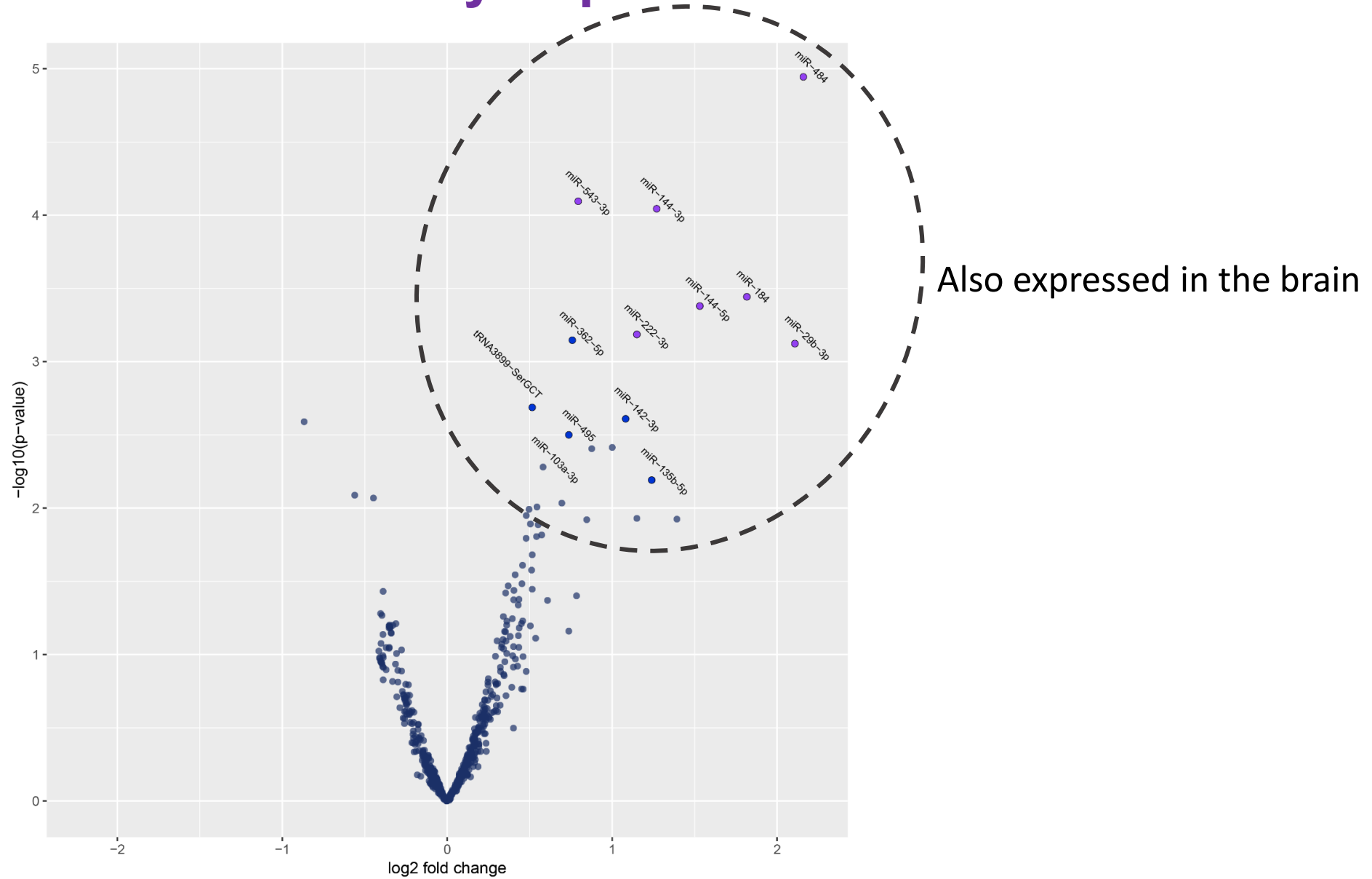
Treated



N-Cad (adherens junctions), DAPI

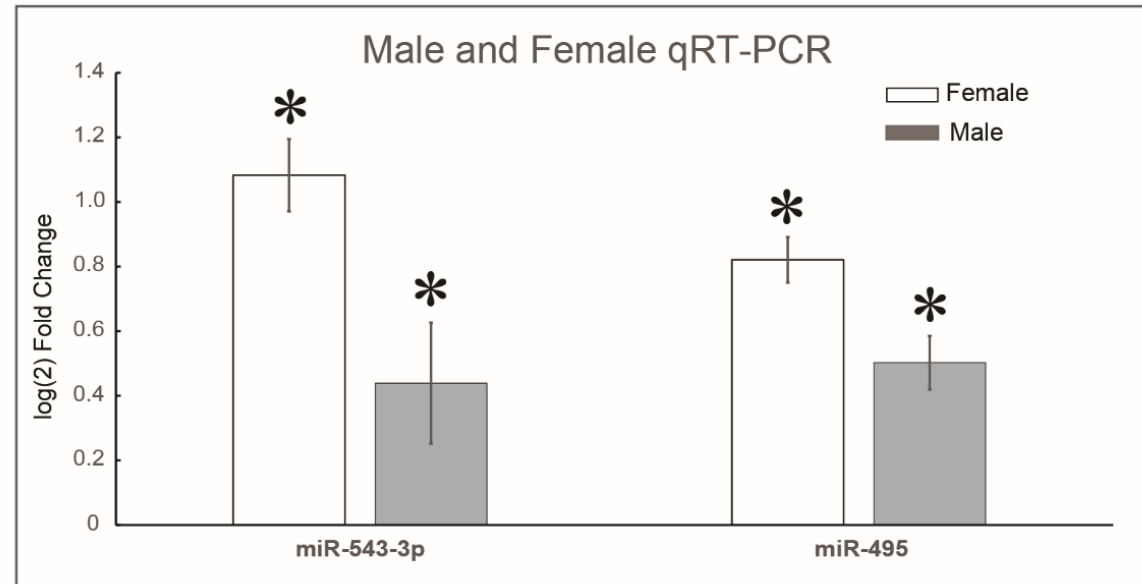
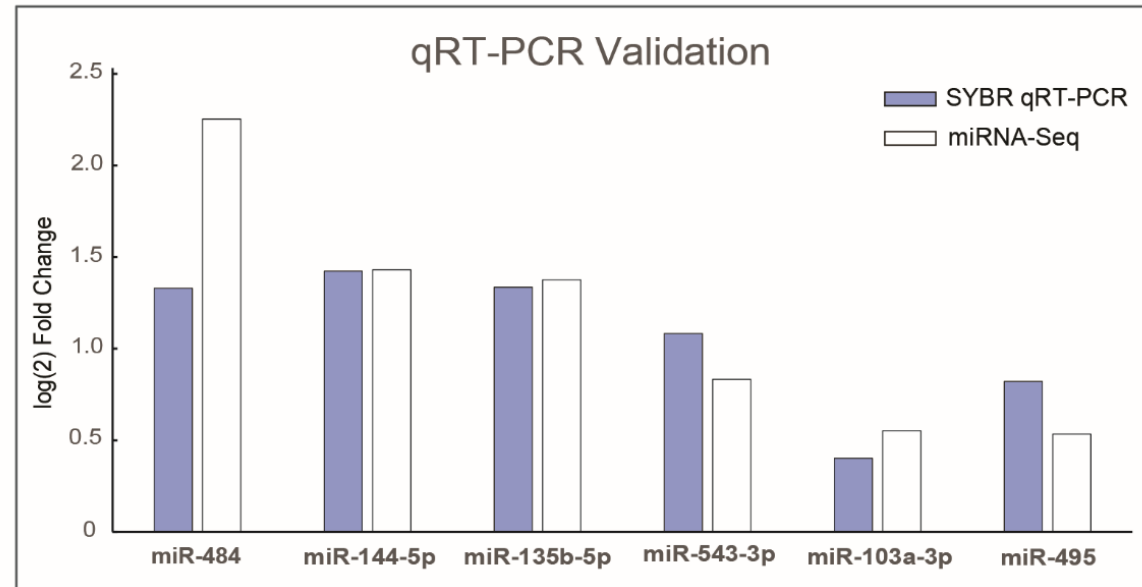


miRNA-Seq Identified Differentially Expressed microRNAs in Sera

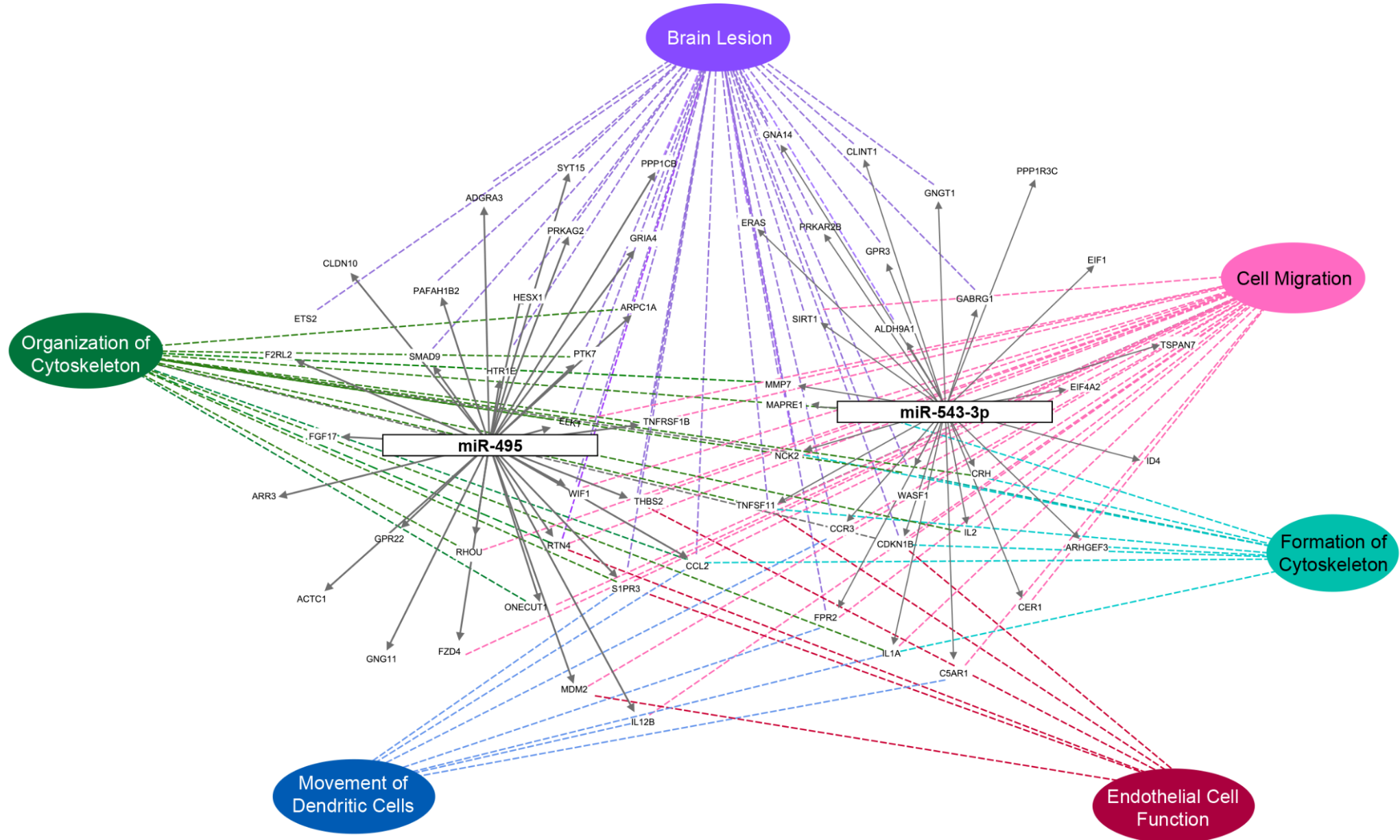


The known microRNAs are perfectly conserved in rat, mouse, and human

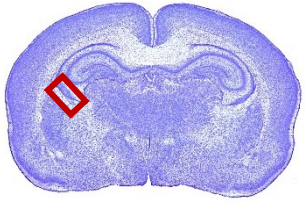
PCR Validation and Sex Differences



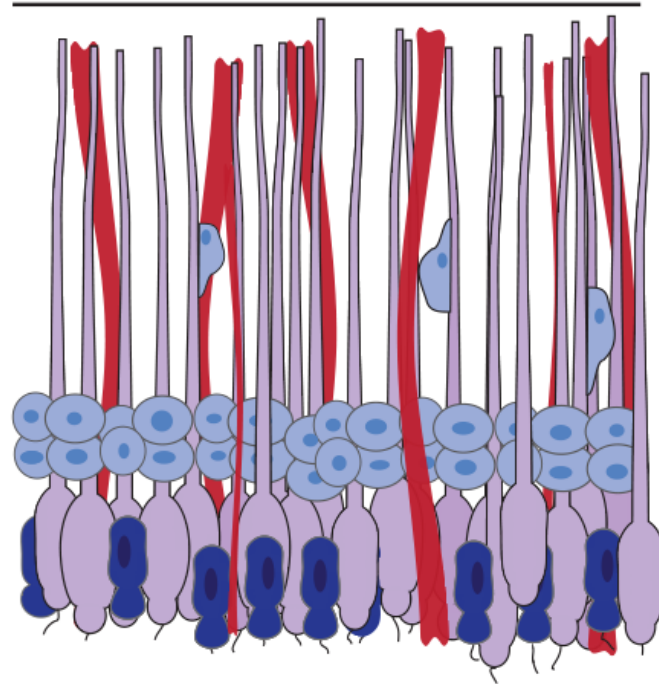
Gene Ontology Analysis of Known Serum microRNAs



Working Model: microRNAs May Bypass the Brain Barriers

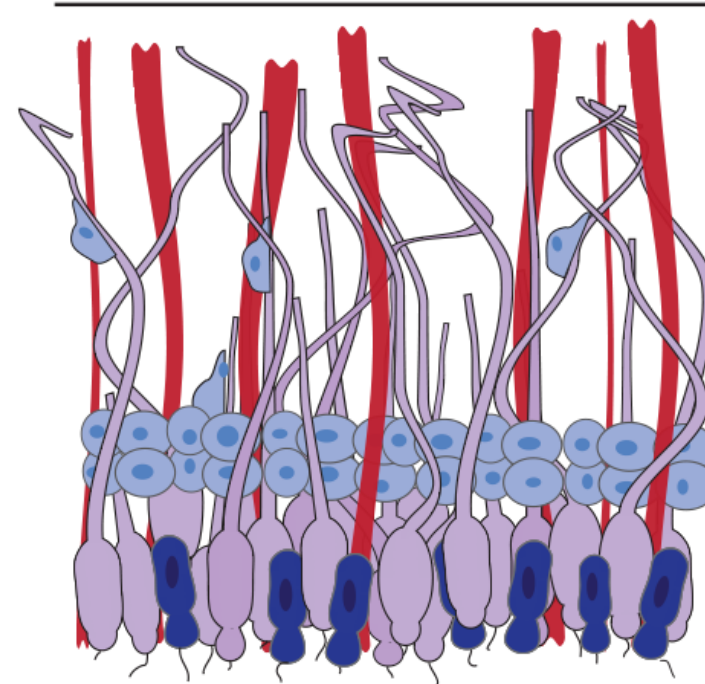


Euthyroid Neonatal Ventricle
(CSF-Brain Barrier)

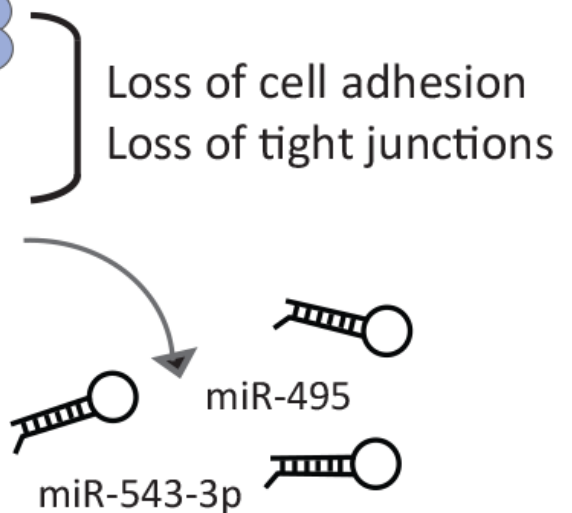


Cerebrospinal Fluid (CSF)

Hypothyroid neonatal ventricle
(CSF-Brain Barrier)

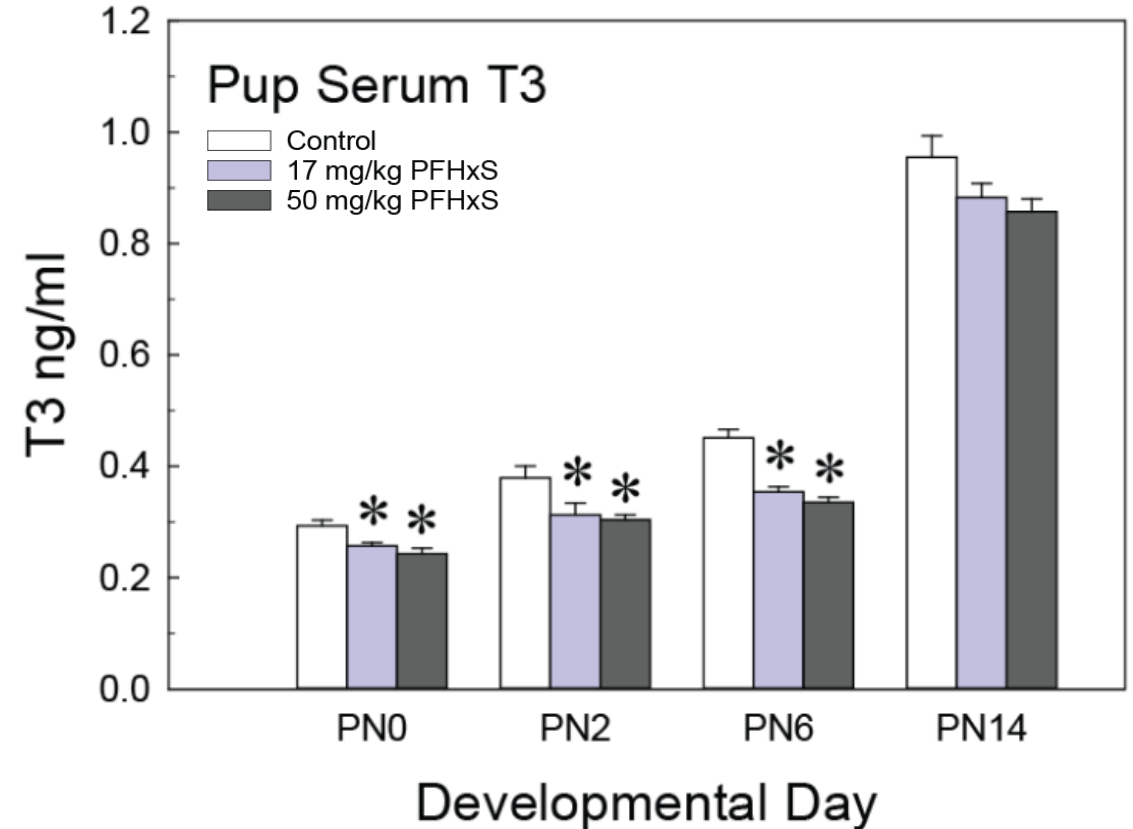
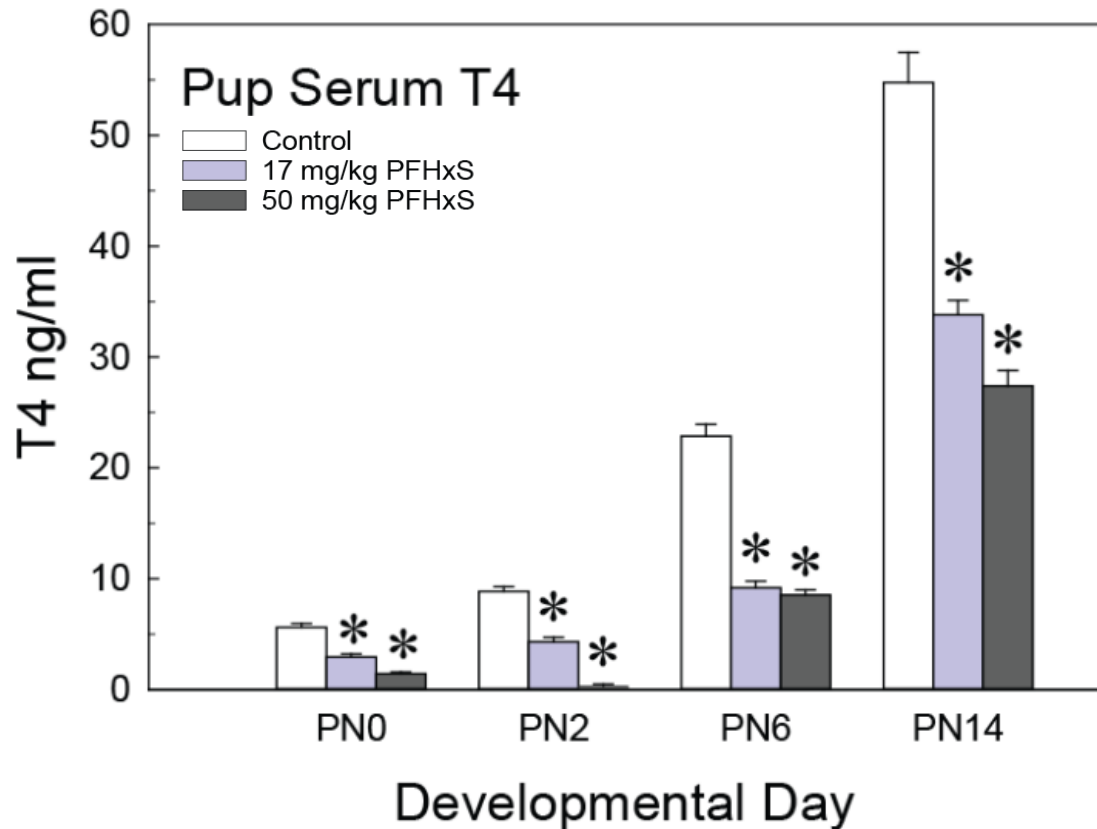
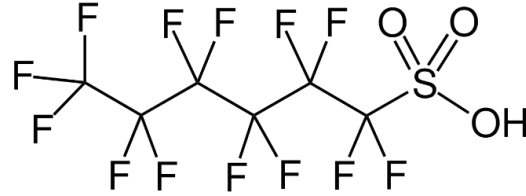


Cerebrospinal Fluid (CSF)



The Future: Analyzing These microRNAs in the Context of Environmental EDCs

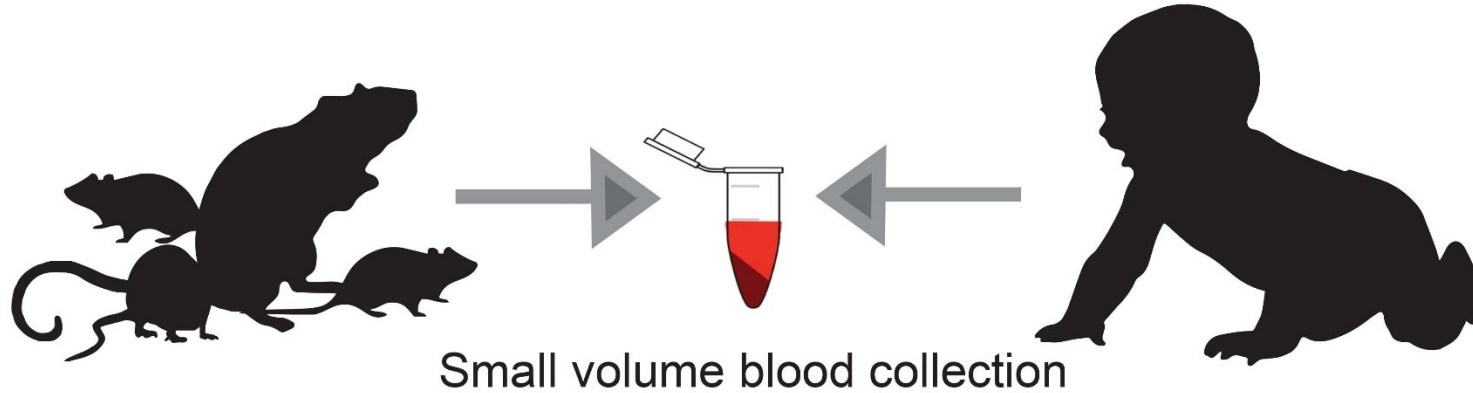
Perfluorohexanesulphonic acid (PFHxS)



The Application: A Potential Non-Invasive Biomarker

Regulatory Toxicology
OECD Tests 421, 426

Population Monitoring
Newborn Screening



Real-time PCR to screen for our discovered microRNA

Final Costs

\$50 per sample†, and can be ran in 384-well format in <8 hours

Acknowledgements

Co-Authors

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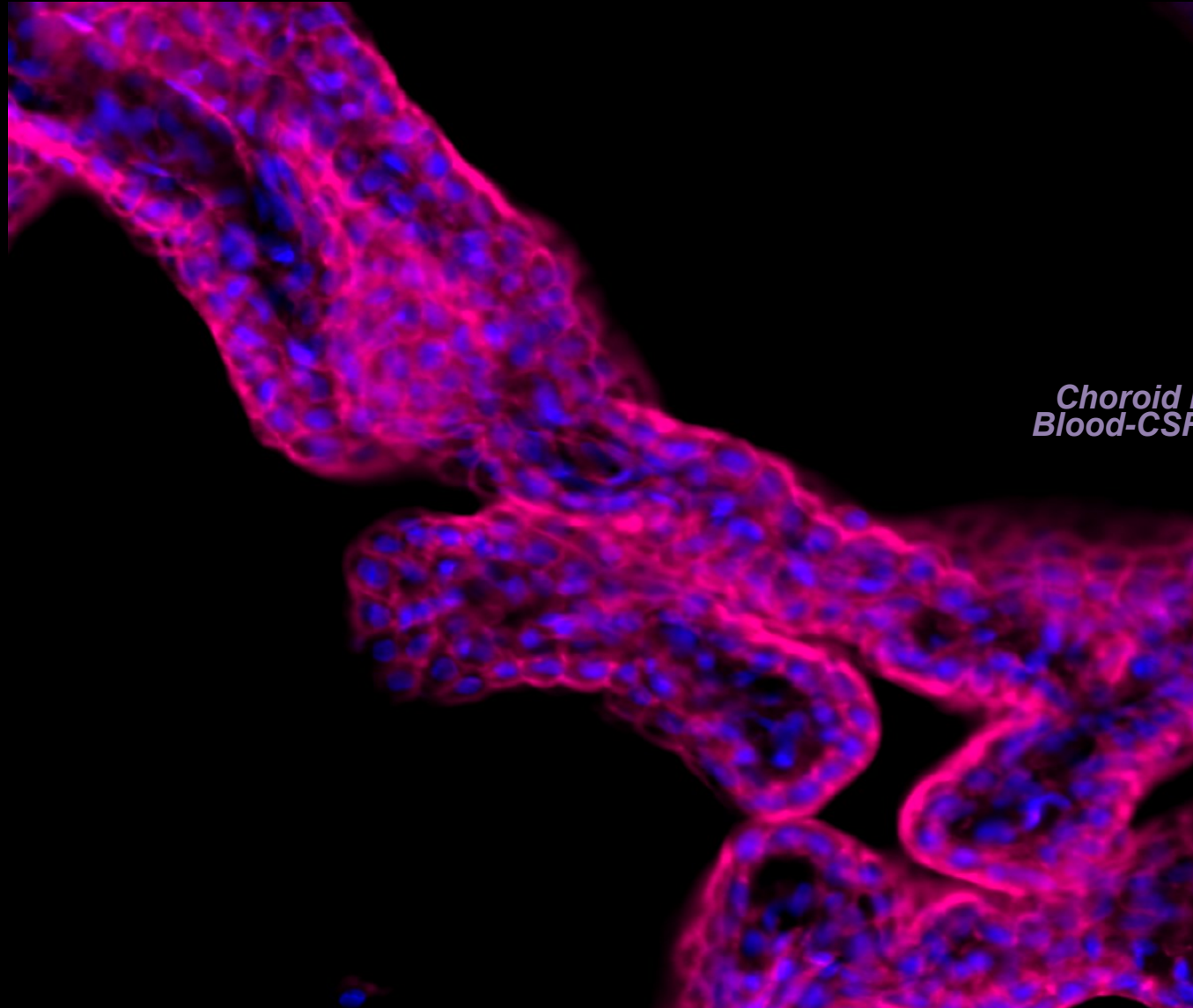
US EPA – ORD

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*Choroid Plexus
Blood-CSF Barrier*