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Classification model of blood-brain barrier development and toxicity

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Horizons and Challenges in Organotypic Culture Models for Predictive Toxicology
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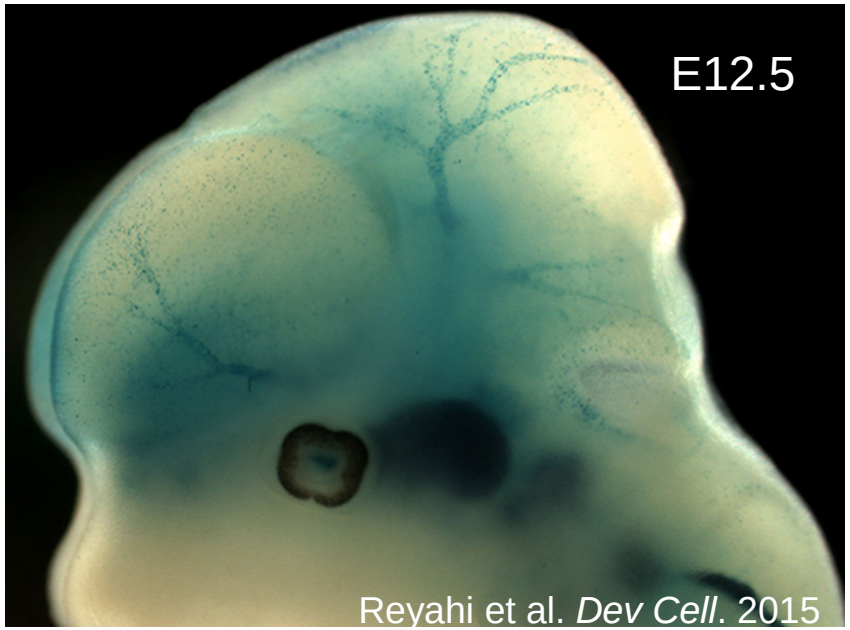
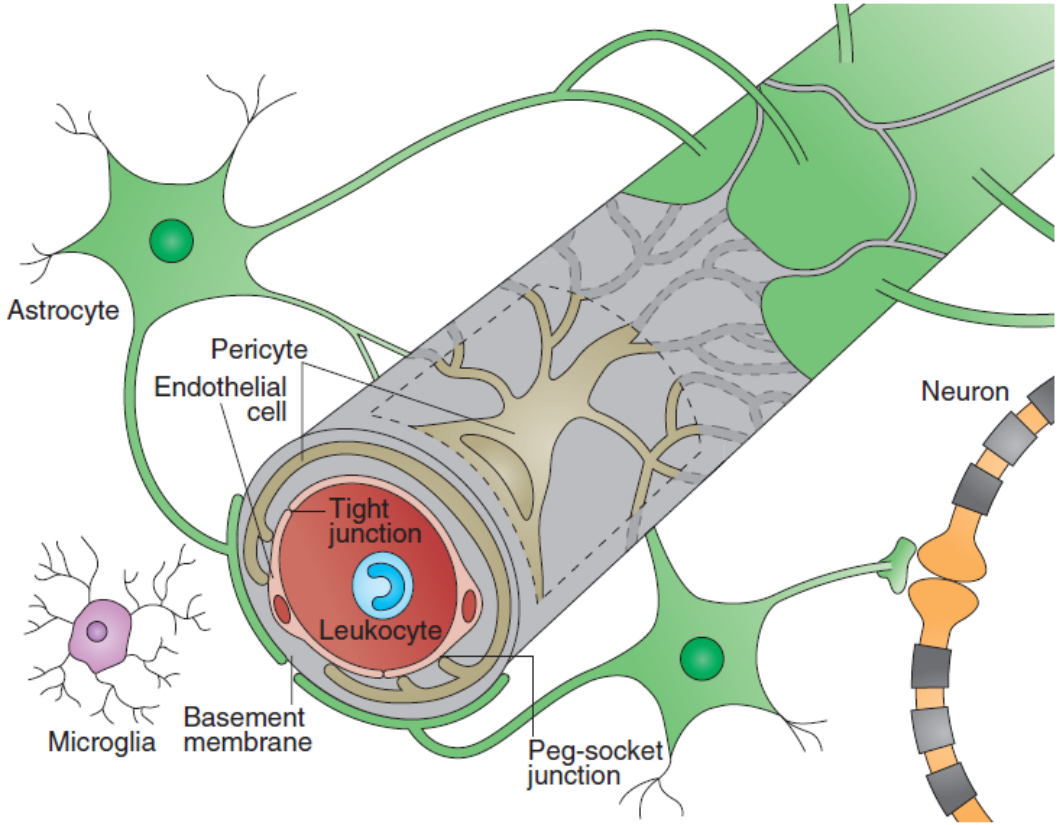
Neurovascular unit (NVU)

Hypothesis: Chemical disruption of NVU development will adversely impact blood-brain barrier (BBB) formation, and lead to abnormal brain development and function

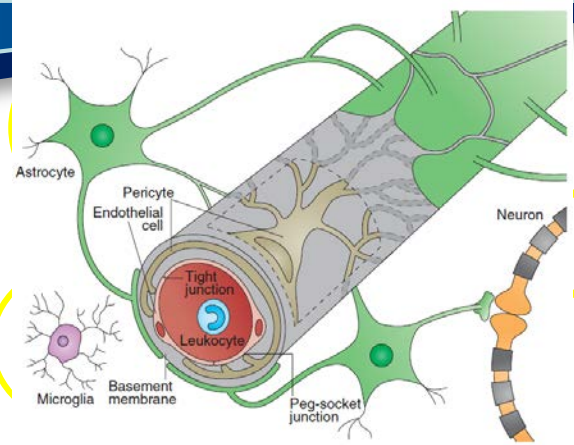


Reyahi et al. *Dev Cell*. 2015



Obermeier et al. *Nat Meth*. 2013

Biowiring diagram of NVU development



Pericyte

Microglia

Chemical

Endothelial cell

Astrocyte

NPC

Saili, et al., Blood-brain barrier development: Systems modeling and predictive toxicology (Review; *in prep*)

Overview

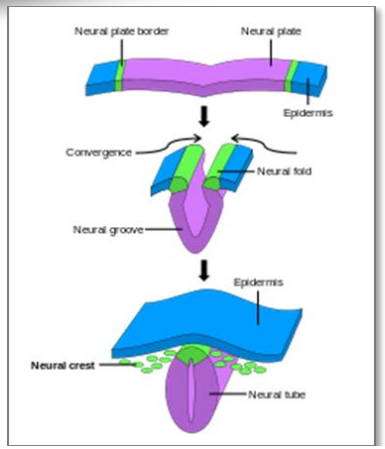
- **Objective:**
  - Identify putative biomarkers of developmental NVU disruption among ToxCast HTS assays
  - Build a pathway based predictive signature to identify putative NVU disrupting ToxCast chemicals (Neuro, Angio, both, or neither)

- **Approach**: Increase the diversity of ToxCast assays to include DevTox assessments that may represent developmental processes or toxicities
  - ArunA (neurogenic), Vala (angiogenic), STEMINA (hESC), OT (endogenesis)

NVU

Early development

Experimental Design



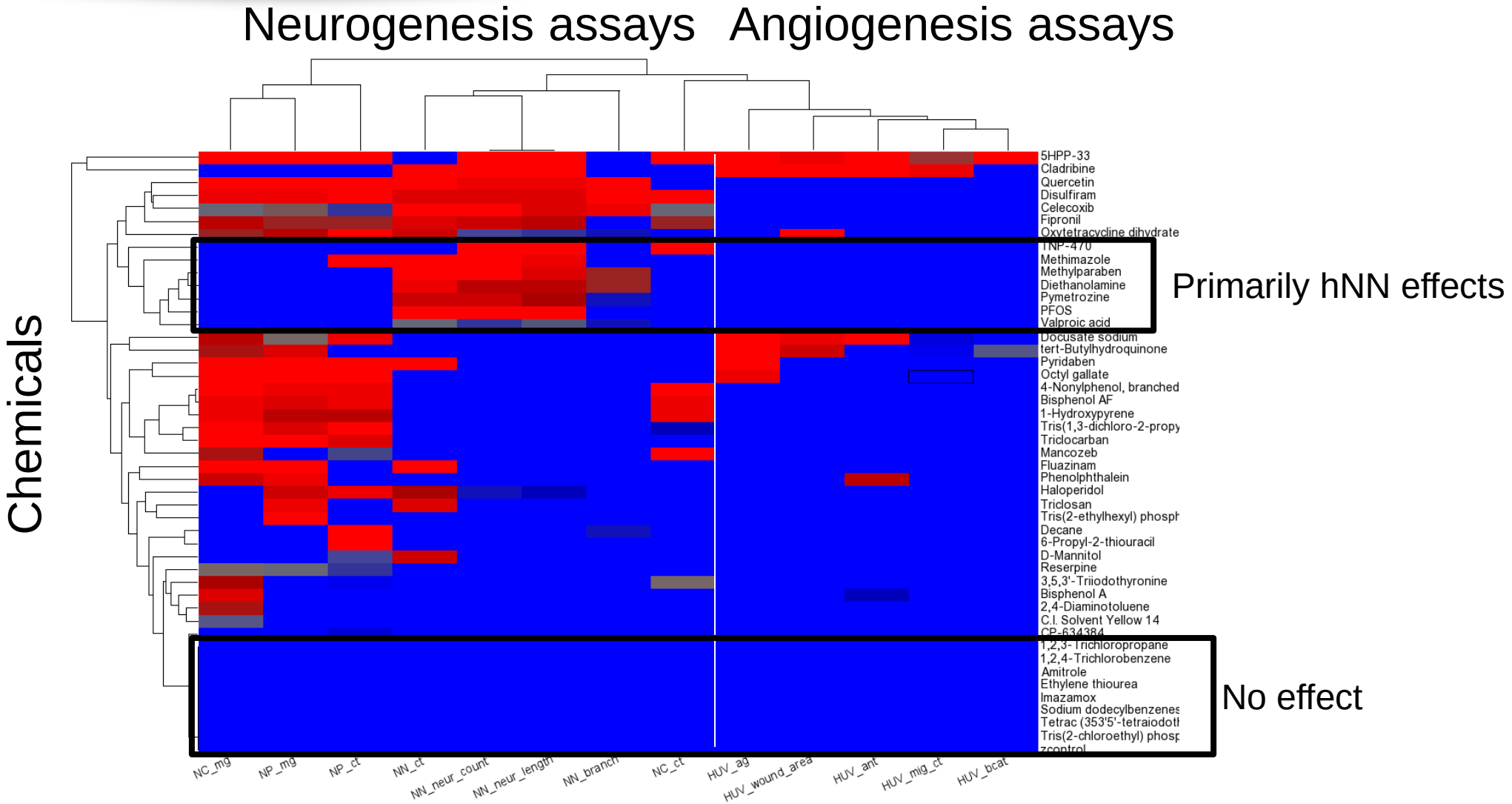
Exposures: 0.1% DMSO control
0.1 - 100 µM (x5 concentrations)

46 ToxCast chemicals

- 5HPP-33 (ref.)
- TNP-470 (ref.)
- flame retardants
- pesticides
- pharmaceuticals
- plasticizers
- pTDCs
- pVDCs

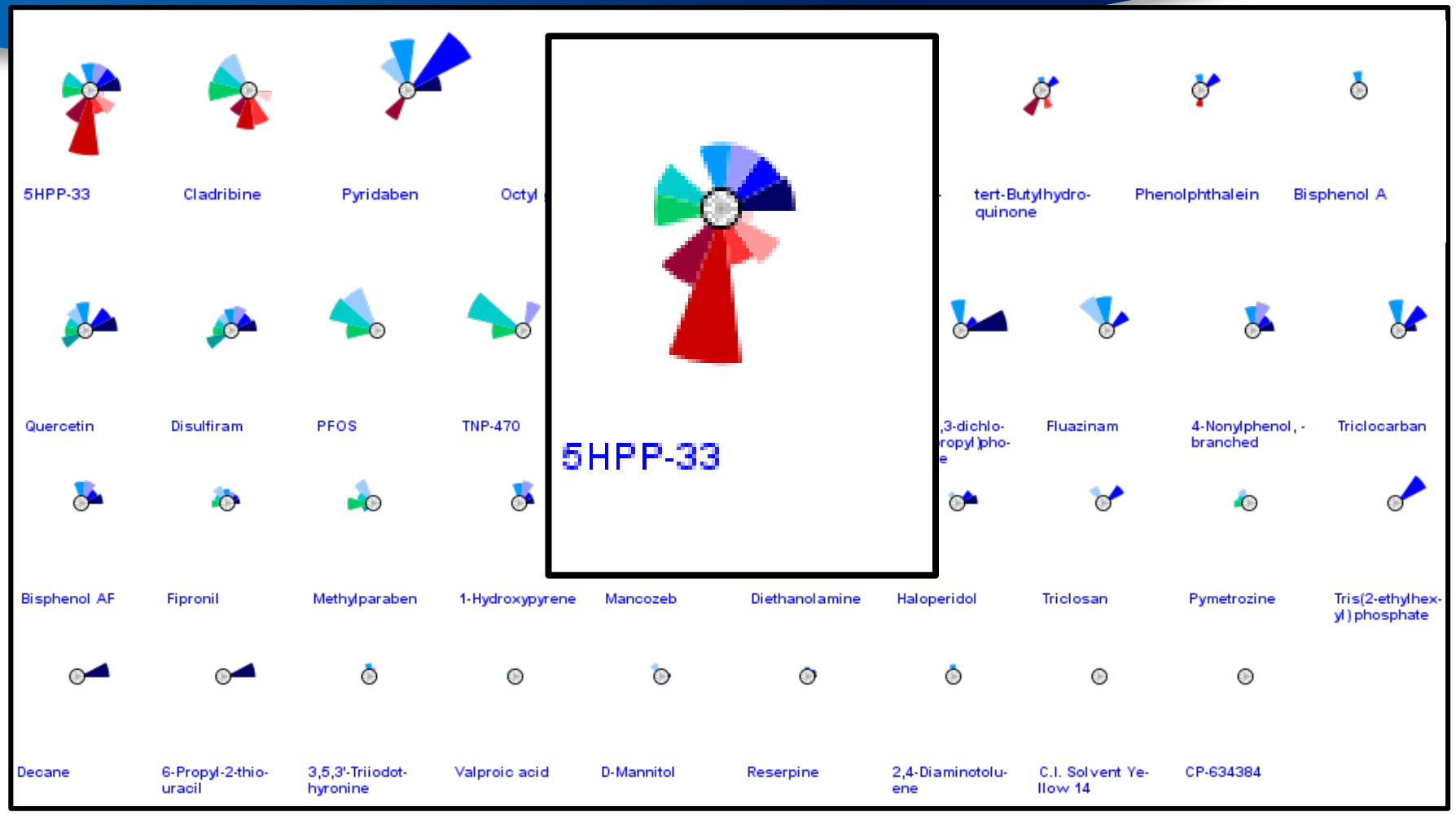
Platform	Cell type	Assay	# of Endpoints
Neurogenesis	hESC (H9)	hNP, hNC, hNN: Cell titer	2
		hNP, hNC, hNN: Cell migration/proliferation	2
		hNP, hNC, hNN: Neurite outgrowth	4
Angiogenesis	HUVEC (iPSC)	Tubule formation agonist	1
		Tubule formation antagonist	1
		Cell migration/proliferation	3

Positive ‘hits’ across 13 assays

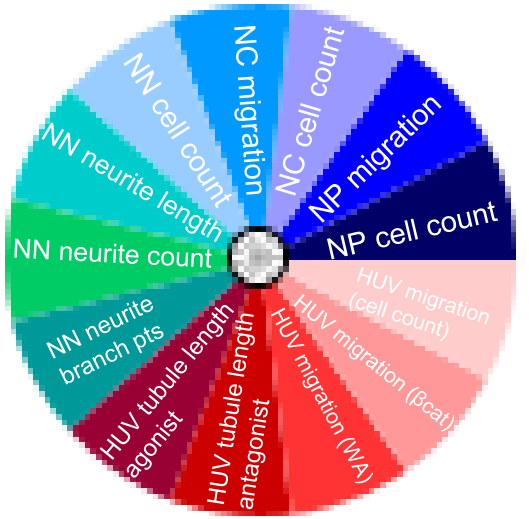


ToxPi ranking of 46 Aruna/Vala chemicals

Angio X9



Neurogenesis Assays x8



Angiogenesis Assays x5

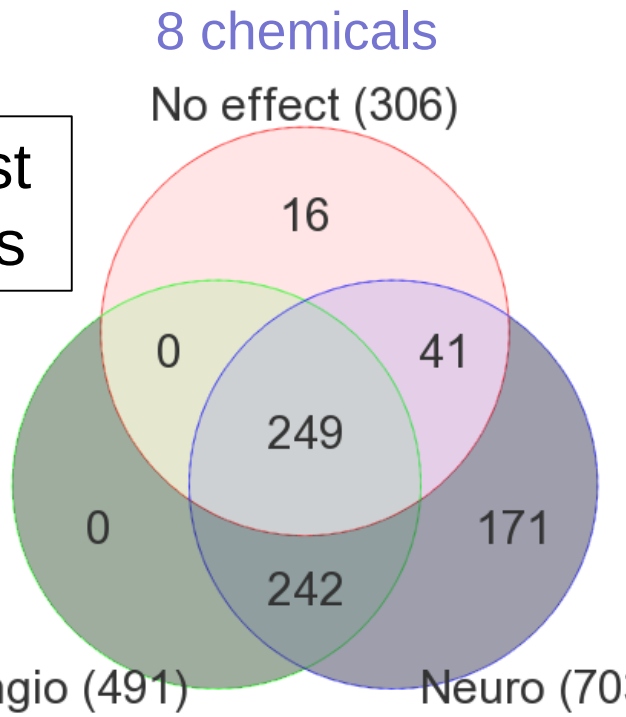
No Effect x8

AC_{20} normalized to control shown as percentage of max potency across all assays

http://comptox.unc.edu/toxpi.php

Building a pathway-based prediction model of NVU toxicity

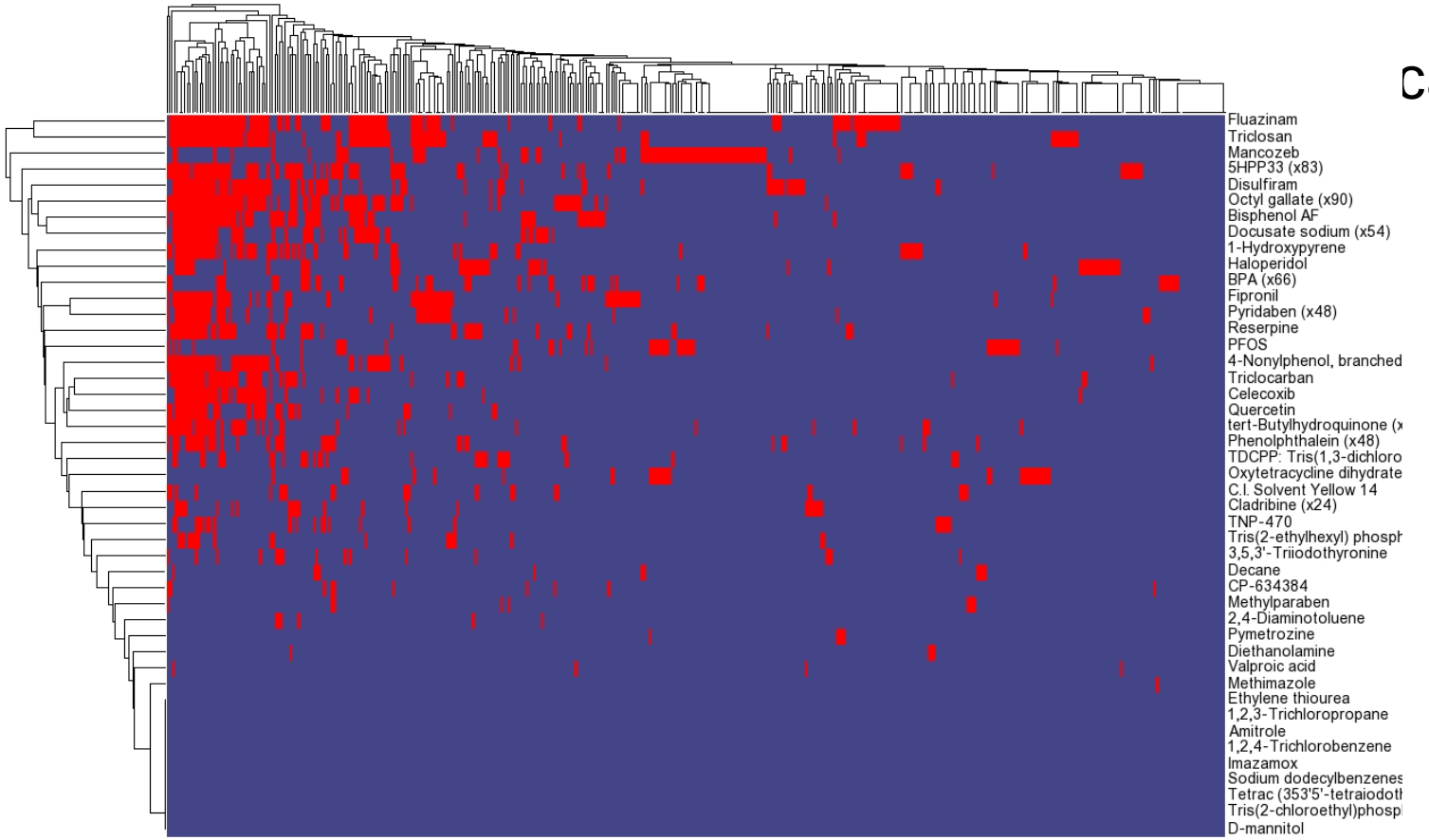
ToxCast assays



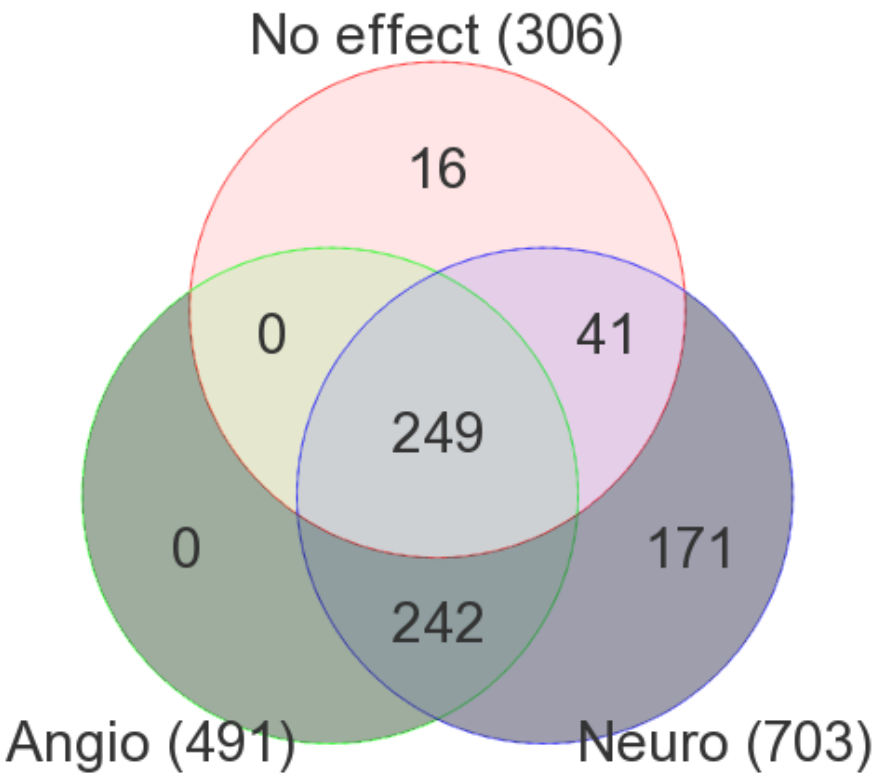
8 chemicals

9 chemicals

38 chemica



Identifying signaling pathways that may mediate toxic effects on the NVU



413 ToxCast assays
(assay_component_endpoint_name)

↓

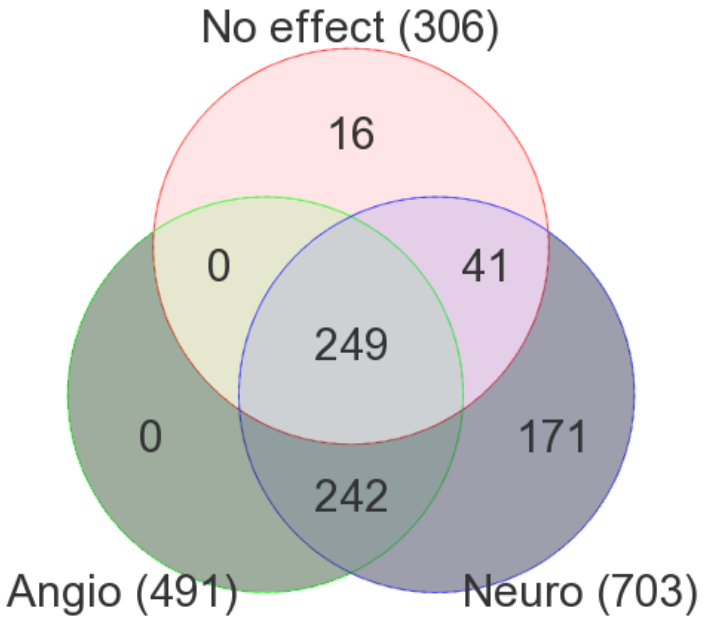
194 associated genes
(tech_target)

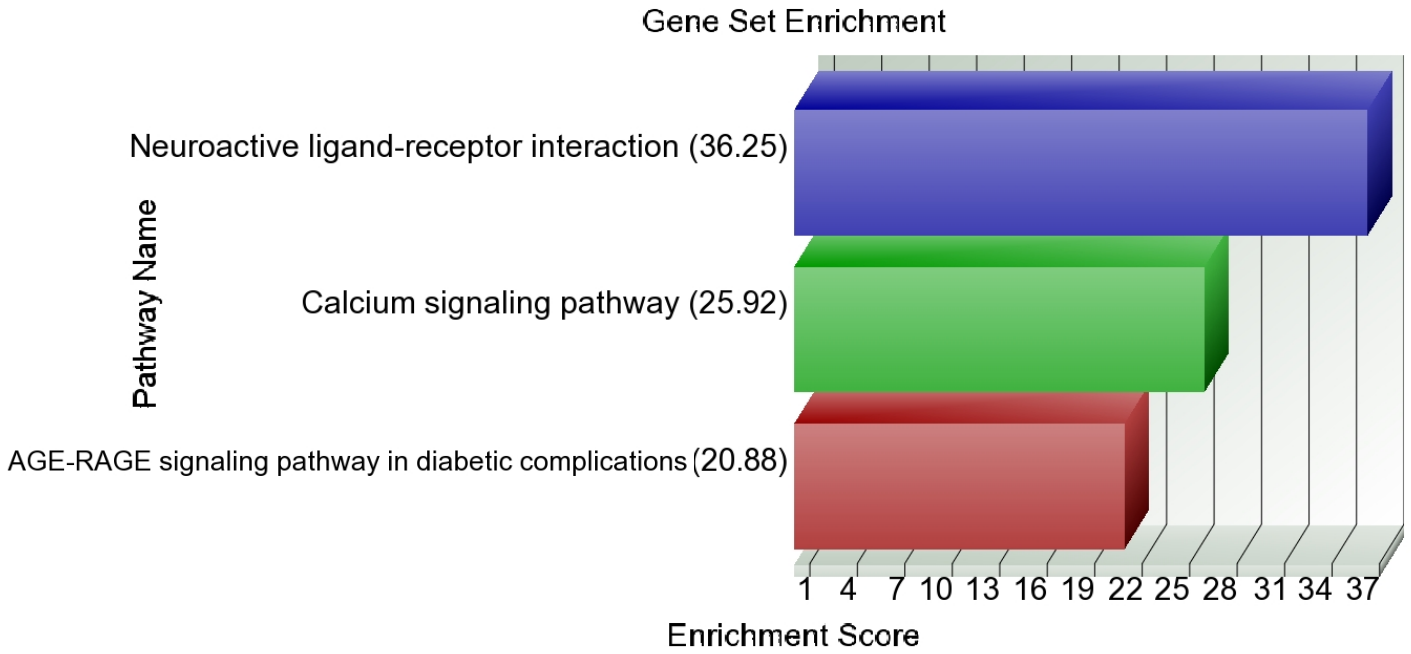
↓

KEGG pathway analysis

Signaling pathways that may mediate toxic effects on the NVU

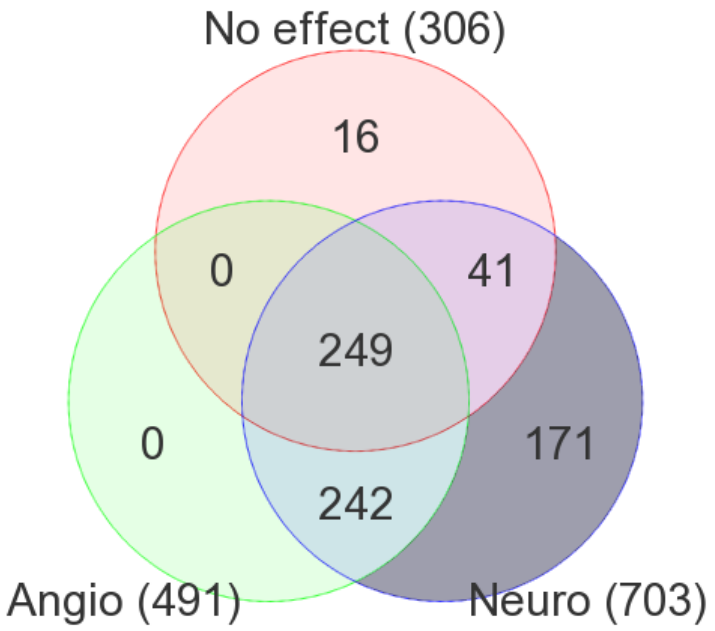
Angiogenesis and/or neurogenesis

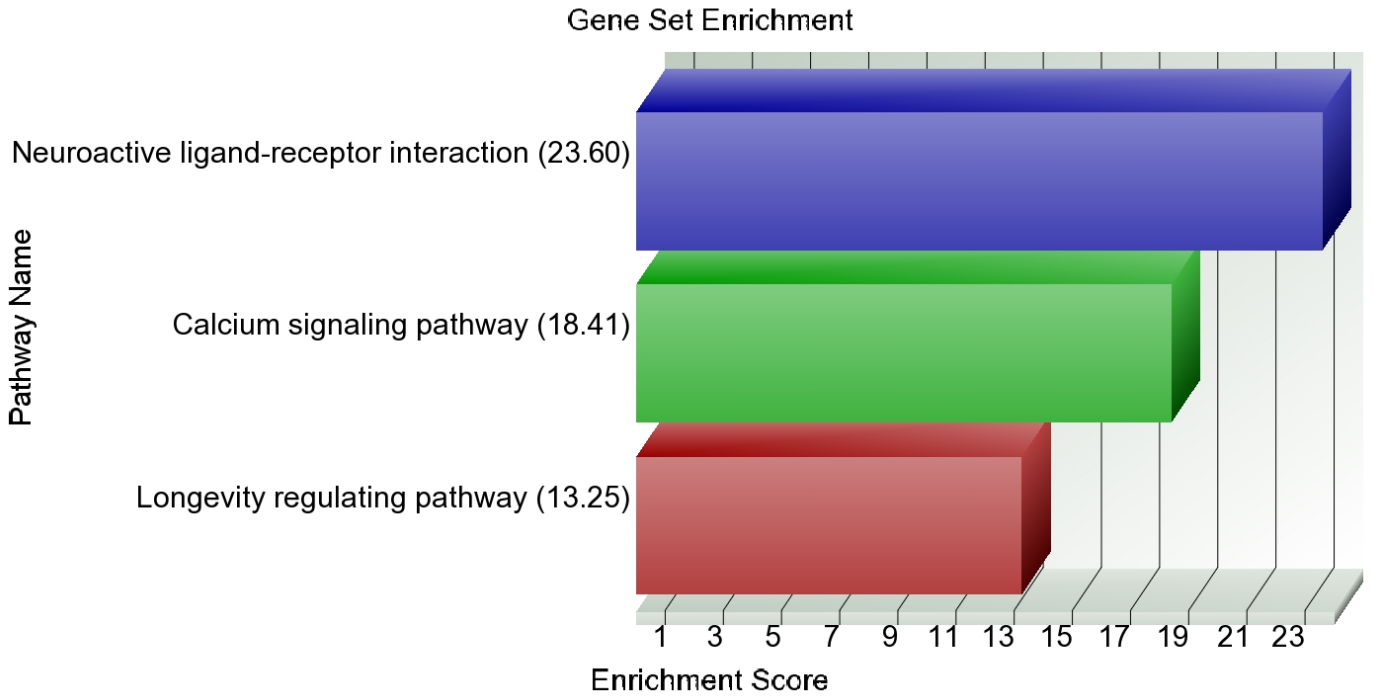




Signaling pathways that may mediate toxic effects on the NVU

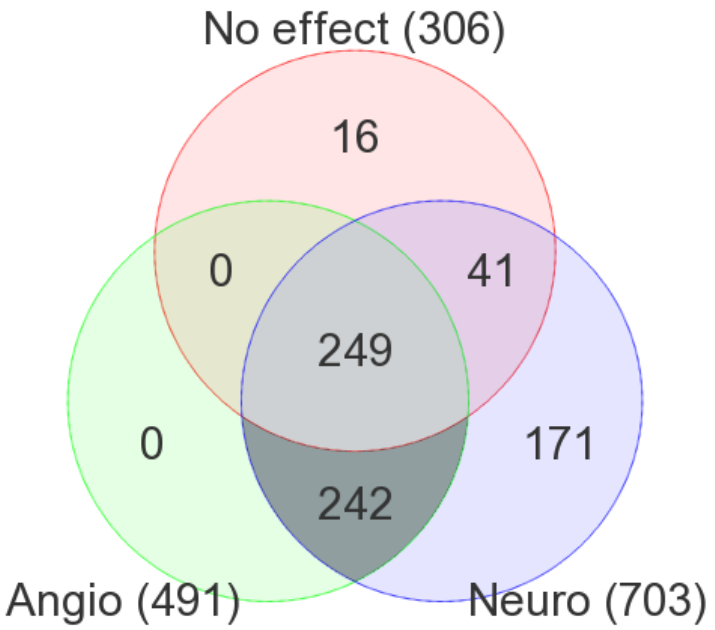
Neurogenesis

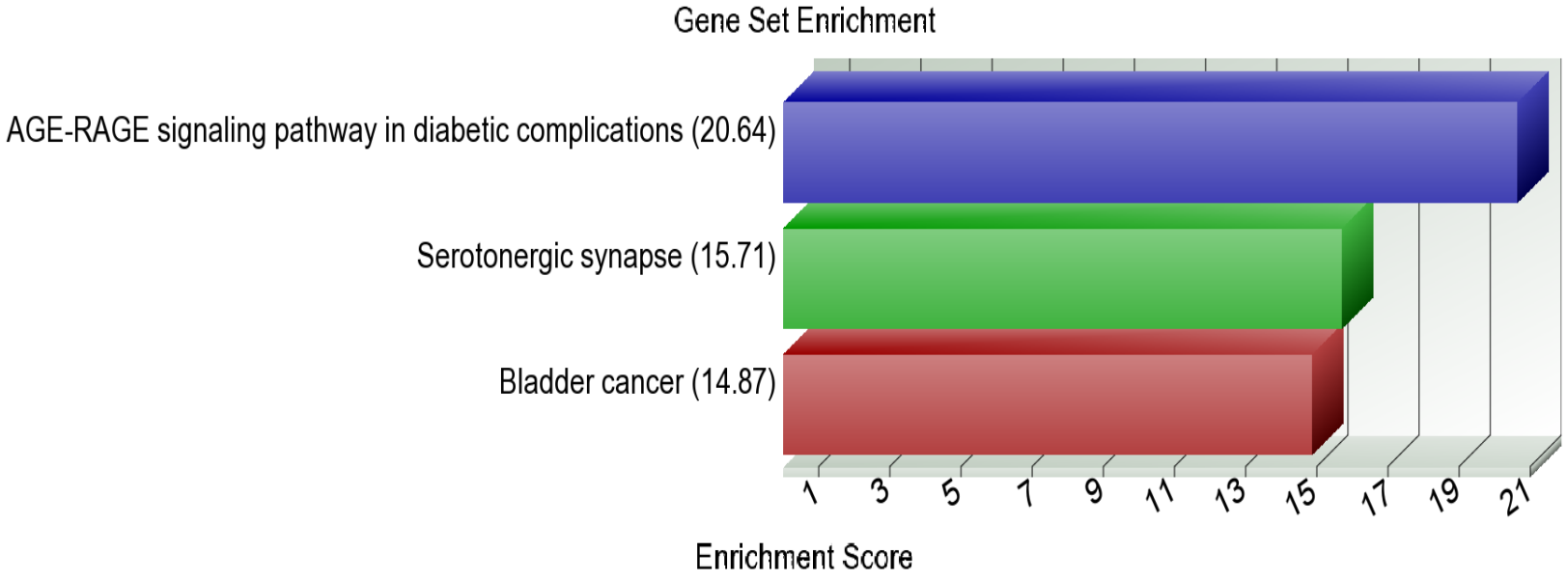




Signaling pathways that may mediate toxic effects on the NVU

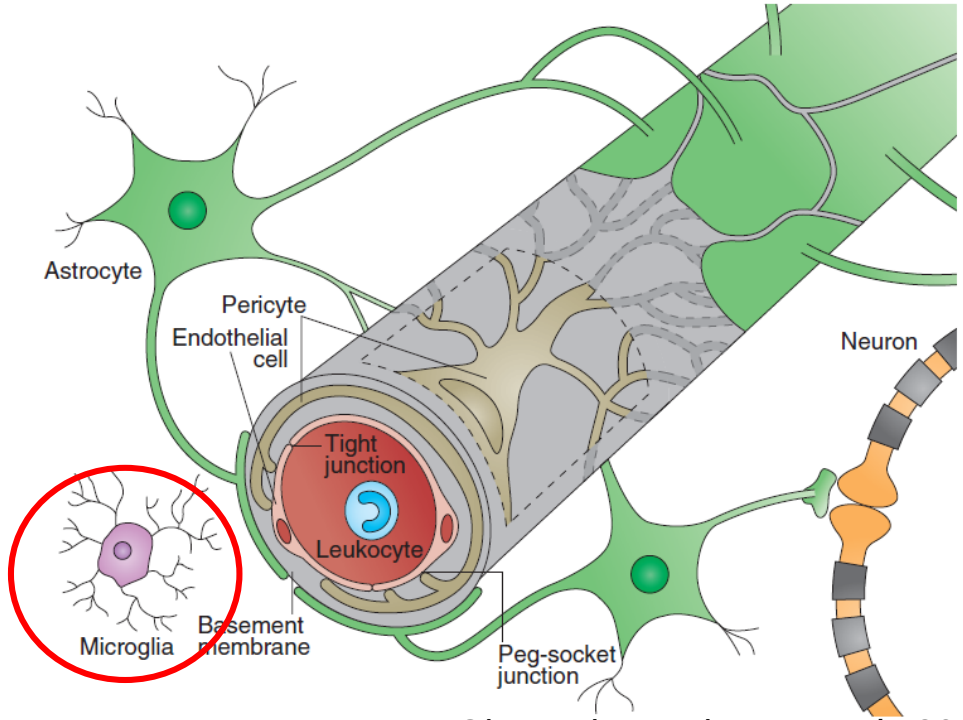
Angiogenesis and neurogenesis





Top Pathway – Top Chemical

Hypothesis: Chemical disruption of NVU development will adversely impact blood-brain barrier (BBB) formation, and lead to abnormal brain development and function



Obermeier et al., *Nat Meth*. 2013

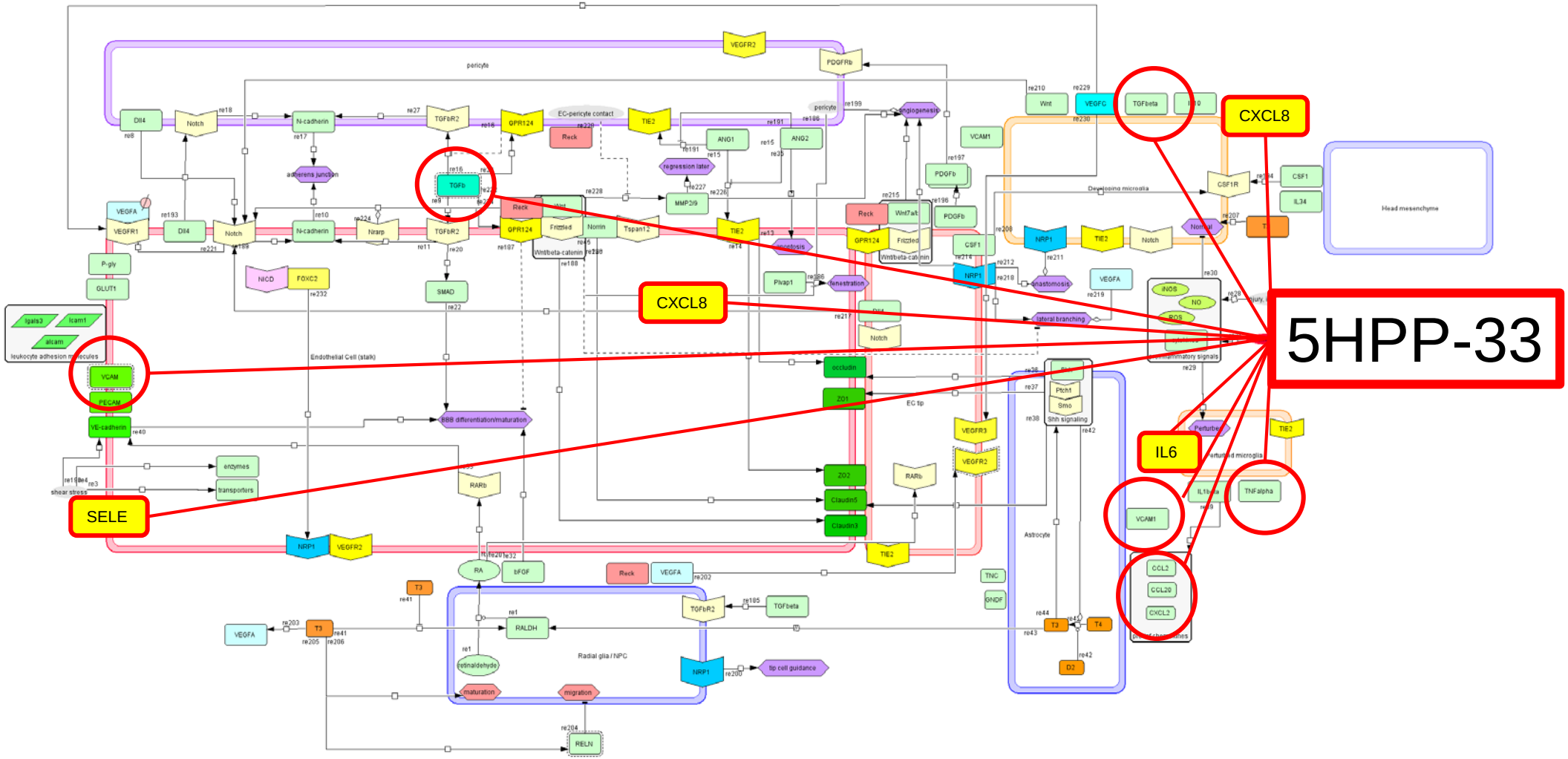
AGE-RAGE signaling pathway in diabetic complications

Immune response	Adhesion	Cell growth/ survival	Coagulation	ECM remodeling
CCL2	SELE	JUN	F3	MMP2
CXCL8	VCAM1	PI3CA	THBD	
IL6	ICAM1	MAPK1		
TNF				
TGFB1				
NFKB1				

(genes from list in pathway)

5HPP-33 gene targets based on ToxCast assay hits

How might microglia mediate impacts on BBB development and function?



Future work

- Focus on role of microglia
  - Cell transducers of inflammatory signals
  - Role in remodeling/anastomosis during angiogenesis
- Test more ToxCast chemicals in these platforms
- Prioritize chemicals for OCM *in vitro* testing

Acknowledgements

- Tom Knudsen (mentor)
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http://www2.epa.gov/sites/production/files/2015-08/documents/virtual_tissue_models_fact_sheet_final.pdf

Thank You

Questions?